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Review

Toll-like receptors and immune cell crosstalk in the intestinal epithelium

Declan P. McKernan*

Pharmacology & Therapeutics, Human Biology Building, School of Medicine, National University of Ireland, Galway, H91 TK33, Republic of Ireland

* **Correspondence:** Email: declan.mckernan@nuigalway.ie; Tel: +35391493826.

Abstract: The intestinal epithelium consists of a barrier one cell thick found along the length of the gastrointestinal tract composed of many cell subtypes such as absorptive enterocytes and secretory Paneth cells, Goblet cells and enteroendocrine cells. Primarily known as a cell layer used to absorb nutrients from the products of digestion and as a protective barrier from infection, this has changed in recent years with numerous discoveries indicating its importance in priming and tolerising immune cells. Toll-like receptors are a family of pathogen recognition receptors that are widely expressed in human cells including the intestinal epithelium and are known primarily as initiators of inflammatory responses. However, recent evidence suggest that they may have a variety of roles and are involved in cross-talk with a variety of cell types. This review discusses TLR signalling pathways in the context of the intestinal epithelial microenvironment, namely innate and adaptive immune cells as well as microorganisms that resident in the lumen of the gut. TLR signalling is not only involved in defence against such microorganisms but also in communicating with the underlying immune cells. This review describes the many mechanisms by which such communication is executed. It also highlights potential sources of variation in such signalling in the general population in particular the effects of genetic variation, diversity of the microbiota, concomitant disease, diet and age.

Keywords: Toll-like receptors; intestinal epithelium; inflammation; innate immunity

1. The gastrointestinal epithelium

The gastrointestinal epithelium is continually replacing itself while at the same time digesting and absorbing food, producing antimicrobial molecules, mucus and communicating with resident microorganisms. The epithelium replaces itself every 4–5 days; it maintains its own stem cell niche at the base of the crypts of Lieberkuhn [1,2]. These stem cells are responsible for generating absorptive cells called enterocytes, responsible for the transport of digested food metabolites from the lumen to the blood circulation and secretory cell types such as Paneth cells, Goblet cells and enteroendocrine cells [3]. The lumen of the GI tract is home (either transiently or more permanently) to trillions of bacteria, fungi and viruses. Most of these microorganisms are not immediately harmful to the host with some intimately involved in host food and xenobiotic metabolism as well as vitamin production. Others produce molecules such as bacteriocins capable of destroying pathogenic bacteria. Many of these microorganisms are in constant communication with host cells directly or indirectly, sometimes releasing effector molecules, other times involving direct cell-to-cell interactions or phagocytosis [4,5].

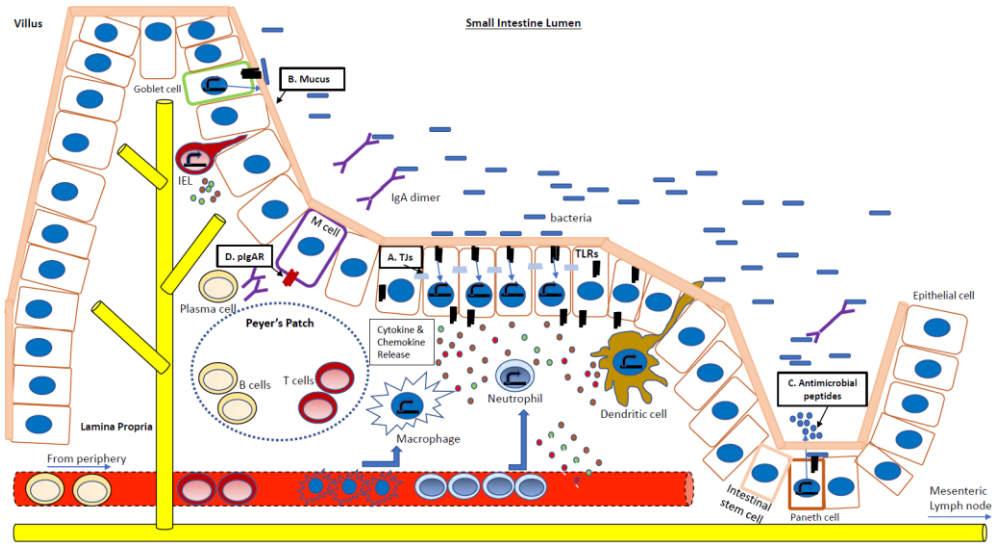

Figure 1. Apical and basolateral communication of intestinal epithelial cells via TLR induced signalling. Apical TLR signalling via microbial interaction in the lumen is responsible for a number of defence mechanisms including A. tight junction (TJs) protein expression; B. Mucus production; C. Antimicrobial peptide production; D. Polymeric Immunoglobulin A Receptor (pIgAR) expression. Basolateral release of cytokines and chemokines results in signalling with distinct populations of innate and adaptive immune cells (indicated in figure) in the lamina propria.

On the basolateral (serosal) side of the epithelial cell layer, there is a substantial number and diversity of immune cells in direct contact with the epithelium. These include antigen presenting cells such as dendritic cells and macrophages [6]. There is also a substantial population of intraepithelial lymphocytes [7] as well as cells located in the underlying lamina propria [8,9] or

discrete follicles such as Peyer's patches [10] that contain innate, innate lymphoid and lymphoid populations [11] (Figure 1). In addition, there are leukocytes that traffic to the intestine where they undergo antigen induced activation and priming [12]. These cells react to threats and defend the host when needed but not to overreact to innocuous antigens present in food, for example. How all of these cells (host and non-host) communicate has not been fully elucidated yet but there have been a number of studies that suggest Toll-like receptors are central to this, in particular in the intestinal epithelium [13–15].

2. Toll-like receptors

Toll-like receptors (TLRs) are a family of pattern recognition receptors [16] and are a key part of the innate immune response. TLRs are capable of recognising pathogen associated molecular patterns [16] present in microorganisms as well as danger associated molecular patterns (DAMPs) released from host cells. PAMPs from microorganism include lipoproteins and lipopolysaccharides (LPS) from the surface of bacterial cells as well as single- and double-stranded nucleic acids from bacteria and viruses. TLRs are present at the cell surface as well as intracellularly in endosomes to deal with different modes of extracellular and intracellular infection. There are ten TLR family members in humans (TLR1-10). TLRs 1, 2, 4, 5 & 6 are localised on the cell surface and thought to respond to bacterial molecular patterns whereas TLRs 3, 7 & 8 are endosomal and deal with viral infections mostly [17]. Encounters with PAMPs results in receptor dimerization and initiates a signalling cascade which culminates in the activation of transcription factors such as nuclear factor kappa light chain enhancer of activated B cells (NFκB) and/or interferon regulatory factor 3 (IRF3) [18] leading to the synthesis and release of cytokines (e.g interleukin 6) and type I interferons (e.g. interferon α/β).

Most TLRs activate a myeloid differentiation primary response 88 (MyD88)-dependent pathway with TLR3 activating a MyD88-independent pathway. TLR4 can activate both pathways. TLR expression seems to be present in most human cells although the expression profile and their localisation differs between tissues. In the intestinal epithelium, TLRs can localise to the apical and/or basolateral surface depending on the family member and cell type [13]. This is thought to influence immune responses particularly when there is a breach of epithelial integrity. The regulation of TLR expression appears to be tightly controlled with restriction of intracellular localisation and negative regulators playing an important role [17]. Loss and gain of function mutations in TLR family members can enhance or reduce susceptibility to certain chronic inflammatory conditions indicating their importance [19]. This review discusses the role of TLR signalling in intestinal epithelial-immune cell cross talk.

3. TLR signalling in intestinal immune defence

Within the intestinal epithelium, the primary role of TLRs is to recognise danger and to warn neighbouring cells of potential invaders as well as convey to them the contents of the luminal environment. However, we now know that TLR signalling can regulate not only innate and adaptive immunity but also metabolism, proliferation, repair and cell death [20]. This is achieved through the

synthesis of multiple different proteins after TLR signalling that are capable of interacting with multiple cell types. This review will discuss some of the mechanisms by which this is achieved with specific examples described below.

As mentioned above, the intestinal epithelium is primarily a barrier to invading bacteria and viruses. The major mechanisms used by intestinal epithelial cells to protect the host include maintenance of tight junctions, mucus production, antimicrobial release and facilitative antibody transport (Table 1). Although it is only one cell thick, this barrier manages to exclude food and microorganism through the use of tight junction proteins that bind neighbouring cells together very tightly. Such proteins include zonula occludens 1 (ZO-1), ZO-2 and claudins. These proteins block the passage of most bacteria between epithelial cells. The regulation of such proteins can vary between homeostasis and infection. Homeostatic TLR activation by commensal luminal bacteria (in particular TLR2 and TLR4) results in a reorganisation of such proteins in a mechanism dependent on protein kinase C, leading to an increase in transepithelial resistance and an increase in IEC survival thus strengthening the barrier [10,21–24]. Connexin 43 is also a key protein involved in gap junctional intercellular communication and has been implicated in a number of barrier diseases including enterocolitis and cancer. TLR2 signalling increases the expression of connexin 43 and prevents the occurrence of spontaneous colonic inflammation [25]. TLR2 signalling has also been implicated in maintaining the integrity of the underlying enteric nervous system as well as in neurochemical coding. As a consequence, this seems to regulate inflammation in the intestine. A possible reason for this may be that in the absence of TLR2 there is an alteration in intestinal motility and this an alteration in the transit of bacteria through the gut [26]. In the case of infection, it seems that TLR4 is also implicated but this time in increasing the epithelial permeability in response to lipopolysaccharide [27]. The response may therefore depend on the species of bacteria involved.

Table 1. TLR-induced defence mechanisms in intestinal epithelial cells.

Defence mechanism	TLR(s) implicated	References
1. Tight junction protein expression ZO 1 & Connexin 43 ↑ in IECs	TLR2	10, 21–25
2. Mucus production TFF3 & mucin 2 ↑ in Goblet cells	TLR2	28–31
3. Antimicrobial peptide/enzyme production RegIIIγ, RELMβ, CRP-ductin, cathelicidin, β-defensin ↑ in Paneth cells	TLR2 & 4	32–36
4. Facilitative antibody transport sIgAR ↑ in IECs	TLR3 & TLR4	42, 43

A second mechanism that the epithelium uses to prevent infection is producing mucus, which is a specific responsibility of Goblet cells. Mucus is composed of glycoproteins and trefoil factor 3 (TFF3). TFF3 was shown to be regulated by TLR2 stimulation via a PI3K/Akt dependent mechanism and that the absence of such signalling can enhance susceptibility to colitis [28,29]. TLR ligands and commensal bacteria have also been implicated in the mechanism for mucin 2 production in Goblet cells recently [30,31]. A third mechanism used is the production of antimicrobial peptides and

enzymes by Paneth cells in particular. These proteins include the C-type lectin regenerating islet-derived protein IIIγ (RegIIIγ), that binds bacterial peptidoglycan; resistin-like molecule β (RELMβ), a modulator of macrophage and T cell responses and also promotes the secretion of mucin 2; CRP-ductin, which agglutinates Gram-positive and Gram-negative bacteria, cathelicidin and β-defensin. Their production is dependent on TLR signalling and induction of degranulation [32–36]. Some of these peptides such as cathelicidins have themselves been reported to regulate TLR activity [37]. During infection with *Salmonella tymphimurium*, it has been shown that the TLR adaptor MyD88 is necessary for the induction of mucus proteins Muc2 and TFF3 from Goblet cells as well as the antimicrobial proteins RegIIIγ and RELMβ. MyD88 knockout mice suffer accelerated tissue damage and colitis following infection [38]. In a *Citrobacter* infection model, IEC MyD88 signalling was shown to promote barrier function as well as the induction of RegIIIγ amongst many other genes and this was thought to contribute to host resistance to infection [39].

The fourth major protective mechanism is facilitative antibody transport. Plasma cells induced by commensal bacteria-loaded DCs in the underlying lamina propria (LP) [8] produce large quantities of polyreactive soluble IgA (sIgA) molecules that can bind many components of different microbial species in the lumen [40]. By binding to such bacteria, it can prevent them from coming into contact with the epithelium. Recently, IgA was shown to regulate the composition and metabolic function of gut microbiota, this is thought to promote colonic homeostasis [41]. The transport of dimeric sIgA from the LP requires the polymeric immunoglobulin receptor (pIgR), which is found on the basolateral surface of intestinal epithelial cells. From here, the sIgA is actively trancytosed into the lumen where it can interact with a subset of commensal and pathogens alike. The expression of the pIgR is regulated by TLR3 and TLR4 stimulation [42,43].

While it seems that TLR signalling predominates in enterocytes, Paneth and Goblet cells, there is also some evidence that enteroendocrine cells express functional TLRs [44] and that TLR signalling may induce enteroendocrine cells (a subset of epithelial cells – close to 1% of the total) to secrete hormones that may induce muscular contraction in the intestine [45] as well as specific chemokines such as CXCL1 [46]. It has also been demonstrated recently that these cells increase expression of PYY, a hormone central to the control of food intake and gut motility, in response to a host of TLR ligands. This study also demonstrated that these responses could be enhanced further in the presence of butyrate (metabolites of commensal bacteria) [47]. The mechanisms mentioned above limit the ability of bacteria to come in contact with either the epithelium or the underlying cells in the lamina propria and in some cases assist in their removal from the lumen. In addition, such mechanisms seem to aid in the selection of microbes for colonisation. Many commensals have evolved mechanisms to bypass many of these defences such as the ability to digest mucus but without the virulence factors associated with pathogens. While certain mechanisms aid in preventing microbes from breaching the epithelial barrier, there are also mechanisms of tolerance which prevent an overreaction to commensal microbes. For example, it has been reported that a tolerance to endotoxin in IECs is developed shortly after birth and this may be dependent on the mode of delivery [48].

4. TLR signalling in immune cell crosstalk

In addition to mechanisms involved in limiting microbial access to the epithelium, TLR signalling is also directly involved in alerting neighbouring and distant immune cells to potential dangers. Most of the mechanisms listed above such as antimicrobial secretion, tight junctions and mucus production deal with the apical surface, communication with immune cells is mostly on the basolateral side of the epithelium in the lamina propria. Examples of intestinal epithelial-immune cell cross talk includes the recruitment of phagocytes, facilitating antigen uptake, inducing the expression of integrins and other adhesion molecules, tolerising antigen presenting cells and switching phenotypes of lymphocytes [49].

Table 2. Effects of TLR-induced signalling to immune cells in the lamina propria.

Target cell	Protein(s) affected	Outcome	References
Neutrophils	IL8	↑ recruitment	50–52
	IL6	↑ degranulation	53
Dendritic cells	CCL20	↑ recruitment	55
	CCL20	↑ extension	64, 65
	TGFβ & TLSP	↑ tolerance	66–68
	Retinoic acid	↑ tolerance	67
Endothelial cells	ICAM1 & VCAM1	↑ extravasation	56
Macrophages	FcγRII	↑ phagocytosis	58
	TNFα, IL12, IL6	↑ Th1 phenotype	59
	IL10	homeostasis	60
	MIP2	↑ infiltration	52
Intraepithelial Lymphocytes	IL-15	↑TCRαβCD8αα population	75, 76
	Occludin	↑ dendrite projection	77
	RegIIIγ	↑ antimicrobial response	79
	IL23/IL22	↑ antimicrobial response	80
Regulatory T cells	TGFβ	↓ inflammation	82
B cells	BAFF	class switch recombination	86
	TLSP/APRIL	class switch recombination	87, 88

The primary cell targets of epithelial TLR signalling include neutrophils, macrophages, dendritic cells and B and T lymphocytes (Table 2). TLR signalling in the epithelium is thought to facilitate immune cell recruitment, extravasation and maturation depending on the nature of the interaction and often dependent on engagement with commensals or pathogens. Following engagement with TLR ligands, epithelial cells can release large amount of the chemokine IL8 known to induce neutrophil infiltration into the mucosa [50–52]. It has been shown that in the absence of TLR signalling, there is a delayed recruitment of neutrophils to the colon leading to increased bacterial colonization in a *Citrobacter rodentium* infection model [53]. In addition, IL6 release from the epithelial cells on engagement with *Salmonella tymphimurium* is thought to stimulate/degranulate neutrophils in proximity in a calcium dependent manner [54]. TLR5 stimulation by pathogenic

bacterial strains such as *Salmonella* but not commensal strains have been shown to mediate the migration of dendritic cells via CCL20 release from epithelial cells [55]. The release of these specific cytokines from the epithelium has also been shown to indirectly increase the expression of adhesion molecules ICAM1 and VCAM1 on endothelial cell walls that facilitate the extravasation of leukocytes [56] as well as increasing expression of ICAM1 on epithelial cells and facilitate neutrophil adhesion [57].

In addition to cell recruitment and facilitating extravasation, epithelial TLR signalling has been shown to prime or activate key cell populations in advance of a required response. TLR2 and TLR4 engagement can mediate phagocytosis and translocation of bacteria across the epithelium as well as induce an increase in intestinal barrier permeability, which may facilitate antigen presentation for priming the immune cells in the LP [10,58]. TLR4 deficient mice have increased bacterial translocation (in particular *Escherichia coli*) to the mesenteric lymph nodes) compared to their wild-type littermates [52].

TLR engagement can influence the polarization of immune responses particularly for monocytes and macrophages in the vicinity of the epithelium. For example, there is an increase in the production of Th1 responses on ligation with TLR4 and TLR9 with release of TNFα, IFNγ, IL12 and IL6. This can also modulate the phenotype of neighbouring T cells and monocytes in a co-culture system [59]. TLR4 signalling has also been shown to generate crosstalk between IECs and macrophages leading to increased expression of IL10 in IECs, which is important in maintaining intestinal homeostasis [60]. TLR8-mediated signalling in IECs has been shown to prime dendritic cells, monocytes and T cells for antiviral responses [61]. In addition, during inflammatory states IECs have been shown to establish gap junction intercellular communication with monocyte/macrophage cells by augmenting the expression of specific protein such as connexins [62]. TLR4 signalling has also been shown to influence macrophage infiltration as well as macrophage inflammatory protein 2 (MIP2) expression in the colon in a DSS model of colitis in mice [52].

Professional antigen presenting cells are in close proximity to the epithelium [63]. TLR signalling has been suggested to promote dendritic cell (DC) extension into the lumen for sampling of the small intestine upon engagement with *Salmonella* species [64,65]. Another mechanism described to prime these cells involves the release of transforming growth factor (TGFβ) and thymic stromal lymphopoietin (TLSP) from epithelial cells in response to engagement with a variety of different commensal bacterial strains via TLRs. Data from studies in co-culture systems suggest that such signalling can drive dendritic cells away from Th1 signalling and towards a more tolerogenic phenotype [66–68]. It has also been reported that Muc2 derived from IECs may able to imprint DCs with anti-inflammatory properties potentially contributing to tolerance of commensal microbes [69]. Other contributors to this mechanism include retinoic acid signalling in DCs, which requires TLR signalling [70–72]. It seems that lamina propria DCs may respond differently to various molecular patterns engaging TLRs and can be quite distinct in their responses and maturation compared to peripheral DCs [73]. This may be partly explained by different levels of TLR expression in these subsets as well as responsiveness to IL10 [74].

Crosstalk between IECs and intestinal intra-epithelial lymphocytes (IELs) is also dependent on TLR4 recognition of Gram-negative commensal bacteria involving the release of the T cell growth factor IL15 [75,76]. These cells, which mostly consist of T cell receptor (TCR)$\gamma\delta^+$ and TCR$\alpha\beta^+$ T

cells, are thought to migrate dynamically within the epithelium. They are able to project dendrites into the luminal space via a mechanism dependent on occludin which is used for the interaction between IECs and the lymphocytes [77]. This process may limit the ability of pathogens (such as *Salmonella typhimurium* and *Toxoplasma gondii*) to cross the epithelium and thus limits systemic disease [78]. IELs can direct an antimicrobial response (via RegIIIγ release) in response to the microbiota in the small intestine which is dependent on TLR signalling in the epithelium [79]. These cells in coordination with IECs are able to change their behaviour depending on the food or microbes present by altering their motility, metabolism and antimicrobial gene expression in a TLR-dependent manner [80].

Regulatory T cells (T_{regs}) are a subpopulation of T cells (that express CD4, Foxp3 and CD25) that play a key role in regulating immune responses to infection and in autoimmunity. *Fusobacterium nucleatum* is a major contributor to periodontal disease and has also been detected in patients with inflammatory bowel diseases (IBD). TLR2/4 signalling has recently been shown to induce T_{regs} and to attenuate the inflammation associated with *Fusobacterium* infection in IECs [81]. It has been shown recently that commensal bacteria can play a role in regulating this particular population of T cells. Commensal *Clostridium* species have been shown to induce $Foxp3^+$ IL10 releasing T_{regs} in the LP of the colon and this is dependent on signals from IECs, particularly TGFβ. This has been shown to reduce the incidence of colitis in mice [82]. Cross talk between IECs and T_{regs} are critical in maintaining homeostasis. Studies using mice deficient in Foxp3 highlight the importance of TLR signalling in T_{reg} induced homeostasis in the gut by restraining tonic microbial-dependent proinflammatory signals in IECs [83]. In contrast, segmented filamentous bacteria [84] colonisation of the small intestine appears to induce Th17 T cells in the LP possibly by serum amyloid A and is protective of *Citrobacter* infection in mice [84]. IEC and IEL cross talk is also evident during *Salmonella* infection, whereby IECs release IL23 in a TLR-dependent manner in response to infection. This in turn is thought to stimulate IL22 release from IELs, which can then stimulate Paneth cells to release the bactericidal protein angiogenin 4 into the lumen [85].

In addition to interacting with antigen presenting cells and T cells, TLR signalling in epithelial cells in response to viral infection for example may be able to influence B cell phenotypes and class switching. Data from tonsillar epithelial cells suggest such interactions involve epithelial TLSP triggering the release of B-cell activating factor (BAFF) [86] from dendritic cells in response to viral RNA and inducing the expression of cytidine deaminase resulting in class switch recombination (CSR) in B cells [86]. In IECs, it was demonstrated that commensal bacteria recruit and trigger LP B cell IgA_2 CSR by releasing a cytokine called a proliferation inducing ligand (APRIL) from DCs in a TLR-dependent manner also via TLSP. This mechanism was shown to be T cell independent [87,88]. Recently, TLR5 activity in the IEC has been shown to be necessary to induce effective antibody responses to a seasonal influenza vaccine. More specifically, it seems TLR5 signalling combined with the microbiota impact on primary and secondary B cell responses [89]. Recently, it was shown that Gram-negative commensal gut bacteria also induce antigen-specific IgG under steady state conditions. This requires TLR4 signalling and is thought to have a protective role in preventing systemic infections by opsonising pathogens [8].

As well as alerting immune cells to potential dangers, TLR signalling has been reported to be involved in the maintenance of gut homeostasis as well as repair. A seminal study by Medzhitov and

colleagues reported that TLR-mediated recognition of commensals in the colon regulated the production of tissue protective factors and TLR signalling protects from mortality caused by intestinal epithelial injury [9]. Recent examples include a key role for TLR1 in the intestinal epithelium. A deficiency in TLR1 was associated with mucosal-associated bacteria, gut permeability and a reduction in wound healing as well as systemic bacteria and an elevated innate immune response [90]. In another report, TLR5 expression in IECs but not DCs was identified as being necessary for homeostasis as mice deficient in IEC TLR5 developed low-grade inflammation, an altered microbiota and increased susceptibility to colitis [91]. TLR9 signalling has also been reported to maintain homeostasis and even protect in certain cases of colitis by conferring intracellular tolerance to subsequent TLR challenges [92]. TLR signalling also influences the development of certain discrete cell populations in the epithelium. For example, TLR4 may regulate the development of mucus producing Goblet cells via Notch signalling in the small intestine [93]. TLR signalling has been linked with regulation in the microbiota composition in particular the numbers of mucus-associated and opportunistic bacteria [94]. Taken together, these findings suggest that TLR signalling in the intestinal epithelium is involved in many diverse activities necessary for survival and immunological defence.

5. TLR regulation and variation

While there is considerable evidence to suggest that TLR signalling plays an important role in immune cell crosstalk, there are a number of questions that arise from the studies cited above. Firstly, how translatable are such findings? The studies described above were mostly conducted with isolated populations or cell lines *in vitro* or using *in vivo* animal models where there is a higher degree of uniformity of conditions. For example, cell culture conditions are often kept standardised across many experiments and rodents are usually fed a very similar diet and housed in similar conditions within studies. Many of the biochemical findings described may be products of this uniformity. However, this may not be the case in the human population. There has already been a number of reports that indicate that there is variation in the genetic sequences that code for TLRs and these can influence individual susceptibility to infection and inflammation [19]. There is also evidence that TLR expression levels are not uniform and that there is also considerable regional variation in not only immune cell populations but the diversity of the microbiota that can induce some of these events [4,95,96]. It is important therefore to consider what factors are likely to induce such differences and how this might explain variation in the general population. Factors such as genetics, concomitant disease, microbiota diversity/density, diet as well as age are plausible sources of variation within the wider population.

Another question which arises from studies cited above is how uniform is TLR expression in the GI epithelium and will there be TLR signalling variation? A very recent study examined the temporal and spatial expression patterns of a subset TLRs in the mouse GI tract. This revealed a distinct pattern of TLR expression in the small intestine and the colon intestinal epithelium. Most notably, the authors reported the restricted expression of TLR5 to Paneth cells in the small intestine and that TLR signalling induced the expression of a distinct subset of defence genes that did not include antimicrobial peptides. These instead were induced indirectly via cytokine signalling in

proximal immune cells [97]. Studies with cell lines have revealed that intestinal epithelial cell TLR expression may be relatively low rendering cells unresponsive to TLR ligands, in particular TLR4 [98].

For many of the cell-based *in vitro* models cited above, there isn't the same degree of cell polarization that is found *in vivo*. Cell localization of protein is a key determinant of TLR signalling. As mentioned above, TLRs can be expressed at the cell surface or intracellularly. For intestinal epithelial cells, this is complicated further by the positioning of cell surfaces at the apical (luminal) and basolateral (serosal) sides. TLR localization is thought to be crucial in determining the extent and type of inflammatory response. Previous studies have demonstrated differential cytokine responses depending on whether receptor signalling occurs from the apical or basolateral side of the epithelium ([99]. This can be determined by chaperone proteins such as UNC93B1 for TLRs 3, 7, 8 & 9, for example [17]. In addition, the polarity of intestinal epithelial cells is an important determinant for some TLRs such as TLR9. It appears the apical and basolateral TLR9 signalling execute different transcriptional responses [92]. Some TLRs do not signal on their own and require accessory proteins to complete their duties. Expression of accessory proteins in intestinal epithelial cells such as CD14 and MD2 and LPS binding protein in the periphery have a central role in determining TLR4 responses, in particular [17].

When considering differences in TLR responses, it is crucial to consider regional differences (some controlled by TLR responses and other which influence them) between the duodenum, jejunum, ileum, caecum and the colon. Such differences include variations in structure (length of or absence of villi), cell types (e.g presence/absence of Paneth cells), cell density (e.g DCs and IELs/T_{regs}), mucus thickness (one versus two layers), concentrations of specific nutrients/metabolites (e.g. vitamin A), microbial density and diversity (which increases towards the colon), production of microbial metabolites (e.g. SCFAs/indoles), presence of follicle associated epithelium (e.g. Peyer's patches/cryptopatches). Since TLR signalling in the intestinal epithelium is so dependent on the interactions with both immune and bacterial cells, such responses can vary along this tract given this variation. It has been suggested that this variation may influence individual susceptibility to chronic diseases such as allergy and inflammatory bowel diseases (IBD) [95,96].

A question which is not often considered when examining TLR signalling, is the role of disease/existing inflammation. Concomitant diseases often influence the trajectory of each other. In particular, inflammation is increasingly described as a risk factor in many disease states. This may be explained by its influence on key signalling pathways. TLR expression has been reported to be altered in inflammatory conditions such as IBD, in particular TLR3 and TLR4 [100]. Specific cytokines have been shown to augment or reduce TLR expression and activity [101]. It seems that Th1 and Th2 cytokines have the opposite effects on TLR3 and TLR4 expression. Th1 cytokines increase their expression in IECs, while Th2 cytokines decrease their expression [102]. In particular, interferon γ augments TLR4 expression and signalling in IEC cell lines in response to lipopolysaccharide (LPS) [103].

Following extensive research carried out recently on the microbiome, a key question that now arises is how these populations affect TLR signalling across human populations showing diversity. Microbiota diversity and density in participants are increasingly reported in studies of a wide variety of disease states. In particular, alterations in the symbiotic relationship between the microbiota and IECs are associated with many diseases of the GI tract. It has been suggested, that these alterations

may manifest at the molecular level and specifically at the level of TLR expression and signalling. As outlined above, interactions between the microbiota and TLRs are essential for maintaining intestinal homeostasis but a recent study has demonstrated that the circadian clock may influence this. This may be explained by the fact that microbiota have been reported to regulate the expression of key circadian clock and nuclear receptor genes in IECs (as well as regulating plasma corticosterone levels). Studies have suggested this could be a key regulator of TLR expression in these cells [104]. There have also been reports that suggest individual bacterial species may differentially regulate TLR expression itself. A recent report demonstrated that individual species of *Bifidobacterium* could down-regulate the expression of both TLR2 and TLR4. In contrast, endotoxin from Enteropathogenic *E. coli* (EPEC) increased expression of both and dramatically decreased transepithelial resistance (TEER) [105].

How the intestinal cell populations interact with their environment is key to homeostasis. Certain molecules produced or metabolised in the luminal microenvironment are thought to influence expression of genes by epigenetic mechanisms. It has been suggested that TLR expression and signalling may be regulated by such mechanisms. Inhibitors of DNA methyltransferase (DNMTi) and histone deacetylases (HDACi) have been shown to inhibit TLR signalling (reviewed in [16]). Microbiota are large producers of short chain fatty acids (particularly those that are resident in the colon). Some of which including butyrate, are known to have HDACi activity [4]. Short chain fatty acids such as butyrate and propionate have been shown to inhibit TLR signalling in intestinal macrophages, dendritic cells and epithelial cells *in vitro* in response to TLR4 and TLR5 ligands [106,107].

Diet is an important contributor to the composition and diversity of the human gut microbiota. This may in part be due to the selection of species that produce specific molecules from digestion that modulate immune homeostasis such as indoles, aryl hydrocarbon (AHR) ligands and SCFAs [4,5]. Specific foodstuffs are thought to influence subsets of immune cells and certain signalling pathways. In particular, it has been shown that human breast milk can have distinctive effects on different TLR signalling pathways. A study using IEC cell lines showed that human milk enhanced IL8 responses to both the TLR4 ligand LPS and the TLR5 ligand flagellin but reduced the responses for TLR2 ligand peptidoglycan and the TLR3 ligand Poly I:C [108]. Bacterial metabolites such as indole 3-propionic acid [109] which is a ligand for the pregnane X receptor [109] regulates mucosal integrity by modulating TLR4 expression and signalling [109]. This metabolite has also been shown to attenuate inflammation, by interfering with cytokine signalling [110].

Finally, age is an important factor in determining responses in particular the early postnatal period. Previous studies have shown that there is an acquisition of tolerance to endotoxin (as mediated via TLR4) in the early postnatal period due to a decrease in TLR4 and its co-receptor MD2 as well as signalling protein IRAK1 within the first four weeks of life [48]. A similar situation appears with TLR5 expression gradually decreasing during this time window [97].

6. Conclusions

The studies described in this review indicate the many roles that intestinal epithelial cells have besides from nutrient absorption. They also indicate the key role Toll-like receptors play in their survival and the numerous interactions these cells have within their microenvironment. Although

many reports have described various mechanisms of how TLR signalling contributes to the defence of the host, we are still uncertain as to the sources and implications of variation in the population. Future studies should focus on this aspect as to date, we have relied on uniform systems involving cells in culture or genetically similar rodents. It is also not clear at present how this information can used for pharmacological benefit. A future challenge will be to translate these findings into developing therapeutics for inflammatory diseases.

Conflict of interest

The author declares no conflicts of interest in this paper.

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