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REVIEW

Helminths and intestinal barrier function

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ABSTRACT

Approximately one-sixth of the worlds' population is infected with helminths and this class of parasite takes a major toll on domestic livestock. The majority of species of parasitic helminth that infect mammals live in the gut (the only niche for tapeworms) where they contact the hosts' epithelial cells. Here, the helminth-intestinal epithelial interface is reviewed in terms of the impact on, and regulation of epithelial barrier function, both intrinsic (epithelial permeability) and extrinsic (mucin, bacterial peptides, commensal bacteria) elements of the barrier. The data available on direct effects of helminths on epithelial permeability are scant, fragmentary and pales in comparison with knowledge of mobilization of immune reactions and effector cells in response to helminth parasites and how these impact intestinal barrier function. The interaction of helminth-host and helminth-host-bacteria is an important determinant of gut form and function and precisely defining these interactions will radically alter our understanding of normal gut physiology and pathophysiological reactions, revealing new approaches to infection with parasitic helminths, bacterial pathogens and idiopathic auto-inflammatory disease.

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Introduction

The epithelial lining of the gut is the interface between the body proper (i.e. lamina propria and mucosa) and the gut lumen and is exposed to a myriad of antigens, a vast microbiota, and, more transiently, a variety of protozoan and helminth parasites. Indeed, the gut is a preferred niche for helminth parasites, providing a sheltered environment, a soft mucosal surface that can be readily abraded to gain access to a rich microvasculature for blood-feeders, and a steady stream of host-ingested nutrients.

The barrier function of the gut is the net outcome of the physical character of the epithelial layer, secreted elements (i.e., HCl, mucus, IgA, anti-microbial peptides, electrogenic ion secretion to create a driving force for directed water movement), and the mucosal immune system that would, for example, attack bacteria that enter the mucosa to prevent their systemic dissemination.[1,2] The mobilization of mucosal immunity in the context of enteric helminth infection is multi-faceted, complex and intriguing but a comprehensive discussion of such is beyond the scope of this commentary: the reader is referred to excellent recent reviews of this topic.[3-8] For the purposes of this review we will use 'epithelial permeability' to denote studies that address the physical properties of the epithelial layer and 'barrier function' is used as a more encompassing term that refers to the many extrinsic (e.g. mucus, IgA, commensal microbiota) components of the intestinal barrier.

Nematodes can cause significant damage in the small or large intestine of their mammalian hosts that would be a significant breech in the epithelial barrier. Recognizing that physical damage caused to the epithelium by tissue- or blood-feeding nematodes or trematodes can increase epithelial permeability, we will not belabor this point, other than to note that secondary bacterial infection, or sepsis, is not a common clinical feature of infection with gastrointestinal nematodes, likely due to the combination of an effective mucosal immune system and the recuperative power of the epithelium. Here, we briefly discuss helminths as a phylum, the nature of the epithelial barrier and then how infection with helminths can affect this directly or indirectly via host immunity.

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Helminth parasites

Helminth parasites are endo-parasites that are classified into 2 major groups: the nematodes (round worms) and platyhelminths (flatworms), with the later subdivided into trematodes (flukes) and cestodes (tapeworms) (Fig. 1A). Parasitic helminths typically exhibit complex life-cycles that involve one or more intermediate hosts for juvenile stages of the worm and a definite host where adults reach sexual maturity (host specificity is the basis of parasitism and while each species of parasite has a preferred definitive host there is promiscuity in the system, with implications for zoonotic disease[9]). It is also safe to say that for every vertebrate species at least one parasitic helminth has evolved. The successful parasite must: (a) recognize its' preferred host and niche therein; (b) be capable of maintaining itself in the preferred nice and be

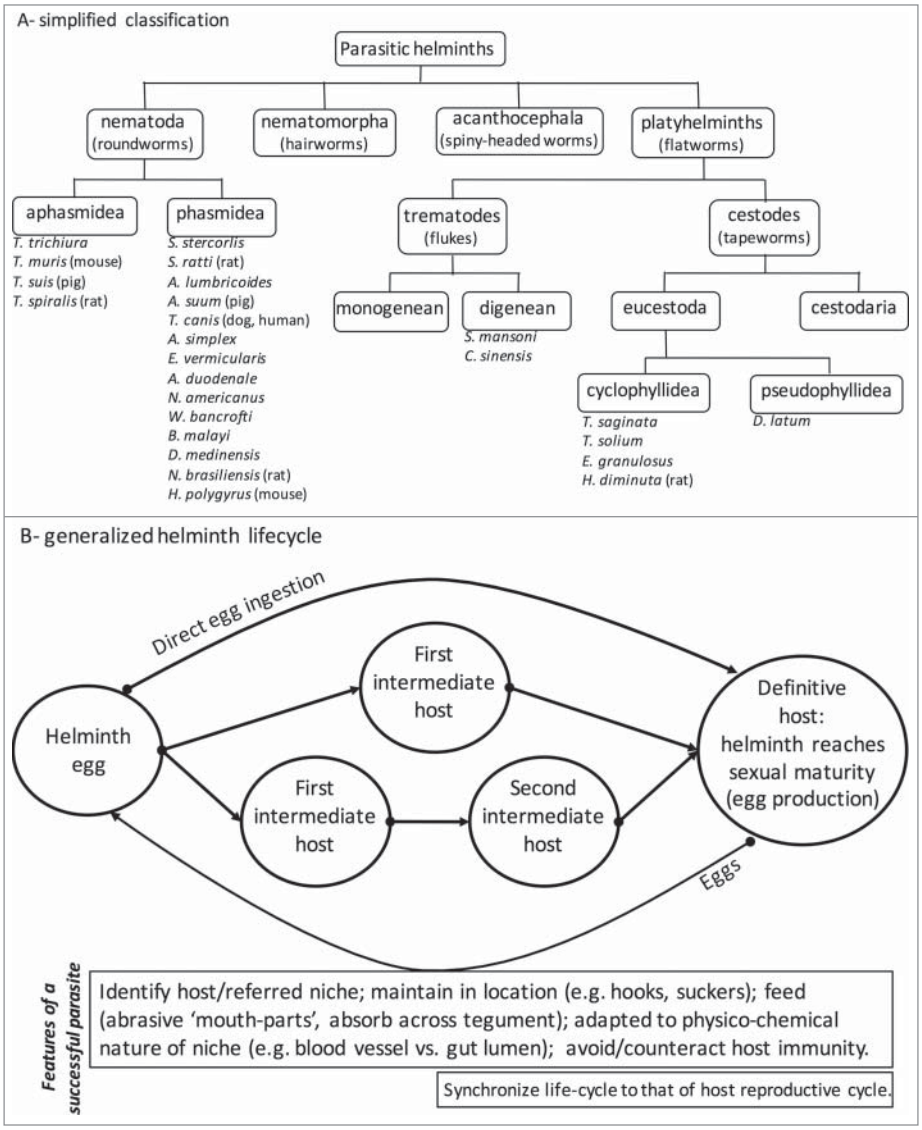

Figure 1. Panel A provides a simplified phylogenic overview of parasitic helminths with typical examples of species that infect humans or are common in laboratory studies (definitive host in parentheses). Detailed classification can be found in "Introduction to Animal Parasitology" by J.D. Smyth, Cambridge University Press, 1994: *Anasakis simplex*, *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Ascaris suum*, *Brugia malayi*, *Diphyllobothrium latum*, *Clonorchis sinensis*, *Dracunculus medinensis*, *Echinococcus granulosus*, *Enterobius vermicularis*, *Heligmosomoides polygyrus*, *Hymenolepis diminuta*, *Necator americanus*, *Nippostrongylus brasiliensis*, *Schistosoma mansoni*, *Strongyloides stercorlis*, *Strongyloides ratti*, *Taenia saginata*, *Taenia solium*, *Toxocara canis*, *Trichinella spiralis*, *Trichuris trichiura*, *Trichuris muris*, *Trichuris suis*, *Wuchereria bancrofti*). In Panel B the generic complexity of the lifecycle of parasitic helminths is shown along with an inset box presenting essential features of successful parasites (synchronization of the parasite life-cycle with host reproductive cycle is not critical but could be advantageous).

adapted to the physico-chemical conditions of that environment; (c) obtain nutrition from the host; (d) avoid or counteract the host attempts to eradicate it; and (e), while not essential, it is beneficial if the parasite can synchronize egg production with the hosts' reproductive cycle (Fig. 1B).

The life-span of a helminth in its definitive host can vary considerably from 2–3 weeks in the case of the nematode *Nippostrongylus brasiliensis* in the rat to 24 months for the cestode *Hymenolepis diminuta* in the rat (basically the life-span of the rat). A similar spectrum can be applied to humans where gastrointestinal nematodes (e.g., *Ascaris lumbricoides*), tapeworms (e.g., *Echinococcus granulosus*) and trematodes (e.g., *Schistosoma mansoni*) can co-exist with the host for years. The longevity of the helminth-mammalian host relationship has lead to the suggestion that these parasites have exerted a major evolutionary selection pressure on host immunity.[10]

Endemic in developing regions of the world, it is estimated that ~1.5–2.0 billion people suffer from infection with parasitic helminths with effects ranging from loss of nutrition, to anemia, to gastrointestinal upset, to stunted growth, to loss of organ function (e.g., blindness with *Onchocerca volvulus* (nematode), elephantiasis with *Brugia malayi* (nematode) and spleno-hepatomegaly with *S. mansoni*) and fatality (neurocysticercosis from *Taenia solium*): death from infection with helminth parasites is the extreme and mild/significant morbidity is the norm. A similar situation exists for domestic livestock, a problem in developing and developed regions of the world, where infection with helminth parasites takes a tremendous toll on productivity (e.g., meat yields),[11] and resistance to anthelminthics is increasing alarmingly.[12] Thus, while helminth biology may be unfamiliar to the reader, helminths are a ubiquitous component of our ecosystem.

The nature of the intestinal barrier

For antigens, microbes or parasites to enter the mucosa several barriers need to be negotiated. If antigens and organisms can run the gauntlet of host-derived stomach acid, proteases, mucus and anti-microbial peptides that are produced throughout the entire gastrointestinal tract, and a washer/sweeper event caused by increased water movement into the lumen of the gut combined with increased peristalsis,[13] they are then faced with the physical barrier of the epithelium (and after that an extensive mucosal immune system). In the absence of ulceration and damage to the epithelial layer, soluble/particulate antigen can cross the epithelium via uptake by specialized microfold (M) cells that overlay lymphoid aggregates,[14] capture by dendritic cell processes that extend between adjacent epithelial cells into the gut lumen,[15] or traversing the epithelium via the paracellular and transcellular pathways.

Paracellular permeability refers to the flux of material between adjacent epithelial cells, where it must pass the apical junctional complex composed of the tight junction (TJ), the adherens junction and desmosomes: once established the TJ is the rate-limiting step in paracellular permeability.[16] The paracellular space is in fact a continuity between the gut lumen and the mucosa and is often considered in terms of a pore pathway, a high capacity, size- and charge-selective route, and a lower capacity leak pathway that allows entry of larger molecules (e.g., 4 kDa dextrans).[17] Research over the last 25-years has defined the molecular composition of the TJ, its' dynamic nature, the mechanisms that regulate its' opening and the impact of microbial pathogens (including protozoans) on enteric epithelial paracellular permeability.[18]

Briefly, the TJ is composed of many transmembrane proteins: TJ-associated MARVEL (MAL and related proteins for vesicle trafficking and membrane) proteins (TAMPs) include occludin, tricellulin (restricted to tri-cellular junctions) and MarvelD3; claudins, a 27-member family, are single-spanning transmembrane proteins that interact homotypically with the extracellular domain of claudins on neighboring cells; and, single transmembrane immunoglobulin-like junction adhesion molecules (JAMs).[17] These proteins reside in cholesterol-rich, detergent-insoluble lipid domains of the plasmalemma and are connected to a peri-junctional ring of actin and myosin via the scaffolding proteins zona occludens (ZO)-1, -2 and -3. The function of TJ and ZO proteins is phosphorylation dependent.[19,20] Thus, TJ opening and closing (i.e., the permeability characteristics or epithelial permeability coefficient) is dependent on: (1) the molecular composition of the tight junction [occludin was the first TJ protein identified[21] but it has emerged that it is dispensable for TJ formation and that claudins are the critical players in TJ permeability[22] where increased expression can enhance the barrier or conversely increase TJ permeability via expression of the pore-

forming claudin-2[23] (and claudin 15) (note that interleukin-13 that is mobilized in response to helminths increases claudin-2 expression[24])]; (2) phosphorylation status of the TJ proteins; (3) cell membrane fluidity;[25] and, (4) contraction/relaxation of the actinomysin ring via control of the F-actin cytoskeleton and the balance of myosin light chain kinase (MLCK) and myosin light chain phosphatase activity (MLCP).[26]

Intuitively, transcytosis through an enterocyte (i.e., the transcellular pathway) would be a more difficult and hazardous route for lumen-derived material to breech the epithelial barrier. Yet, evidence is accumulating demonstrating that transcellular permeation of antigen and microbes can be increased in epithelial monolayers exposed to metabolic stress, inflammatory cytokines (e.g., interferon-γ) and bacterial pathogens such as *Campylobacter jejuni*.[27-29] While helminths are too big to reside within an epithelial cell this should not deter consideration of the transcellular pathway as a route by which helminth-derived antigen could enter the body.

Why consider epithelial permeability in the context of infection with enteric helminth parasites?

This question needs to be considered from helminth and host perspectives. With respect to tissue- and/or blood-feeding helminths, destruction of the epithelial barrier is essential for survival. Evoking a 'washer/sweeper' event would assist the caudal movement of helminth eggs to facilitate dissemination and hence continuation of the life cycle: infection of the intestine with parasitic nematodes (e.g., *Trichinella spiralis*) is associated with altered neuro-muscular function and electrogenic Cl^- flux into the gut lumen.[30] At the same time a "washer/sweeper" effect could contribute to expulsion of the worm burden alleviating any detrimental effect of a parasitized gut:[13,31] this can be an example of the elegant co-evolution that characterizes the host-parasite relationship – the host wins by removing the parasite and the parasite wins by completing its' life cycle.

A similar argument can be advanced for the passage of helminth-derived antigens or excretory/secretory (E/S) products into the mucosa. With a parasite such as the tapeworm *H. diminuta* that lacks teeth, hooks and abrasive structures and causes no overt damage to the gut, the host most likely recognizes the presence of the worm by epithelial or immune cell detection of worm products. This can result in the release of alarmins from the enterocyte (e.g., interleukin (IL)-25), the promotion of T helper-2 (TH2) type immunity and upregulation of effector mechanisms (e.g., IL-5 evoked eosinophils) aimed at worm destruction and expulsion.[32,33] However, the corollary of this is that helminths are adept at manipulating immunity in their hosts to meet their own needs,[34] and do so via the release of molecules that may need to gain access to target cells in the mucosa – a leaky epithelial barrier would facilitate this.

Increased epithelial permeability could have additional host benefits and anti-worm effects, for example allowing increased nutrient uptake into the mucosa could benefit the host to meet the energy requirements needed to defend itself against the parasite. Easing the passage of complement, antibody and putative effector cells (eosinophils, macrophages) into the gut could be invaluable in the attack on lumen-dwelling helminths.

Increased epithelial permeability triggered by infection with helminth parasites

Direct effects on epithelial permeability are typically assessed using cell lines grown as polarized monolayers on filter supports[35] or analysis of tissues mounted in Ussing chambers[36] (use of isolated loops of intestine for *in vivo* or *ex vivo* studies (*ex vivo* the loops can be inverted) are less frequently used[37]). Studies with monolayers add the product/drug/agent of interest to either the apical or basal side of the epithelia and then monitor transepithelial resistance (TER) or apical-to-basal flux of marker molecules (e.g., ^{51}Cr-EDTA, FITC-dextrans) over time, typically up to 72-hours post-treatment. Studies with Ussing-chambered tissue use the same approach but are more short-term, seldom extending beyond 4 hours because of issues with tissue viability. Tissue can be retrieved at various time-points post-infection with helminth parasites for assessment in Ussing chambers. Under these circumstances altered epithelial permeability could be due to the host immune response to the helminth and not to direct effects of the helminth or its' E/S products on the epithelium.

Helminths and their products do directly interact with enteric epithelial cells and indeed the enterocytes synthesis of IL-25 and thymic stromal lymphopoietin (TSLP) is important in the initiation of TH2-dominiated immunity

that can result in worm expulsion.[32,33,38-41] Studies in which helminth E/S products are added directly to epithelial monolayers and permeability subsequently assessed are scarce. The E/S products from the nematodes *Haemonchus contortus* and *Teladorsagia circumcincta* when applied to the apical surface of monolayers of the human colon-derived Caco2 epithelial cell line lowered the TER by ~20% 2 hours post-treatment.[42] Caco2 monolayers typically have TER values of 250–300 $\Omega.cm^2$ and so in the absence of data on other markers of paracellular permeability it is difficult to determine the functional significance of the E/S-evoked 20% drop in TER. The TER gradually recovered over a 24-hour period but remained lower than that in time-matched naïve monolayers; immunolocalization at 4 hours post-treatment revealed less peri-junctional staining of ZO-1 and occludin, with the latter showing a diffuse cytoplasmic distribution.[42]

Hiemstra *et al.* showed that a glycan component of E/S products from the nematode *Trichuris suis* dose-dependently decreased the electrical impedance (analogous to TER) across monolayers of the mouse cecal CMT93/69 epithelial line and increased the flux of FITC-dextrans (10–100 kDa): this was associated with reduced mRNA for claudin-4 and a claudin-like protein the authors designated epithelial membrane protein-1 (but not claudin-3 or ZO-1) and was not accompanied by increased epithelial cell death.[43] This study also showed that the barrier defect permitted *T. suis* E/S products to cross the epithelial layer. This is noteworthy from 2 contrasting perspectives: first, infection with *T. suis* has been promoted as a therapy for inflammatory bowel disease[44] and here increases in epithelial permeability could exaggerate the disease. In this context, the magnitude of the increase in intestinal permeability in individuals infected with the nematode *Anasakis simplex* correlated with worse disease.[45] Alternatively, the passage of *T. suis* E/S products across an epithelial layer driven by the presence of the E/S products themselves could be a component of the anti-inflammatory effect of *T. suis,* as the E/S products would now be positioned to interact with resident immune cells or those recruited to the gut.[46]

Data on the direct effect of trematodes and cestodes on enteric epithelial permeability are limited. As adults, *S. mansoni* lodge in mesenteric blood vessels and to complete their lifecycle eggs must 'breakthrough' the tissue and enter the gut lumen that may be facilitated, at least in part, by the spine on the egg. This process must disrupt the epithelial barrier but neither the mechanism of the transepithelial passage nor any physiologic/pathophysiological consequences of this are well understood. *S. mansoni*, and other flukes (e.g., *Clonorchis sinensis* (human), *Fasciola hepatica* (cattle)), infect or affect the liver with implications for bile flow and bile salt formation:[47] bile acids can directly affect epithelial permeability and electolyte transport,[48,49] but we are unaware of data in support of the possibility that infection with these flatworms affect epithelial permeability indirectly via bile salts.

We reported that mice (non-permissive host) infected with 5 cysticercoids of the rat tapeworm, *H. diminuta*, displayed a small, statistically-significant increase in ionic conductance (the reciprocal or TER) across jejunal tissue mounted in Ussing chambers at 5 d post-infection (dpi.) that returned to control levels by 8 dpi.[50] Infection with helminth parasites can result in less severe disease in animal models of colitis.[51] The first study in this area used *H. diminuta* and dextran sodium sulfate (DSS)-induced colitis:[52] while improvement in colon function was observed in the infected mice, ion conductance across segments of colon in Ussing chambers was not different between control, *H. diminuta*-infected (11 dpi.) or DSS ± *H. diminuta*-treated mice. This is an unusual finding and may, in this instance, reflect on the technique used to assess epithelial barrier – measurements of TER or conductance in Ussing-chambered tissue consider the whole tissue and so increases in epithelial permeability could be off-set by increased tissue thickness due to edema or hyperplasia of the outer muscle layers in inflamed tissue.

Infection with helminth parasites reduces the severity of colitis in murine model systems,[51] and this can be accompanied by a preservation of epithelial barrier function. For example, downregulation of colonic levels of ZO-1 and occludin mRNA and protein and the increase in bacterial translocation to the blood, spleen and mesenteric lymph nodes observed in tri-nitrobenzene sulphonic acid (TNBS)-induced colitis were reduced in mice treated with 10,000 freeze-killed eggs of the trematode *Schistosoma japonicum* (given by intra-peritoneal injection).[53] However, it is unclear whether the worm antigen directly affected the epithelium or enhanced epithelial barrier function occurred

via suppression of the inflammation: the latter being the more likely of the 2 possibilities.

Infection with gastrointestinal nematodes increases epithelial permeability,[54] which in the acute stages is likely due to the significant damage that species such as the nematodes *N. brasiliensis* and *Trichinella spiralis* do to the gut either directly or as a consequence of a cell-mediated immune responses:[37] the villus atrophy can resemble that observed in celiac disease. Increases in epithelial permeability after expulsion of the parasite (the post-infectious state) are driven by host cells, principal among these being mast cells.

In the late 1970s it was shown that 10 or 11 dpi. with *N. brasiliensis*, loops of rat jejunum displayed increased leakiness to lactulose and mannitol (makers of paracellular permeability).[37] Subsequently increases in gut permeability as assessed by ^{51}Cr-EDTA and ovalbumin were shown at 4, 10 and 35 dpi., the latter being ~2 weeks after expulsion of the worm and when the jejunum is characterized by mast cell hyperplasia.[55,56] Work by Miller *et al.* linked helminth-induced mast cell hyperplasia and activation, and the release of proteases with the increase in gut permeability.[57-60] Similarly, mast cells are important in the increases in gut permeability that occur as a consequence of anaphylaxis due to challenge of previously infected rats with worm antigen.[55,57] cKit$^+$ mast cells have been implicated in the increased intestinal permeability observed 9–10 dpi. with *T. spiralis*, which was accompanied by reduced expression of occludin.[58] This barrier defect was enhanced in *T. spiralis*-infected IL-9 transgenic mice that have increased numbers of mucosal mast cells[58] (increased IL-9 is common following infection with helminth parasites[61] and IL-9-knockout mice treated with TNBS displayed increased colonic mRNA and protein levels of claudins 4 and 7, occludin and JAM-A[62]). In the *T. spiralis* post-infectious paradigm, the increase in lactulose permeation across the gut correlated with reduced expression of claudin-1 protein in the ileum.[63]

More recent studies corroborate the participation of mast cells in nematode-evoked increases in epithelial permeability. Thus, 30 dpi. with *T. spiralis* when there is no histological evidence of damage in the jejunum of rats, there is a ~2-fold increase in tissue conductance and increased fluxes of 4- and 40-kDa FITC-dextrans across jejunal segments in Ussing chambers.[64] This epithelial permeability defect was insensitive to *in vitro* tetrodotoxin treatment and hence occurred independent of neuronal fast sodium channels. A time-course analysis confirmed increased jejunal permeability at 14 and 30 dpi. with *T. spiralis* and correlated this with increased numbers of mucosal, but not connective tissue-type, mast cells and increased mRNA expression of the rat mast cell proteases (MCP) 1, 2, 4, 5, 8, 9 and 10 in jejunal mucosa-submucosa at 6 and/or 14 dpi., but not 30 dpi.[54] PCR-analysis of the same tissues revealed reduced expression of occludin at 2, 6 (when conductance is not significantly altered), 14 and 30 dpi. A role for mouse MCP1 in the increased permeability observed 8 weeks after infection with the trematode *S. mansoni* was ruled out using mMCP1$^{-/-}$ mice.[65]

Given that the 2 most consistent findings observed in humans having irritable bowel syndrome (IBS) are increased gut permeability and mastocytosis,[66] one wonders if helminth therapy aimed at treating autoinflammatory disease[34] might predispose an individual to IBS-like symptoms.

The adaptive immune response that follows infection with helminth parasites can also participate in the increase in gut permeability. For instance, infection with the nematode *Heligmosomoides polygyrus*, a parasite of the mouse duodenum, increases colonic permeability at 7 pdi. characterized by ballooning of the paracellular space, increased transcytosis of the marker protein horse-radish peroxidase (HRP), and loss of the epithelial adherence junction protein, E-cadherin. These findings were not apparent in severe combined immunodeficient mice (SCID), but were recapitulated when SCID mice were re-populated with T cells.[67] In addition, mice lacking signal transducer and activator of transcription (STAT)-6, which is critical in IL-4 signaling, did not display the *H. polygyrus*-induced barrier defect.[67] Likewise, the drop in TER observed in muscle-free preparations of jejunum from nematode-infected Balb/c mice was not seen in STAT-6$^{-/-}$ animals.[68] The drop in TER in secondary infections with *H. polygyrus*, was accompanied by a small increase in the epithelial expression of the pore-forming claudin-2, and the barrier defect was absent in IL-13Rα1$^{-/-}$ mice.[69] Increased IL-13 production following infection with helminths can be from innate[70] or adaptive immune cells,[71] and while IL-13 has been

shown to directly decrease the barrier function of epithelial monolayers *in vitro*,[24,72] it is unclear if helminth-evoked IL-13 targets the epithelium directly or via other immunoregulatory activities.

With respect to TH2-type cytokines mobilized in response to infection with helminths, many of these have the potential to affect epithelial barrier function. For instance, IL-6 was shown to lower Caco-2 epithelial monolayer TER, increase Na^+ (but not macromolecule) permeability and the expression of claudin-2.[73] As another example, helminth-evoked IL-5 is critical for eosinophil development and eosinophils can directly increase epithelial permeability,[74] or indirectly via mast cells[75] (Fig. 2). However, the host-parasite interaction is so exquisitely balanced that the net effect of any given cytokine *in vivo* is difficult to fully ascertain and will depend on the target cell, temporal kinetics of cytokine production and the microenvironment in which the cytokine operates. Thus, the prototypic TH2 cytokine, IL-4 added to monolayers of human colon-derived epithelial monolayers increased paracellular permeability,[76] while reciprocally, one can hypothesize that alternatively activated macrophages induced by IL-4 production after helminth-infection[77] could, via their tissue reparatory capacity, enhance epithelial barrier function.

Collectively, the available evidence suggests that helminths can increase gut permeability by abrading the epithelium directly, that defined helminth-derived products may affect the structure of the tight junction (in general there is a paucity of data here), and that immune activity during or after infection can significantly compromise or enhance epithelial permeability (Fig. 2).

Helminth and host-derived factors impact on intestinal barrier function

Defining the intestinal barrier as mechanisms that prevent material in the lumen entering the circulatory system, there is a vast literature on helminth-evoked changes in intestinal barrier function: goblet/mucus, trefoil factors,[78] Paneth cells/defensins/antimicrobial peptides, serotonin, and mucosal immunity are all components of the barrier that can change significantly (cell number and/or function) following infection with parasitic helminths and each is worthy of a focused review.

Enhanced epithelial cell turnover may contribute to the hosts' anti-helminth defenses[79-81] and this has the concomitant benefit of clearing any microbe-infected enterocytes. Stimulated electrogenic ion transport creates a driving force for water movement that can lubricate the epithelial surface and may assist in the expulsion of intestinal helminths:[82] water is important in the physical properties of mucus.[83,84]

Intestinal goblet cell hyperplasia is perhaps the most prominent gut characteristic of infection with gastrointestinal parasitic helminths[3,85] and increases in mucin production, type (e.g., Muc5a) and glycosylation can be critical components of the epithelial barrier that aids the expulsion of helminths, such as the nematode *N. brasiliensis*[86-89] and the trematode, *Echinostoma caproni*;[90] indeed, helminths in their own

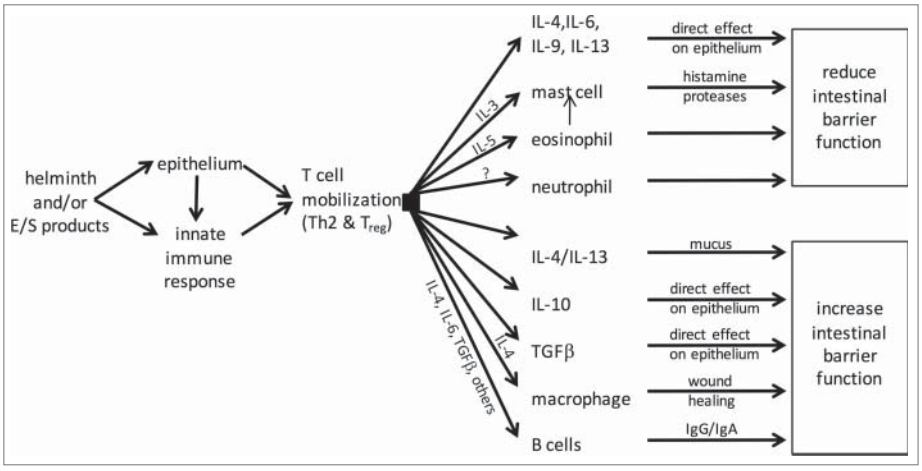

Figure 2. Schema showing the variety of possible mechanisms by which T cell activation following helminth infection could affect epithelial permeability and intestinal barrier function (E/S, helminth-derived excretory/secretory products; IL, interleukin; TGF, transforming growth factor; Th2, T-helper cell type-2; T_{reg}, regulatory T cells).

defense may release proteases to degrade mucus.[91] The advent of mucin-gene knockout mice positions the field to unequivocally test the role of mucus in the expulsion of a range of parasitic helminths. The goblet cell/mucus response can be driven by the helminth-evoked TH2 response, principally IL-4 and IL-13[92,93] and IL-25[94] (Fig. 3) and potentially by contact with the worms or their E/S products: the latter needs to be explored on a species-by-species basis. The potential of helminth-evoked changes in the enteric nervous system[95] and the regulation of mucus production by acetylcholine should also be pursued.[96] Finally, recent work has shown that goblet cells can sense their microenvironment and pass this information to dendritic cells;[97] the implications of this for barrier function and host-parasite interactions are poorly understood.

Paneth cells are a significant source of anti-microbial peptides in the mammalian small intestine and infection with nematodes and *S. mansoni* was shown to induce Paneth cell hyperplasia,[98] while *Toxocara canis* (nematode) infection increased the number of secretory granules in Paneth cells.[99] Helminth-regulation of anti-microbial peptides has implications for the overall barrier function of the gut as this will affect the composition and structure of the commensal microbiota. For instance, *N. brasiliensis* infection has been associated with reduced expression of the mRNA of the anti-microbial peptides, lysozyme-1 and RegIIIγ.[89] In contrast, expulsion of the nematode *Trichuris muris* from mice was accompanied by increased expression of the anti-microbial peptide, angiogenin-4,[84] with goblet cells identified as a source of angiogenin-4 in the colon of infected mice.[100] Reduced numbers of segmented filamentous bacteria (SFB) in the gut of *N. brasiliensis*-infected mice have been reported (Table 1):[89] SFB are important stimuli of TH17 cells and should this be a general outcome of infection with helminths, the question arises are there any short or long-term consequences to the host to a reduced TH17-TH1 axis in terms of gut homeostasis or vulnerability to microbial pathogens. For example, mice infected with *H. polygyrus* have an impaired response to concurrent infection with the bacterial pathogen, *Citrobacter rodentium* that the investigators related to mobilization of alternatively activated macrophages in the co-infected mice.[101,102]

Entrochromaffin cells (ECs) within the enteric epithelium are the body's major source of serotonin.[103] Increases[104] and decreases[103] in intestinal ECs and serotonin have been demonstrated after infection with helminths, with early studies linking serotonin to

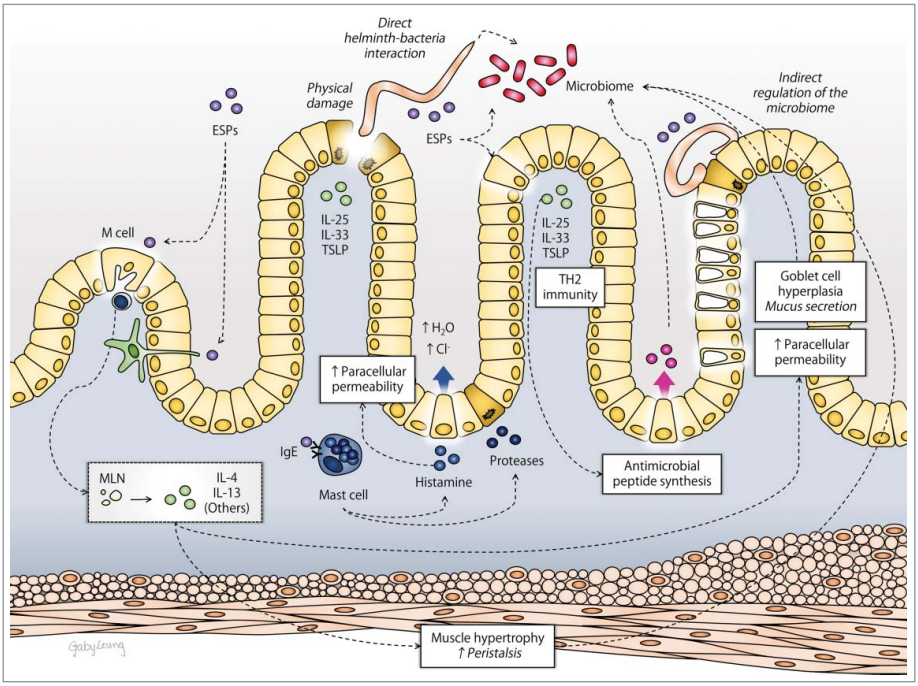

Figure 3. Schematic overview of the mechanisms by which infection with intestinal parasitic helminths (worm or their excretory/secretory products (ESP)) can directly or indirectly impact the barrier function of the gut (IL, interleukin; MLN, mesenteric lymph nodes; TH2, T-helper cells type 2; TSLP, thymic stromal lymphopoietin).

Table 1. Changes in the gut microbiota associated with infection with helminth parasites.

Species	Host	Location	Diversity	class level change in relative abundance	Ref.
			Nematodes		
Necator americanus	Human	Small intestine	NC	NC	115
			↑ species richness	NC	116
Trichuris trichuria	Human	Small intestine	NC	↓ Clostridia	117
Trichuris trichuria	Macaques	Small intestine	↑ α diversity	↑ Bacteroidia	118
			↑ α and β diversity	↑ Chlorophyta, ↓ Bacteroidia	119
Trichuris suis	Pig	Proximal colon	NA	↑ Fibrobacter & Clostridia	120
			NC	↑ Deferribacteres	121
Heligmosomoides polygyrus	Mouse	Small intestine	NA	↑ Bacilli (Lactobacillus)	122
Trichinella spiralis	Mouse	Small intestine	NA	↑ Bacilli (Lactobacillus)	123
			NC	↑ Clostridia (Lachnospiraceae)	112
			increased (?)	↑ Gammaproteobacteria	124
			NA	↑ Bacilli (Lactobacillus) & Clostridia (Clostridiales) ↓ Bacilli (Turicibacteraceae)	106
Nippostrongylus brasiliensis	Mouse	Ileum	NC α diversity	↓ Fimicutes (Lactobacillaceae) (↓ segmented filamentous bacteria) ↑ Bacteroides & Actinobacteria	89
Trichuris muris	Mouse	Large intestine	↓ α and β diversity	↑ Bacilli (Lactobacillus)	125
			↓ α diversity	↓ Bacteriodia	126
			NA	↑ Clostridia (Lachnospiraceae) ↓ Bacteriodia	127
			Cestodes		
Hymenolepis diminuta	Rat	Cecum	NC	↑ Clostridia, ↓ Bacilli	128
	Rat	Small intestine	↓aerobic bacteria	NA	92

Note. NC, no statistical change reported; NA, no data available; ↑ increased abundance; ↓ decreased abundance

worm expulsion,[105] possibly via its ability to elicit electrogenic Cl^- secretion into the gut to create a driving force for directed water movement.[30]

This sampling of studies aptly illustrates the magnitude of changes in the amount and function of factors that comprise the extrinsic component of the intestinal barrier (noting we have not addressed changes in antibody or complement) that can accompany, or follow, infection with parasitic helminths. The challenge is to understand the consequences of these changes for gut barrier function in a host-parasite specific manner, and the putative implications for host interaction with microbes and concomitant inflammatory disease.

Helminth-microbial interactions at the epithelial barrier

The mammalian intestine is home to trillions of bacteria from a diverse array of species. Layer on top of this virus and fungi, protozoan and helminth parasites and a complex ecosystem of microbiota and macrobiota emerges. Intuitively, one can accept that the interplay between these species and the host is critically important in controlling gut function: yet, knowledge of inter-species communication or cross-kingdom interactions in the regulation of gut function is rudimentary.[106]

A normal commensal microbiota is considered an extrinsic component of the intestinal barrier. The commensal bacteria can produce bacteriostatic factors to influence other bacteria in the gut and may prevent pathogen colonization by niche-exclusion via competition for nutrients and space. The release of bacterial molecules (e.g., PAMPS: pathogen-associated molecular patterns), may keep the gut primed to respond to invasion by pathogens.

Recent studies have begun to catalog changes in the gut bacteria that occur following infection with helminth parasites, typically those that seek to establish in the intestine (Table 1).[107,108] It is unclear if helminth-regulation of the enteric microbiota is a direct effect, since they are in the same location, or indirect via the host anti-worm immune response; for example, IL-25 mobilized in response to helminths could suppress synthesis of IL-22 (and *vice versa*[109]) which is an important regulator of the epithelial response to bacteria, promoting mucin and anti-bacterial peptide synthesis,[110,111] and under certain circumstances may aid worm expulsion from the gut.[88]

Functional consequences of perturbation of the enteric microbiome are largely unknown, with a notable exception being that the suppression of airways

inflammation observed in *H. polygyrus*-infected mice was dependent on bacteria-derived short-chain fatty acids.[112] Intriguingly, the decreased barrier function reported following infection and rejection of *T. spiralis* was ameliorated by treatment with probiotics:[63] the probiotic treatment also resulted in lower expression of pro-inflammatory cytokines, returning us to the conundrum of whether the probiotic effect was by interaction at the level of the enterocyte or the immune system. The possibility that infection with helminths that do not localize to the gut could affect the composition of the enteric microbiota should be assessed: we are unaware of any data in this area.

Thus, the stage is set to delve into analyses of the functional outcome of helminth-induced changes in microbiota content and diversity for the host, the bacteria and the helminth. How important is the commensal bacteria to intestinal barrier function following infection with helminth parasites? What happens to the bacterial metabolome? Is the energy status of the host impacted by helminth regulation of the microbiota? Is the host more vulnerable to opportunistic bacterial infections because of the helminth? Does the altered microbiome impact the course of the helminth-infection[113] and any associated pathology? How important is the microbiota to helminth-modulation of disease? These questions and many more await rigorous research efforts. Understanding the interplay between the triumvirate of host epithelium (or immune system), helminth and bacteria (or virus or fungi) is a new frontier in enteric biology that will revolutionize awareness of the control of gut form and function, and how we manage digestive disease.[106]

Concluding comments

The epithelial surface of the gut is the largest contact site with the outside world and is recognized as a dynamic regulated barrier composed of 6 cell phenotypes (goblet, tuft, enteroendocrine, M-cell, Paneth and transporting enterocyte), the activation of which directs mucosal immunity. To date the there has been little commentary on the barrier defect due to the physical damage caused by infection with abrasive helminths, with more information on the mechanism(s) of host immunopathology following primary or secondary infections with parasitic helminths, and anaphylactic reactions due to exposure to worm antigen in previously infected animals (Fig. 3). Data on the direct effect of helminths or their E/S products on epithelial permeability is lacking. Given the contribution of the epithelium to innate immunity, we suggest that focused efforts to define how helminths affect epithelial permeability will reveal novel aspects of host-parasite interactions and new ways to combat infection with gastrointestinal parasites. Finally, we underscore that the gut is an ecosystem and that the myriad of cells that reside there or are recruited in response to infection form an integrated circuit: while challenging, we contend that the holistic approach of integrated neuroimmunophysiology[114] needs to be applied if we are to understand the complex regulation of intestinal barrier function (or indeed any aspect of gut function) and the consequences of perturbed barrier function for gut homeostasis and disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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