TLR4 signalling in the intestine in health and disease

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Abstract

The colonic epithelium is lined along its apical membrane with ∼1014 bacteria/g of tissue. Commensal bacteria outnumber mammalian cells in the gut several fold. The reason for this degree of commensalism probably resides in the recent recognition of the microbiome as an important source of metabolic energy in the setting of poorly digestible nutrients. As in many themes in biology, the host may have sacrificed short-term benefit, i.e. nutritional advantages, for long-term consequences, such as chronic inflammation or colon cancer. In the present review, we examine the role of TLR (Toll-like receptor) signalling in the healthy host and the diseased host. We pay particular attention to the role of TLR signalling in idiopathic IBD (inflammatory bowel disease) and colitis-associated carcinogenesis. In general, TLR signalling in health contributes to homeostatic functions. These include induction of antimicrobial peptides, proliferation and wound healing in the intestine. The pathogenesis of IBD, ulcerative colitis and Crohn’s disease may be due to increased TLR or decreased TLR signalling respectively. Finally, we discuss the possible role of TLR signalling in colitis-associated neoplasia.

TLR (Toll-like receptor) signalling in the intestinal mucosa in health

Commensal bacteria have a significant role in the maintenance of IEC (intestinal epithelial cell) homoeostasis. For example, germ-free mice have reduced IEC proliferation compared with colonized mice [1,2]. Germ-free mice that are colonized with a single bacterial species undergo global intestinal transcriptional responses to colonization [3–5]. On the basis of these observations, IECs may recognize luminal bacteria directly or indirectly. TLR signals may be important for maintenance of intestinal epithelial homeostasis. TLRs are members of a conserved IL (interleukin)-1 superfamily of transmembrane receptors that recognize PAMPs (pathogen-associated molecular patterns). The interaction of TLR4 with its ligand LPS (lipopolysaccharide) results in the recruitment of the adaptor molecule MyD88 (myeloid differentiation factor 88) and phosphorylation of the IRAK (IL-1 receptor-associated kinase) followed by TRAF6 [TNF (tumour necrosis factor) receptor-associated factor 6] [6]. Recruitment of TRAF6 leads to the phosphorylation of IκBα [inhibitor of NF-κB (nuclear factor κB)] kinases (mainly IκKβ in the case of TLR4 signalling) and release of NF-κB. After TRAF6 activation, the MAPK (mitogen-activated protein kinase) pathway can be activated through ECSIT (evolutionarily conserved signalling intermediate in Toll pathways) and TAK1 [TGF-β (transforming growth factor β)-activated kinase] [7].

The understanding of TLR signalling in the intestine has been limited by the lack of good antibodies to examine expression and localization of TLR expression and the inability to study functional signalling of TLR in vivo. Nevertheless, the expression and function of several TLRs have been examined in the intestine. Human IECs normally express TLR3 and TLR5, whereas TLR2 and TLR4 are only barely detectable [8–11]. However, TLR4 is up-regulated in both Crohn’s disease and ulcerative colitis, whereas the expression of TLR2 and TLR5 remains unchanged [8]. We have shown that inflammatory cytokines such as IFN (interferon) γ and TNFα increase expression of TLR4 and MD-2 (myeloid differentiation protein 2), resulting in increased LPS responsiveness [11,12]. In addition, expression of TLR4 and TLR2 is increased in lamina propria macrophages in IBD (inflammatory bowel disease) [13]. Therefore TLR4 signalling may be increased in the setting of chronic colitis at the level of both the IECs and lamina propria macrophages.

Investigators have examined the ontogeny of TLR expression and function [14]. They have shown that TLR4/MD-2 is present in fetal, neonatal and adult IECs. In spite of its presence, it is only functional in fetal IECs. Following vaginal delivery and colonization of the fetal gastrointestinal tract, TLR4 signalling is down-regulated in IECs. The reason for down-regulation of signalling was post-transcriptional down-regulation of IRAK-1. Thus IECs acquire tolerance following colonization, presumably to permit colonization without chronic inflammation.

Key words: cancer, colitis, Crohn’s disease, inflammatory bowel disease (IBD), intestine, Toll-like receptor (TLR)

Abbreviations used: AOM, azoxymethane; APC, adenomatous polyposis coli; CA, colitis-associated cancer; COX-2, cyclo-oxygenase 2; DSS, dextran sodium sulfate; EGR, epidermal growth factor receptor; IBD, inflammatory bowel disease; IBT, intestinal epithelial cell; IκK, IκKB (inhibitor of nuclear factor κB) kinase; IL, interleukin; IRAK, IκK-1 receptor-associated kinase; LPS, lipopolysaccharide; MD-2, myeloid differentiation protein 2; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor κB; PGE2, prostaglandin E2; SAR, single-stranded RNA; SRA, single-stranded RNA receptor; TLR, Toll-like receptor; TGF-β, transforming growth factor β; TNF, tumour necrosis factor; TRAF6, TNF-receptor-associated factor 6.

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The data on the role of TLRs in IBD come from two broad areas. First, the use of animal models of inflammation in TLR-deficient systems, and, secondly, in examining TLR polymorphisms in patients with IBD. With respect to the latter, we and others have described that TLR4−/− mice have increased susceptibility to bleeding and bacterial translocation following DSS (dextran sodium sulfate)-induced mucosal injury [15, 16]. Underlying this more severe clinical phenotype is the fact that TLR4−/− mice have a decreased ability to secrete chemokines that are responsible for recruitment of neutrophils and, to a lesser extent, monocytes to the intestinal mucosa [15] (Figure 1). This in part permits escape of bacteria from the gut and to the mesenteric lymph nodes and spleen. TLR2−/− mice share some features with TLR4−/− mice with respect to increased rectal bleeding and weight loss following DSS injury, but the underlying pathology is distinct.

In examining why TLR4−/− mice had increased bleeding, we performed studies in which we administered DSS for 7 days and then allowed mice to recover for 7 days [17]. We found that TLR4−/− mice had significantly reduced proliferation and increased apoptosis following DSS injury compared with wild-type mice. COX-2 expression by IECs and lamina propria macrophages was also very blunted in TLR4−/− mice. Macrophages are known to up-regulate COX-2 in response to LPS. We showed that IEC lines up-regulate COX-2 expression in a TLR4- and MyD88-dependent fashion. We found that LPS potently stimulated COX-2 in IECs, whereas peptidoglycan, an agonist of TLR2, had a much weaker effect [17]. PGE2 supplementation of TLR4−/− mice resulted in improvement in clinical signs and symptoms of colitis and restoration of proliferation and apoptosis to wild-type values. We also demonstrated that LPS stimulates phosphorylation of EGFR (epidermal growth factor receptor) in IECs. EGFR activation can be blocked by COX-2 inhibitors, suggesting a COX-2-dependent pathway. Given the importance of COX-2–PGE2 and EGFR signalling in colorectal cancer and CAC (colitis-associated cancer), our findings support an important role for TLR4 in the development of CAC.

TLR5, one of the receptors for flagellin, may also be important for recognition of mucosal injury. Rhee et al. [18] have described that, under normal conditions with an intact epithelial barrier, administration of flagellin has no effect. Conversely, following DSS-induced mucosal injury, flagellin induces increased inflammation. These data highlight differences between the expression and function of TLR signalling in health or following injury.

Other TLRs have been examined for their role in intestinal inflammation. Oral or systemic administration of CpG oligodeoxynucleotides, the ligand for TLR9, ameliorates inflammation in several animal models of colitis [19], but not in TLR9−/− mice [20]. Recent work demonstrates that TLR9 signalling along either the apical membrane or basolateral membrane activates distinct signalling pathways [21]. For example, TLR9 signalling on the apical membrane where it would come into contact with CpG DNA from commensal bacteria results in dampened NF-κB activation, whereas basolateral signalling activates NF-κB. These data highlight yet another way in which TLR signalling is regulated in the mucosa based on cell polarity.

TLRs and human inflammatory bowel disease

With respect to studying human IBD and expression of TLRs, many TLRs will be up-regulated once inflammation is established, since pro-inflammatory cytokines and NF-κB can induce the TLR promoters. It is therefore hard to conclude that TLRs play a causal role in inflammation, rather it appears that they are merely part of the diathesis that occurs when the tissue is no longer in homoeostasis. Moreover, our own work would suggest that TLR signalling is important for repair of the injured gut so the increase in TLR expression may serve a protective role. TLR polymorphisms have been examined as candidate genes in IBD. Several studies have found the D299G polymorphism of TLR4 to be associated with either ulcerative colitis or Crohn’s disease or both [22–27]. Crohn’s disease has been associated with a TLR9 polymorphism, which is interesting given the animal data...
that TLR9 may be anti-inflammatory in certain contexts [28]. SNPs (single nucleotide polymorphisms) in TLR1, TLR2 and TLR6 have been examined in IBD. Although none of the SNPs was involved in disease susceptibility, all were associated with more colonic disease extent in ulcerative colitis and Crohn’s disease [29]. TLR1 R80T and TLR2 R753G SNPs were associated with pancolitis in ulcerative colitis. These association studies highlight the role of an abnormal innate immune response in the pathogenesis of IBD.

Role of bacteria in the development of CAC

IBD is characterized by a dysregulated immune response to commensal bacteria in the genetically susceptible host. Colorectal cancer is one of the most serious complications of IBD, accounting for increased mortality in these disorders [30–36]. The clearest link between inflammation and colon cancer is seen in IBD [37]. The severity of inflammation correlates with the risk of colorectal cancer in patients with IBD [38,39]. Therefore focusing on the relationship between chronic inflammation and carcinogenesis may elucidate the pathogenesis of CAC.

Mouse models of colitis are useful to understand the role of bacteria in the development of CAC. Several rodent models of IBD require commensal bacteria or specific bacteria (*Helicobacter hepaticus*) for initiation of colitis and development of dysplasia or cancer [37,40,41]. Germ-free rats given carcinogens are protected from colonic dysplasia and cancer [42–44]. Long-term or repeated cycles of DSS treatment can induce chronic colitis, followed by dysplasia and cancer in rodents [45–47]. As in human IBD, the incidence of neoplastic lesions in this model is associated with the severity of mucosal injury. AOM (azoxymethane) is a colonic genotoxic carcinogen that has been used extensively for the investigation of colorectal carcinogenesis in rodents because it enhances the incidence of dysplastic lesions in the DSS model [48–51]. AOM intercalates in the DNA, but the action of reactive oxygen or nitrogen species resulting in oxidative DNA damage is necessary to induce dysplasia in C57BL/6 mice [52–55].

Part of the difficulty of understanding the contribution of bacterial signalling to CAC is that commensal bacteria are required for initiation of colitis [37,40,56,57]. Germ-free IL-10 mice do not develop cancer, but they also do not develop inflammation. Similarly, animals knocked-out for TGF-β [58] or double knocked-out for TCRβ and p53 do not develop colonic inflammation and do not develop colon cancer under germ-free conditions [56]. Therefore the independent contribution of bacterial recognition and signalling in the development of CAC could not adequately be explored. Since bacteria are recognized by TLRs, animals deficient in TLR signalling may permit the dissection of inflammation compared with carcinogenic pathways.

TLR signalling in cancer

A recent study by Xiao et al. [59] highlights the role of TLR signalling in colonic tumorigenesis. The SIGIRR (single-Ig IL-1 receptor-related molecule) acts as a negative regulator of TLR signalling. SIGIRR−/− animals demonstrate increased intestinal inflammation and increased tumorigenesis following treatment with AOM–DSS. Restitution of SIGIRR expression in the epithelium reduces inflammation and tumours, suggesting a role for epithelial TLR signalling in tumour development. Their findings are the mirror image of our findings to date with TLR4−/− mice. A small Croatian study has shown that patients with colorectal cancer are more likely to carry a microsatellite GT polymorphism in the TLR2 gene and are slightly more likely to carry the D299G allele of the TLR4 gene [60].

A recent study crossing MyD88−/− mice with the murine model of familial Apc (adenomatous polyposis coli), the Apc<sup>Min/+</sup> mouse, found that the absence of MyD88 protects against small intestinal polyposis [61]. The mechanism by which this occurs is not clear, since the small bowel is usually very low in bacterial content, arguing against a TLR-dependent role in this animal model. MyD88 serves as an adaptor for many upstream pathways, including IL-1 and IL-18, which may also play a role in colon cancer.

The role of TLR signalling in tumour progression has largely focused on the effect of TLR signalling on anticancer immunity [62–66]. With respect to anticancer immunity, there is evidence that TLRs suppress cancer growth [62–65]. Blocking TLR4 signalling in colon cancer cells results in a reduction of tumour growth in a subcutaneous implant model [69]. A recent study in a model of lung inflammation leading to cancer suggests that TLR4 expression protects against lung cancer [70]. These results suggest that TLR signalling may act as a double-edged sword, enhancing host anticancer immunity, but promoting tumour cell growth.

TLR4 activates key pathways involved in colorectal cancer

Considerable evidence suggests that mucosal COX-2 expression is associated with the development of sporadic colorectal cancer [71–73]. Supporting a role for COX-2 in colon cancer development, COX-2 inhibitors decreased the incidence of adenomas in patients with prior adenomas [74,75]. COX-2 expression by macrophages infiltrated inside the tumour has been thought to be an early event in colorectal carcinogenesis [76]. COX-2 can induce cellular transformation, inhibit apoptosis, indirectly promote free radicals and promote angiogenesis mainly through production of PGE<sub>2</sub> [77–79].

COX-2 expression is elevated in the inflamed mucosa of IBD as well as in CAC lesions in IBD patients [80,81]. Likewise, anti-inflammatory mediators, especially 5-aminosalicylates, can reduce the development of colorectal dysplasia and cancer in IBD [82,83]. A case-control study demonstrated a stronger chemopreventive effect of 5-aminosalicylic acid on CAC (by 75%) than NSAIDs (non-steroidal anti-inflammatory drugs) in sporadic colorectal cancer [84]. Downstream of COX-2, PGE<sub>2</sub> can activate the EGFR pathway [85]. Increased EGFR tyrosine phosphorylation in the rectal mucosa of patients with ulcerative colitis has...
Figure 2 | Model of TLR4-mediated neoplasia

In the setting of acute injury, TLR4 expression is increased. TLR4 signalling in response to LPS induces COX-2 expression and PGE2 production. Through a variety of pathways, TLR4 stimulates wound healing. In chronic injury such as that seen in IBD, the stimulus to proliferate may culminate in colon cancer. APCs, antigen-presenting cells; EMT, epithelial-mesenchymal transition.

been reported [86]. EGFR phosphorylation results in a variety of biological events, including induction of proliferation and protection against apoptosis [87–90]. PGE2 may mediate EGFR phosphorylation through induction of ligands, such as amphiregulin, or through intracellular mediators such as cAMP, Src or PI3K (phosphoinositide 3-kinase) [91].

Most TLR signalling results in NF-κB activation, and this input is important to maintain tissue homeostasis. The transcription factor NF-κB has the potential to promote both inflammation and cell survival, and the role of NF-κB in colon carcinogenesis has been well established [92]. Karin and colleagues have generated mice with a conditional ablation of IKKβ in either IECs or myeloid cells and examined their effect on CAC using the AOM–DSS model [92]. Deletion of IKKβ in IECs results in a dramatic reduction in the number of adenomas, whereas deletion of IKKβ in myeloid cells has a modest effect on adenoma numbers, but a greater effect on adenoma size. Importantly, deletion of IKKβ in IECs has no effect on the underlying inflammation. These data suggest that NF-κB may have differential roles in inflammation and carcinogenesis and these effects may be cell autonomous or through cell–cell interactions. NF-κB is necessary for normal cell survival [93]. Because of the central role of NF-κB, it may be impractical to target NF-κB in the prevention or treatment of CAC. Given these compelling circumstantial data, our ongoing studies are examining the role of TLR4 in human and murine colitis-associated cancer (Figure 2).

Summary

TLRs play an important role in the intestinal mucosa in both health and disease. Harnessing their power to protect against bacterial invasion under certain circumstances will be important in human gastrointestinal pathogens, which kill millions of people in the developing world. At the same time, Western countries suffer from chronic ailments, including IBD and colon cancer, which may relate to inappropriate TLR signalling. Finding strategies to enhance or suppress TLR signalling may be essential in the treatment of certain gastrointestinal conditions.

References
