

# Intestinal epithelial cells: at the interface of the microbiota and mucosal immunity

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## Summary

The intestinal epithelium forms a barrier between the microbiota and the rest of the body. In addition, beyond acting as a physical barrier, the function of intestinal epithelial cells (IECs) in sensing and responding to microbial signals is increasingly appreciated and likely has numerous implications for the vast network of immune cells within and below the intestinal epithelium. IECs also respond to factors produced by immune cells, and these can regulate IEC barrier function, proliferation and differentiation, as well as influence the composition of the microbiota. The mechanisms involved in IEC–microbe–immune interactions, however, are not fully characterized. In this review, we explore the ability of IECs to direct intestinal homeostasis by orchestrating communication between intestinal microbes and mucosal innate and adaptive immune cells during physiological and inflammatory conditions. We focus primarily on the most recent findings and call attention to the numerous remaining unknowns regarding the complex crosstalk between IECs, the microbiota and intestinal immune cells.

**Keywords:** bacterial; epithelial cell; gut; microbiota; mucosal immunology.

## Introduction

The mucosal surface of the gastrointestinal tract consists of a single layer of intestinal epithelial cells (IECs) that provide an interface for immune cells to detect and respond to environmental substances. These include food components and pathogenic or commensal microbial species of Archaea, bacteria, fungi, viruses and parasites, with around  $10^{11}$  bacteria colonizing the human gastrointestinal tract.<sup>1</sup> This creates an enormous source of potential immune stimuli; however, under homeostatic conditions the immune cells in and underlying the mucosa develop and function in a controlled manner, balancing inflammatory and regulatory responses to prevent overreaction to innocuous luminal antigens. During pathogenic infection, immune cells are mobilized to fight and clear invading microbes. Although the mechanisms that regulate intestinal immune responses during health and disease are still being elucidated, dialogue between intestinal microbes, IECs and innate and adaptive immune cells is increasingly appreciated to play a major role.

The IEC monolayer is composed of a number of cell types that differentiate from epithelial stem cells residing in the crypts. IEC types include goblet cells that produce

mucin glycoproteins and form mucus, absorptive enterocytes, enteroendocrine cells, Paneth cells at the bottom of intestinal crypts that secrete antimicrobial peptides (AMPs), microfold (M) cells involved in antigen capture and presentation to immune cells, and tuft cells that promote type 2 immunity to intestinal parasites.<sup>2,3</sup> Single-cell RNA sequencing has further defined the behaviour and characteristics of each IEC cell type,<sup>4</sup> and a recent study identified two subtypes of tuft cells that change in frequency during helminth infection.<sup>5</sup> Together, IECs form the boundary between the internal body and the outside environment, and studies in germ-free mice have demonstrated that microbial colonization of the intestinal lumen influences IEC metabolism, proliferation, survival, barrier function and communication with immune cells.<sup>6</sup> IECs are the main cell type in direct contact with stimuli from the luminal microbiota and are critical players in microbe–host interactions. As such, in addition to epithelial cell-mediated defence mechanisms, IECs also coordinate the development and maturation of downstream immune responses from immune cells residing in the lamina propria and underlying lymphoid tissues. These immune cells help to contain microbes at the mucosa and maintain intestinal homeostasis.

Although much is known about the immune cell populations in the gut, less is known about the mechanisms by which IECs regulate the development and maturation of immune cells during homeostasis and how this is disrupted during different disease states. In addition, the stimuli from commensal bacterial species recognized by IECs and the receptors and signalling pathways involved are not thoroughly understood. In this review, we describe the role of IECs as important communication hubs and modulators that shape and coordinate the activity of both microbes and immune cells. We place special emphasis on the most recent findings and highlight the many open questions regarding the complex network of interactions between IECs, the microbiota and intestinal immune cells.

### Microbiota–IEC crosstalk

The intestinal epithelium is a highly dynamic tissue that provides both physical and chemical barriers to protect the intestinal mucosa and peripheral organs from commensal microbes or invading pathogenic microorganisms. In addition to forming a barrier, IECs also detect a myriad of signals from intestinal microbes, allowing fine tuning of IEC proliferation and homeostatic functions (Fig. 1). Likewise, IEC programmes can influence the composition of the intestinal microbiota in a number of ways.

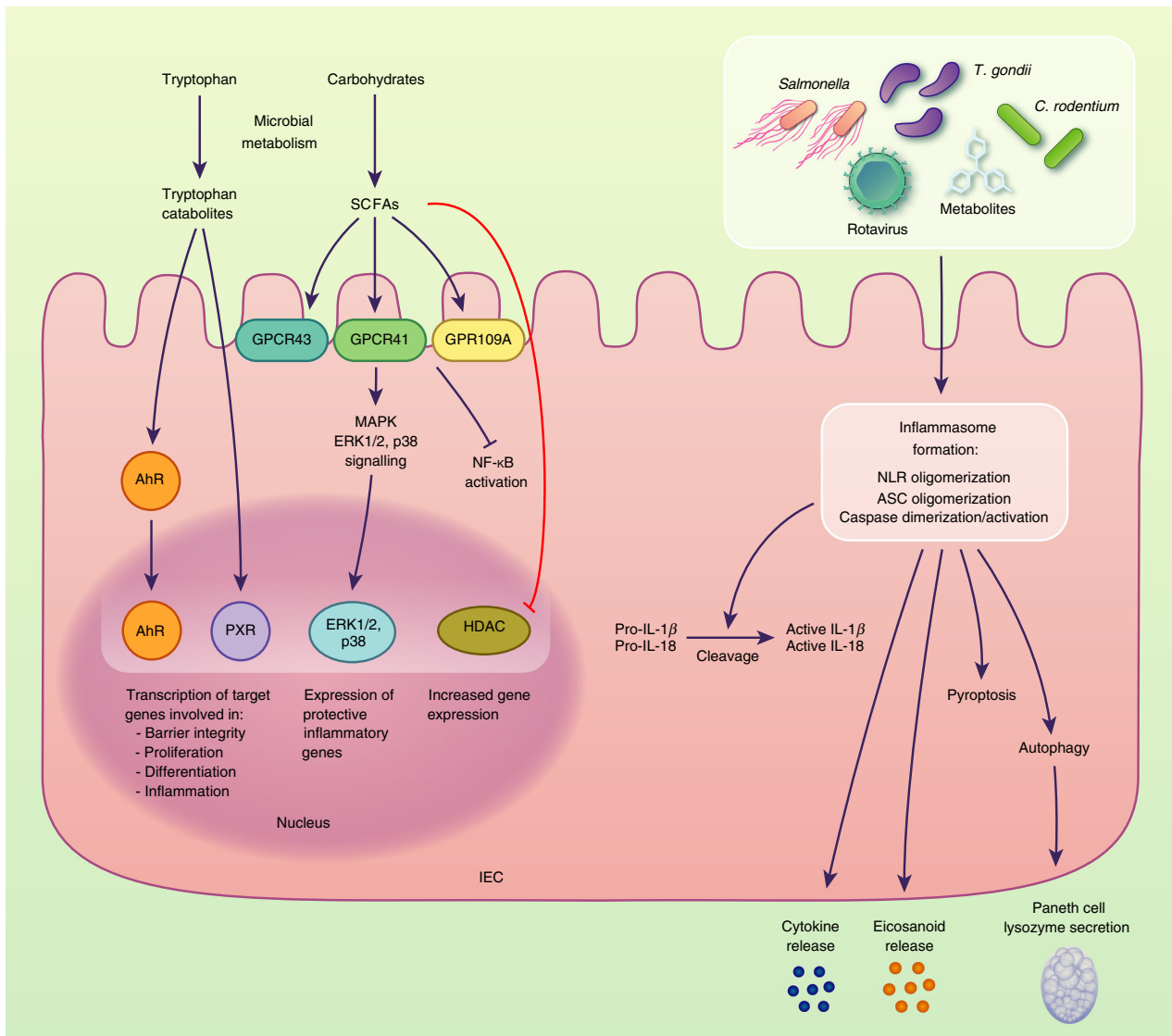
### Microbial regulation of IEC growth and function

Intestinal epithelial cells possess a number of mechanisms to sense and respond to the presence and activity of intestinal microbes. IECs express pattern recognition receptors (PRRs) to specifically detect molecular patterns from commensal and pathogenic gut microbes, and these have been extensively described in previous reviews.<sup>7–9</sup> Following the detection of intestinal microbes, IECs enhance various components of the intestinal barrier to protect underlying host tissues from bacterial infiltration. These include AMP production, mucus secretion, tight junction integrity, and IEC growth and differentiation. IECs secrete a range of AMPs, many through PRR/MyD88-dependent mechanisms, that accumulate in the mucus layer and possess broad antimicrobial activities.<sup>10,11</sup> Indeed, during *Citrobacter rodentium* infection, MyD88 signalling solely in IECs was recently shown to be sufficient to enhance IEC barrier integrity and increase production of RegIII $\gamma$  and immunomodulatory acute-phase protein serum amyloid A1 (SAA1).<sup>12</sup> Goblet cells secrete mucin glycoproteins to create the viscous mucus layer, and the importance of mucus in protection against invading microbes was recently highlighted in a study showing that the discontinuous mucus layer in the mouse caecum and corresponding uncovered areas of the epithelium form hotspots for *Salmonella* infection.<sup>13</sup> A number

of bacterial species have been shown to modulate mucin secretion by goblet cells. For example, commensal *Ruminococcus gnavus*<sup>14</sup> and *Lactobacillus rhamnosus*<sup>15</sup> stimulate the production of mucins, while pathogenic microbes including adherent and invasive *Escherichia coli* promote a less effective mucus barrier.<sup>16</sup> In a recent study, Amuc\_1100, a membrane protein from commensal *Akkermansia muciniphila*, was shown to interact with the PRR Toll-like receptor 2 (TLR2) to increase intestinal barrier function, namely mucus thickness and tight junction protein (TJP) expression.<sup>17</sup>

Although PRR-mediated mechanisms of sensing microbial products are the most extensively studied, IECs also use a number of other pathways. For example, inflammasomes have been shown to play an important role in IEC-sensing of microbial stimuli and damage-associated molecular patterns and in triggering protective barrier responses.<sup>18–21</sup> The NAIIP-NLRC4 inflammasome has recently been implicated in the IEC response to *Salmonella* infection *in vivo*, enabling pro-inflammatory programmes that result in production of cytokines and the hormone-like eicosanoid prostaglandin E<sub>2</sub>, as well as lytic cell death and the expulsion of infected IECs.<sup>22</sup> The autophagy pathway has also been shown to be critical for maintaining intestinal epithelial integrity in response to microbes, and a recent study demonstrated that release of lysozyme by Paneth cells during bacterial infection is mediated through an autophagy-based alternative secretion pathway.<sup>23</sup> Although mechanisms of microbial modulation of and sensing by IECs continue to be uncovered, many pathways remain incompletely characterized.

Microbial metabolites produced by bacterial fermentation of dietary components are also important signals detected by IECs. For example, tryptophan catabolites, detected by pregnane X receptor (PXR)<sup>24</sup> and the aryl hydrocarbon receptor (AhR),<sup>25,26</sup> drive a multitude of anti-inflammatory and protective barrier functions. IEC AhR sensing of dietary components and tryptophan catabolites contributes to the maintenance of intestinal barrier integrity by inducing IEC differentiation from crypt stem cells<sup>26</sup> and mitigating inflammatory responses.<sup>27</sup> PXR was recently shown to respond to indole 3-propionic acid, a tryptophan metabolite produced by commensal *Clostridium sporogenes*, and mice deficient for PXR exhibited increased epithelial inflammatory injury and decreased tight junction protein expression. In contrast, germ-free mice colonized with *C. sporogenes* and dosed with L-tryptophan exhibited decreased intestinal permeability and increased expression of detoxifying PXR target genes.<sup>24</sup> In addition to serving as a major energy source for enterocytes, microbiota-derived short-chain fatty acids (SCFAs) have also been implicated in the regulation of most IEC functions including cell turnover,<sup>28</sup> tight junction protein expression,<sup>29</sup> and inflammasome- or hypoxia-inducible factor (HIF)-mediated epithelial



**Figure 1.** Intestinal epithelial cells (IECs) sense microbial stimuli through a number of different mechanisms that regulate IEC gene transcription and inflammatory responses. For example, tryptophan catabolites and short-chain fatty acids (SCFAs) produced as a result of microbial metabolism trigger the activation of AhR, PXR, ERK1/2 and p38 that directly regulate the expression of target genes. The inflammasome complexes in IECs reported to respond to microbial stimuli include NLRP3, NAIP-NLRC4, NLRP6 and NLRP9b, which trigger cell death pathways and the release of inflammatory cytokines and mediators.

integrity.<sup>30,31</sup> SCFAs can directly influence gene transcription by binding to and inhibiting histone deacetylases (HDAC) or through binding to the metabolite-sensing receptors GPR41, GPR43 and GPR109A.<sup>32</sup> Indeed, a recent study showed that optimal expression of AMPs requires IEC-sensing of SCFAs via GPR43. Using *Gpr43*<sup>-/-</sup> mice and enteroids, investigators observed that the AMPs RegIIIγ and β-defensins 1, 3 and 4 were reduced in the absence of GPR43 or downstream mammalian target of rapamycin (mTOR) and signal transducer and activator of transcription 3 (STAT3) activation.<sup>33</sup>

Microbes also induce a number of non-barrier functions in IECs, including changes in metabolism and the

biosynthesis of signalling molecules. For example, early during *Citrobacter rodentium* infection, IECs have been shown to exhibit changes in cholesterol and carbon metabolic pathways, suggesting that IEC metabolism is reprogrammed to meet increased cellular energetic demands during tissue repair.<sup>34</sup> Some enterochromaffin cells, a subtype of enteroendocrine cell, have been shown to secrete serotonin (5-hydroxytryptamine, 5-HT) in response to mechanosensing via the mechanotransducer Piezo2,<sup>35</sup> and 5-HT is an important regulator of enteric nervous system development, and gastrointestinal tract motility and inflammation.<sup>36</sup> In addition to mechanosensing, a recent study demonstrated that several

metabolites from a consortium of commensal spore-forming bacteria (predominantly Clostridial species) promote 5-HT biosynthesis by colonic enterochromaffin cells in colonized mice.<sup>37</sup> In response to microbes, IECs also secrete a number of cytokines and effector molecules including interleukin-25 (IL-25) and SAA.<sup>38,39</sup> These effectors regulate the development and function of intestinal immune cells, as described in the next section of this review. Collectively, these recent findings indicate that a broad range of IEC functions are affected by sensing of intestinal microbes (Table 1); however, it is worth noting that many of these studies were performed in the context of pathogenic microbial infection. Further studies are required to identify additional stimuli from commensal microbes and characterize commensurate IEC responses at steady state.

### Influence of IECs on diversity and function of the intestinal microbiota

Although the effects of IEC–microbe crosstalk on IECs are beginning to be elucidated, the effects of this interaction on the gut microbiota are substantially less characterized. Still, numerous recent studies have indicated that IECs also have an impact on the microbial populations residing in the gut. Autophagy is particularly well studied in maintaining the function of Paneth cells and protecting against pathogenic bacteria.<sup>40–44</sup> Recently, disruption of IEC autophagy has also been shown to dramatically alter the composition of the gut microbiota and reduce intestinal microbial alpha-diversity in mice.<sup>45</sup> Another recent study showed that serotonin production by enterochromaffin cells modulates gut microbial composition,<sup>46</sup> and AMPs secreted by IECs have been broadly reported to influence the composition of intestinal gut microbes.<sup>47–49</sup> The NLRP6-inflammasome is also highly expressed by IECs, and previous studies have shown that NLRP6 helps to maintain eubiosis of the intestinal microbiota.<sup>50,51</sup> However, recent studies of *Nlrp6*<sup>-/-</sup> and *Asc*<sup>-/-</sup> mice co-housed with wild-type littermates report that the NLRP6 inflammasome does not affect gut microbial diversity,<sup>52,53</sup> highlighting that non-genetic confounding factors may impact *in vivo* studies investigating causal relationships between host gene deficiencies and alterations in the microbiota.<sup>54</sup> Indeed, whereas previous studies eliminated a role for nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and NOD2 in shaping microbiota composition based on polymerase chain reaction for 10 targeted bacterial groups in co-housed littermates of different genotypes,<sup>55</sup> NOD2 signalling in IECs was recently strongly implicated in specifically controlling the colonization and growth of commensal *Bacteroides vulgatus*.<sup>56</sup> In this recent study, although wild-type animals co-housed with *Nod2*<sup>-/-</sup> mice acquired the overabundance of *B. vulgatus* characteristic of knockout mice, this was diminished upon re-separation.

Given these conflicting observations, a strong case has been made for using crosses and littermate controls as a superior alternative (or addition) to co-housing.<sup>57</sup> Still, the effects of IEC PRRs on the composition of the intestinal microbiota remain contentious and, importantly, the mechanisms behind many of the microbiota alterations observed have not been fully uncovered.

Microbial gene expression is also influenced by IECs through several mechanisms. For instance, a recent study using IEC-specific TLR4 knockout (TLR4<sup>IEC-KO</sup>) mice demonstrated that TLR4 influences the composition and function of intestinal microbes, including the expression of microbial genes involved in the metabolism of lipids, amino acids and nucleotides.<sup>58</sup> TLR4<sup>IEC-KO</sup> mice developed metabolic syndrome, and lysozyme and genes regulated by peroxisome proliferator-activated receptors were down-regulated, suggesting a mechanism by which intestinal TLR4 may influence the microbiota. In another study, attaching and effacing enterohaemorrhagic *E. coli* was shown to require mechanosensing of IECs to express the locus of enterocyte effacement that encodes its type 3 secretion system, and this was responsible for forming lesions in the gastrointestinal tract.<sup>59</sup> In addition, a recent study reported that miRNA is released by IECs into the intestinal lumen where it enters bacterial species such as *Fusobacterium nucleatum* and *E. coli* and regulates their gene expression and growth.<sup>60</sup>

The main nutrient source for gut microbes is typically diet-derived components including polysaccharides or glycans. However, some gut microbes can also use host glycans on mucin proteins and the surface of IECs, providing an alternative energy source when dietary glycans are reduced.<sup>61–63</sup> For example, several commensal Clostridiales members use the mucin-associated sugars fucose and sialic acid as energy sources, promoting their colonization of the gut.<sup>27</sup> Glycans are also ligands for bacterial attachment, and some gut microbial species such as *Ruminococcus gnavus* are hypothesized to target mucin glycans to assist their spread and persistence in niches in the intestinal lumen.<sup>64</sup> Together, these studies demonstrate the multitude of interactions between microbes and IECs that can trigger various IEC programmes and shape the microbial ecosystem in the gut.

### IEC–immune cell crosstalk

Intestinal epithelial cells possess a number of independent barrier functions to control and/or kill gut microbes, they also mediate crosstalk between the microbiota and intraepithelial and subepithelial immune cells by responding to microbial metabolites and coordinating immune responses. This is achieved by a number of known and unknown mechanisms including the secretion of chemokines, cytokines and other immunomodulatory molecules (Fig. 2), as well as the transport of microbial

Table 1. Gut microbial stimuli that interact with intestinal epithelial cells (IECs)

IEC sensor/ signalling pathway	Microbial stimuli	Microbial species used	<i>In vivo/in vitro</i> and study details	IEC response	References
Toll-like receptor 9, nuclear factor- $\kappa$ B	Unmethylated CpG bacterial DNA <sup>1</sup>	<i>Citrobacter rodentium</i> (DBS100), <i>Salmonella typhimurium</i> (ATCC 14028), <i>Helicobacter pylori</i> (PMSS1)	<i>In vivo</i> , <i>Thr9</i> <sup>-/-</sup> mice	Decreases intestinal inflammation and damage following bacterial challenge	140–143 <sup>1</sup>
Caspase-3/7-mediated apoptosis	Enterotoxins (TcdA and TcdB)	<i>Clostridium difficile</i> (VPI10463)	<i>In vivo</i> and <i>in vitro</i> intestinal organoids; <i>Casp3</i> <sup>IEC-KO</sup> mice	Restricts <i>C. difficile</i> growth <i>in vivo</i>	144
NAIP/NLRC4 inflammasome	Flagellin <sup>1</sup> Unknown <sup>2</sup>	<i>Salmonella Typhimurium</i> , <i>Citrobacter rodentium</i> <sup>2</sup>	<i>In vivo</i> , <i>Casp1</i> <sup>-/-</sup> , <i>Casp8</i> <sup>-/-</sup> , <i>Nlr4</i> <sup>-/-</sup>	Protects against enteric pathogen invasion; expulsion of pyroptotic IECs and release of eicosanoid and interleukin-18 (IL-18)	21,22,145 <sup>1</sup>
Toll-like receptor 4, peroxisome proliferator-activated receptor (PPAR)	Free fatty acids <sup>1</sup>	Commensal gut microbes	<i>In vivo</i> , <i>Thr4</i> <sup>IEC-KO</sup>	Prevents development of metabolic syndrome; regulates expression of lysozyme and PPAR-controlled genes	58,146, 147 <sup>1</sup>
P2X7/NLRP3 inflammasome	Ligands include extracellular ATP and K <sup>+</sup>	<i>Toxoplasma gondii</i>	<i>In vitro</i> , FHs 74 Int cells	IL-1 $\beta$ secretion and inhibition of parasitic proliferation	18,148, 149 <sup>1</sup>
NLRP6 inflammasome	Unknown	<i>Citrobacter rodentium</i>	<i>In vivo</i> , <i>Nlrp6</i> <sup>-/-</sup> , <i>Asc</i> <sup>-/-</sup> , <i>Casp11</i> <sup>-/-</sup>	Orchestrates goblet cell mucin granule exocytosis	19
Nlrp9 inflammasome	dsRNA <sup>1</sup>	Rotavirus EW	<i>In vivo</i> , <i>Nlrp9</i> <sup>IEC-KO</sup> <sup>-/-</sup>	Restricts rotavirus infection by IL-18 production and pyroptosis	20
Aryl hydrocarbon receptor	Tryptophan indole derivatives	Lactobacilli, Clostridiales members	<i>In vivo</i> , <i>Ahr</i> <sup>-/-</sup>	IL-22 production; resistance to enteric pathogens; maintenance of intestinal homeostasis and barrier functions	25–27
Receptors GPR41, GPR43 and GPR109; HDAC inhibition; mTOR, STAT3, ERK and MAPK signalling MyD88 signalling	short-chain fatty acids	Various microbes including <i>Bacteroides</i> spp.	<i>In vivo</i> , <i>GPR41</i> <sup>-/-</sup> , <i>GPR43</i> <sup>-/-</sup> , <i>GPR109</i> <sup>-/-</sup> , <i>in vitro</i> murine intestinal organoids	Protective inflammatory responses during pathogen infection; secretion of AMPs, chemokines and cytokines; controls IEC turnover and barrier functions; RALDHI expression and vitamin A metabolism	28–31,33,150–152
GPCR and ERK/MAPK signalling	Various TLR ligands	<i>Citrobacter rodentium</i>	<i>In vivo</i> , <i>MyD88</i> <sup>-/-</sup>	Secretion of AMPs, control of bacterial infiltration, enhanced barrier integrity	12
Various cellular stresses including nutrient deprivation, infection with microbes <sup>1</sup>	Pili, novel 3000 MW molecule Autophagy	<i>Lactobacillus rhamnosus</i> (CNCM 1-3690), <i>Ruminococcus gnavus</i> (E1) <i>Helicobacter hepaticus</i> , <i>Salmonella Typhimurium</i> , <i>Pasteurellaceae</i> family	<i>In vivo</i> , <i>in vitro</i> HT29-MTX cells <i>In vivo</i> , <i>Atg161</i> <sup>-/-</sup> , <i>in vitro</i> <i>Atg161</i> <sup>-/-</sup> organoids	Expression of glycoproteins and mucus production by goblet cells; cytoprotective responses Control inflammation-induced apoptosis, necroptosis and maintains intestinal barrier, lysozyme secretion by Paneth cells, promotes bacterial clearance	14,15 23,43,45,153–155 <sup>1</sup>

Table 1. (Continued)

IEC sensor/signalling pathway	Microbial stimuli	Microbial species used	<i>In vivo/in vitro</i> and study details	IEC response	References
Cellular forces	Mechanosensors/ mechanotransducer Piezo2	Clostridial species	<i>In vivo, in vitro</i>	Serotonin release by enterochromaffin cells	35,37
Peptidoglycan components: muramyl dipeptide <sup>1</sup>	Nod2	<i>Bacteroides vulgatus</i> , <i>Enterococcus faecium</i>	<i>In vivo, Nod2</i> <sup>-/-</sup> and <i>in vitro</i>	Restriction of bacterial growth or dissemination, expression of inflammatory genes, goblet cell function	56,156,157 <sup>1</sup> , 158
Pregnane X receptor (PXR)	Indole 3-propionic acid	<i>Clostridium sporogenes</i>	<i>In vivo, Nr1h2</i> <sup>-/-</sup> , <i>Nr1h2</i> <sup>-/-</sup> , <i>Tlr4</i> <sup>-/-</sup> , <i>Pxr</i> <sup>-/-</sup>	Regulation of intestinal permeability and intestinal inflammation, defence against intracellular pathogens	24,159

<sup>1</sup>A finding from a different or additional study.

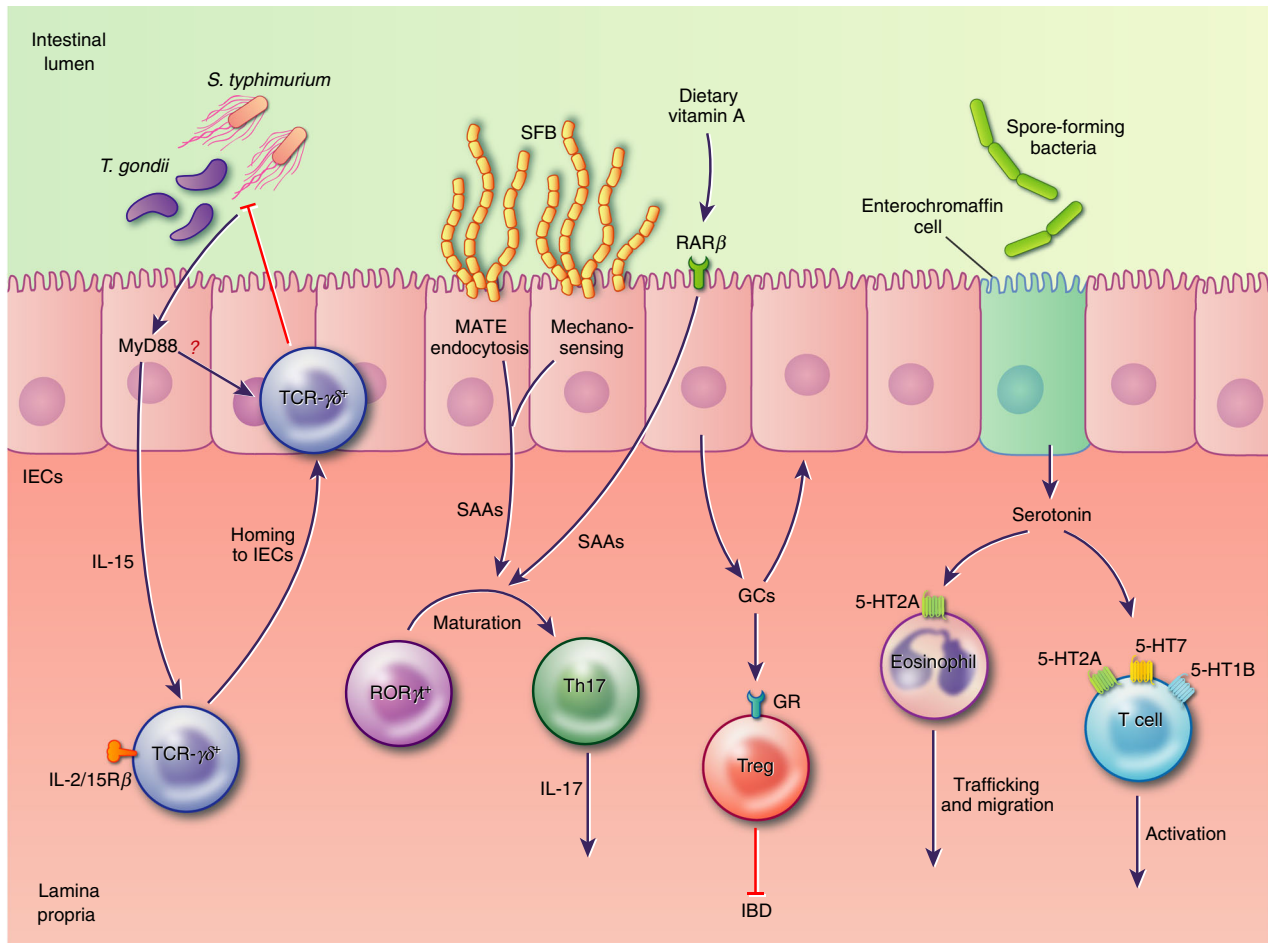
<sup>2</sup>While type 3 secretion system components expressed by *C. rodentium* are thought to provide the stimulus triggering NLR4 inflammasome formation *in vivo*, this has yet to be demonstrated.

antigens and metabolites to underlying immune cells in the lamina propria. Reciprocally, intestinal immune cells support a number of important IEC functions (Fig. 3).

### IEC secretion of immunomodulatory molecules

Among the immunomodulatory molecules that are produced by IECs, thymic stromal lymphopoietin, transforming growth factor (TGF)- $\beta$ , retinoic acid and IL-10 have been shown to impact a broad range of immune cells and have each earned their own detailed reviews.<sup>65–68</sup> In addition to these well-described modulators of immune cell function, IEC production of IL-15 has recently been shown to be required for the homing of protective T-cell receptor- $\gamma\delta$ -positive (TCR- $\gamma\delta^+$ ) intraepithelial lymphocytes (IELs) to the epithelium of the small intestine.<sup>69</sup> The TCR- $\gamma\delta^+$  IEL surveillance behaviour, antimicrobial responses and protection against pathogens such as *Salmonella* Typhimurium and *Toxoplasma gondii* are dependent on MyD88 signalling in IECs;<sup>70,71</sup> however, the mechanisms of IEC–IEL communication required for these functions are still unknown. In response to colonization by adherent microbes, IECs secrete SAAs, which promotes the functional maturation of retinoic acid-related orphan receptor  $\gamma$ t-positive (ROR $\gamma$ t<sup>+</sup>) T cells to IL-17-secreting T helper type 17 (Th17) cells.<sup>72,73</sup> This has been hypothesized to occur via mechanosensing of microbial contact, and a recent study has shown that in the case of segmented filamentous bacteria (SFB), the transfer of SFB antigens through IECs via microbial adhesion-triggered endocytosis (MATE) plays a pivotal role.<sup>74</sup> Another recent study shows that epithelial sensing of dietary vitamin A through retinoic acid receptor  $\beta$  is also required for IEC expression of SAAs.<sup>75</sup>

Perhaps less appreciated, glucocorticoids (GCs) and neurotransmitters are also abundantly produced by epithelial cells in the gut. GCs are well-known for their general anti-inflammatory effects, but beyond their production in adrenal glands, crypt IECs have been shown to release GCs in response to anti-CD3-mediated T-cell activation, and IEC synthesis of GCs has been shown to control local inflammation and disease severity in a 2,4,6-trinitrobenzene sulphonic acid colitis model.<sup>76,77</sup> As almost all vertebrate cells express glucocorticoid receptors (GRs) the effects of GCs are pleiotropic; however, T-cell-specific responses to GCs have been shown to be involved in T-cell homeostasis, and regulatory T (Treg) cell-specific GR deficiency was recently shown to impair Treg cell capacity to prevent the induction of disease in a mouse model of inflammatory bowel disease.<sup>78,79</sup> In addition, a recent study of mice with diminished GR responses revealed an interferon-specific gene signature in the gut that was abrogated by antibiotic treatment, indicating a role for the microbiota.<sup>80</sup> While information regarding intestinal production of GCs continues to emerge, the stimuli involved and immune cell effects have yet to be fully elucidated.



**Figure 2.** In response to microbial stimuli, intestinal epithelial cells (IECs) secrete factors that modulate various immune cell functions. In the small intestine these include interleukin-15 (IL-15), required for the recruitment of protective T-cell receptor (TCR)  $\gamma\delta^+$  intraepithelial lymphocytes (IELs) to the epithelial layer, and serum amyloids, which induce the differentiation of IL-17-secreting T helper type 17 cells. In the small and large intestine, glucocorticoids and serotonin promote anti-inflammatory responses by immune cell populations, including lymphocytes and eosinophils, modulating inflammation and the development of disease pathology.

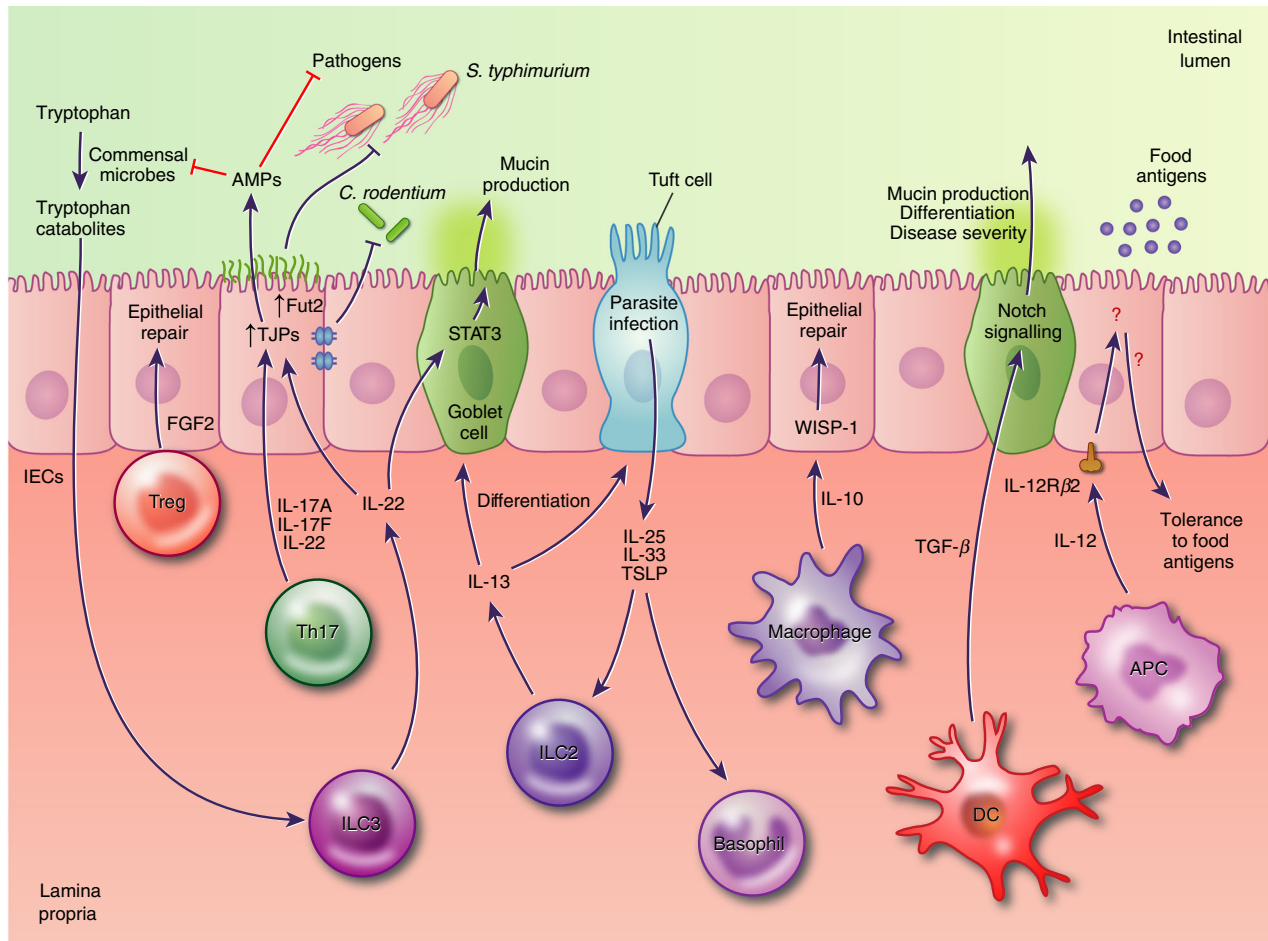
Similarly, although a monoamine neurotransmitter, serotonin is primarily produced in the intestines by enterochromaffin cells. As discussed earlier, a recent study using germ-free mice colonized with spore-forming bacteria identified a role for metabolites from commensal microbes in promoting serotonin biosynthesis by colonic enterochromaffin cells.<sup>37</sup> Although the effects of serotonin on intestinal immune cells have not been completely characterized, most immune cells express the serotonin transporter (SERT), and there is evidence that functions as diverse as T-cell activation, eosinophil trafficking and tumour necrosis factor- $\alpha$ -mediated inflammation are modulated by serotonin.<sup>81–83</sup>

#### IEC transport of microbial antigens and metabolites

An important mechanism by which intestinal epithelial cells direct adaptive immune responses to gut microbes is

by antigen sampling and presentation to immune cells underlying the epithelium. Specialized M cells are concentrated in the follicle-associated epithelium that overlies the luminal surface of Peyer's patches and isolated lymphoid follicles of the small intestine. M cells directly take up antigens and intact microorganisms from the intestinal lumen and transport them in a unidirectional way for presentation to resident immune cells. Antigen sampling by M cells is likely to be the key initiator of intestinal IgA responses to commensal bacteria as mice with impaired M cell differentiation display decreased faecal secretory IgA.<sup>84</sup>

In addition to M cells, goblet cells contribute to antigen sampling by forming goblet cell-associated antigen passages (GAPs) to deliver intestinal lumen antigens to CD103<sup>+</sup> dendritic cells in the lamina propria.<sup>85</sup> Regulation of GAPs may constitute a dynamic means of modulating intestinal immune responses. While small intestine



**Figure 3.** Immune cells contribute to the regulation of intestinal epithelial cell (IEC) differentiation and barrier function. For example, type 3 innate lymphoid cells (ILC3) secretion of interleukin-22 (IL-22) regulates IEC secretion of antimicrobial peptides (AMPs) and mucins, tight junction formation, and surface protein glycosylation, assisting in resistance to pathogenic microbes. Tolerance to food antigens is reported to involve IEC responsiveness to IL-12; however, the subsequent IEC signalling pathways and immune cell types that mediate this response are not currently known.

goblet cells form GAPs in response to acetylcholine, colonic goblet cell sensing of commensal microbes via MyD88 decreases their acetylcholine responsiveness and formation of GAPs to limit inflammatory immune responses to commensals.<sup>86</sup> Timed control of GAPs during the pre-weaning phase has been implicated in Treg cell-mediated tolerance towards commensal bacteria,<sup>87</sup> and during *Salmonella* infection IL-1 $\beta$  inhibits GAP formation, leading to decreased bacterial dissemination.<sup>88</sup>

Enterocytes also participate in antigen presentation by several processes. These include presentation of lipid antigens to natural killer T cells via expression of CD1d, and IEC CD1d expression has been shown to suppress pro-inflammatory natural killer T-cell functions thereby reducing intestinal inflammation.<sup>89</sup> In addition, MHC class II has been shown to be constitutively expressed by IECs in the upper villi of the small intestine, and surface expression appears to be increased in patients with inflammatory bowel disease and in response to interferon- $\gamma$ .<sup>90–92</sup>

Reciprocally, IEC antigen presentation was shown to promote interferon- $\gamma$  secretion by CD4<sup>+</sup> T cells in co-cultures of normal T cells with IECs from patients with inflammatory bowel disease;<sup>93</sup> however, more recent studies suggest that interferon- $\gamma$ -induced MHC class II expression on IECs plays a more anti-inflammatory role by promoting a tolerogenic ratio of Treg cells to effector CD4<sup>+</sup> T cells.<sup>94,95</sup> Still, the role of IEC antigen presentation in shaping intestinal immunity has not been thoroughly explored, and the intimate contact between the epithelium and commensal microbes provides ample opportunity for IECs to curate intestinal T-cell responses.

### Immune cell contributions to IEC differentiation and function

In addition to IECs regulating immune cell functions, several intestinal immune cell types influence IEC homeostasis and inflammatory responses (Fig. 3). For example,



in response to microbial metabolites such as tryptophan catabolites, type 3 innate lymphoid cells (ILC3s) produce cytokines that regulate barrier functions of IECs.<sup>25</sup> ILC3s secrete IL-22, which promotes IEC homeostasis and repair, and can induce AMPs to control the growth of both pathogenic and commensal microbes.<sup>96–98</sup> IL-22 also affects the glycosylation of IEC surface proteins by inducing fucosyltransferase 2 (Fut2) expression, thereby enhancing host protection against *Salmonella* Typhimurium.<sup>99</sup> Mucin production by IECs is also increased by IL-22 through the activation of signal transducer and activator of transcription STAT3,<sup>100</sup> and tight junction proteins such as claudin-2 have recently been shown to be up-regulated by IL-22, inducing diarrhoea and facilitating clearance of *Citrobacter rodentium* in a mouse model of enteric infection.<sup>101</sup>

Beyond ILC3s and IL-22, some other lymphoid cells also contribute to IEC responses. During parasitic infection, IECs secrete a number of cytokines that promote the expansion and activation of group 2 innate lymphoid cells (ILC2s) and basophils, including IL-33 and thymic stromal lymphopoietin (TSLP), and IL-25 produced by tuft cells.<sup>2,5,102,103</sup> Reciprocally, activated ILC2s secrete IL-13, which promotes tuft and goblet cell differentiation and parasite clearance.<sup>3,104</sup> The signature cytokines secreted by Th17 cells (IL-17A, IL-17F and IL-22) can also induce IEC-mediated AMP secretion and reinforce IEC tight junctions.<sup>105–108</sup> In addition, production of fibroblast growth factor 2 (FGF2) by Treg cells has recently been shown to synergize with IL-17 to enhance mechanisms of intestinal epithelial repair.<sup>109</sup> IEC responsiveness to tumour necrosis factor also promotes mucosal repair and healing in individuals with Crohn's disease, human cells and mouse models.<sup>110</sup>

Myeloid cells also play key roles in IEC differentiation and function. For instance, perturbations to macrophage–IEC interactions lead to aberrant differentiation of IEC subtypes. Using CSF1R blockade to deplete macrophages that localize to the intestinal crypt epithelium, a recent study found that absence of macrophages results in reduced Lgr5<sup>+</sup> intestinal stem cells, lysozyme-expressing Paneth cells and Peyer's patch M cells, and increased goblet cell density.<sup>111</sup> Macrophages have also been shown to be the likely source of IL-10 in a colon biopsy-induced injury model, and in this model, macrophage IL-10 induced epithelial synthesis of the pro-repair WNT1-inducible signalling protein 1 to mediate IEC proliferation and mucosal wound healing.<sup>112</sup> In DCs, transforming growth factor- $\beta$  signalling has been suggested to control goblet cell numbers, mucus production and disease severity in dextran sulphate sodium colitis via Notch signalling, although the effects of DC dysfunction and involvement of other immune cell types were not fully investigated in this study.<sup>113</sup> More recently, IL-12 responsiveness via IL-12R $\beta$ 2 on IECs has been shown to play a

protective role in food allergy; however, the precise mechanism of protection is once again unknown.<sup>114</sup>

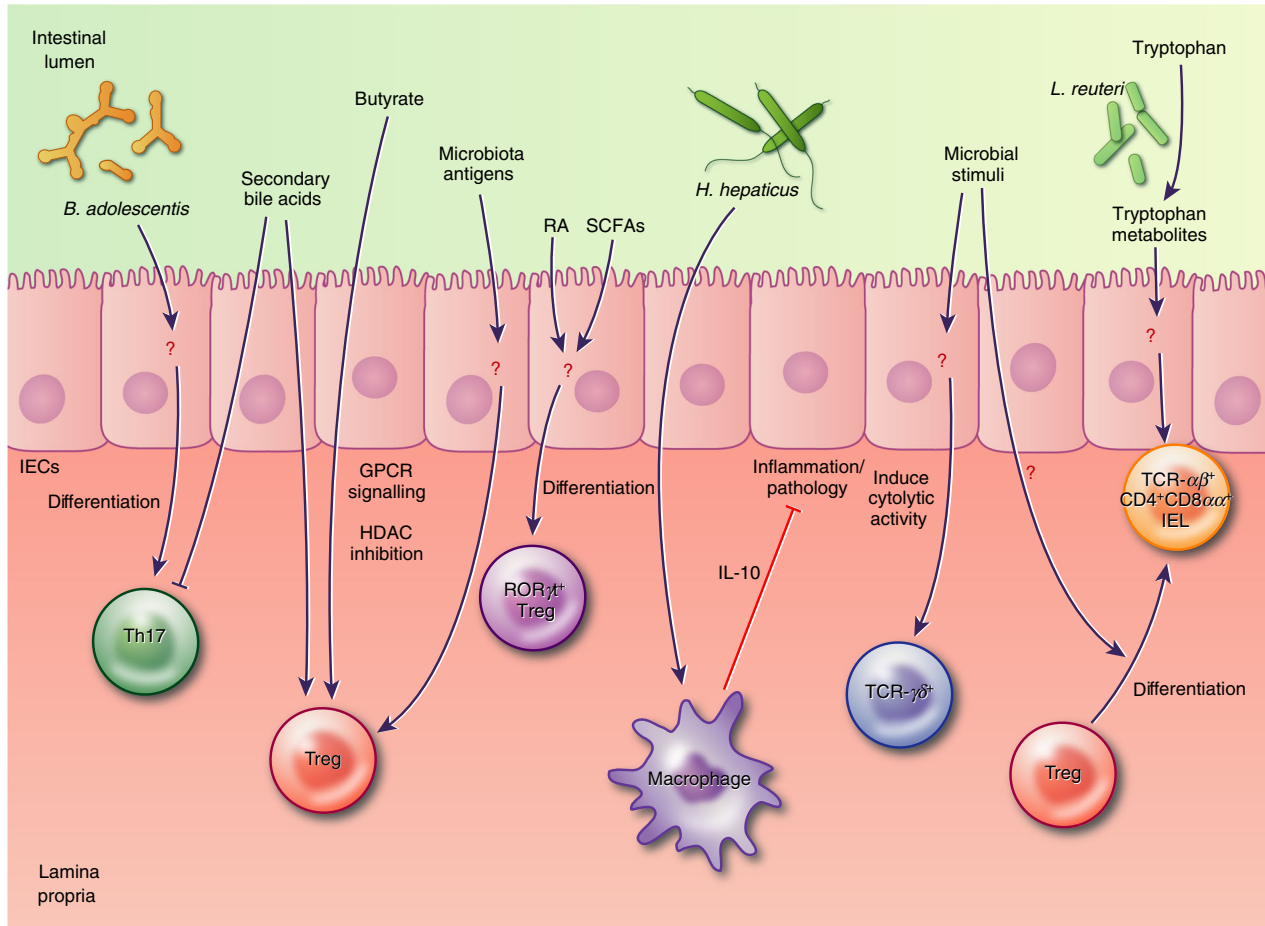
### Immune cell–microbiota crosstalk

Due to limited direct contact, most immune cell–microbiota communication is likely mediated, at least to some extent, by IECs; however, the contributions of IECs to many microbiota–immune cell interactions have yet to be fully realized. Nevertheless, a growing body of work has revealed the importance of commensal microbes for the proper development and function of immune cells (Fig. 4), and immune cells reciprocally shape the microbial habitat and microbiota diversity.

### Microbiota modulation of intestinal lymphocytes

As mentioned earlier, the proper development of IL-17-secreting Th17 cells requires SAA production by IECs in response to microbial adhesion and specifically MATE in response to SFB adhesion. The human symbiont *Bifidobacterium adolescentis*, which closely associates with the gut epithelium, is also reported to induce Th17 cells in the murine intestine with a transcriptional programme distinct from SFB, suggesting that Th17 accumulation can also be promoted by another mechanism.<sup>115</sup> Although precise roles for IECs have not been completely defined, roles for commensal microbial metabolites and antigens also continue to emerge for the generation and function of Treg cells. In three seminal studies, commensal-derived butyrate was shown to drive induction of peripheral Treg cells in the colon.<sup>116–118</sup> A later study also showed a role for recognition of antigens from commensal microbes in intestinal Treg cell differentiation. Transfer of naive transgenic T cells specific for commensal antigens into mice with a normal microbiota resulted in robust Foxp3 induction in these cells.<sup>119</sup> At weaning, the intestinal microbiota induces a vigorous immune response associated with the generation of ROR $\gamma$ t<sup>+</sup> Treg cells in an SCFA and retinoic acid-dependent manner, and inhibition of this response leads to later immunopathologies including colitis.<sup>120</sup>

ROR $\gamma$ t<sup>+</sup> Treg cells specific for *Helicobacter hepaticus* have also been shown to mediate tolerance to this commensal pathobiont,<sup>121</sup> and a polysaccharide from the same species induces anti-inflammatory IL-10 secretion in intestinal macrophages.<sup>122</sup> However, *Helicobacter* specificity itself does not dictate an anti-inflammatory programme. A recent study demonstrated that the same *Helicobacter*-specific T cells differentiate to Treg cells during homeostasis and effector T cells during colitis.<sup>123</sup> *Helicobacter bilis* colonization, on the other hand, has previously been shown to induce persistent immune reactivity to other commensal bacteria.<sup>124</sup> Collectively, these studies suggest the importance of antigen-independent



**Figure 4.** Microbial modulation of intestinal immune cells is reported to involve both direct interaction of lymphocytes and antigen-presenting cells (APCs) with microbial stimuli, as well as relatively uncharacterized indirect interactions via intestinal epithelial cells (IECs). These interactions involve many subsets of intraepithelial and lamina propria T cells and microbial metabolites like short-chain fatty acids, tryptophan catabolites and secondary bile acids, as well as currently undefined microbial antigens.

contextual cues during T-cell activation in the gut for determining T-cell fates. Indeed, two secondary bile acids, generated by commensal bacteria transformation of primary bile acids, were recently shown to inhibit Th17 differentiation and promote Treg cell induction.<sup>125</sup> Identifying the full spectrum of contextual cues will be integral for understanding how intestinal T cells are programmed.

In addition to conventional T cells, IELs have proved to be markedly influenced by the commensal microbiota. For example, TCR- $\alpha\beta^+$  IELs are almost absent in germ-free mice,<sup>126,127</sup> and TCR- $\gamma\delta^+$  IELs have impaired cytolytic activity.<sup>128</sup> The mechanisms of this control are still under investigation, but they likely involve transmission of signals through the IECs. The gut microbiota is also an important factor in the generation of TCR- $\alpha\beta^+$  CD4<sup>+</sup> CD8 $\alpha\alpha^+$  IELs. In a recent study, introduction of tryptophan-metabolizing *Lactobacillus reuteri* in mice given a diet rich in tryptophan was sufficient to induce TCR- $\alpha\beta^+$  CD4<sup>+</sup> CD8 $\alpha\alpha^+$  IEL differentiation.<sup>129</sup> Another

study has demonstrated microbiota-dependent conversion of lamina propria Foxp3<sup>+</sup> Treg cells into TCR- $\alpha\beta^+$  CD4<sup>+</sup> CD8 $\alpha\alpha^+$  IELs upon homing to the intestinal epithelium.<sup>130</sup> The ability of epithelial cells and microbial metabolites to contribute to the induction of this IEL subset is also still being elucidated.

### Immune cell effects on the intestinal microbiota

Although historically met with scepticism and comparatively understudied, the influences of intestinal immune cells on the microbiota are also gaining appreciation. Evidence that the adaptive immune system shapes microbial composition and diversity in the gut has been provided using sequencing of bacteria in multiple intestinal loci in Rag-deficient mice that lack B and T cells.<sup>131</sup> However, while ILCs are present in Rag-deficient mice, there is evidence that their number and function are altered,<sup>132</sup> complicating the conclusions that can be drawn from these animals about the role of B and T cells. Further studies

have identified an important role for polyreactive IgA in facilitating the induction of bacteria-specific IgA, and differences in these significantly influence colonization by commensal microbes.<sup>133</sup> Indeed, *Bacteroides fragilis* has now been shown to permit binding of IgA to facilitate its ability to occupy a privileged intestinal niche in close proximity to IECs.<sup>134</sup> Very recently, an important role was identified for commensal-specific IgG that results from epithelial disruption in the gut. Responsiveness to these IgGs in intestinal macrophages via activating FcγRs drives intestinal inflammation and colitis.<sup>135</sup> Although the effects of these IgGs on microbiota composition have not yet been characterized, future studies may define functions for both intestinal IgA and IgG in modulating commensal microbial communities.

Immune cells in the gut are tasked with maintaining a balance of physiological inflammation and tolerance. The resulting intestinal immune cell programmes regulate the microbial ecosystem in the gut in a manner that allows for beneficial colonization and deters invasive pathogenic infection. For example, Foxp3<sup>+</sup> Treg cells have been shown to support microbiota diversity both by suppressing inflammation and facilitating IgA selection in Peyer's patches.<sup>136</sup> Conversely, a lack of peripheral Treg cells leads to increased type 2 immune responses and disruption of microbial niches for IEC border-dwelling bacteria,<sup>137</sup> highlighting the importance of these T cells in shaping the intestinal microbial environment. In addition to composition and diversity, the evolution of commensal bacterial species has also been shown to be influenced by host adaptive immunity. In the intestines of Rag-deficient mice, the rate and predictability of *E. coli* adaptation are altered in comparison with wild-type hosts.<sup>138</sup> Taken together, these studies bring new insight into the intimate interdependence of the intestinal microbiota and immune system and open additional questions about the mechanisms involved and contribution of IECs.

## Conclusion

Due to the anatomical location of IECs between the intestinal microbiota and the host intestinal tissues, it is reasonable to predict that IECs play an important role in controlling the interaction between the luminal microbiota and underlying immune cells. Indeed, recent literature has highlighted the ability of IECs to contribute to shaping both host intestinal immunity and gut microbial composition. However, despite recent progress in the field, several challenges remain to be addressed and overcome.

Demonstrating that IEC-secreted factors are induced in response to microbe-derived signals, and the effects of these factors on immune cells, has proved difficult. Most IEC-derived cytokines are also produced by other cell types, therefore IEC involvement *in vivo* is usually

inferred but not definitively demonstrated. Knockout mice for certain receptors or effector molecules expressed by IECs have yielded further insight into the roles of IECs as direct sensors of microbial signals; however, few studies have employed IEC-specific genetic ablation *in vivo*. Studying the impact of microbe–IEC signalling on the function of immune cell subsets is also limited due to the difficulty in isolating and manipulating these cell types; the lifespan of IECs is extremely short as they are renewed every 2–6 days.<sup>139</sup> Although *in vitro* models have provided valuable insight into IEC signalling pathways and production of effectors, they remain unable to recapitulate the complexity of the intestinal environment, and interpretation of these studies is consequently limited. By further elucidating the mechanisms involved in microbe–immune crosstalk at the intestinal epithelium, we can better understand the role of IECs in regulating host immunity during homeostasis as well as during states of dysbiosis and disease.

## Disclosures

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