Review

The intestinal epithelial barrier in the control of homeostasis and immunity

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In the intestine, multiple interactions occur with the external world. Thus, the intestinal mucosal barrier has to tolerate millions of microorganisms that commonly inhabit the gut, degrade and absorb food, and establish tolerance or immunity, depending on the nature of the encountered antigens. Recent findings have highlighted that intestinal epithelial cells are not simply a barrier, but also are crucial for integrating these external and internal signals and for coordinating the ensuing immune response. Here, I review these findings and show how epithelial cells harmonize information that comes from inflammatory and non-inflammatory components of the microbiota to preserve intestinal homeostasis. If dysregulated, this immunomodulatory function of epithelial cells might contribute to the development of intestinal inflammation.

The gastrointestinal (GI) tract is an extremely complex organ

Every region of the digestive tract has a specialized function, from digestion and absorption of nutrients in the upper tract and small intestine, to digestion of complex molecules, and water and salt absorption in the large intestine. These functions are linked to specialized epithelial barriers and to the associated microbiota and immune cells, therefore, different regions of the GI tract vary morphologically and cellularly. However, common characteristics are found in the intestinal epithelial barrier. These include: the presence of a single epithelial cell (EC) layer that lines the mucosa; tight junctions (TJs) between ECs that seal the barrier; a mucus layer that overlies the epithelium that can vary in size in different regions of the intestine; and the presence of associated immune cells. Most of the differences observed in the epithelial barrier, such as the size of the mucus layer and the composition of associated immune cells, are probably the result of the presence of the microbiota. Indeed, the mucus is thicker in areas where the microbiota is more abundant, and mice that are raised under germ-free conditions (i.e. in the absence of the microbiota) display reduced and smaller gut-associated lymphoid tissue (reviewed in [1]).

In this review, I do not focus on the physical properties of the epithelial barrier and its associated permeability, which have been comprehensively reviewed elsewhere [2]. Instead, I review recent data on the different interactions of ECs with the mucus and the microbiota from the luminal side and with immune cells from the basolateral side, and how these responses are important to maintain immune homeostasis of the gut.

The epithelial barrier, not just ECs

An efficient epithelial barrier is composed of physical, cellular and chemical components. These three elements are thoroughly interconnected with each other and defects in any of these compartments can affect the function of the barrier, by leading to increased epithelial permeability or to dysbiosis, that is, altered composition of the microbiota, which eventually leads to inflammatory disorders. The epithelium is composed of four cell types: absorptive enterocytes, goblet cells, Paneth cells and enterochromaffin cells [3] (Figure 1). Enterocytes and goblet cells both produce mucin glycoproteins, which are the components of the glycocalyx and the loose mucus layer, whereas Paneth cells are responsible for the production of antimicrobial peptides (Table 1). Enterochromaffin cells represent the most abundant neuroendocrine cells in the gut. They are considered to be the first 'sensors' of the luminal content, which they reach with a very thin luminal extension. The function of enterochromaffin cells is not discussed here.

Mucus as an immune regulator

Mucus provides the first protection against luminal microorganisms (for a review, see [4]). Indeed, the mucus physically separates the intestinal lumen from the epithelium, thus limiting access of the microbiota to the apical side of the epithelium [5]. In addition, IgA antibodies can attach to the mucus and provide anchorage for bacteria. The mucus is composed of two layers, of which the inner one is devoid of bacteria [5]. Mice deficient for mucin (Muc)2 display a reduced inner mucus layer that leads to direct contact of the microbiota with the epithelial layer [5]. This might foster inflammation and could explain why these mice spontaneously develop colitis [6], and are more susceptible to intestinal tumors [7]. Indeed, mice with a missense mutation in the Muc2 gene (Winnie mice) display increased epithelial permeability and spontaneous intestinal inflammation due to endoplasmic reticular stress[8]. Winnie mice have increased numbers of $CD11c^+$ cells in the lamina propria (LP) that produce more inflammatory cytokines such as interleukin (IL)-12p40, IL-6, Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES) and macrophage inflammatory protein 1α [9]. The authors also have reported a reduced production of thymic stromal lymphopoietin (TSLP) [9]. This is an EC-derived cytokine that is involved in the regulation of

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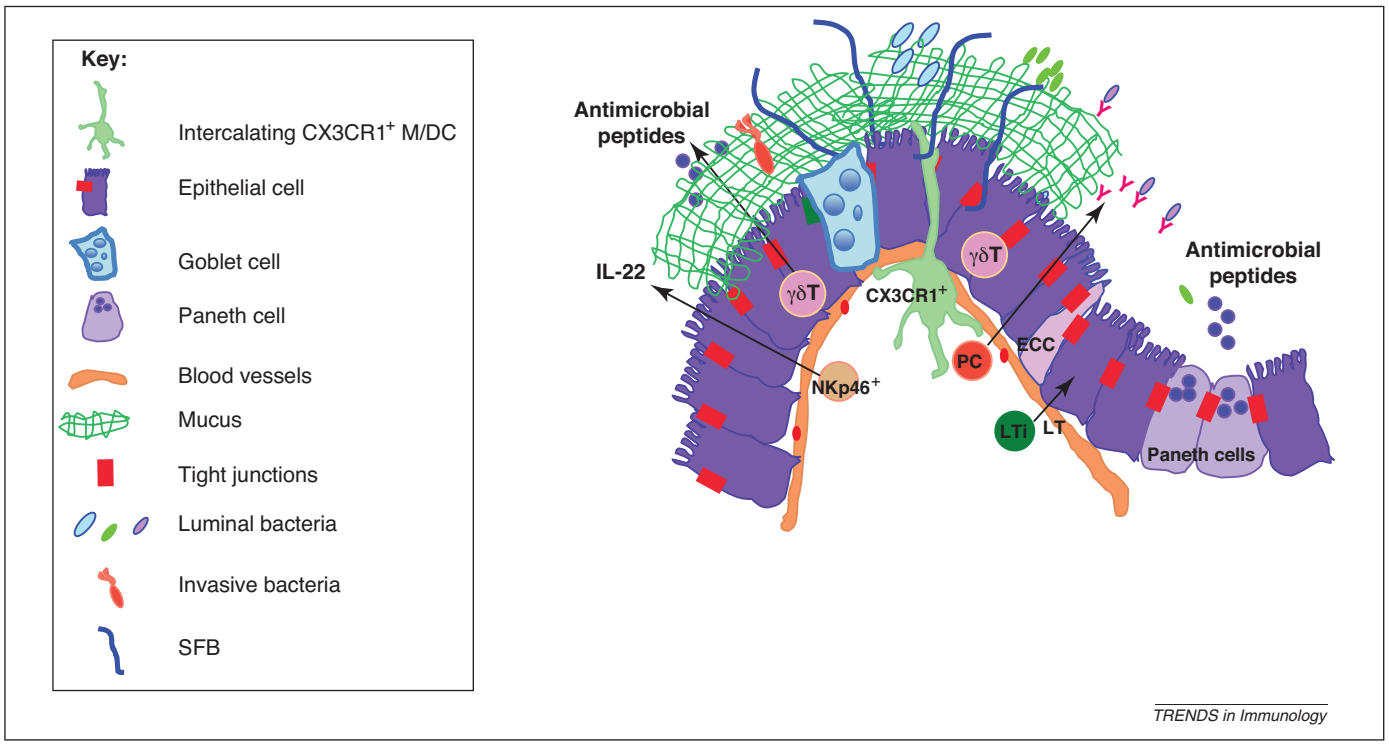

Figure 1. The intestinal barrier is composed by a cellular structural component (ECs, goblet cells, Paneth cells and enterochromaffin cells) and associated intraepithelial immune cells (γδ T cells and CX3CR1+ M cells/DCs). Other immune cells also participate in the barrier function, such as RORγt+ cells (NKp46+ and LTi). Goblet cells and ECs produce mucins that form mucus. The mucus physically separates the microbiota from the epithelium, but some bacteria can elude it, such as SFBs, and pathogenic invasive bacteria such as *Salmonella*. IgA that is released by plasma cells also contributes to epithelial defense by excluding bacteria from the epithelium. Paneth cells and γδ T cells are the principal producers of antimicrobial peptides that limit bacterial growth and shape the microbiota. RORγt+ cells produce cytokines that participate in control of bacterial infections.

the inflammatory potential of dendritic cells (DCs), which are professional antigen-presenting cells that are required for the priming and polarization of naïve T cells [10,11]. This could account for or be the consequence of altered production of inflammatory cytokines by DCs. Indeed, Winnie mice display a skew towards Th17 and Th1 inflammatory responses in the colon that are probably involved in the worsening of colitis in old age [9]. Along the same line, mice in which DICER, an enzyme that is involved in the maturation of miRNA, is knocked down only in intestinal ECs, have a defect in goblet cell differentiation, and in the production of the antimicrobial peptide RELMb and of TSLP. Similar to Winnie mice, these mice can mount only a non-productive Th1 response and become more

Table 1. Cells and mediators of specific functions in the epithelial barrier.

Cell type	Factor	Function
Goblet cells and enterocytes	Mucins forming the mucus layers	Separates the microbiota from epithelial cells [5,91] Provides anchorage to the microbiota [4] Provides nutrients to the microbiota [92]
Paneth cells	Antimicrobial peptides	Control of microbial growth [13] Shape of the microbiota composition [14] Protection against pathogens [13]
γδ T lymphocytes	Antimicrobial peptides	Control of microbial growth [16]
CX3CR1+ DCs	Express TJs	Uptake of commensal or pathogenic bacteria [17,18,46]. Exit into the intestinal lumen [23]. Mediate Th17 T cell differentiation [19,20,84] or restimulate Treg cells [85].
CD103+ DCs	TGF-β, RA	Induce Foxp3+ Treg cell differentiation [93]. Imprint T cells with gut homing properties in the mouse [77] and human [78] system
RORγT cells (NKp46 and LTi)	IL-22	Epithelial cell repair and antimicrobial activity [24–30].
RORγT cells (NKp46 and LTi)	LT	Induces the release of CXCL-1/CXCL-2 from EC and the recruitment of neutrophils, macrophages (only after infection) [31]
Enterocytes	MyD88, TLR4, TLR5, NEMO	Protection against colitis [49–51]
		Epithelial cell viability [53]
Enterocytes	Nlrp3, caspase 1	Production of IL-18 and protection against colitis [59,60], but controversial [57,58]
Enterocytes	TGF-β, RA TSLP (in humans)	Inhibition of Th1 differentiation [10] Conditioning of tolerogenic CD103+ DCs [76]
Enterocytes	BAFF, APRIL	Induce DCs to produce BAFF and APRIL via TSLP [86]. Promote IgA class switching in B cells [86,87].

susceptible to parasites [12]. Hence, defects that affect the function or differentiation of goblet cells and the production of mucus result in gut inflammatory disorders and dysfunction.

Antimicrobial peptides, not only a reinforcement of the barrier

Paneth cells that are found primarily in the crypts of Lieberkhun in the intestinal villi are secretory cells that are specialized in the production of antimicrobial peptides (AMPs). These include defensins, lysozymes and cathelicidins (for an extensive review, see [13]). Emerging evidence has shown that defensins are not only required to fight invading pathogens, but participate in shaping the composition of the microbiota. Mice deficient for α-defensins, for instance, or overexpressing human Paneth cell α-defensin 5 (DEFA5) display completely different microbiota composition, even if the total number of bacteria is unchanged [14]. Most striking, mice that overexpress DEFA5 display much reduced colonization by segmented filamentous bacteria (SFBs) [14]. Hence, an important balance of AMPs can control the overgrowth of some bacterial species such as SFB that, as we will see later, are involved in shaping adaptive immune responses.

Intraepithelial immune cells: phagocytes and more

In the epithelium, it is possible to find intraepithelial immune cells (Figure 1). Some are just in contact with ECs but do not gain access to the intestinal lumen, such as intraepithelial γδ and αβ lymphocytes, and RAR-related orphan receptor (ROR)γt$^+$ lymphoid tissue inducer (LTi) and Nkp46 innate immune cells; others have direct access to the lumen, such as DCs or neutrophils after infection (Table 1). The most abundant intestinal intraepithelial lymphocytes bear the γδ T cell receptor [15]. γδ T cells play a major role in limiting the entrance of commensal bacteria after epithelial injury via the release of AMPs that are induced by the microbiota [16]. DCs express tight junction proteins and can intercalate between ECs for direct uptake of antigens and bacteria across the intestinal lumen [17]. These cells express CXC3 chemokine receptor 1 (CX3CR1) [18], and have the capacity to induce Th17 cell-type responses *in vitro* [19,20]. However, because they are unable to enter the lymphatics to reach the mesenteric lymph nodes [21], they are considered more like macrophages [22]. Consistently, CX3CR1$^+$ cells have been shown to exit the intestinal lumen following *Salmonella* infection and might therefore participate in bacterial killing, as do macrophages [23]. NKp46$^+$RORγt$^+$ cells release IL-22, which is required for EC repair and antibacterial activity [24–29]. This cytokine is already produced by RORγt$^+$ innate lymphoid cells before birth, which suggests that it is produced independently of bacterial colonization of the gut [30].

ECs and immune cells closely interact with each other to preserve epithelial barrier integrity. The lymphotoxin (LT)–LT β receptor (LTbR) axis is one example [31]. After *Citrobacter rodentium* infection, RORγt$^+$ cells produce LT that binds to ECs and induces the release of chemokines that are involved in neutrophil and macrophage recruitment [chemokine (C-X-C motif) ligand (CXCL)-1 and CXCL-2]. Recruited neutrophils are involved in bacterial clearance [31]. This response precedes the adaptive response and renders mice that lack B and T cells more susceptible to *C. rodentium* infection. Hence, epithelial barriers are equipped with immune cells that can confer a first line of protection against invading pathogens through the release of cytokines, chemokines, AMPs and other soluble mediators that are involved in recruitment of phagocytes or in direct bacterial containment and killing.

Interaction of ECs with the external world: microbes

Microbe–epithelial barrier interaction can lead to different immunological outcomes

Although the mucus separates the microbiota from the epithelial layer, some components of the microbiota can penetrate the mucus and make contact with the epithelium. One such group of microorganisms are the SFBs. SFB colonization of germ-free mice is sufficient to induce the development of the mucosal immune system, including broad activation of T helper cells and, in particular, Th17 cells [32,33]. Consistently, mice that overexpress DEFA5, which display much reduced colonization by SFBs, show reduced Th17 skewing [14]. Other components of the microbiota, such as *Bacteroides fragilis* or a still unknown *Clostridium* species, can protect mice from experimental colitis via the induction of IL-10-producing T regulatory (Treg) cells [34,35]. Treg cells are fundamental in the control of autoimmunity because they can inhibit the proliferation of effector T cells and release non-inflammatory cytokines [36]. Although SFBs can directly contact the epithelium and presumably also DCs, *B. fragilis* can interact with our immune system after TJ disruption (as described below). Alternatively, as the principal effector of the tolerogenic response induced by *B. fragilis* is the bacterial polysaccharide A, it is possible that this crosses the mucus layer for direct interaction with the epithelium and/or DCs, but this remains to be established. Hence, microorganisms within the microbiota (or as described by Mazmanian and Lee, pathobionts [1]) share characteristics that are typical of inflammatory or non-inflammatory bacteria, depending on their representation within the microbiota and on their capacity to drive preferentially different adaptive immune responses [1], presumably via interaction with ECs.

Microbial entrance across the epithelium

Crossing of the epithelium is a strictly controlled process. ECs are sealed by the presence of TJs between the cells, but although being impermeable to bacteria, the barrier is not tight and is very dynamic [37]. Thus, unless being exploited by pathogens, the paracellular route is commonly excluded to microorganisms. When TJs are not properly formed, epithelial permeability is increased and mice become more susceptible to colitis [38]. Entrance of microbes occurs primarily at the level of specialized ECs called M cells that lack the organized brush border of ECs. M cells are scattered in the follicle-associated epithelium of Peyer's patches, but they have been shown to be present also in intestinal villi [39]. Recent data have demonstrated that M cells are not fully permissive and endocytosis of some enterobacteria is receptor dependent [40], even for those

bacteria such as *Salmonella* that are equipped with invasion type three secretion systems (a syringe-like apparatus that allows the injection of virulence factors that induce cytoskeleton rearrangements and bacterial engulfment). M cells have been shown to express on their apical surface glycoprotein 2 (GP2), which is required for the internalization of $FimH^+$ bacteria [40]. FimH is a component of type I pili and is expressed by a subset of pathogenic and commensal enterobacteria. GP2 expression is restricted to M cells and not to ECs [40]. As bacteria such as *Salmonella*, *Shigella* and *Yersinia* can induce their own phagocytosis by ECs [41] – a characteristic that is conferred by type three secretion systems – it remains to be addressed whether their entrance also requires receptor-mediated endocytosis and, if so, which are the receptors involved. Microorganisms such as *Clostridium difficile*, *B. fragilis*, *Vibrio cholerae* and *Clostridium perfringens*, can target TJ proteins, either directly or via the release of elaborated toxins, to disorganize TJs, which allows their penetration via the paracellular route [42]. By contrast, viruses can exploit receptor-mediated endocytosis in ECs and probably also DCs [43–45]. Finally, microorganisms can cross the epithelium also via transepithelial dendrites exposed by DCs [46]. However, whether bacteria enter DCs via receptor-mediated endocytosis remains to be established.

Microbial sensing by ECs is required to preserve homeostasis

Intestinal ECs and immune cells in the barrier express a series of pattern recognition receptors, including Toll-like receptors (TLRs), nucleotide-binding site and leucine-rich repeat containing receptors (NLRs), and retinoic acid inducible gene-I (RIG)-like receptors (RLRs) (for a comprehensive review, see [47]). Intestinal ECs in mice reared under germ-free conditions display reduced expression of TLRs, which suggests that their expression is somehow controlled by the microbiota [48].

TLRs and the myeloid differentiation primary response protein 88 (MyD88) adaptor protein

Recent evidence has shown that TLRs might be involved in protection and pathogenesis of intestinal inflammatory disorders, depending on the cells that express them, and on the model of experimental colitis. Initial studies have suggested a protective role for TLRs in dextran sodium sulfate (DSS) colitis development, because mice that ubiquitously lack the MyD88 adaptor protein, which is downstream of most TLRs (although also some cytokines such as IL-1 and IL-18), or that lack TLR4 that recognizes bacterial lipopolysaccharide or TLR5 that recognizes flagellin, are more susceptible to acute DSS colitis [49–51]. However, recent data indicate that the MyD88 pathway can also have a pathogenic role in a chronic infectious model of colitis [52]. This effect is attributed to bone-marrow-derived cells, because chimeras that lack MyD88 only in bone marrow cells do not develop colitis after *Helicobacter hepaticus* infection. By contrast, MyD88 expression in non-hematopoietic cells is required for host survival in a Rag-deficient background (i.e. mice that lack B and T cells) [52]. This is particularly interesting because it suggests a protective role of the MyD88 pathway in non-hematopoietic cells, and a pathogenic role in hematopoietic cells (at least in this model of *H. hepaticus*-dependent colitis). Consistently, mice in which NEMO, which is required for nuclear factor (NF)-κB activation downstream of TLR signaling, is knocked out only in ECs that develop chronic intestinal inflammation [53]. ECs that lack NF-κB undergo apoptosis and this leads to increased permeability of the barrier and translocation of the microbiota across the epithelium [53]. Hence, microbial recognition by ECs is important for preservation of intestinal homeostasis. Similarly, forkhead box O4 (Foxo4) protein that is a negative regulator of NF-κB is required to maintain intestinal homeostasis [54]. Colons of mice deficient for Foxo4 have increased production of inflammatory cytokines and chemokines and much reduced epithelial permeability [54]. The latter is probably due to a direct effect of Foxo4 deficiency in ECs, because it has been observed also in Foxo4-knocked down ECs *in vitro* [54]. However, it is not known how Foxo4 in epithelial and immune cells participates to maintain intestinal homeostasis.

NLRs

The NLR protein NLR family, pyrin domain containing 3 (NLRP3) recruits caspase 1 and apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) to form the inflammasome that is responsible for production of the inflammatory cytokines IL-1β and IL-18 [55]. IL-1β has been implicated in the pathology of Crohn's disease (CD) [56], however, contrary to what is expected, but still controversial [57,58], mice deficient for Nlpr3 or caspase 1 are more susceptible to DSS colitis and colitis-associated intestinal tumorigenesis [59,60]. The cells protective for each event are different. Expression of Nlrp3 in non-hematopoietic cells is required for protection against colitis [59], whereas expression in bone marrow cells is required to protect against tumorigenesis [60]. IL-18 is strongly upregulated in patients with CD [61], however, it is not clear whether it has a protective or pathogenic role. Apparently, Nlrp3-deficient mice reconstituted with IL-18 during colitis display reduced disease severity, which suggests that at least part of the protective role of Nlpr3 in colitis is mediated by IL-18 [59]. Thus, ECs by sensing bacteria release IL-18 that is a crucial mediator in the repair of the mucosal barrier and protection against colitis (Figure 2).

Other receptors

ECs also express receptors for small peptides such as N-formylated peptides and muramyl-dipeptide that are associated with the bacterial cell wall. One example is the intestinal dipeptide transporter hPepT1 (human peptide transporter 1) that mediates the transport of these bacterial products into the cytosol of colonic epithelial cells [62]. Here, they can activate NLR proteins, such as NOD2 (nucleotide-binding oligomerization domain containing 2), and the NF-κB pathway [63]. However, the expression of these receptors occurs only in inflamed colonic ECs, thus leading to amplification of the inflammatory response by inducing cytokine secretion. Hence, receptors expressed at steady-state might participate towards epithelial barrier integrity, whereas receptors upregulated during inflammation might contribute to disease severity.

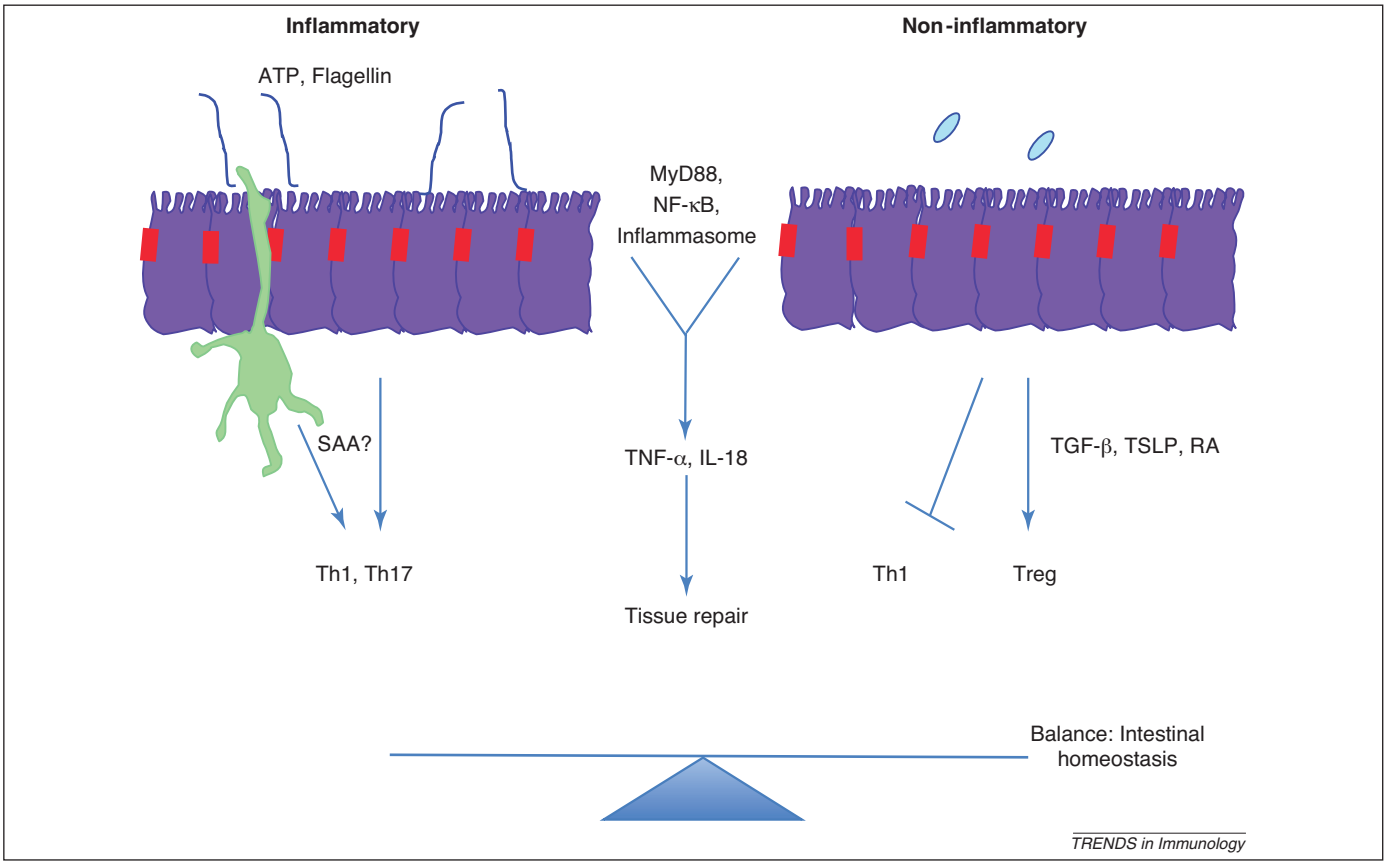

Figure 2. ECs sense signals coming from the external world, and in particular, the microbiota. Components of the microbiota can be more inflammatory or non-inflammatory in nature. One example of the first class is SFBs, and an example of the second class is *B. fragilis*. SFBs favor the development of adaptive immunity, and in particular, inflammatory Th17 cells maybe via the release of serum amyloid A (SAA). Inflammatory mediators, such as Flagellin or ATP that is released by bacteria, or by necrotic cells, may promote Th1 or Th17 differentiation by acting on ECs or directly on DCs. The non-inflammatory component can act on ECs directly to induce the production of modulators of DC function such as TSLP, RA and TGF-β. An initial innate inflammatory response to the microbiota is required to activate the MyD88 pathway, NF-κB and the inflammasome for production of EC repair cytokines such as TNF-α and IL-18.

Interaction of ECs with probiotics

Probiotics are classified as bacteria that have beneficial effects on the host. Most of them are derivatives of the microbiota, therefore, understanding how probiotics interact with the host can shed light on how the microbiota interacts with the host. The mechanisms of action of probiotics have recently started to be unraveled [64]. Similar to the microbiota, probiotics can also be classified as inflammatory or anti-inflammatory, depending on their capacity to stimulate immune and non-immune cells [65]. Probiotics can help preserve intestinal homeostasis by downmodulating the immune response and inducing the development of Treg cells [66–68]. However, recently, a new mechanism of action has been proposed based on the hypothesis that CD susceptibility is dependent on a defective initial innate immune response [69]. It has been demonstrated that a mixture of probiotics named VSL#3, can induce NF-κB nuclear translocation in ECs, followed by release of TNF-α, and that this correlates with reduced epithelial permeability and susceptibility to CD-like ileitis in SAMP1/YitFc mice that spontaneously develop the disease [70]. Although unexpected, this observation is particularly interesting, because it has been recently shown that TNF-α can stimulate EC proliferation, and only when in combination with IFN-γ does TNF-α induce EC apoptosis [71]. Hence, it is possible that by upregulating TNF-α, probiotics participate in epithelial barrier regeneration. Hence, the interaction of inflammatory bacteria with ECs might be beneficial to stimulate innate immunity that protects against chronic inflammation. However, the same bacteria cannot ameliorate overt disease in mice [70], and I would not be surprised if they could even be deleterious, as shown in other systems by the use of inflammatory probiotics [65].

Also, food components might participate to control intestinal homeostasis via immunomodulatory activity on ECs. Colostrum, for instance, downregulates the NF-κB pathway in a mouse intestinal epithelial cell line (mICc12) [72].

Interaction of ECs with the internal world: immune cells

Delivery of microbial signals

As mentioned above, the microbiota is required for the development of the mucosal immune system. For instance, the microbiota is important for the spontaneous proliferation of microbiota-specific T cells, and this is dependent on MyD88-induced IL-6 [73] by DCs. Hence, ECs have somehow to deliver this information to underlying immune cells. This can happen either via the direct delivery of the microbial component (as a microbe associated molecular pattern) or as a signal elaborated within the ECs and released either as a soluble factor, or as a membrane-bound signal. The microbiota is required for the formation of DC extension into the lumen, but this information is first

elaborated by ECs [46]. Indeed, EC and not myeloid cell expression of MyD88, TLR2 and TLR4 is required for DCs to extend their protrusions into the lumen, which suggests that TLRs are first sensed by ECs that then release signals that are not TLR-dependent to underlying immune cells [46]. It is important to note that the number of DC extensions varies according to which part of the intestine is analyzed. In particular, in the duodenum, DC extensions are constitutively formed, whereas in the distal ileum, they are induced only in response to pathogen exposure [46]. Whether this is caused – under steady state – by the presence of a thicker mucus that does not allow epithelium/microbe interaction and therefore also the formation of DC protrusions, is not known.

EC control of immune cell function

At steady state, ECs play an important role in driving non-inflammatory DCs [74]. As mentioned above, human ECs release TSLP that inhibits IL-12 production by DCs in response to bacteria, and drives Th2-polarizing cells [10]. In mice, impaired NF-κB signaling by IKK-β (inhibitor of NF-κB kinase subunit β) deletion in intestinal ECs results in reduction of TSLP expression and upregulation of DC-derived IL-12p40 [75]. This is associated with inability to drive Th2 cells and to control *Trichuris* infection [75]. Human and mouse ECs both also release transforming growth factor (TGF)-β and metabolize vitamin A to retinoic acid (RA) which is sufficient, in mice at least, to drive the development of tolerogenic DCs that are characterized by expression of $CD103^+$. Newly generated $CD103^+$ DCs are capable of inducing forkhead box P3 $(Foxp3)^+$ Treg suppressor cells, which are protective against colitis [76]. In addition, $CD103^+$ DCs can release RA and imprint T cells with gut homing properties in the mouse [77] and human [78] system. LP DCs are also characterized by the constitutive activity of β-catenin, which is required for production of RA, TGF-β and IL-10 by DCs [79], hence, it would be interesting to know whether ECs can activate the Wnt signaling pathway that leads to β-catenin activation in DCs. Probiotics can control the release of these mediators and hence can shape the response of DCs indirectly via action on ECs [65,80]. Indeed, incubation of ECs with probiotics, and in particular with *Lactobacillus paracasei* B21060 results in induction of immunomodulatory mediators by ECs, and control of the proinflammatory response of DCs [65]. Hence, ECs are at the crossroads between bacteria and immune cells and can be shaped by the first to control the second. Notably, ECs isolated from patients with CD display much reduced expression of TGF-β, RA and TSLP, and fail to control the DC proinflammatory response and tolerogenic properties [10,11]. Thus it would be particularly interesting to evaluate whether dysbiosis (i.e. disequilibrium of inflammatory and non-inflammatory components of the microbiota) that is characteristic of inflammatory bowel disease patients [81] can account for differences in the capacity of ECs to respond to bacteria and control the function of immune cells.

An intriguing and unresolved question is whether ECs in different regions of the gut have diverse ability to drive tolerogenic responses. For instance, a recent study has shown that a *Clostridium* species drives the development of adaptive Treg cells in the colon of germ-free mice, whereas in the small intestine, the number of Treg cells is not changed between mice reared under germ-free or conventional conditions [35]. This suggests that in mice, ECs in the small intestine might be prone to induce tolerogenic responses by default, whereas in the colon, these responses are driven by some components of the microbiota, but this remains to be established.

As mentioned above, $CX3CR1^+$ and $CD103^+$ antigen-presenting cells seem to have opposite functions; $CX3CR1^+$ cells induce the development of Th17 cells, whereas $CD103^+$ DCs induce Treg cell development. How can these cell types coexist in a non-inflammatory environment? Is there any conditioning of ECs on $CX3CR1^+$ DCs? It is known that $CD103^+$ and $CX3CR1^+$ cells are derived from different blood precursors [82,83]. In particular, $CD103^+$ DCs are derived from pre-DCs, whereas $CX3CR1^+$ DCs are derived from monocytes [82,83]. It is possible that these precursors respond differently to the above-mentioned conditioning factors. In addition, $CX3CR1^+$ cells are more responsive to bacterial components such as ATP [19] and flagellin [84], for example, and hence become more inflammatory. However, the concept that $CX3CR1^+$ cells are inflammatory is challenged by a recent study that has shown that they can actually restimulate Treg cells [85]. Hence, under some circumstances $CX3CR1^+$ cells also have anti-inflammatory properties, which leaves the function of these cells an open question.

Not much is known about the interaction of ECs with other immune cells, but some axis that is involved in their crosstalk has been identified. As mentioned, $ROR\gamma t^+$ cells produce LT that binds to ECs via LTbR, and induces the release of chemokines that are involved in neutrophil and macrophage recruitment (CXCL-1 and CXCL-2) [31]. Hence, it is probable that other such interactions occur. In addition, recognition of bacteria through TLRs could also account for the production of B-cell-activating factor of the TNF family (BAFF, also known as BLyS) and a proliferation-inducing ligand (APRIL) by intestinal ECs [86–88]. BAFF and APRIL are CD40-independent IgA class switch recombination-inducing signals that can act directly on B cells. Intriguingly, ECs further amplify BAFF and APRIL production by stimulating DCs via TSLP; at least in humans [86,87]. Ultimately, BAFF and APRIL induce IgA class switching by activating B cells in cooperation with cytokines released by DCs or other cell types, including IL-10 and TGF-β1 [86,87].

Finally, ECs can integrate signals that come from the host and from bacteria. Recent evidence has shown that ATP, a stress signal that is released during necrosis, cooperates with the bacterial component flagellin (FliC) to worsen intestinal inflammation [89]. ECs activated with FliC and ATP release more of the proinflammatory cytokine IL-8, which is involved in neutrophil recruitment, and decrease levels of chemokine (C–C motif) ligand (CCL)20 that is involved in the recruitment of several immune cells, including Treg cells [89]. The two cytokines are regulated by different pathways: IL-8 upregulation is regulated by NF-κB, whereas CCL20 downregulation is controlled by the extracellular signal-regulated kinase (ERK)1 and ERK2 pathway. During inflammation and

after luminal translocation of neutrophils, P2XR7 are translocated from the apical to the basolateral membrane of intestinal ECs and this could be a mechanism to reduce inflammation, because it could impede ATP–receptor interaction [90]. Consistently, ATP exacerbates DSS colitis, and mice die after receiving DSS plus ATP and flagellin [89]. Given the important role of ATP to drive Th17 cell differentiation by acting on a subset of LP DCs [19], it would be interesting to test the Th17 skewing in these mice and to assess the cooperative effect of ATP on intestinal ECs and DCs.

Conclusions and future perspectives

It is becoming clear that ECs play a major role in integrating all the signals that come from the external and internal world to preserve intestinal immune homeostasis under steady-state conditions (Figure 2). Gut microorganisms can have more inflammatory or anti-inflammatory properties, which induce different outcomes that, when balanced, contribute to intestinal homeostasis. In case of dysbiosis, one of the two responses can take over the other and lead to intestinal inflammatory disorders. The role of ECs in these responses remains to be fully understood: although it is clear that ECs are involved in the induction of tolerogenic immune cells and that these responses can be amplified by non-inflammatory components of the microbiota. However, it is unknown whether ECs also actively participate to the development of adaptive Th1 and Th17 responses. In particular, it is not known whether ECs contribute to the development of inflammatory CX3CR1^{+} antigen-presenting cells, or whether the lack of a tolerogenic response leads to inflammatory responses or both. Understanding how ECs participate in shaping the immune response could be crucial to find new therapeutic targets in intestinal inflammatory disorders.

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