Trends in Parasitology

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Review

The Intestinal Epithelium at the Forefront of Host–Helminth Interactions

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Gastrointestinal helminth infection still constitutes a major public health issue, particularly in the developing world. As these parasites can undergo a large part of their lifecycle within the intestinal tract the host has developed various structural and cellular specializations at the epithelial barrier to contend with infection. Detailed characterization of these cells will provide important insights about their contributions to the protective responses mediated against helminths. Here, we discuss how key components of the intestinal epithelium may function to limit the initial establishment of helminths, and how these cells are altered during an active response to infection.

Highlights

Previously, research on the mucosal immune response to gastrointestinal helminth infection has been limited by a lack of knowledge around defined subpopulations of intestinal epithelial cells (IECs) such as tuft cells and enteroendocrine cells.

Recent advances in single-cell sequencing technology have allowed for more detailed identification of IEC subsets and their potential roles during helminth infection.

The characterization of IEC subsets has led to advances in our knowledge of helminth–IEC interactions (e.g., tuft cell activation by helminths, which elicits a potent type 2 immune response).

This review is a call to arms for the need to dissect the mechanisms as to how helminths initiate an immune response through these subsets, as well as the need to use modern sequencing technology to further characterize IEC subsets.

The Host Immune Response to Helminth Infection

Soil-transmitted gastrointestinal (GI) nematodes are multicellular organisms which have successfully coevolved with their human hosts over millennia, infecting around 2.5 billion people globally [1]. In countries of low socioeconomic standing, where access to adequate healthcare and sanitation is limited, GI nematode infection is endemic and has significant clinical implications for children and other vulnerable populations if the infection remains chronic [2]. As such, rodent helminths offer a tractable model to study these infections in a laboratory setting (Box 1). Helminth infection is associated with the release of type 2 cytokines, IL-4, IL-5, and IL-13, from adaptive $CD4^+$ **T helper type 2** (**Th2**) **cells** (see Glossary), and innate immune cells including **type 2 innate lymphoid cells** (**ILC2s**), eosinophils, basophils. and mast cells [3]. The resulting type 2 cytokine milieu, in addition to the participation of macrophages and antibodies, mediates a protective response that drives parasite expulsion known as the 'weep and sweep'. This is characterized by goblet cell hyperplasia, increased mucus production, intestinal permeability (the 'weep') [4,5], and smooth muscle contractility (the 'sweep') [6,7] to facilitate parasite expulsion. The innate and adaptive intestinal immune response can be initiated by the release of epithelial 'alarmins', including adenosine triphosphate (ATP) [8,9], IL-25 [10–13], IL-33 [14,15], and thymic stromal lymphopoietin (TSLP) [16,17], following the recognition of infection or by helminth-induced damage of the intestinal barrier.

The intestine is a complex organ consisting of specialized epithelium, nerves, immune cells, blood, lymphatic vessels, smooth muscle, and a commensal microbiome (Box 2; Figure 1, Key Figure). The intestinal epithelium mediates protection from helminths in several ways: through its architecture it creates an impermeable barrier; through innate recognition of parasite infection it communicates with key immune effector cells to support the stereotypic 'weep and sweep' response; and through barrier repair it overcomes any untoward effects of damage resulting from the increased tissue entry of luminal bacterial. This review focuses on the diverse roles of intestinal epithelial cells (IECs) during helminth infection including: (i) their importance as a physical barrier between the environment and tissues and (ii) their active role as both initiators and effectors of the host defense.

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Box 1. Laboratory Models of Helminth Infection

The main species of helminths that infect people are the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and the hookworms (*Necator americanus* and *Ancylostoma duodenale*) [1]. Owing to the complex lifecycle of helminth parasites it is very difficult to study human infection under laboratory conditions. The organ niches that these parasites reside in (often lung and intestine) are not readily accessible, and the areas that are affected globally by high burdens of helminth infection often have limited medical research capacity [1,2]. As such, the use of rodent laboratory models of helminth infection has provided fundamental insights regarding host defense and protective type 2 immunity. The models frequently discussed in this review are: the rat parasite *Nippostrongylus brasiliensis*, the closest model to human hookworm infection which migrates through the skin and lung during its natural lifecycle [75]; *Heligmosomoides polygyrus,* a small intestinal nematode which lives chronically within its murine host and has a completely enteric lifecycle [75]; *Trichuris muris*, the murine counterpart to the human whipworm *T. trichiura* [76]*, which* resides in the cecal and colonic epithelium during its lifecycle; and *Trichinella spiralis,* the causative agent of the zoonotic infection trichinellosis which occupies both intestinal and muscle niches throughout its lifespan [77].

The First Barrier – The Role of the Intestinal Epithelium in Preventing the Establishment of Helminth Infection

The small intestinal barrier consists of finger-like projections known as villi (which are absent in the large intestine), separated by epithelial invaginations known as crypts (Figure 1A). The intestinal crypts constitute a niche of multipotent leucine-rich-repeat-containing G-protein-coupled receptor 5 (Lgr5)$^+$ intestinal stem cells (ISCs), their rapidly proliferating transit-amplifying (TA) daughter cells, and supportive Paneth cells [18]. This niche facilitates the constant renewal of the epithelial barrier and generates specialized secretory cells as well as absorptive enterocytes, whose characteristics and function are outlined in Table 1. In the small intestine, epithelial cell renewal (occurring approximately every 3–5 days in mice, and 5–7 days in humans) involves the shedding of mature enterocytes and other differentiated cells which migrate towards the apex of the villi [19].

Lgr5$^+$ stem cells within the crypt give rise to goblet cells (which synthesize mucins, trefoils, and resistin-like molecules (RELMs)), tuft cells (chemosensory cells), enteroendocrine cells (which secrete hormones and neuropeptides), nutrient-absorbing enterocytes (which constitute a majority of the epithelial layer) and Paneth cells (a reservoir for antimicrobials which also supports the stem cell niche) [20,21] (Table 1). Lgr5$^+$ cells are controlled by Wnt and Notch signaling, which are critical pathways in epithelial tissues governing cellular proliferation and fate determination. Due to advances in **single-cell RNA sequencing** (**scRNAseq**) technology, distinct heterogeneity within the tuft cell lineage in the small intestine is now recognized [22], revealing tuft-1 and tuft-2 subtypes (Table 1). The same study also provided further definition of enteroendocrine cell (EEC) subsets, and note that the ratios of both tuft-2 cells and EECs were altered in response to enteric helminth infection. Other cell types present in the small intestine include rare microfold 'M' cells,

Box 2. The Composition of the Intestine

The small and large intestines represent a major part of the GI tract which acts as an essential entry point for the absorption of fluids and nutrients, supporting digestion and functioning as a selectively permeable barrier against the external luminal environment. The innermost layer of the intestinal tract, which faces the lumen, is composed of a cohesive single cell layer of columnar epithelial cells, covered by an outer mucin-rich glycocalyx that forms both a molecular filter and robust barrier [25]. The thickness of the mucus barrier differs along the length of the intestinal tract, with a more heterogeneous, thin and permeable single layer in the proximal small intestine which is specialized in nutrient uptake, and a thicker double layer overlying the distal colon where a larger load of microbes is also found [78,79]. The immediate subepithelial layer is known as the lamina propria, a loose layer of connective tissue which consists of fibroblasts and immune cells, as well as nerves, capillaries, and lymphatic vessels which are supplied by the deeper submucosal layer. The final layer, known as the muscularis externa, is composed of an inner circular layer and an outer longitudinal layer, which contract in a coordinated manner to facilitate peristalsis. The intestinal tract is also residence to significant populations of commensal microbiota. These factors work in tandem to support the host, with any disruption of these factors – such as microbial dysbiosis, or barrier damage from invading pathogens such as helminths – having significant consequences for host nutrition, metabolism, and inflammation.

Glossary

Excretory/secretory (E/S) products: a variety of molecular products, including proteins, nucleic acids, carbohydrates (amongst others), that are excreted or secreted by helminths. E/S products have a range of biological functions that can support the parasite lifecycle, enhance infectivity, communicate with host cells, and modulate immunity.

G-protein-coupled receptors (GPCRs): one of the largest and most diverse families of membrane receptor proteins that detect a wide range of physicochemical stimuli and use distinct GTP-binding regulatory proteins known as G proteins to mediate signal transduction within cells.

Interferon-gamma (IFN-γ): a pleiotropic cytokine which is classically associated with the type 1 immune response to intracellular pathogens and also plays a role in antitumor immunity and regulation of inflammation and apoptosis.

Single-cell RNA sequencing (scRNAseq): a tool for dissecting the transcriptome of heterogeneous populations at a single-cell level, which is not normally possible with 'bulk' RNAseq.

T helper type 2 (Th2) cells: adaptive CD4$^+$ T lymphocytes that are typically associated with allergy and parasite infection and release a broad spectrum of cytokines, including IL-4, IL-5, IL-9, IL-10, and IL-13, that facilitate type 2 immunity.

Type 2 innate lymphoid cells (ILC2s): a rare subset of innate immune cells which lack the expression of surface markers typically used to describe other lymphocytes. These are found at mucosal sites, can respond to epithelial alarmins, and are critical early reservoirs of type 2 cytokines such as IL-5 and IL-13.

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Key Figure

The Intestinal Epithelial Response to Helminth Infection

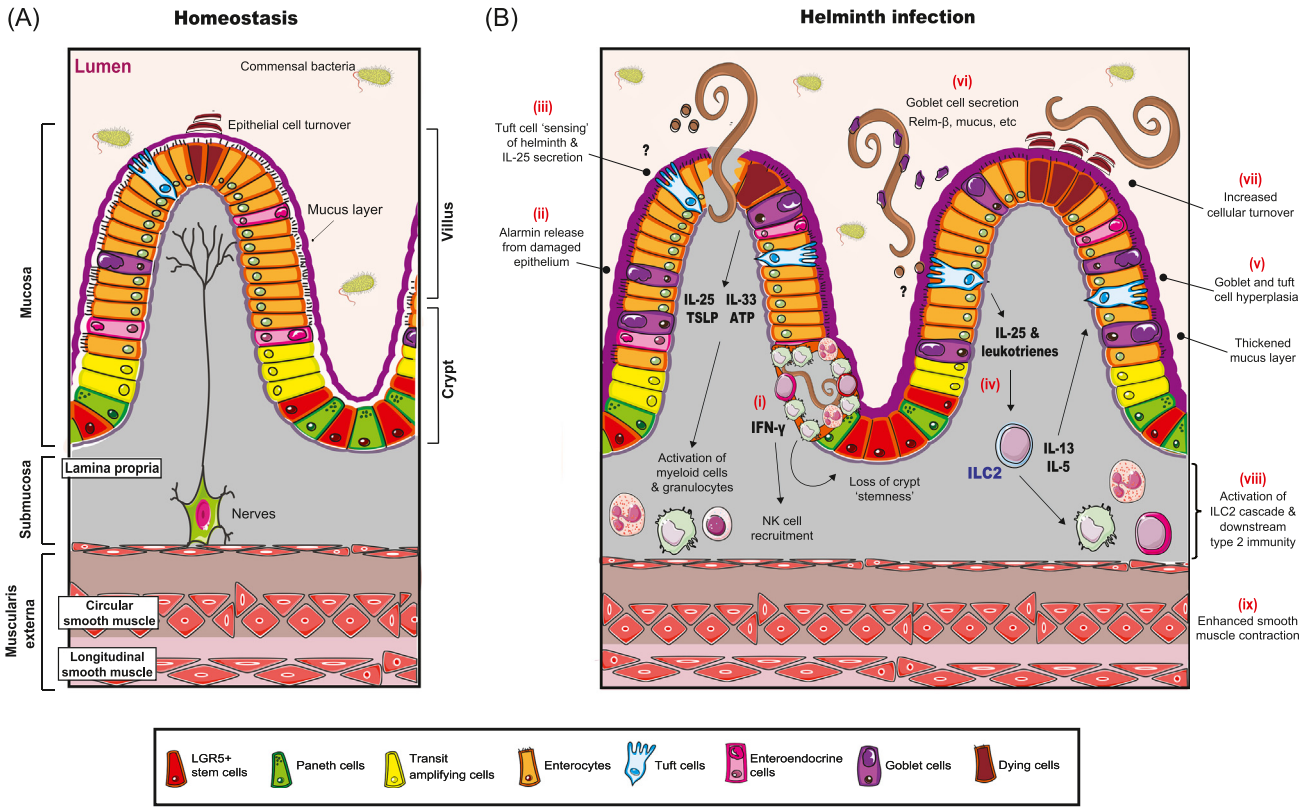

Figure 1. (A) Representative schematic of the small intestine during homeostasis, highlighting the major layers of the intestine: mucosa, submucosa, and muscularis externa, including their mucus layer and the presence of commensal microbiota (green) (Box 2). Multipotent Lgr5^{+} stem cells reside in the crypt base; they continually proliferate, giving rise to transit-amplifying progenitor cells that differentiate into highly specialized epithelial cells with dynamic functions. Also found in the crypt base are Paneth cells; these support the stem cell niche and secrete a range of antimicrobial peptides. Absorptive enterocytes constitute the major cell type present in the intestinal epithelium and are interspersed with various secretory cells. These include goblet cells, which facilitate the formation of the mucosal barrier by secreting mucins and other protective molecules; enteroendocrine cells, which principally secrete hormones and are associated with nervous control; and tuft cells, a rare chemosensory cell population which utilize taste receptor pathways for detection of metabolites and secrete effector molecules such as IL-25. (B) Representative schematic of the small intestine during helminth infection. (i) In some helminth infections, larvae are encysted in the intestinal submucosa by infiltrating immune cells that form a granuloma. Granulomas express an IFN-γ signature that drives the early recruitment of natural killer (NK) cells and induces a reparative dedifferentiation program in closely associated crypts to repopulate the intestinal stem cell (ISC) niche. (ii) Helminths can breach the intestinal barrier, causing cell death that leads to the release of alarmins, such as IL-25, IL-33, Tslp, and ATP, which activate innate effector cells, including mast cells, macrophages, eosinophils, basophils, and ILC2s, to drive a type 2 immune response. (iii and iv) Tuft cells detect the presence of helminths (through ligand-receptor interactions which are yet to be fully characterized), releasing IL-25 and leukotrienes to potently activate ILC2s. (v) In a feed-forward mechanism, ILC2-derived IL-13 will then promote tuft and goblet differentiation and hyperplasia. (vi) The expanded goblet cell population releases Relm-β, which facilitates larval trapping, and increases mucus production to inhibit parasite colonization and support expulsion. (vii) IL-13 signaling also enhances epithelial cell turnover, potentially displacing parasites from their intestinal niche. (viii) Beyond the acute epithelial response, ILC2-derived cytokines potentiate downstream effector responses, including eosinophil recruitment, macrophage activation, enhanced Th2-associated immunity, and smooth-muscle hypercontractility (ix) to mediate eradication of the helminth parasite, whilst driving repair of the epithelial barrier. Abbreviations: ATP, adenosine triphosphate; IFN-γ, interferon gamma; ILC2, type 2 innate lymphoid cell; Lgr5, leucine-rich repeat-containing G-protein-coupled receptor 5; Relm-β, resistin-like molecule beta; Tslp, thymic stromal lymphopoietin. Images are adapted from Servier Medical Art by Servier (http://smart.servier.com/) and modified by the authors under the following terms: Creative Commons Attribution 3.0 Unported (CC BY 3.0).

Table 1. Intestinal Epithelial Subsets and General Function[a]

Intestinal epithelial cell lineage	Subtypes and known markers	General function	Refs
Lgr5^{+} stem cell	Lgr5, Olfm4	Provides rapid ongoing replacement of all epithelial cell types in homeostasis and drives restoration following injury	[18–20,22]
Paneth cell	Lyz, DefensinA1, Mptx2	Production of antimicrobial peptides and defensins (e.g., Reg3y and lysozyme) and support of stem cell niche through secretion of Wnt ligands, EGF, and Notch ligands required for stem cell maintenance	[18–22]
Enterocyte	Enterocyte (small intestine, Alpi), Colonocyte (large intestine)	Predominant cell type in the intestine. Provides a barrier to intestinal insult, absorption of nutrients across the apical barrier membrane, as well as secretion of antimicrobials	[19,22]
Enteroendocrine cell	Pan EEC identifiers: Prox1, Gpbar1, Gpr119, Sct, Chromogranin A Enterochromaffin cells (5-HT), I cells (Cck), D cells (Sst), K cells (GIP), S cells (Sct), M cells (Mln), L cells (Glp-1 and PYY), N cells (Nts)	Secretion of specific hormones and neuropeptides in response to nutrients and other luminal components such as bacterial metabolites. Altered expression of unique subsets along the length of the intestine – aid in intestinal motility, satiety, barrier function, homeostasis, and immunity	[20,22,62–66]
Goblet cell	Muc2, Clca3	Creates a 'sticky' barrier to commensals, pathogens, and other luminal factors. Single thin layer (small intestine) or thicker double layer (large intestine). Augmented production of mucus and other effector molecules, e.g., Relm-b and trefoil factors in response to pathogens such as helminths	[4,5,20,22,55,58,59]
Tuft cell	Pan tuft cell identifiers: Prox1, Gfi1b, Pou2f3, Dclk1, IL-25 Tuft-1 (see markers above) and Tuft-2 (Tslp)	Neuromodulatory functions (tuft-1) and immunomodulatory functions (tuft-2). Involved in the detection of microbial metabolites through broad array of GPCRs and secrete a number of effector molecules such as neurotransmitters (acetylcholine), eicosanoids (e.g., cysteinyl leukotrienes and prostaglandin D2), and alarmins (Tslp and IL-25)	[10–12,22,43–46]
Microfold (M) cell	Spib, Gp2	Located in the follicle-associated epithelium. Nonciliated, uptake and transfer of luminal antigens to basal immune compartments	[9,22,23]

[a]Abbreviations: 5-HT, serotonin; Alpi, intestinal alkaline phosphatase; Cck, cholecystokinin; Clca3, calcium-activated chloride channel 3; Dclk1, doublecortin-like kinase 1; EEC, enteroendocrine cell; EGF, epidermal growth factor; Gfi1b, growth-factor-independent 1B transcriptional repressor; GLP-1, glucagon-like peptide 1; GIP, glucose-dependent insulinotropic peptide; Gp2, glycoprotein-2; Gpbar1, G-protein-coupled bile acid receptor 1; GPCR, G-protein-coupled receptor; Gpr119, G-protein-coupled receptor 119; IL-25, interleukin-25; Lgr5, leucine-rich repeat-containing G-protein-coupled receptor 5; Lyz, lysozyme; Mln, motilin; Mptx2, pentaxin; Muc2, mucin 2; Nts, neurotensin; Olfm4, olfactomedin 4; Pou2f3, POU Class 2 homeobox 3; Prox1, Prospero homeobox protein 1; PYY, peptide YY; Relm-b, resistin-like molecule beta; Spib, Spi-B transcription factor; Sct, secretin; Sst, somatostatin; Tslp, thymic stromal lymphopoietin.

which are located overlying intestinal lymphoid follicles known as Peyer's patches; these cells facilitate the transport of luminal antigens to basal immune compartments and are typically associated with bacterial infections [23]. Although M cells play a crucial role in host responses to many gastrointestinal pathogens, the number and function of these cells were shown to be unchanged following infection with the rodent helminth *Heligmosomoides polygyrus* [24], and mice lacking M cells through deficiency in the transcription factor Spi-B were resistant to infection with the same nematode [9]. Nevertheless, further studies will be required to discern whether these cells contribute to host responses against other helminths.

Tight Junctions

Other aspects of the intestinal barrier include intrinsic features such as paracellular junctional protein complexes known as tight junctions (TJs). TJs are formed between neighboring cells, segregating the apical region of the membrane from the basolateral region. TJs consist of a network of transmembrane proteins, including claudins and occludin, which interact with the actin cytoskeleton to allow ion transport and the movement of water into the lumen in addition to supporting epithelial cell organization [25]. Increased intestinal permeability may be a common feature of

some enteric nematode infections, as similar observations were made in *H. polygyrus* and *Nippostrongylus brasiliensis*-infected mice [26]. Decreased TJ integrity and claudin-2 expression were also demonstrated in secondary *H. polygyrus* infection [5]. These effects may not always be localized as the presence of *H. polygyrus* in the small intestine resulted in increased mucosal permeability in the colon, which was shown to be mediated through activated Th2 cells [27]. Infection with the small intestinal nematode *Trichinella spiralis* alters occludin expression within jejunal TJs, a mechanism suggested to be mediated through mast cell proteases [28]. The disruption of TJs contributes to parasite expulsion in this model by allowing fluid leakage across the epithelial barrier [28], a phenomenon that contributes to the 'weep' part of the 'weep and sweep' response. It may also be beneficial to the host through the rapid transport of parasite antigens to local immune cells and lymphoid tissue sites, by facilitating the entry of primed immune cells and other effector molecules into the lumen, and by supporting increased nutrient absorption.

Intestinal Mucus

Another intrinsic barrier component and a key player in the defense against enteric pathogens is the intestinal mucus. A heterogenous mucus layer overlies the intestinal epithelium during homeostasis and impedes the ingress of commensals, pathogens, and other large particulates towards the IEC monolayer and deeper compartments within the mucosa and submucosa (Box 2). The mucus layer also provides an anchor point for antimicrobial peptides, including Reg3γ and Relm-β [29,30]. Mucus is composed primarily of glycoproteins, known as mucins, which are synthesized by goblet cells in aggregates to form a viscoelastic gel layer. The highly O-glycosylated mucin, Muc2, represents the major mucin source within the intestine, which is shown to play a role in antigen sampling and immune tolerance [31]. A thickened mucus layer is a common attribute of gastrointestinal helminth infection, whereby type 2 cytokines are shown to drive upregulation of goblet cells and mucin expression (discussed in more detail later).

The Intestinal Microbiome

Lastly, another significant feature of the homeostatic intestine is the host microbiota. A number of studies have now demonstrated a crucial role for the intestinal microbiota in supporting nutrient uptake and metabolism, pathogen defense, and in shaping immune cell development and responses (reviewed elsewhere [32]). Intriguingly, a number of studies demonstrate that the microbiome may actually be necessary for the establishment of helminth infection [33,34]. This is supported by studies showing that specific microbial taxa influence the hatching of *Trichuris muris* eggs [35], and enhance *H. polygyrus* infection following administration in mice [36]. This relationship is not unidirectional as a number studies in murine models and humans have shown that helminth infection impacts microbiome diversity [34,36–39]. This may occur through the release of parasite effector molecules, as *H. polygyrus* **excretory/secretory** (**E/S**) **products** exhibit antimicrobial activity [34]. Other features of helminth infection, such as enhanced mucus production, may also limit the colonization of pathogenic species such as *Bacteroides* whilst increasing the abundance of protective *Clostridialis* species, as observed during a model *T. muris* infection [37]. Importantly, the researchers noted similar changes in the gut microbiome of humans from helminth-endemic regions, correlating to *T. trichiura* worm burden. This shows a complex relationship whereby helminths may control the outgrowth of specific microbial populations, while relying on the presence of a yet unspecified microbiome for fitness. Alterations in either the microbiome or helminth infection can have significant implications for host immunity [40], and are likely to impact strongly on the nature and responsiveness of the epithelium. Advances in metagenomic analyses will support further exploration of these multidirectional interactions between host, microbiome, and helminth.

In summary, the intestinal epithelium and lumen (containing both epithelial products and commensal bacteria) can shape the ability of parasite helminths to invade and establish residence in

the intestine. Whilst these mechanisms exist prior to infection they can also be altered in response to helminthic challenge. It is likely that we are only at the beginning of understanding how these cells can utilize their significant capacity for self-renewal and secretion to prevent and respond to helminth infection

The Second Wave – Recognition of Helminths

Sounding the Alarm: The Damaging Presence of a Helminth

The damage caused by helminths to the intestinal epithelium can be substantial [26,27] resulting from direct invasion of larval stages through the tissue barrier or potentially through feeding. Whilst the initial events which drive the antihelminth cascade remain poorly understood, activated and/or damaged epithelial cells are shown to release various danger signals such as ATP, IL-25, IL-33, and Tslp (Figure 1B). These alarmins in turn drive the type 2 immune response to the invading helminths (see earlier: The Host Immune Response to Helminth Infection).

During the lifecycle of *H. polygyrus*, infective larvae emerge though the epithelium of the small intestine into the lumen, which is shown to cause the release of extracellular ATP from damaged cells [9]. Cell-surface ectonucleotidases convert ATP to adenosine, which binds to the A2B adenosine receptor (A2BAR) and triggers upregulation of IL-33 in epithelial cells. Importantly, A2BAR$^{-/-}$ mice fail to mount a protective type 2 immune response to infection, which is correlated to inhibition of IL-33 release, activation of ILC2s, and recruitment of myeloid cells [8]. Mucosal mast cells also respond to extracellular ATP, releasing IL-33 to facilitate a protective type 2 response to infection [9]. IL-33-deficient mice exhibit defective type 2 responses to infection with *N. brasiliensis* and were unable to efficiently expel the parasite [41]. This was linked to the ability of IL-33 to induce IL-13 production by both ILC2 and Th2 cells, which in turn increases production of the antihelminth effector molecule, Relm-β, by goblet cells. Interestingly, exogenous administration of IL-33 drove Th2-mediated expulsion of *T. muris* and induced T cell-independent alterations in crypt architecture and epithelial proliferation within the cecum [15].

Sensing the Invader: The Central Role of the Tuft Cell in Initiating Antihelminth Type 2 Immunity

More recently, a rare type of epithelial cell, known as the tuft cell, has established itself as an essential player in initiating type 2 immune responses following recognition of protozoa and helminths at mucosal sites [10–12]. Morphologically, tuft cells are characterized by a 'tuft' of blunt apical microvilli. These cells lie in close proximity to local cholinergic neurons and express signaling components of the taste-chemosensation pathway, including transient receptor potential cation channel subfamily M member 5 (Trpm5) and the subunit α-gustducin (Gnat3) [42]. Howitt and colleagues observed that intestinal tuft cells fail to expand during experimental infection with the protozoan *Tritrichomonas muris* when mice lack either of these signaling components [12]. They propose a mechanism in which tuft cells detect the presence of the symbiont through **G-protein-coupled receptors** (**GPCRs**) and trigger the release of IL-25, driving ILC2 activation, which act in a positive feedback loop to further increase tuft cell numbers and potentiate the type 2 immune response. Importantly, whilst expansion of tuft cells in response to enteric nematodes *H. polygyrus, N. brasiliensis*, and *T. spiralis* was abrogated in *Trpm*$^{-/-}$ mice, these cells were unaffected in *Gnat3*$^{-/-}$ mice. This suggests that helminths may mediate differential upstream activation of this cascade. Other receptors expressed on IL-25^{+} Trpm5^{+} tuft cells, such as succinate receptor 1 (Sucnr1), activate a type 2 immune response in the intestine to commensal protists [43,44]. Whilst Sucnr1 was shown to be essential for mediating tuft cell detection of the metabolite succinate from the tritrichomonad *Tritrichomonas rainier*, it played a redundant role during *N. brasiliensis* infection. [44]. This indicated that another receptor likely plays a role in the tuft cell response to helminths. More recently, Luo *et al*. demonstrated that intestinal tuft cells upregulate expression of another GPCR, bitter taste receptor type 2 (Tas2rs), during *T. spiralis* infection

[45]. They showed that coculture of larvae, adult worm extracts, or E/S products with intestinal villous explants activates tuft cell taste-signaling. Activated tuft cells then release IL-25 in a Trpm5-dependent manner, suggesting that Tas2rs may be critical to sensing and initiating type 2 immunity during helminth infection.

Currently, there are still a number of open questions regarding how helminths are recognized by cells at innate barrier sites. The advent of scRNAseq has allowed for the identification of at least two subtypes of tuft cells that exist in the intestinal epithelium, which may also allude to heterogeneity in tuft cell receptor recognition of helminths within the intestine and other sites [22]. Tuft cells are found to be the major source of IL-25, which signals through the IL17RA/IL17RB heterodimeric receptor on ILC2s and Th2 cells. In a feed-forward manner, ILC2- and Th2-derived IL-13 can then further expand epithelial tuft and goblet cell populations in the intestine and enhance type 2 immunity to drive helminth expulsion [10,11]. A recent study from McGinty and colleagues demonstrated that tuft cells in the small intestine also secrete cysteinyl leukotrienes, which act in concert with IL-25 and/or IL-33 to drive optimal type 2 responses during helminth infection [46]. Intriguingly, the authors show that the release of cysteinyl leukotrienes from tuft cells is not driven by succinate or following colonization of succinate-producing *Tritrichomonas musculis*, indicating that there may be distinct pathways of recognition and response by tuft cells to individual intestinal commensals or helminth parasites. The characterization of tuft-1 and tuft-2 subtypes may also allude to specific functional roles of these cells. Although both subtypes are identifiable by constitutive expression of transcriptional regulators growth-factor-independent 1b (*Gfi1b*), POU class 2 homeobox 3 (*Pou2f3*) and double cortin-like kinase 1 (*Dclk1*) [11,22], and IL-25, only tuft-2 cells express significant levels of Tslp. Notably, the tuft-2 cell population was specifically expanded in the intestines of *H. polygyrus*-infected mice [22]. Whilst Tslp was shown to play a critical role in mediating Th2 polarization and expulsion of *T. muris* through dendritic cell activation [16,17], this cytokine was expendable for generating normal Th2 responses to *H. polygyrus* and *N. brasiliensis* [17]. Tslp has also been shown to limit Th17-driven mucosal inflammation following bacterial colonization in the intestine [47]. It could therefore be speculated that Tslp may be released to ameliorate inflammation caused by helminth damage and bacterial breach of the barrier. Interestingly, exogenous IL-25 and *N. brasiliensis* infection were both shown to modify intestinal levels of segmented filamentous bacteria, known drivers of Th17-mediated inflammation [48]. Thus, it will be important to determine if expansion of a Tslp-enriched tuft cell subset is conserved in other enteric nematode infections where significant barrier damage occurs. It will also be of critical importance to identify the precise molecular cues from helminths which drive the specific expansion of tuft cells and to understand how these may activate other cells within the epithelium. It is unclear whether the same signaling pathways exist in humans, although a recent report does describe increased tuft cell numbers and IL-25 production in colonic biopsies from patients suffering diarrheal irritable bowel syndrome [49].

The Third Act – Epithelial Cells as Key Players in Helminth Expulsion

The innate and adaptive type 2 immune responses which are generated following epithelial cell activation by helminths mediate a range of cellular and physiological changes at the epithelial cell interface (Figure 1B), highlighting the constant interplay between epithelial cells and immune cells in driving parasite expulsion.

Renew, Weep, and Sweep

As discussed earlier, epithelial cell turnover and secretion is a continual process that occurs in the homeostatic intestine and it is possible that these processes help to prevent the establishment of helminths when they first enter the intestine. Although this hypothesis has not been formally tested it is clear that these processes are altered in response to helminth infection, after which

they contribute to parasite clearance. This is true for both trematode [50] and nematode [51] infection, with the latter being dependent on IL-13 signaling. Indeed, the association of IL-4 and/or IL-13 signaling, and the downstream transcriptional regulator Stat6, has long been appreciated to play a key role in antihelminth immunity [52]. Constitutive activation of *Stat6* in IECs resulted in increased IEC turnover, increased numbers of tuft and Paneth cells, and goblet cell hyperplasia [53]. The proliferation of mucus-producing goblet cells is characteristic of a number of helminth infections and contributes, together with fluid leakage across the epithelial barrier, to the 'weep' part of the 'sweep and weep' response. Goblet cell hyperplasia is driven predominantly by IL-13 [4,5], with alarmin cytokines such as IL-25 [10,11] playing an indirect role through activation of the ILC2 cascade. Other cytokines, such as IL-22, were shown to directly alter mucin expression in the intestinal epithelium. In the same study, the authors show that IL-22-deficient mice exhibit defective goblet cell responses and worm expulsion, even in the presence of intact type 2 immunity [54]. The composition of mucus is also shown to contribute to the efficacy of host defense at this critical barrier interface. Elevated Muc2 expression (as observed in resistant strains of mice) has been correlated with both barrier permeability and expulsion of the nematode *T. muris* [55]. The same group found another mucin, Muc5ac, was also upregulated in an IL-13-dependent manner in resistant mice, and was essential for expulsion of *T. muris* and other nematodes, including *T. spiralis* and *N. brasiliensis* [56]. IL-13 has also be shown to promote specific glycosylation of the intestinal mucus barrier, protecting it against degradation by *T. muris* E/S *products* [57].

Goblet cells produce the cysteine-rich secretory molecule Relm-β, which is shown to impact the fitness of *N. brasiliensis* and *H. polygyrus* through larval trapping or impeding parasite feeding [58]. Goblet cells also secrete immunoregulatory glycoproteins known as trefoil factors (TFFs). *Tff2* and *Tff3* expression was elevated in the intestines of *N. brasiliensis*-infected mice, with higher adult worm burdens observed in *Tff2* and *Tff3* knockout mice [59,60]. Tff2 has been shown to be necessary to drive tissue repair in the lung, another major mucosal site, which is damaged during earlier migration of *N. brasiliensis* larvae, augmenting both IL-33 and Muc5ac expression within the lung [59].

Another key component of host defense against enteric helminths is smooth muscle hypercontractility, or the 'sweep', with peristaltic movements stimulating the expulsion of parasites from the intestinal tract in response to neurotransmitters or nerve stimuli. Mice with defects in epithelial-derived IL-25 [13] or the downstream Stat6 signaling cascade [6,7] were unable to mediate a hypercontractile responses to *N. brasiliensis*, resulting in delayed worm expulsion. IL-4/IL-13 signaling was shown to enhance the expression of the serotonin receptor, 5-HT2A [7], and the M3 muscarinic acetylcholine receptor (M3R) [61] in the intestine. Importantly, M3R-deficent mice had muted smooth muscle contractility in response to *N. brasiliensis* infection, highlighting the important role of type 2 cytokines in mediating the protective antihelminth 'sweep' response. It is also possible that the epithelium signals directly to the enteric nervous system to regulate peristalsis as sensory afferent nerves extend into the villi and have been reported to make contact with tuft cells [42]. However, little information is available as to whether direct interactions between these cells occur, nor whether this changes in response to helminth infection.

Enteroendocrine Cell Responses and Paneth Cells – Not Just Antimicrobial?

Interestingly, epithelial cells may also contribute to the 'sweep' mechanism of parasite expulsion through their ability to produce neurotransmitters. Intestinal EECs are specialized epithelial cells that are traditionally thought to constitute at least eight subtypes based on the hormones they produce (see Table 1 for further details), and comprise <4% of cells in the small intestinal epithelium [22]. These include enterochromaffin cells (which produce serotonin, or 5-HT), I cells

(producing Cholecystokinin (Cck)), and L cells (secreting glucagon-like peptide 1). The production of hormones and cytokines by EECs in response to pathogen-associated molecular patterns (PAMPs) highlights the importance of these cells within the mucosal barrier where notable changes are observed in these cells in clinical inflammatory bowel disease (reviewed in [62]). Interestingly, alterations of intestinal enterochromaffin cells and serotonin levels have been described in helminth infection [63] and may act in concert with the IL-13-induced upregulation of 5-HT2A mentioned above [7]. The concept of EECs contributing to intestinal motility is further supported by more recent evidence illustrating the direct innervation of Cck^{+} EECs by vagal nerves in the small intestine [64], Expansion of the I-cell EEC subtype during *T. spiralis* infection was also reported to alter the balance of Th1/Th2 responses in mice which resulted in parasite expulsion [65]. Whilst recent data point to a more immediate role of L cells in responding to bacteria following barrier damage [66], the contributions of these cells and other EEC cell types in helminth infection have yet to be revealed. Whether the effect of helminths on EECs occurs directly, or indirectly via the microbiome, is not yet clear. This offers an exciting area for further study, particularly with regard to their involvement in intestinal contractility and potential contribution in the 'sweep' response during helminth infection.

Whilst Paneth cells are typically associated with antimicrobial function in the intestine, their role in helminth infection is less clear. Earlier studies observed Paneth cell hyperplasia in mice during *T. spiralis, N. brasiliensis, H. polygyrus*, and *Schistosoma mansoni* infection, which correlated with a Th2-like response [67]. In support, Walsh and colleagues noted an increase in Paneth cell numbers in the crypt base during *T. spiralis* infection, along with a shift in the epithelial proliferative zone moving up the crypt–villus axis [68]. Lastly, Gerbe *et al.* showed that exogenous IL-4 and IL-13 treatment drove Paneth cell expansion in tuft cell-deficient *Pou2f3*$^{-/-}$ mice [11]. The role of Paneth cells in antimicrobial responses, as well as their contributions in maintaining the intestinal epithelial stem cell niche, cannot be understated [21]. During helminth infection these cells may expand to cope with the presence of infiltrating pathogenic bacteria as a result of barrier damage, whilst supporting the increased requirement for specific epithelial cells that mediate antihelminth responses.

Not Quite Resolved – Repair of the Epithelium

The return to a homeostatic state following helminth infection is a multifaceted process. Immune-mediated mechanisms of repair following mucosal injury are well documented [69], and there is increasing appreciation of the specific contributions of the epithelial compartment in this process. An important study in 2018 by Nusse *et al.* described a role for **interferon-gamma** (**IFN-γ**) in promoting epithelial stem cell regeneration during *H. polygyrus* infection [70]. They observed an early IFN-γ gene signature in larval-associated granulomas within the intestine, which drove intestinal crypt remodeling. It was noted that intestinal stem cell markers (including Lgr5) were lost in proliferative crypts associated with larval granulomas. In turn, these cells revert to a fetal-like phenotype through upregulation of Sca-1, acquiring the enhanced capacity for renewal and differentiation that fetal cells typically display, allowing for the potential regeneration of the intestinal epithelium. Although not typically associated with helminth infection, the induction of type 1-associated cytokines may have important implications in barrier protection, mediating an antimicrobial response to invasive bacteria whilst initiating an epithelial repair program. In concert, Gentile *et al.* recorded a similar IFN-γ signature in *H. polygyrus*-associated granulomas which they demonstrated to be necessary for the recruitment of natural killer (NK) cells in the early stages of infection [71]. Abrogation of NK cells did not alter worm fitness, but the researchers did observe an increase in vascular injury, thus indicating an unexpected role for NK cells in mediating protection of epithelial tissue in response. Notably, the expression of IFN-γ in duodenal tissue of *H. polygyrus*-infected mice falls to baseline levels by day 14 postinfection, with concurrent

upregulation of type 2 genes that are associated with parasite expulsion [71]. This suggests an interesting mixture of type 1 and type 2 immune responses that are defined by kinetics, providing an important nuance to the prevailing dogma of type 2-dominated immunity during antihelminth responses. Interestingly, Cliffe and colleagues demonstrated a role for IFN-γ in driving epithelial cell apoptosis in the cecum of *T. muris*-infected mice, which is proposed to counteract the hyperproliferative response of the epithelium during chronic helminth infection [72]. Whilst these studies of Gentile *et al.* [71] and Cliffe *et al.* [72] appear to indicate contradictory roles for IFN-γ, this could be attributed to differences in both the specific tissue niche these parasites occupy during their lifecycle and the kinetics of infection. IFN-γ expression peaks around day 6 during *H. polygyrus* infection, whereas elevated IFN-γ (in addition to cecal epithelial cell apoptosis) is observed at day 42 in a chronic *T. muris* infection. The epithelial response to different helminths will require further characterization in order to determine the contribution and balance of type 1 and type 2 immunity during early and later stages of infection. Other cytokines and factors undoubtedly contribute to repair of the intestinal epithelium during helminth infection as the ability of the intestinal barrier to respond to injury is complex, relying on the plasticity of epithelial stem cells and their response to cues in the surrounding microenvironment. A report by Chandrakesan and colleagues suggests that tuft cells regulate DNA damage following radiation-induced epithelial injury via Dclk1 [73]. It will be interesting to see if tuft cells are able to 'sense' these alterations and whether they employ similar mechanisms during helminth-mediated damage of the barrier.

Concluding Remarks

Until recently there was considerably less attention paid to the subpopulations of cells comprising the intestinal epithelial barrier during helminth infection. It is becoming increasingly clear that these cells are critical determinants of defense, with recent advances in scRNAseq highlighting the heterogeneity of these cells and their individual contributions following infection. Whilst great attention has been paid to the crosstalk between epithelial cells and the immune compartment, comparatively little is known about the interactions between IECs and the subepithelial stroma during helminth infection, which is shown to be important in mediating epithelial cell regeneration in other contexts [74] (see Outstanding Questions). Further research will be vital in unveiling novel epithelial cell contributions to host immunity, barrier defense, and repair. Investigations of how helminths impact on the epithelium have already yielded important new discoveries, such as the discovery of tuft cells specialized for IL-25 production, and these findings inform not only our understanding of host–helminth interactions but also of the complex biology of the intestine and its role in guarding against a variety of environmental and pathogenic stressors.

Outstanding Questions

Do EECs play a functional role during helminth infection? If so, how are they activated?

Does the detection of microbial metabolites by intestinal epithelial cell lineages influence the response to a concomitant helminth infection?

What are the elusive molecular cues from helminths that activate tuft cells?

Does the heterogeneity of IEC subsets and receptor recognition impact subsequent responses to infection?

Is the release of IL-33 just a damage response, or can it be specifically released following helminth recognition?

Could tuft cells and EECs participate in the 'sweep' response through afferent nerve activation?

Is crosstalk between epithelial cells and stromal cells an important mediator of barrier repair following helminth infection?

What are the therapeutic advantages of targeting tuft cells?

How are tuft cell and EEC lineages altered during human helminth infection?

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