Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function

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Abstract | A single layer of epithelial cells lines the small and large intestines and functions as a barrier between commensal bacteria and the rest of the body. Ligation of Toll-like receptors (TLRs) on intestinal epithelial cells by bacterial products promotes epithelial cell proliferation, secretion of IgA into the gut lumen and expression of antimicrobial peptides. As described in this Review, this establishes a microorganism-induced programme of epithelial cell homeostasis and repair in the intestine. Dysregulation of this process can result in chronic inflammatory and over-exuberant repair responses, and it is associated with the development of colon cancer. Thus, dysregulated TLR signalling by intestinal epithelial cells may explain how colonic bacteria and inflammation promote colorectal cancer.

Lamina propria
The layer of mucosal tissue directly under the mucosal epithelial cell surface of the gastrointestinal tract, in which mucosal effector immune cells reside.

Goblet cells
Mucus-producing cells found in the epithelial cell lining of the intestine and lungs.

Enteroendocrine cells
Specialized endocrine cells of the gastrointestinal tract that differentiate from pluripotent stem cells. They are characterized by their ability to produce hormones such as serotonin, somatostatin, motilin, cholecystokinin, vasoactive intestinal peptide and enteroglucagon.

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Good fences make good neighbours. Robert Frost, 1914

The intestinal epithelium provides a physical barrier that separates the many trillions of commensal bacteria in the intestinal lumen from the underlying lamina propria and deeper intestinal layers. The intestinal epithelium is composed of four cell lineages that arise from a pluripotent stem cell progenitor[1]: absorptive enterocytes, which make up most intestinal epithelial cells (IECs), mucus-producing goblet cells, hormone-producing enteroendocrine cells and Paneth cells, which produce antimicrobial peptides or lectins (FIG. 1). Stromal cells, B cells (especially IgA-producing plasma cells), T cells, macrophages and dendritic cells of the lamina propria lie directly beneath the intestinal epithelium (FIG. 1). In addition, specialized subsets of T cells (intraepithelial lymphocytes) and some dendritic cells localize between the IECs, making them strategically positioned to sample the luminal contents[2–4]. Beneath the lamina propria there are two layers of innervated smooth muscle that generate the peristaltic waves that propel nutrients along the digestive tract.

It was originally thought that the cells lining the intestine functioned only to keep bacteria from invading the body. However, it is now clear that IECs have a complex and mutually beneficial relationship with the gut flora. The bacteria metabolize food (especially carbohydrates) that cannot be fully digested in mammals; in turn, the IECs metabolize the short-chain fatty acids that are produced by bacterial fermentation of undigested carbohydrates and use them as a source of energy.

In this Review, I highlight recent studies that show how the intestinal epithelium takes advantage of the signals provided by bacteria to maintain a functional barrier. In particular, I focus on the role of Toll-like receptor (TLR) recognition of commensal- and pathogen-associated molecular patterns (PAMPs) from the gut flora in shaping the function of IECs in the small intestine and colon. Although I use the conventional term PAMP, the molecular patterns that are actually recognized by TLRs in the intestine are generally not from pathogens but from the commensal flora. TLR signalling has been shown to be involved in epithelial cell proliferation, IgA production, maintenance of tight junctions and antimicrobial peptide expression — which are functions that are crucial for maintaining a healthy epithelial barrier. Despite this clear beneficial role for TLR signalling in the intestinal epithelium, it can also trigger pro-inflammatory responses by underlying lamina propria immune cells[5,6] and, if dysregulated, may precipitate the development of colon cancer, thereby linking bacteria and chronic inflammation to cancer. A better understanding of the pathway from dysregulated TLR signalling to cancer may lead to the design of new therapies to prevent or treat colorectal cancer.

Sensing bacteria by the intestinal epithelium

IECs are structurally and functionally polarized, with an apical surface facing the intestinal lumen and a basolateral surface facing the underlying basement membrane and the lamina propria. The polarized structure of IECs

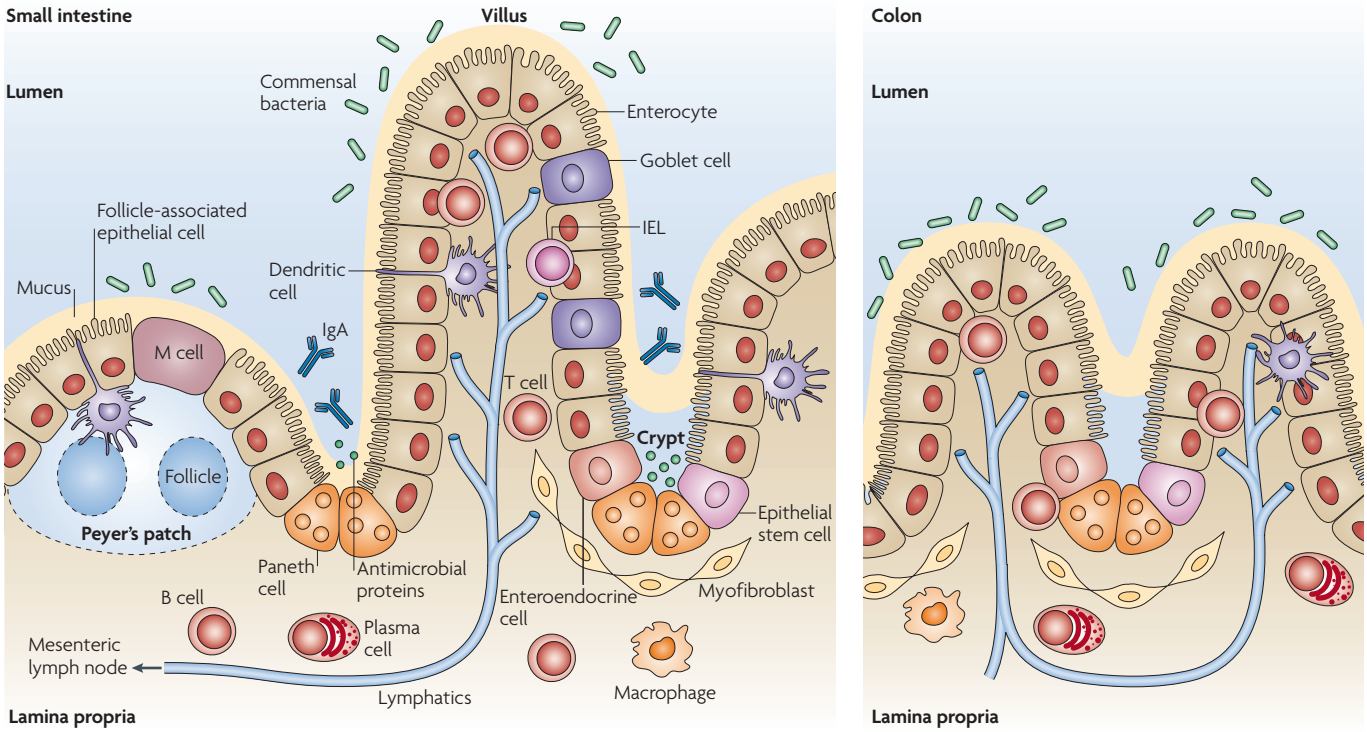

Figure 1 | **Anatomy of the intestinal immune system.** A single layer of intestinal epithelial cells (IECs) provides a physical barrier that separates the trillions of commensal bacteria in the intestinal lumen from the underlying lamina propria. The IECs lining the lumen are bathed in nutrients, commensal bacteria, IgA and goblet cell-produced mucus. Epithelial stem cells proliferate and give rise to daughter cells with the potential to proliferate. These IECs then differentiate into villous or colonic enterocytes, which absorb nutrients (small intestine) and water (colon). In addition to differentiated enterocytes and goblet cells, progenitor IECs differentiate into both enteroendocrine cells, which secrete enteric hormones, and Paneth cells at the base of the small intestinal crypts. Beneath the IECs, the lamina propria is made up of stromal cells (myofibroblasts), B cells (especially IgA-producing plasma cells), T cells, macrophages and dendritic cells. Certain subsets of T cells and dendritic cells localize between the IECs. The small intestine has regions of specialized epithelium termed follicle-associated epithelium and microfold (M) cells that overlie the Peyer's patches and sample the intestinal lumen. IEL, intraepithelial lymphocyte.

Paneth cells
Specialized cells that are generally only found at the base of small intestinal crypts, although they can also be seen in the human colon in chronic inflammatory conditions. Paneth cells produce antimicrobial peptides or lectins, including REG3 proteins and defensins.

Tight junction
A belt-like region of adhesion between adjacent epithelial or endothelial cells that regulates paracellular flux. Tight junction proteins include the integral membrane proteins occludin and claudin, in association with cytoplasmic zonula occludens proteins.

Laser capture microdissection
A method used to isolate specific subpopulations of cells under direct microscopy of the tissue. A laser is used to separate the desired cells from the tissue on a microscope slide and the cells of interest are then captured for subsequent analysis.

is established through exclusive targeting of certain membrane proteins to either the apical or basolateral surface and is maintained under normal conditions by tight junction complexes. This, together with a layer of mucus on the apical surface, establishes a barrier that is generally thought to be impermeable to commensal bacteria and other intestinal microorganisms in the gut lumen. However, it is likely that a low number of intestinal bacteria, and perhaps other microorganisms such as viruses and fungi, traverse the epithelium under steady-state conditions, as gut flora-derived microorganisms can be cultured from the spleens of mice, especially from mice that lack TLR signalling ability[7]. Disruption of the epithelial barrier, such as that which occurs during intestinal ulceration or infection with a pathogen, can allow PAMPs to access the basolateral surface.

Given the juxtaposition between IECs and the gut flora, how does the intestinal epithelium respond to luminal PAMPs that are largely derived from non-pathogenic commensal microorganisms? Consistent with the known function of TLRs, indiscriminate recognition of PAMPs by TLRs on the epithelium would be expected to trigger an inflammatory response. Such a response to PAMPs from commensal, non-pathogenic bacteria would disadvantage the host. Instead, the intestinal epithelium seems to tolerate the presence of luminal PAMPs and does not mount an acute inflammatory immune response. Moreover, the epithelium requires these signals for its normal function and for sensing a breach in its barrier.

TLR expression in the intestine. To explore the relationship between IECs and commensal bacteria, studies aimed to determine whether the intestinal epithelium expresses TLRs under normal physiological conditions and, if so, whether there is regional and spatial localization of TLR expression in the intestine. Expression of TLRs specifically by IECs is difficult to determine using whole intestinal tissues because they contain many other cell types, including macrophages, dendritic cells, B cells, T cells and stromal cells, that can express TLRs. So, immunohistochemistry, enzymatic separation of IECs and laser capture microdissection of the intestinal epithelium were used to show that TLR2 and TLR4 are expressed at low levels by IECs in normal human colon tissues[8–10]. TLR3 seems to be abundantly expressed in normal human small intestine and colon, whereas TLR5 is expressed predominantly in the colon[8]. Almost all TLRs are expressed, at least at the mRNA level, in the human colon (TABLE 1); the expression of

Table 1 | **Expression and localization of Toll-like receptors in the intestine**

	Expression in the small intestine (and method of detection)		Expression in the colon (and method of detection)		Cellular localization		Functions in intestinal epithelium		
TLR	**Mouse**	**Human**	**Mouse**	**Human**	**Mouse**	**Human**	**Mouse**	**Human**	**Refs**
1	RNA	Protein (IHC)	ND	RNA and protein (IHC)	ND	ND	ND	ND	10,22, 44,141
2	RNA and protein	RNA and protein (IHC)	RNA and protein (IHC)	RNA and protein (IHC)	Apical in villi and crypts; apical and basolateral in FAE	Basolateral in ileal crypts (fetus); low in adult ileum or colon, mainly in crypts	Chemokine and cytokine production; RELA phosphorylation; ZO1 redistribution and TFF3 expression	Preserved tight junction structure; increased TFF3 expression	8,10,22, 24,25,44, 71,127, 141–148
3	RNA	Protein*	RNA	RNA and protein (IHC)*	ND	Basolateral in ileum and colon; top of colonic crypts	Blocking of TLR3 reduces IL-15 production	ND	8,10,22, 141,147, 149
4	RNA and protein (IHC and WB)‡	RNA and protein (IHC and WB)§	RNA and protein (IHC)	RNA and protein (IHC)	Apical in terminal ileum; basolateral in colon (low); intracellular in fetal small intestine	Basolateral in ileal crypts (fetus); basolateral in colon (low); apical in active Crohn's disease (ileum and colon)	Cell growth, chemokine and cytokine production, phagocytosis and translocation of bacteria, and uptake of microparticles by M cells; expression leads to increased TNF production, apoptosis and NF-κB activation; lack of expression leads to decreased TNF production and protects against NEC	ND	8,10,22,24, 25,40,69, 89–91, 94,114, 141–143, 145–148, 150–155
5	RNA and protein (IHC)	Protein*	ND	RNA and protein (IHC)*	Basolateral in ileum and colon; apical in FAE (small intestine)	Basolateral and intracellular in colon	Chemokine expression	Chemokine expression	8,10,22, 24,26,27, 29,30,89, 138,141, 156
6	RNA	ND	ND	RNA	ND	ND	ND	ND	10,44,141
7	RNA	ND	ND	RNA	ND	ND	ND	ND	10,141
8	RNA	ND	ND	RNA and protein (IHC)	ND	Top of colonic crypts in ulcerative colitis and Crohn's disease; not in normal intestine	ND	Chemokine secretion	10,45,141
9	RNA and protein (IHC)	Protein (IHC)	Protein (IHC and WB)	RNA and protein (IHC)	Apical and basolateral (ileum); in granules and cytoplasm of Paneth cells	Apical and basolateral in colon	Protects against NEC; signalling causes degranulation of Paneth cells	IL-8 secretion (whole biopsy)	10,13,20, 24,28,94, 141,146, 157
10	ND	ND	ND	NE	ND	ND	ND	ND	10
11	RNA	ND	ND	ND	ND	ND	ND	ND	146
12	ND	ND	ND	ND	ND	ND	ND	ND	–
13	ND	ND	ND	ND	ND	ND	ND	ND	–

FAE, follicle associated epithelium; IHC, immunohistochemistry; IL, interleukin; M cell, microfold cell; NEC, necrotizing entercolitis; ND, not determined; NE, not expressed; NF-κB, nuclear factor-κB; TLR, Toll-like receptor; TFF3, trefoil factor 3; TNF, tumour necrosis factor; WB, western blot; ZO1, zonula occludens 1.
*REF. 22 did not find expression. ‡REF. 141 did not find expression. §REF. 148 did not find expression.

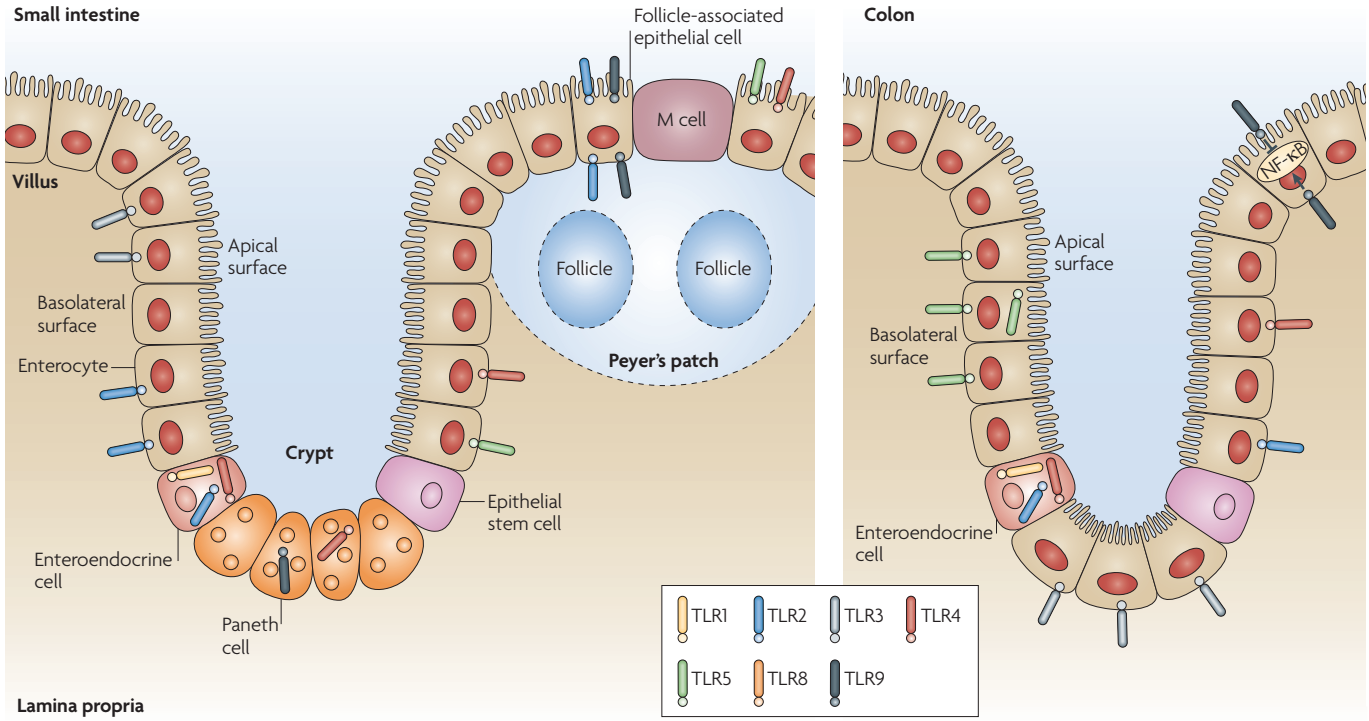

Figure 2 | **Polarized expression of Toll-like receptors by intestinal epithelial cells.** The small intestine and colonic epithelium is composed of a single layer of polarized epithelial cells with apical and basolateral surfaces. Toll-like receptor (TLR) expression by intestinal epithelial cells (IECs) also shows a polarized pattern. TLRs can also be present in endosomes, where they are thought to interact with their ligands (not shown). In the human small intestine, the expression of TLR3, TLR4 and TLR5 has been shown on the basolateral surfaces of villus enterocytes. In mice, the follicular-associated epithelial cells that neighbour microfold (M) cells and overlie the Peyer's patches express TLR2 and TLR9 on both the apical and basolateral surfaces. Enteroendocrine cells also express several TLRs, including TLR1, TLR2 and TLR4, but it is not clear whether they are present on apical or basolateral surfaces. In the human colon, TLR3 and TLR5 are abundantly expressed, whereas TLR2 and TLR4 expression is low. TLR9 has been shown to have contrasting effects on nuclear factor-κB (NF-κB) following apical or basolateral ligation. A detailed description of the localization of TLR expression in the intestine is provided in TABLE 1. This schematic is based on *in vivo* and *in vitro* expression by human epithelial cells. The TLR expression pattern by follicle-associated epithelium is based on data from mouse studies[24].

TLR1, TLR2, TLR3, TLR4, TLR5 and TLR9 has also been detected in IECs of the human small intestine[10] (TABLE 1). These studies must be interpreted with caution because of the limitations of immunohistochemistry or RNA-based studies and the inability to properly address TLR function using these techniques.

IECs from patients with inflammatory bowel diseases have higher expression of TLRs, especially TLR4, and similar or lower expression of TLR2, TLR3, TLR5 and TLR9 than IECs from control individuals[8,11–13]. Inflammatory cytokines have been shown to regulate the expression of TLRs by IECs[12,14–16]. Early studies showed that interferon-γ (IFNγ) and tumour necrosis factor (TNF) induce the transcription of *TLR4* and its co-receptor *MD2* (also known as *LY96*)[12,15]. Cytokine-mediated induction of TLRs may allow their selective expression during times of danger perceived by the host[17]. Conversely, interleukin-4 (IL-4) and IL-13 decrease the responsiveness of IECs to the TLR4 ligand lipopolysaccharide (LPS), suggesting that these T helper 2 (T_H2)-type cytokines decrease the expression of TLR4 by IECs[16,18]. Studies comparing germ-free mice with conventionally housed mice indicated that commensal bacteria induce the expression of certain TLRs (TLR2, TLR3, TLR4 and TLR5)[19]. However, this study assessed TLR expression levels in mucosal scrapings, which may have been contaminated by other cell types. Using immunohistochemistry, TLR9 was shown to be expressed on the apical surface brush border of the colon of mice with conventional flora but not that of germ-free mice[20].

TLR expression by cell lineage in the intestine. TLR signalling by IECs could be regulated to prevent deleterious inflammation through restricted expression of particular TLRs according to cell lineage. Enteroendocrine cells of the intestine secrete various hormones, such as serotonin, vasoactive intestinal peptide and somatostatin, that regulate fluid and electrolyte secretion, motility, blood flow and food intake[21]. One study showed that TLR1, TLR2 and TLR4 are co-expressed in human and mouse colonic and ileal crypts (FIG. 2) and co-localize with serotonin, a marker of the enteroendocrine cell lineage[22]. *In vitro* stimulation of an enteroendocrine cell line with LPS resulted in the secretion of somatostatin[22]. This cell lineage also expresses TLR5 and TLR9 and in response to flagellin and CpG-containing oligodeoxynucleotides

Crypts
Tubular invaginations of the intestinal epithelium. At the base of the crypts, there are Paneth cells, which produce bactericidal defensins, and stem cells, which continuously divide and are the source of all intestinal epithelial cells.

CpG-containing oligodeoxynucleotides
Synthetic DNA sequences that include a cytosine–guanosine sequence and certain flanking nucleotides, which have been found to induce innate immune responses through interaction with TLR9.

(CpG ODNs) releases cholecystokinin, a hormone that induces contraction of the gall bladder and small intestine[23]. This combination of responses suggests that TLR expression by enteroendocrine cells has a role in the development of diarrhoea in response to pathogens and may facilitate the elimination of pathogens. The role of TLR signalling in enteroendocrine cell function during pathogenic infection has not yet been addressed.

In the study described above[22], as well as other studies[8,9,25,144,148], the expression of TLRs by IECs is generally low, but during intestinal inflammation it increases; it occurs throughout the colonic crypts in patients with ulcerative colitis and Crohn's disease and on the villi of patients with ileal Crohn's disease[8]. It is probable that IECs have the potential to upregulate the expression of TLRs following the appropriate cytokine signals or other stimuli.

In the mouse small intestine, the expression of TLR2 is found in both the follicle-associated epithelium (FAE) and the IECs of the villi and crypts[24]. TLR2 and TLR9 expression is found on both apical and basolateral sides of the FAE but only the apical side of the villous IECs. TLR5 is also present on the apical side of the FAE and villous IECs, but TLR4 expression is low. Stimulation with TLR2 ligands or LPS induced increased particle uptake by the FAE cells, supporting a functional role for TLR2 and TLR4 in the FAE, despite low TLR4 expression by these cells. These data highlight cell specific differences in TLR expression in the intestinal epithelium. Data on human FAE are not available.

Spatially restricted TLR expression by polarized IECs. In addition to the possibility that TLR expression is restricted to specific cell lineages of the intestinal epithelium, TLR signalling in the intestine is also thought to be regulated spatially — that is, regulated apical or basolateral expression (FIG. 2; TABLE 1). TLR2 and TLR4 were shown to be expressed on the basolateral surface of ileal crypt enterocytes in the human fetus[25]. Although TLR2 and TLR4 expression is low in normal adult colon[8], TLR4 is highly expressed on the apical side of colonic IECs from patients with active Crohn's disease, but not those with ulcerative colitis[8]. Analysis of polarized human IECs *in vitro* indicated that TLR5 is expressed only on the basolateral surface, where it can trigger the production of cytokines and chemokines, such as IL-8 and CC-chemokine ligand 20 (CCL20), in response to basolateral flagellin[26–28]. Consistent with the hypothesis that TLR5 functions only on the basolateral surface, luminal flagellin could activate TLR5 only after injury to the epithelial barrier induced by the chemical dextran sodium sulphate (DSS)[27]. However, another study indicated that functional TLR5 is present on the apical surface of mouse ileum and induces epithelial cell production of KC (also known as GROα), the mouse homologue of IL-8, in response to commensal-derived flagellin[29]. TLR5-deficient mice develop spontaneous colitis and have a large increase in the density of luminal bacteria[30]. However, it is not clear whether these observations are due to the absence of TLR5 expression by the epithelium or by the lamina propria immune cells[31].

Other TLRs also seem to be polarized in IECs. Depending on the polarized cell line, basolateral or apical exposure of T84 cells or Caco-2 cells, respectively, to LPS results in activation of nuclear factor-κB (NF-κB) and IL-8 secretion[11,32]. Apical exposure to TLR2 and TLR4 ligands resulted in TLR activation in another polarized IEC line[10]. TLR2 expression has been shown on the apical surface of polarized IECs *in vitro*[33]. In human fetal small intestine, TLR2 is expressed on the basolateral surface[25]. A comprehensive overview of the expression of TLRs in cell lines is provided in Supplementary information S1 (table).

Apical expression of TLR9 is found in the mouse colon following colonization with commensal flora but not in the germ-free state[20]. Moreover, polarized IECs respond differently to apical or basolateral exposure to the TLR9 ligand CpG ODNs, suggesting that not only the localization but also the function of TLRs can be polarized[28]. In immune cells, TLR9 interacts with CpG ODNs in endosomes[34,35]. Unlike immune cells, TLR9 expression was detected on both the apical and basolateral surfaces of polarized IECs, and signalling was not inhibited by endosomal inhibitors[20,28]. Apical exposure of IECs to CpG ODNs resulted in a gene expression programme that was characterized by inhibition of NF-κB and expression of the WNT protein receptor Frizzled 5, which is important for the differentiation of Paneth cells, whereas basolateral exposure of IECs to CpG ODNs led to the activation of NF-κB and JNK1 or JNK2, and the secretion of IL-8. Moreover, apical activation of TLR9 inhibited the inflammatory response of IECs to basolateral CpG ODNs and other PAMPs, which indicates a state of cross-tolerance. Mechanistically, both apical and basolateral exposure to CpG ODNs resulted in the polyubiquitylation of inhibitor of NF-κBα (IκBα) but only basolateral CpG ODNs led to the degradation of IκBα that is required for the release and activation of NF-κB[28].

It is worth noting, however, that differing results have been reported in studies of TLR9 in human cells. Studies using isolated human colonic IECs reported the presence of *TLR9* mRNA but little TLR9 protein, and there was no observed secretion of IL-8 in response to CpG ODNs[13]. It is possible that human IECs respond to TLR9 activation but do not secrete IL-8. Other groups have shown TLR9 protein in mucosal tissue biopsies and IECs, as well as an increase in IL-8 secretion in response to bacterial or CpG ODNs[13,20,28,36]. In a study using mucosal biopsies from patients with ulcerative colitis, exposure to CpG ODNs decreased the production of IL-1β[37]. The cell types involved in the response to CpG ODNs were not elucidated.

The divergent responses following apical or basolateral TLR triggering are consistent with the hypothesis that inflammatory and potentially destructive TLR responses to PAMPs should only occur when there has been a breach in the epithelial barrier. In this way, invasive bacteria that can penetrate the epithelial barrier (generally pathogenic bacteria) elicit a pro-inflammatory response, whereas bacteria that cannot cross the barrier (generally non-pathogenic bacteria) remain on the apical face and elicit a homeostatic, anti-inflammatory response.

Crohn's disease
A form of chronic inflammatory bowel disease that can affect the entire gastrointestinal tract but is most common in the colon and terminal ileum. It is characterized by transmural inflammation, strictures and granuloma formation, and is thought to result from an abnormal T cell-mediated immune response to commensal bacteria. Symptoms include abdominal pain, rectal bleeding, diarrhoea and weight loss.

Villi
Projections into the lumen that have an outer layer that mainly consists of mature, absorptive enterocytes and mucus-secreting goblet cells.

Follicle-associated epithelium
(FAE). The epithelium that overlies mucosal lymphoid tissues, such as the Peyer's patches and the isolated lymphoid follicles in the intestine. Lymphoid tissues induce the differentiation of normal intestinal epithelium into FAE, which is specialized in antigen capture and transport.

WNT proteins
Glycoproteins related to the *Drosophila melanogaster* protein Wingless, a ligand that regulates the temporal and spatial development of the embryo. WNT-mediated signalling has been shown to regulate cell fate determination, proliferation, adhesion, migration and polarity during development. WNT and its downstream signalling molecules also have been implicated in tumorigenesis and have causative roles in human colon cancers.

Cross-tolerance
A state of unresponsiveness in which cells exposed to a TLR ligand show tolerance to subsequent challenge with the same stimulus and also to subsequent challenges with other stimuli that signal through one or more different TLRs.

Intracellular expression of TLR4 by IECs. TLR4 signalling is generally thought to occur at the plasma membrane following the binding of LPS to MD2–TLR4 complexes[38,39]. However, in an epithelial cell line derived from the mouse small intestine, TLR4 was found in the Golgi apparatus and was shown to co-localize with internalized LPS[40]. However, IECs of the small intestine were not studied in the polarized state that they display in the native intestine. Inhibition of LPS internalization or Golgi function in the mouse IEC line prevented LPS-mediated secretion of CXC-chemokine ligand 2 (CXCL2; also known as MIP2) or activation of NF-κB, respectively[41]. The observation that internalization of LPS is required for signalling led to the suggestion that this may prevent nonspecific activation of apical TLRs by commensal bacteria and that the intracellular localization for TLR4 may differ between IECs and immune cells, as is the case for TLR9 (described above). TLR localization may also change following stimulation with a ligand[33]. Unfortunately, because normal IECs cannot be cultured for prolonged periods, it is difficult to study the function of TLRs in the normal epithelium in isolation. Therefore, most studies of TLR function in the intestine have been carried out using short-term cultures of isolated IECs or using immortalized or cancer cell lines that show certain aspects of normal IECs; these studies must be interpreted with caution.

Negative regulators of TLR signalling in IECs. Expression of molecules that inhibit TLR signalling can also avoid deleterious TLR activation in IECs. Toll-interacting protein (TOLLIP) is an intracellular protein that inhibits TLR2 and TLR4 signalling through its effect on IL-1R-associated kinases (IRAKs)[42,43]. Tollip was found to be expressed by IECs *in vitro*[44], especially following stimulation with LPS or lipotechoic acid, and inhibited TLR activation, a phenomenon termed LPS tolerance[10]. IECs from patients with inflammatory bowel disease failed to upregulate Tollip expression, suggesting that this may contribute to chronic inflammation[45]. Single immunoglobulin IL-1R-related molecule (SIGIRR; also known as TIR8), which is a negative regulator of IL-1R, IL-33R, TLR4 and TLR9 signalling[46], was highly expressed by IECs and when deleted, made animals susceptible to intestinal inflammation[47,48]. The other molecule that seems to negatively regulate NF-κB activation is peroxisome proliferator activated receptor-γ (PPARγ). PPARγ expression was induced by TLR4 signalling in the intestinal epithelium, and, therefore, it may serve to dampen TLR-mediated inflammation[49]. Finally, IEC expression levels of MD2 (which is required for binding LPS[38,50]) were kept low[9,32], possibly owing to degradation by trypsin produced by the pancreas[51], thereby limiting LPS hyperresponsiveness.

Sensing intestinal injury through TLRs

TLRs regulate proliferation in response to chemically induced injury. The intestinal epithelium is normally replaced every 5 days[1]. This is achieved by the proliferation of intestinal stem cells at the base of the crypts of Lieberkühn that give rise to daughter cells with the potential to proliferate further or differentiate into the cell lineages that constitute the intestinal epithelium: absorptive enterocytes and secretory progenitors that give rise to goblet cells, Paneth cells or enteroendocrine cells depending on the milieu of WNT, Notch and Hedgehog factors[1,52]. Underlying the base of the colonic and small intestinal crypts are blood vessels and myofibroblasts, which express markers of both fibroblasts and smooth muscle; together, these elements are referred to as the stem cell niche. One of the observations made during early studies of germ-free mice is that the proliferation of IECs is nearly half the rate of that of mice with conventional flora[53–55]. This observation suggests that the bacterial flora induces IECs to proliferate. Given that bacterial products are recognized through TLRs, investigators have studied IEC proliferation in mice lacking TLRs or the TLR adaptor molecule myeloid differentiation primary-response protein 88 (MYD88).

Under normal conditions (no injury), proliferation of IECs in MYD88- or TLR4-deficient mice seems to be similar to wild-type mice based on the uptake of bromodeoxyuridine by dividing cells[56]. IEC barrier function, as measured by electrical resistance, permeability and proliferation, is also normal in mice deficient in all TLR signalling (that is, mice lacking both MYD88 and TIR-domain-containing adaptor protein inducing IFNβ (TRIF; also known as TICAM1))[7]. These observations seem to contradict the finding of decreased proliferation in germ-free mice and suggest that other pattern recognition receptors, such as nucleotide-binding oligomerization domain proteins (NODs), are involved in the proliferation of IECs in response to commensal flora or other factors derived from the flora; for example, short-chain fatty acids[57].

DSS causes injury to colonic epithelial cells and allows access of luminal bacteria to the lamina propria, leading to an acute inflammatory response[58]. Mice deficient in MYD88, TLR2 or TLR4 are more susceptible to DSS-induced injury than wild-type mice[56,59,60]. During DSS-induced injury, MYD88- or TLR4-deficient mice have greatly decreased proliferation and increased apoptosis[59,61,62]. Similar observations were made using an antibody antagonist of TLR4 or an LPS antagonist[63,64]. Treatment of wild-type mice with broad-spectrum antibiotics[59] or an antibody antagonist of TLR4[63] made the animals similarly susceptible to DSS-induced injury to those with TLR deficiency, suggesting that both the bacterial flora and TLRs are required for optimal epithelial cell proliferation. Other studies have identified that, in addition to PAMPs, hyaluronic acid, which is generated following DSS-induced injury, can be a TLR4 ligand and induce epithelial protection[65]. In the resting state, some deficiency of TLR signalling is tolerated for normal proliferation, however, in the absence of TLR4 or broader defects, such as lack of MYD88, the proliferative burst required to repair injury is impaired.

So what mechanism links TLRs to epithelial cell proliferation? TLR4 is required for the induction of cyclooxygenase 2 (COX2) expression following DSS-induced injury[66] (FIG. 3). In turn, COX2 leads to IEC production of prostaglandin E_2 (PGE_2) and induction of amphiregulin, an epidermal growth factor (EGF) family member[67]. IECs normally express the EGF receptor (EGFR) and

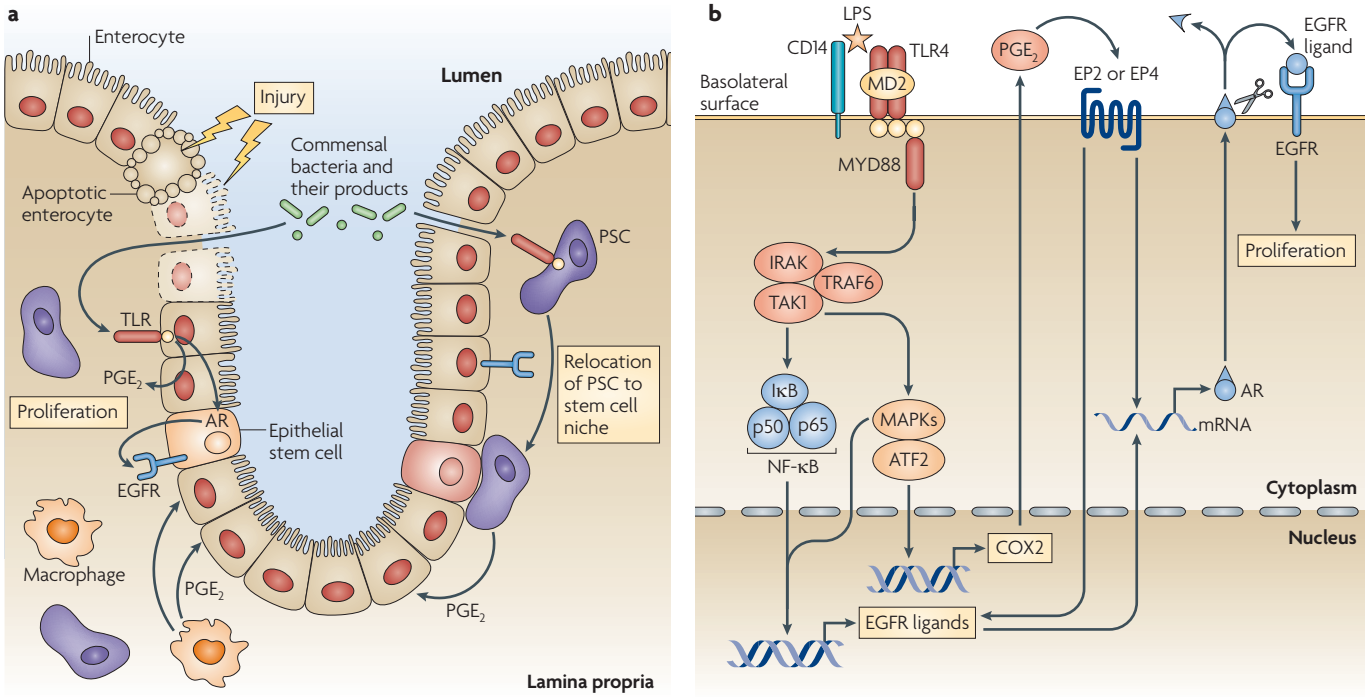

Figure 3 | **Toll-like receptors promote proliferation of intestinal epithelial cells. a** | Studies of intestinal epithelial cells (IECs) that lack Toll-like receptor 2 (TLR2), TLR4 or the signalling adaptor myeloid differentiation primary-response protein 88 (MYD88) indicate a key role for TLR signalling in the proliferation of IECs in response to injury. Damage to the intestinal epithelial barrier can lead to exposure of TLRs on the basolateral surface to bacterial products. TLR signalling in IECs might promote the proliferation of epithelial stem cells located at the base of colonic crypts by inducing the production of ligands for epidermal growth factor receptor (EGFR), such as amphiregulin (AR), and prostaglandin E_2 (PGE_2) by IECs. PGE_2 production can also be induced by TLR signalling in macrophages and cyclooxygenase 2 (COX2)-expressing mesenchymal stromal cells (PSCs). PSCs undergo repositioning to the stem cell niche in response to injury by which they can promote stem cell proliferation. **b** | Recognition of lipopolysaccharide (LPS) by TLR4 and its cofactors CD14 and MD2 triggers signalling through MYD88, IL-1R-associated kinases (IRAKs), TNFR-associated factor 6 (TRAF6) and TGFβ-activated kinase 1 (TAK1), resulting in the activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPKs). In IECs, this leads to the release of EGFR ligands, such as AR, which can activate EGFR signalling leading to proliferation. TLR4 signalling by IECs can also induce the expression of COX2 and the secretion of PGE_2, which, through its receptors EP2 or EP4, can further stimulate the expression and release of AR by IECs. ATF2, activating transcription factor 2; IκB, inhibitor of NF-κB.

Septic shock
A systemic response to severe bacterial infections or Gram-negative bacterial endotoxins (such as LPS) that leads to a hyperactive and dysregulated network of inflammatory cytokines, affecting vascular permeability, cardiac function and metabolic balance, which leads to tissue necrosis, multiple-organ failure and death.

Necrotizing enterocolitis
(NEC). A gastrointestinal disease predominantly affecting premature low-birth-weight infants. NEC involves infection and inflammation that causes destruction of the intestine. Although the pathophysiology of NEC is not completely defined, increasing evidence indicates that immaturity of intestinal innate immune function of the premature gut, characterized by over-exuberant IL-8 responses of intestinal epithelial cells to LPS, is a key factor.

secrete (especially in response to injury) EGFR ligands, such as transforming growth factor-α (TGFα), EGF, amphiregulin, β-cellulin, heparin-binding (HB)-EGF and epiregulin, which induce the proliferation and migration of IECs[68]. Blocking amphiregulin prevented LPS-mediated EGFR phosphorylation in IECs, suggesting that TLR4 signalling can activate a programme of proliferation through the induction of growth factors[69].

In addition to production of PGE_2 by IECs in response to TLR4 activation, other cell types may secrete PGE_2 and promote epithelial cell proliferation. These include COX2-expressing macrophages that are recruited to the site of injury by IECs[56,67] (FIG. 3). Another way in which TLR signalling regulates IEC proliferation is by inducing the movement of mesenchymal stromal cells to a position adjacent to the IEC stem cell. COX2-expressing mesenchymal stromal cells are repositioned from the mid-crypt region to sites adjacent to the proliferating colonic epithelial progenitor cells after DSS-induced colitis[54,62] (FIG. 3). In the absence of MYD88 or COX2, the mesenchymal stromal cells do not undergo repositioning and do not express secreted factors responsible for the induction of IEC proliferation. No differences were seen between wild-type and MYD88-deficient mice in the absence of injury. It was also shown that exogenous administration of dimethyl PGE_2, a longer-acting synthetic derivative of PGE_2, restored epithelial cell proliferation in MYD88- and COX2-deficient mice[62]. These data suggest that activation of epithelial cell or mesenchymal stromal cell TLRs results in a complex response, characterized by the secretion of PGE_2, EGFR ligand expression and EGFR activation, leading to the induction of IEC proliferation (FIG. 3). Thus, following DSS-induced injury, the absence of TLRs impairs several aspects of epithelial barrier repair, including the direct effect of TLR signalling on the IECs (amphiregulin induction and EGFR phosphorylation) and the indirect effect of myofibroblast- and macrophage-expressed COX2 inducing release of PGE_2.

Other TLRs also have a protective effect against DSS-induced injury in the intestine. Systemic administration of flagellin and the TLR2 ligand tripalmitoyl-*S*-glyceryl cysteine-serine$_4$-lysine (Pam3CSK4) protects

against DSS-induced colitis[70,71]. Systemic, but not oral, administration of the viral double-stranded RNA mimic polyinosinic–polycytidylic acid (polyI:C) decreases the severity of DSS-induced colitis through TLR3 (REF. 72). However, this effect may not be due to IEC recognition of polyI:C but instead due to an effect on gut dendritic cells or macrophages.

A similar reduction in DSS-induced colitis was observed following oral or systemic administration of the TLR9 ligand CpG ODN[73,74]. The protective mechanism seems to be mediated through the production of type I IFNs by splenocytes in response to TLR9 activation[75], as well as TLR9-mediated inhibition of NF-κB in IECs[28]. These and other studies support a model in which epithelial TLRs sense injury in the intestine and limit the extent of damage through promoting decreased apoptosis, dampening of pro-inflammatory pathways and causing increased epithelial cell proliferation[76–78].

Radiation-induced injury. Patients with different types of solid and haematopoietic malignancies can be treated with radiation therapy, but one of its well-known rate-limiting toxicities is damage to the intestine and bone marrow. Because IECs are among the most rapidly dividing cells in the body, they are highly susceptible to radiation-induced injury. Finding a way to limit intestinal injury could allow consistent delivery of radiation therapy, instead of having to interrupt treatment when patients develop diarrhoea, and this might prevent the long-term sequelae of radiation proctitis, such as rectal bleeding and pain[79–81]. Early clues that TLRs have a role in epithelial cell repair following radiation-induced injury came from studies in which LPS was systemically administered to mice before radiation therapy; this protected against radiation-induced IEC apoptosis through induction of COX2 and PGE_2 (REF. 82). We now think this phenomenon is due to TLR signalling. It is important to note that TLR ligands may not act directly on IECs to prevent radiation-induced damage but might have an indirect effect through lamina propria macrophages or myofibroblasts[83].

A recent study has shown that administration of recombinant flagellin, a ligand for TLR5, before total body irradiation of mice could protect against subsequent intestinal injury[84]. A similar protective effect was seen in rhesus macaques[84] and was shown to be associated with decreased apoptosis of IECs and vascular endothelial cells in the lamina propria and the induction of cytokines, such as granulocyte colony-stimulating factor (G-CSF), IL-6 and TNF, which may have a protective effect on IECs. Flagellin mediates its protective effect both through epithelial cells and haematopoietic cells[85]. Whether systemic delivery of any PAMP is a viable strategy to treat human disease is questionable given that it might be expected to induce a septic shock response. Nevertheless, this strategy is promising for limiting radiation-induced injury and was seemingly not harmful to mice or rhesus macaques. In the future, manipulation of the intestinal flora or use of TLR agonists might be beneficial to prevent radiation-induced IEC damage[81,86].

Necrotizing enterocolitis. A gastrointestinal complication known as necrotizing enterocolitis (NEC) can occur in premature babies and is associated with necrosis of large segments of the small bowel, requiring extensive surgery and sometimes leading to long-term disability[87,88]. Studies of the role of TLRs in NEC in newborn mice and humans indicated that NEC is associated with increased expression of TLR4 in the intestinal mucosa[89,90]. Mice expressing a non-functional form of TLR4 were protected from the development of NEC compared with their wild-type (C3H/HeOUJ) littermates. Given that TLR4 signalling is decreased in mice following colonization of the gastrointestinal tract after a vaginal delivery[91], it is possible that this decrease in TLR4 signalling fails to occur in premature infants who develop NEC[92]. Studies of a mouse model of NEC revealed that TLR4 activation results in decreased IEC proliferation through a β-catenin-dependent pathway in isolated small intestinal IECs[93]. TLR9-deficient mice had increased severity of NEC and neonatal administration of CpG ODNs decreased the severity of NEC in wild-type mice[94]. Thus, TLR4 signalling seems to be harmful in NEC, whereas TLR9 signalling seems to be beneficial; at least part of the mechanism is the direct anti-proliferative effect of TLR4 on the injured epithelium. Increased TLR2 expression has also been described in NEC and positively correlates with the degree of mucosal damage[95]. Clinical trials of probiotics (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) showed decreased risk of NEC in low-birth-weight preterm infants[96]. If inhibition of TLR4 and activation of TLR9 are beneficial, oral administration of selective PAMPs could be used to treat preterm infants. As with radiation enteritis, the beneficial or harmful effects of TLR signalling may be mediated by both effects on the epithelium and the lamina propria.

TLRs regulate barrier function

Tight junctions. Studies of germ-free mice that are subsequently colonized with the commensal organism *Bacteroides thetaiotaomicron* have shown that the intestinal epithelium activates a complex programme that is characterized by the expression of genes involved in enhancing mucosal barrier function, including those encoding tight junction proteins[97]. Adjacent IECs are held together through interactions between tight junction proteins, such as zonula occludens 1 (ZO1) and claudins, which block the passage of bacteria and most PAMPs between IECs. Studies have been carried out to determine whether bacterial recognition by TLRs changes tight junction protein expression by IECs. Indeed, the activation of TLR2 in a model epithelium was shown to result in the phosphorylation of protein kinase Cα (PKCα) and PKCδ, which in turn leads to the reorganization of ZO1 in tight junctions[98]. During *Citrobacter rodentium*-induced colitis, epithelial cell expression of TLR2 protects against apoptosis and maintains ZO1 at the apical tight junction region[99]. Treatment of IECs in culture with TLR2 ligands resulted in increased transepithelial resistance, a measure of the strength of the tight junctions between IECs, suggesting an increase in barrier function. Studies showed that the treatment of mice with TLR2 ligands during recovery

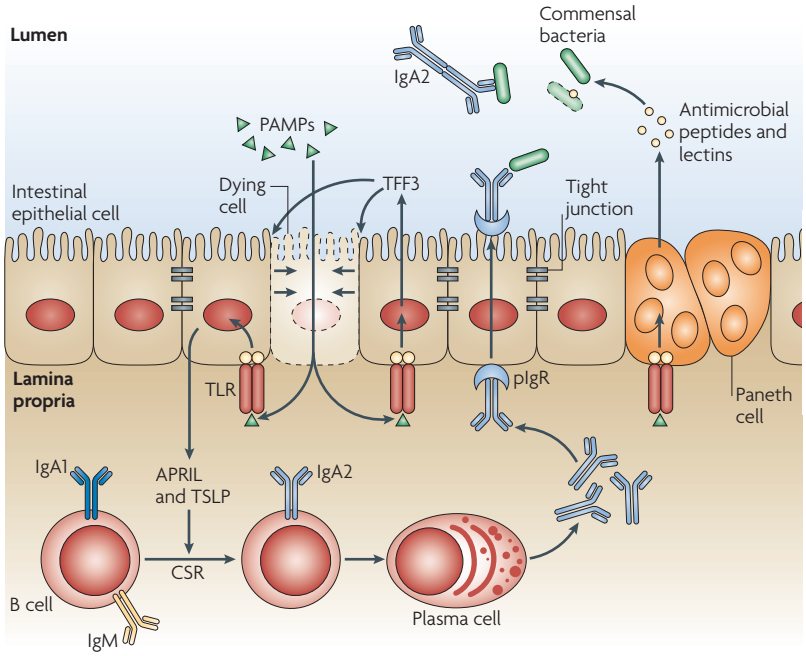

Figure 4 | **The role of Toll-like receptors in epithelial barrier function.** Toll-like receptor (TLR) stimulation of intestinal epithelial cells (IECs) leads to the expression of a proliferation-inducing ligand (APRIL) and thymic stromal lymphopoietin (TSLP), which are cytokines that promote class switch recombination (CSR) of IgM and IgA1 to protease-resistant IgA2. IgA2 binds bacteria at the apical surface of IECs to prevent bacterial invasion. Activation of TLR2 also stimulates the production of trefoil factor 3 (TFF3), which promotes the movement of cells necessary to repair gaps in the epithelial monolayer. In response to TLR stimulation, Paneth cells secrete microbicidal peptides and lectins, such as α-defensins and regenerating islet-derived protein 3γ (REG3γ), respectively. PAMPs, pathogen-associated molecular patterns; pIgR, polymeric immunoglobulin receptor.

from DSS-induced colitis led to decreased IEC apoptosis and improved tight junction function through ZO1 reorganization[71]. The investigators postulated that early disruption of the tight junctions during DSS-induced injury promotes apoptosis, and both these events can be ameliorated by TLR2 signalling. Although the data suggest that TLRs, especially TLR2, are important during injury to increase barrier function, TLR signalling does not seem to be required for the maintenance of barrier function during resting states. MYD88 and TRIF double-deficient animals do not have higher paracellular permeability or electrical conductance than wild-type littermates[7]. These data highlight how manipulation of specific TLRs can have a beneficial effect to minimize intestinal damage by tightening the barrier formed by the epithelial cells.

Antimicrobial peptides and lectins. In addition to forming an impermeable cellular layer, the intestinal epithelium has other strategies that locally inhibit bacterial invasion, including the production of antimicrobial peptides such as defensins that permeabilize the cell walls of bacteria. Although the expression of β-defensins can be induced in IECs by TLR signalling[100], Paneth cells are the main source of antimicrobial peptides, such as α-defensins (also known as cryptdins)[101], angiogenin 4 (REF. 102) and regenerating islet-derived protein 3γ (REG3γ)[103] (FIG. 4). REG3γ specifically targets Gram-positive bacteria because it binds to their surface peptidoglycan[103,104]. Previous studies had suggested that in isolated Paneth cells, bacteria or bacteria-derived PAMPs, such as LPS, lipoteichoic acid, lipid A and muramyl dipeptide, result in α-defensin secretion[105]. Expression of α-defensins is normal in MYD88-deficient mice[106] and is lower in mice that lack the intracellular pattern recognition receptor NOD2 than wild-type mice[107]. TLR9-deficient mice have decreased expression of Frizzled 5 and therefore have decreased expression of α-defensins in the small intestine[28].

Hooper *et al.*[103] have shown that bacterial colonization of germ-free mice with *B. thetaiotaomicron* results in the induction of REG3γ expression by Paneth cells. MYD88-deficient mice have undetectable levels of REG3γ, suggesting a role for TLRs in REG3γ induction by commensal bacteria[108]. However, conclusive evidence that Paneth cells use TLRs to respond to bacterial products had been lacking until recently. Hooper and colleagues[109] used an elegant approach involving Paneth cell-specific transgenic expression of MYD88 (in otherwise MYD88-deficient mice) to show that Paneth cells directly recognize bacterial PAMPs in a MYD88-dependent manner and induce expression of the antimicrobial lectins REG3γ, REG3β, CRP-ductin (also known as DMTB1) and resistin-like molecule-β (RELMβ). Furthermore, in the absence of MYD88 or Paneth cells, there is a significant increase in the dissemination of both commensal and pathogenic bacteria to mesenteric lymph nodes[108,109]. The defective production of all antimicrobial lectins seen in MYD88-deficient mice could not be reproduced by deletion of any single TLR, suggesting that many TLRs contribute to the induction of antimicrobial genes[109]. TLR2, however, regulates the expression of REG3β, and TLR2-deficient animals are more susceptible to infection with *Yersinia pseudotuberculosis*[106]. Treatment of mice with broad-spectrum antibiotics decreased the expression of REG3γ, an effect that was ameliorated by oral administration of LPS[110]. These data support the hypothesis that TLR signalling in Paneth cells is crucial for expression and secretion of antimicrobial proteins and lectins, which limit the invasion of commensal and pathogenic bacteria. Broad-spectrum antibiotics may have the unintended effect of decreasing the production of antimicrobial lectins and allowing colonization of the intestine with pathogens[108].

Shaping the mucosal immune response. Studies have shown that the interaction of IECs with the bacterial flora shapes innate and adaptive immune responses in the lamina propria. IgA production is an essential feature of mucosal immunity to infection[111]. Every day, the small intestine produces several grams of IgA that is transported across the epithelium. In humans, two types of IgA — IgA1 and IgA2 — are produced in the lamina propria. The production of IgA1, but not IgA2, is T cell dependent and antigen specific. By contrast, class switching to IgA2 can be induced by IECs[112], and IgA2 is highly resistant to bacterial proteases and therefore has a longer half-life in the luminal milieu than IgA1 (FIG. 4). Class switching to IgA2 was found to require TLR activation of human IECs, which induced the secretion of a proliferation-inducing ligand (APRIL),

a B cell-stimulating factor that is structurally related to CD40 ligand. APRIL binds to transmembrane activator and calcium-modulating cyclophilin-ligand interactor (TACI) on B cells to induce plasma cell survival and class switching[113]. These findings in human cells have been partially reproduced in a mouse model in which a constitutively active form of TLR4 is expressed in the intestinal epithelium[114]. These mice have a higher number of IgA-producing plasma cells in the lamina propria and increased IgA in the faeces. Moreover, APRIL expression was substantially increased in the epithelium of these mice, indicating that TLR signalling in the intestinal epithelium activates a programme to promote local production of IgA.

Epithelial TLR signalling enhances the ability of dendritic cells to emit projections into the lumen to sample commensal and pathogenic bacteria[115]. Epithelial cell-specific deletion of NF-κB signalling or TGFβ-activated kinase 1 (TAK1), both of which are activated by TLRs, results in chronic intestinal inflammation because of dysregulated TLR signalling by lamina propria immune cells and abnormal epithelial barrier function[116–118]. Bacteria-induced signalling by IECs results in the production of cytokines such as thymic stromal lymphopoietin (TSLP), TGFβ and IL-25, which limit pathogenic T_H1 and T_H17 cell responses in the intestine[119–123] and serve to decrease TLR responsiveness in dendritic cells[124]. During infection with *C. rodentium*, TLR2 and TLR4 signalling by lamina propria cells seems to have a detrimental role rather than a protective one[5]. Thus IECs respond to commensal bacteria in a manner that limits a deleterious pro-inflammatory immune response in the lamina propria.

Trefoil factor. As mentioned above, a layer of mucus separates the luminal bacteria from direct contact with IECs. This mucus is composed of mucin glycoproteins and trefoil factor 3 (TFF3), which are secreted by goblet cells in the small and large intestines (FIG. 4). When the intestinal epithelium has a defect such as an ulcer, rapid reshaping, stretching and migration of the epithelial cells on the edges of the ulcer seal the defect in the barrier — a process known as restitution[68]. Ample evidence supports an important role for TFF3 in wound healing and repair of the intestinal mucosa, which is generally mediated by epithelial cell migration[125]. Administration of recombinant human TFF3 is effective in treating models of colitis, and TFF3-deficient mice are more susceptible to DSS-induced colitis[126]. Cario and colleagues[127] have shown in goblet cell lines and in normal IECs derived from mice or humans that the TLR2 ligand Pam3CSK4 induced the expression of TFF3. In addition, TLR2-deficient mice were shown to have decreased colonic production of TFF3, which might explain their increased apoptosis and susceptibility to colitis after DSS treatment. Conversely, treatment of wild-type mice with Pam3CSK4 protected against DSS-induced injury (less rectal bleeding and improved histology), decreased apoptosis and hastened the repair of ZO1 tight junction structures. These data highlight another way in which the bacterial flora interacts with the epithelium through the induction of a glycoprotein that is essential for epithelial barrier restitution.

TLR signalling and colorectal cancer

As described, epithelial cell proliferation and restitution is required for maintenance of a healthy intestine; however, if dysregulated, they can also contribute to malignancy. Colorectal cancer is the third most common cause of cancer-related deaths in the United States (National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) Program). Most colon cancers are associated with mutations in the adenomatous polyposis coli (*APC*) gene, which results in activation of the WNT pathway and dysregulated nuclear translocation of β-catenin[128]. To mimic sporadic cancer and familial adenomatous polyposis, multiple intestinal neoplasia (Min) mice, which have a point mutation in *Apc*, have been used[129,130]. One important limitation of this model is that the mice mainly develop tumours in the small intestine rather than the colon. Although $Apc^{Min/+}$ mice housed in germ-free conditions have only a slight decrease in tumour development compared with conventionally housed $Apc^{Min/+}$ mice, mouse chow contains PAMPs such as bacterial LPS and peptidoglycan making it difficult to determine the role of TLR signalling in tumour development in this context[131]. The effect of decreased TLR signalling on tumour number and size in the Min mouse model has been studied by crossing $Apc^{Min/+}$ mice with MYD88-deficient mice. It was found that the overall incidence of intestinal tumours was similar in $Apc^{Min/+}$ mice and MYD88-deficient × $Apc^{Min/+}$ mice, but the tumours were significantly smaller in the absence of MYD88, with few macroscopically visible tumours[132]. Among the genes implicated in this observation is *Cox2*, the expression of which was lower in the tumour tissue of the mice lacking MYD88 than controls. COX2 promotes tumour growth through the induction of PGE_2 and other effects to stabilize β-catenin[133]. These findings suggest that intestinal TLRs contribute to the development of sporadic colon cancer.

Patients with ulcerative colitis and Crohn's disease are at increased risk of developing colon cancer and this risk is proportional to the degree of inflammation[134]. The link between inflammation and cancer is well known[135,136], but the molecular mechanism underlying the observation is poorly understood. Consistent with a role for TLRs in the development of colitis-associated cancer, mice deficient in TLR4 were protected against the development of neoplasia induced by the carcinogen azoxymethane (AOM) and DSS[69]. Furthermore, bone marrow chimeras showed that TLR4 expression by the epithelium, rather than haematopoietic cells, was required for neoplasia[67]. Accordingly, increased TLR signalling by IECs increases the risk of inflammation-associated neoplasia[48,137]. Mice that lack the negative regulator SIGIRR develop increased inflammatory responses to AOM and DSS, and they have higher susceptibility to colitis-associated neoplasia[48,137]. The cancer phenotype of these mice was thought to result from epithelial cell TLR signalling, as transgenic epithelial cell expression of SIGIRR protected against neoplasia in the mutant mice[48]. These studies suggest that TLR signalling by the epithelium leads to increased proliferation of IECs, increased inflammation and promotes the development of inflammation-associated neoplasia.

Finally, there may be additional roles for TLRs in tumour growth. Using a model of implanted TLR5-expressing colonic cancer cells, TLR5 stimulation by flagellin was found to induce antitumour immunity and to decrease tumour size, whereas knockdown of TLR5 expression increased tumour size[138]. By contrast, TLR4 activation can protect tumour cells from lysis by antitumour cytotoxic T cells and NK cells[139]. Taken together, specific TLRs may subvert or promote immune responses against tumours and this gives additional impetus to study TLR inhibition as a strategy to treat cancer. For example, the anti-proliferative activity of rapamycin — which is best known as an immunosuppressant for organ transplantation — may also be a useful anticancer drug. Rapamycin decreases TLR4-mediated PGE_2 production and decreases the expression of TLR4 by colon cancer cells[140].

Conclusion

The evidence from examining the relationship between the bacterial flora and the intestinal epithelium leads us to conclude that it is a both beneficial and mutualistic interaction. In health, distinct TLRs seem to be expressed by enteroendocrine cells, goblet cells and Paneth cells, in which the TLRs are involved in protection from pathogens (through the induction of IgA and antimicrobial peptides), repair of epithelial cell injury (through the induction of proliferation, tight junctions and resistance to apoptosis) and cell migration (through the induction of TFF3). Chronic inflammation probably increases the epithelial cell expression of TLRs; although in humans it is not clear whether this is a cause or effect of pro-inflammatory cytokine secretion. Given that TLR- and MYD88-deficient mice are not protected from the induction of inflammatory disease and, in fact, develop more severe disease than their wild-type littermates, it seems that most TLR signalling (at least in the epithelium) has a beneficial role in maintaining intestinal homeostasis. However, through an as-yet-unknown mechanism, TLR4 expression (and possibly the expression of other TLRs) can be upregulated in colon cancer cells in patients with ulcerative colitis. The pathways that are downstream of TLRs that culminate in proliferation and recruitment of inflammatory cells during injury can be usurped to support cancer development. The potential to use PAMPs or TLR agonists and antagonists to treat intestinal disorders, such as inflammatory bowel disease or cancer, remains an attractive possibility. We must first, however, be sure we know how to strike the right balance.

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Competing interests statement
The author declares no competing financial interests.

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