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REVIEW ARTICLE

Toll-like receptor 4 (TLR4) antagonists as potential therapeutics for intestinal inflammation

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Abstract

Gastrointestinal inflammation is a hallmark of highly prevalent disorders, including cancer treatment–induced mucositis and ulcerative colitis. These disorders cause debilitating symptoms, have a significant impact on quality of life, and are poorly managed. The activation of toll-like receptor 4 (TLR4) has been proposed to have a major influence on the inflammatory signalling pathways of the intestinal tract. Inhibition of TLR4 has been postulated as an effective way to treat intestinal inflammation. However, there are a limited number of studies looking into the potential of TLR4 antagonism as a therapeutic approach for intestinal inflammation. This review surveyed available literature and reported on the *in vitro, ex vivo* and *in vivo* effects of TLR4 antagonism on different models of intestinal inflammation. Of the studies reviewed, evidence suggests that there is indeed potential for TLR4 antagonists to treat inflammation, although only a limited number of studies have investigated treating intestinal inflammation with TLR4 antagonists directly. These results warrant further research into the effect of TLR4 antagonists in the intestinal tract.

Keywords Acute inflammation · Chemotherapy · Chronic inflammation · Crohn's disease · Inflammatory bowel disease · Intestinal mucositis · Lipopolysaccharide · Radiation · TLR4 antagonists · Ulcerative colitis

Introduction

Inflammation of the intestinal tract can result in acute or chronic manifestations of intestinal diseases; it may cause irritation, exposure to bacteria, and a dysregulation of the homeostatic balance. This leads to a range of debilitating symptoms that may affect patients' quality of life. Current treatment modalities used for intestinal inflammation are associated with a range of disadvantages including poor efficacy and unwanted side effects. The incidence rates for intestinal inflammation have been steadily increasing around the world for the last 50 years with an increased prevalence most notable in newly industrialized nations [1, 2]. Factors such as cell types, immunological abnormalities, tissue specificity and genetic/environmental factors are involved in the pathogenesis of intestinal inflammation.

Toll-like receptors (TLRs) are type 1 transmembrane proteins belonging to the wider family of pattern recognition receptors, and are responsible for the recognition of a variety of molecular signals, including endogenous damage and pathogen-associated signals, damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), respectively. Both immune (dendritic cells, monocytes, mast cells, macrophages) and non-immune cells (fibroblasts, epithelial cells) express these pattern recognition receptors [3]. The pattern recognition receptor-ligand binding between DAMPs and PAMPs prompts a downstream signalling cascade, which results in the recruitment of leukocytes [3]. TLRs activate downstream signalling pathways, which originates from the toll-interleukin receptor (TIR) domain–containing adaptor proteins such as myeloid differentiation

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primary-response protein 88 (MyD88), TIR-domain–containing adaptor protein (TIRAP), TIR domain–containing adaptor protein inducing interferon-β (TRIF) and TRIF-related adaptor molecule (TRAM) [4]. These adaptor molecules are essential to produce inflammatory cytokines, type 1 interferons, chemokines and co-stimulatory molecules. At present, 13 human TLRs have been identified and are located on various cellular compartments (including extracellular membrane, endosome and golgi apparatus) with each TLR responding to specific stimuli [4, 5].

In a healthy intestinal tract, enterocytes coexist with luminal and mucosa-associated commensal bacteria without the initiation of inflammatory responses. However, the exact mechanism behind bacterial tolerance within the intestinal tract is still largely unknown. TLRs play a pivotal role in immune tolerance to intestinal microbes [6]. These immune tolerance and responses are organized by Peyer's patches, mesenteric lymph nodes and the lamina propria [7]. These lymphoid organs are populated with dendritic cells, which produce interleukin 10 (IL-10) in turn, transforming T cells into transforming growth factor-β (TGF-β) [7]. The production of these cytokines leads to immune tolerance and homeostasis, as well as unnecessary inflammation [7]. This suggests that TLR4 signalling has an effect not only on immune responses but also on the balance of the intestinal microbial ecosystem [8, 9].

In contrast, during conditions of stress (such as disease) in the intestinal tract, inflammatory cytokines are released from enterocytes and mucosal immune cells responding to the stimulation of TLRs [10]. This leads to apoptosis and reduced proliferation of enterocytes, which in turn promotes translocation of bacteria into the lamina propria, exacerbating intestinal inflammation [10]. One of the most well-characterized TLRs is TLR4, which has been shown to be involved in homeostasis, apoptosis, intestinal inflammation and inflammatory bowel disease [11]. The focus of this review will therefore be based on the role of TLR4 antagonism in inflammatory conditions with the purpose of generating a hypothesis to support the use of TLR4 antagonists in intestinal inflammation.

Toll-like receptor 4 activation and signalling

In 1997, toll proteins in *Drosophila* were discovered to mediate protection against fungal infections [12]. Toll proteins in *Drosophila* were activated by fungi and Gram-positive bacteria, which do not contain lipopolysaccharide (LPS). They do, however, trigger a toxic shock response that is similarly induced by LPS [12]. This then led to research focusing on the now established TLR4-LPS signalling cascade. This early work also suggests a much broader role of TLR in homeostasis, tissue repair and immune defence [13].

TLR4 is an intra- and extracellular receptor expressed on endosomes and cytoplasmic membranes, which recognizes PAMPs (flagellin and LPS) and DAMPS (calprotectin, S100A8/9 HMGB1 and HSP70) through its co-receptors MD2 and CD14 [14, 15]. In addition, TLR4 has recently shown to be activated by certain pharmacological agents, including chemotherapeutic agents (paclitaxel). TLR4 is located on many different cell types (endothelial cells, lymphocytes, cardiac myocytes and glial cells) throughout the body [16–18]. In the intestine, TLR4 is expressed on antigen-presenting cells such as macrophages and dendritic cells, and on enterocytes and lymphocytes [19]. TLR4 consists of leucine-rich repeats (LRRs) with a horseshoe-like shape made up of 839 amino acids. The complex ligand specificity of the TLR4/MD2 complex is composed of two antiparallel β sheets, which form a large hydrophobic pocket in MD2 [20]. LPS is able to bind to this hydrophobic pocket through its lipid chains, which are completely buried in the MD2 hydrophobic pocket [20]. However, one of these lipid chains is partially exposed to the outer surface, which allows some interaction with TLR4 [20]. These hydrophilic and hydrophobic interactions between LPS and the TLR4/MD2 complex mediate the dimerization of extracellular domains in the TLR4, thus triggering a downstream signalling cascade leading to the release of pro-inflammatory cytokines [20]. A study by Abreu et al. [21] discovered that increases in TLR4 expression alone would not result in a reaction from LPS without the accompanying expression of MD2. In the study, they challenged different intestinal epithelial cell lines (Caco-2, T84, HT-29) with LPS and found that a decreased expression of TLR4 and MD2 correlated with intestinal epithelial protection against pro-inflammatory gene expression in response to bacterial LPS. It was concluded that careful regulation of both TLR4 and MD2 is necessary to maintain homeostasis in the intestinal tract due to it being continuously exposed to high concentrations of bacteria.

Upon stimulation, TLR4 will activate two signalling pathways, the TRIF-dependent pathway (Fig. 1) and the MyD88-dependent pathway (Fig. 2). In the TRIF-dependent pathway, TLR4 heterodimers recruit TRAM, which is needed to activate TRIF, resulting in the binding of TRIF with TNF receptor–associated factor 3 (TRAF3) and TRAF6 for binding with RIP, a receptor-interacting serine-threonine kinase 1 protein. Subsequently, this leads to the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). The TRIF-activated pathway leads to the activation of interferon regulatory transcription factor 3 (IRF3) by TANK-binding kinase 1 (TBK1) and inhibitor of NF-κB-kinase complex stimulation (IKK), which results in the production of type 1 interferons and anti-inflammatory cytokines (such as IL-10).

In the MyD88 signalling pathway, TLR4 heterodimers will bind to MyD88, which results in the formation of IRAK (interleukin 1 receptor–associated kinases) and TRAF6

complexes [14]. Formation of IRAK and TRAF6 complexes leads to a downstream signalling cascade. Various other complexes such as TAK1, TAB1/2/3, MAP kinases and IκB will be phosphorylated or activated to allow the translocation of NF-κB into the nucleus, ultimately driving the transcription of cytokine genes (such as TNFs, ILs and chemokines) to regulate pro-inflammatory responses [14, 22].

Dysregulation of TLR4 signalling has been linked to the development of a variety of inflammatory diseases. Studies have investigated functional genetic variants of TLR4 and their impact on LPS signalling response. A study by Hold et al. found that cells carrying TLR4 D299G and T399I variants, when stimulated with LPS, had a sixfold lower expression of NF-κB compared to wild-type TLR4 [23]. Ferwerda et al. demonstrated that patients carrying a variant at position 299 (Gly) but not at position 399 (Ile) had a stronger pro-inflammatory cytokine response with increased TNF-α levels in whole blood samples when stimulated with LPS compared to patients carrying wild-type TLR4 alleles at both positions [24]. Weinstein et al. showed that the same *TLR4* variants in patients with acute ischemic stroke are associated with worse neurological outcomes and alterations in systemic markers of inflammation [25]. This dramatic difference in cytokine expression caused by dysregulation of TLR4 signalling due to genetic polymorphisms will affect a person's ability to respond to LPS leading to a dysregulated immune response to infection. Considering that TLR4 downstream signalling plays a pathological role in inflammation, using antagonists or inhibitors to target TLR4 signalling may be beneficial in treating inflammatory disorders.

TLR4-mediated intestinal inflammation

An important component of immunity and host-microbial interactions in the intestinal tract is the recognition of DAMPs,

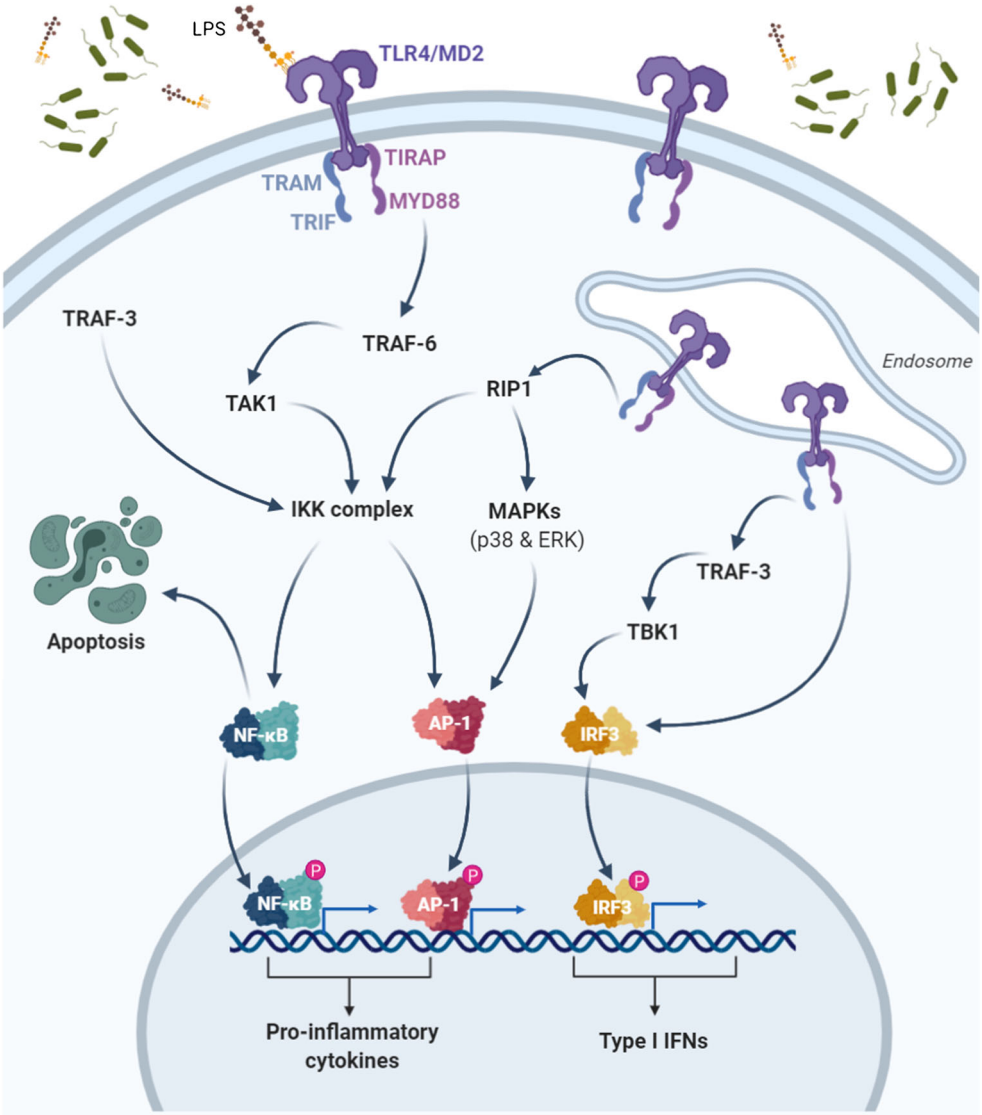

Fig. 1 Pathogen-associated molecular pattern toll-like receptor 4 signalling pathway in an enterocyte. *LPS* Lipopolysaccharide, *TLR* Toll-like receptor, *TIRAP* TIR domain–containing adaptor protein, *TRAM* TRIF-related adaptor molecule, *MyD88* Myeloid differentiation primary-response protein 88, *IKK* Inhibitor of NF-κB-kinase complex, *TRIF* TIR -domain–containing adaptor protein inducing interferon-β, *TBK1* TANK-binding kinase 1, *NF-κB* Nuclear factor-kappaB, *IRF3* Interferon regulatory transcription factor 3

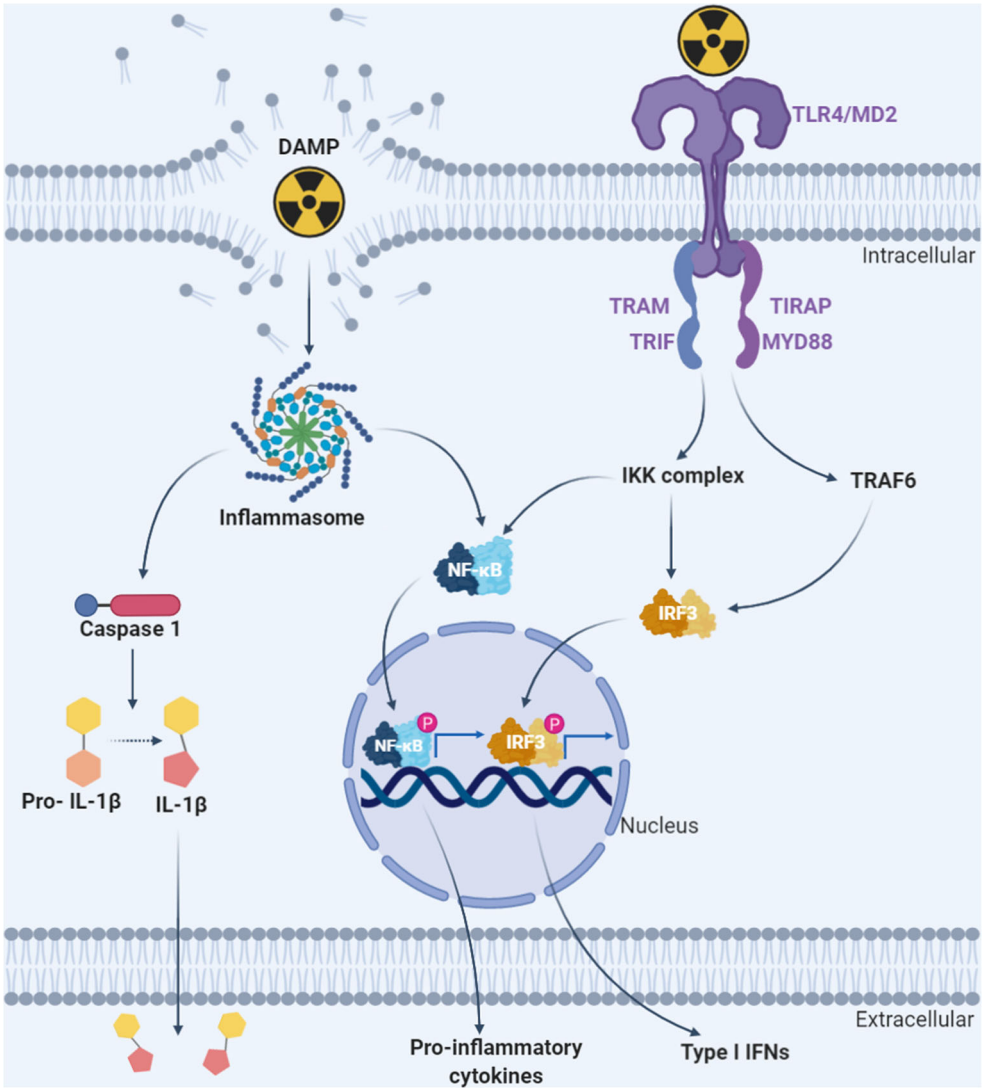

Fig. 2 Toll-like receptor 4 activation by damage-associated molecular patterns from tissue damage leads to a downstream signalling pathway, which induces inflammatory gene expression. *DAMP*s Damage-associated molecular patterns, *TLR* Toll-like receptor, *TRAM* TRIF-related adaptor molecule, *TRIF* TIR -domain–containing adaptor protein inducing interferon-β, *TIRAP* TIR domain–containing adaptor protein, *MyD88* Myeloid differentiation primary-response protein 88, *IKK* Inhibitor of NF-κB-kinase complex, *IRF3* Interferon regulatory transcription factor 3, *NF-κB* Nuclear factor-kappaB, *IL* Interleukin

PAMPs and endogenous ligands by TLR4 expressed on the enterocytes and antigen-presenting cells. Any imbalance from this interaction may contribute to the pathogenesis of inflammation within the intestinal tract [26]. There has also been substantial evidence indicating the involvement of TLR4 in intestinal inflammatory diseases such as ulcerative colitis (UC), Crohn's disease (CD) and intestinal mucositis (IM). It was reported that, in the colonic mucosa of patients with UC and CD, a significant increase in TLR4 mRNA and protein expression was observed compared to healthy controls [27, 28]. However, this may be due to the increased influx of TLR4 expressing innate immune cells. There is also mounting evidence that TLR4 polymorphism is associated with the development of UC and CD, whereby the allele frequencies of the TLR4 Asp299Gly polymorphism were discovered to be significantly higher in UC and CD patients [29].

There have also been several studies using animal models of acute intestinal inflammation. TLR4 expression is strongly upregulated in animal models induced with colitis [30]. Animal models with TLR4 knocked-out were observed to be protected from colitis or colon tumorigenesis by preventing the downstream signalling pathways that induce colitis [30]. For example, TLR4 knockout (KO) mice induced with acute colitis had a decrease in COX-2 expression, prostaglandin production and NF-κB signalling, which lead to a significant reduction of acute inflammatory cells and therefore significantly reduced acute inflammation in the intestinal tract [31]. Other studies have observed an increase in pathogenic *E. coli* and a decrease in beneficial intestinal microbiomes (*Bifidobacterium* spp. and *Lactobacillus* spp.) in a DSS-induced mouse model, which may be associated with an increase in the TLR4 expression observed in the colon of the animals [8], while another study using TLR4 KO mice showed a reduction in pathogenic *E. coli* compared to the DSS-induced wild-type mice, which showed a tenfold increase in pathogenic *E. coli* [9]. These TLR4-deficient mice

also displayed reduced disease activity index and histopathological scoring.

It therefore stands to reason that, by inhibiting TLR4, a protective effect from intestinal inflammation will be induced. Recent research has shown that, by inhibiting TLR4 using antagonists such as paeoniflorin, monoclonal antibodies and CRX-526, DSS-induced intestinal inflammation was attenuated with a significant reduction in disease activity and histopathological scoring [32–34]. However, other studies have discovered that there was no protective effect observed in the clinical symptoms and histology scores when blocking TLR4 during chronic intestinal inflammation [35] despite the opposite results observed during acute intestinal inflammation [31], most likely due to the low involvement of innate immune cells in chronic compared to acute inflammation [36].

TLR4 antagonists as a potential therapeutic alternative for treatment of intestinal inflammation

Inflammatory bowel disease (IBD) (consisting of UC and CD) is known as a non-specific, chronic gastrointestinal inflammatory disorder [37], with periods of disease activation and remission, and in some cases progressive disease [38]. It is considered an autoimmune disease due to the combined effect of genetic factors and abnormal immune responses to the intestinal bacteria and other foreign substances [37]. In addition, one of the most serious complications that IBD patients encounter is colorectal cancer, which accounts for increased mortality rates associated with UC [39]. The severity of inflammation in the intestinal tract also correlates with the risk of colorectal cancer in patients with IBD [39].

Severe intestinal inflammation leads to a range of debilitating symptoms that significantly affects patient quality of life. Current treatment modalities for intestinal inflammation are associated with a range of disadvantages including poor efficacy and unwanted side effects. The TLR4 signalling cascade also plays an important role in intestinal inflammation, with its extracellular and intracellular components being attractive therapeutic targets for the treatment of both acute and chronic intestinal inflammation. The link between intestinal inflammation and colon cancer also offers the possibility of identifying and developing novel ways to prevent cancer.

The incidence rates for IBD have been steadily increasing around the world for the last 50 years with the majority of cases occurring in westernized and industrialized countries [1, 40]. IBD is a chronic and lifelong condition that has no cure and requires a lifetime of care. It has a significant effect on patient quality of life [41, 42]. However, UC and CD are clinically distinct diseases and known to have different anatomical, clinical and histological features [43].

Presently, the etiology and pathogenesis for IBD remain largely unknown [43], with a plausible hypothesis for the etiology of IBD being the unregulated activation of both the body's innate and adaptive immune systems, potentially in response to resident gut microbes. This immune response may be mediated by the innate immune receptor TLR4 in response to luminal antigens (fungi, bacteria) in the intestinal tract [43]. Treatment approaches include aminosalicylates, corticosteroids and antibiotics [44, 45].

As previously stated, patients with IBD are at higher risk of developing colorectal cancer, and a common complication of cancer treatment is intestinal mucositis (IM), occurring in 40% of patients who receive a standard dose of chemotherapy and 100% of patients who are receiving high doses of chemotherapy [46, 47]. IM is the ulceration and inflammation of the mucosa in the intestinal tract caused by chemotherapy and radiation for cancer, and is an acute form of intestinal inflammation [48].

Unfortunately, the gastrointestinal tract is particularly susceptible to the devastating effects of chemotherapy and radiation. TLR4 is the main receptor that detects DAMPs and responds to tissue damage in the intestinal tract [49]. The cytotoxic effects of chemotherapy and radiation on both normal and malignant cells cause the release of DAMPs. This produces a sustained innate immune activation, which develops into the mucosal inflammation seen in patients with repeating cycles of chemotherapy and radiation treatments [49]. While mucositis has been recognized as a major dose-limiting toxicity for decades, there is yet to be an effective treatment to manage intestinal inflammation. Pre-clinical studies have focused on inhibition of inflammation via multiple mechanisms, or accelerating healing with growth factors [50].

IM has a significant impact on the quality of life of patients whereby the average number of days a patient suffering from IM needs to be hospitalized is 3 times more than the 4 days required by patients not suffering from IM [51]. This increased length of stay increases the strain on hospital resources [51]. Patients also suffer from symptoms such as vomiting, abdominal pain and severe diarrhea. In certain cases, the symptoms of IM will cause patients to require a dose reduction, delay or even discontinuation of their regimen, which will affect the patient's survival [52]. To date, studies have shown a link between TLR4/MD2 signalling and the development of IBD and IM [34, 53]. TLR4 antagonists show potential as therapeutic agents in both the settings. However, the majority of studies have focused mainly on sepsis models as well as diseases and infections unrelated to the development of intestinal inflammation, leaving a significant gap in the literature.

TLR4 is overexpressed in both UC (fold increase: 2.33) and CD (fold increase: 1.71) [27, 54, 55]. In IBD, abnormal signal transmission mediated by the upregulation of TLR4 promotes the sustained release of inflammatory cytokines (IL-6, TNF-α). This, in turn, develops and persists as intestinal inflammation. Only low levels of TLR4 and MD2 are expressed on the intestinal epithelium and very little was known about their regulation on intestinal epithelial cells. However, it was established that, during inflammation, expression of both TLR4 and MD2 is increased. It was later discovered by Abreu et al. [56] that the expression of TLR4 and MD2 in the intestinal tract is also regulated by immune-mediated signals. There was an increase in TLR4/MD2 expression when the intestinal epithelial cell lines (T84, HT-29) were exposed to pro-inflammatory cytokines (IFN-γ, TNF-α) highlighting the potential link between the innate and adaptive immune systems in intestinal epithelial cells only in response to pathogenic organisms. Another study by Ungaro et al. has also shown that the inflammation in IBD is decreased in TLR4-deficient mice [35]. However, the study also found that TLR4-deficient mice were unable to undergo mucosal healing and demonstrated decreased epithelial cell proliferation [35]. This shows that TLR4 serves as a mediator for both mucosal healing and inflammation in the intestinal tract.

A similar pattern can also be observed in IM. A study by Wardill et al. has shown that genetic deletion of TLR4 from mice was able to improve chemotherapy-induced gut toxicity and pain [53]. TLR4 KO mice had reduced diarrhea and weight loss compared to wild-type mice [53]. The TLR4 KO mice also exhibited a muted inflammatory response, with no significant increase in IL-1β, IL-6 or TNF-α, compared to their wild-type counterparts [53].

These studies point out the critical role of TLR4 in regulating inflammation in the intestinal tract, and by targeting and inhibiting TLR4, the outcome of intestinal inflammation and its consequence may be prevented. However, careful selection of TLR4 elimination vs. selective or temporary inhibition as a therapeutic is needed since TLR4 has beneficial effects for mucosal healing and homeostasis.

TLR4 antagonists

Targeting TLR4 could represent a potential approach to regulate immune responses and treat inflammation. However, any potential therapeutic agent must be able to block the harmful effects of TLR4 activation without negatively affecting the host's defence functions. Currently, many different antagonists are being investigated for their potential in managing inflammatory-based diseases and settings, summarized in Table 1.

Naturally occurring

The first naturally occurring TLR4 antagonist discovered was from a photosynthetic Gram-negative bacterium that was non-pathogenic, known as *Rhodobacter sphaeroides* [57]. The LPS produced from this bacterium, known as *Rhodobacter sphaeroides* lipid A (RsDPLA), was non-toxic towards murine and human cells and was able to compete with toxic LPS for binding sites. RsDPLA was also able to interact with the TLR4/MD2 complex found in rodents and humans with antagonistic effects [58]. Further in vitro and in vivo studies on the LPS produced by *Rhodobacter sphaeroides* and other bacteria/cyanobacteria have shown potent antagonistic activity of this type of LPS in murine and human cells as well as preventing endotoxic shock in mice.

Additionally, traditional Asian medicines produced from plants, including curcumin, turmeric and a variety of herbs, provide a rich and natural source of molecules which are being investigated for bio-activities that act as TLR4 antagonists [59, 60]. The modulation of TLR4 using herbal extracts promoted a large area of research to determine their pharmacological potential. It was found that certain bio-actives from bacteria or plants had a positive relationship against sepsis and septic shock [61–63]. These bio-actives were also discovered to have positive relationships against inflammatory diseases such as Alzheimer's, arthritis and inflammatory bowel diseases [35, 64, 65]. A summary of research conducted on some of these naturally sourced TLR4 antagonists can be found in Table 1. Although the main focus of this study is on the therapeutic potentials of TLR4 antagonists on intestinal inflammation, there are a limited number of studies, which used intestinal inflammation as a disease model.

Synthetic

Although there are many plant-based products capable of targeting and inhibiting TLR4 in vitro and in vivo in both rodent and human models, these do not possess the necessary stability and target specificity to be considered a potential therapeutic option compared to products and molecules extracted from microorganisms [59], which was why the molecules produced from microorganisms such as *Rhodobacter sphaeroides* have been used as a model to create synthetic antagonists. RsDPLA was used to design the synthetic TLR4 antagonists eritoran (E5564) and E5531 [80, 81]. E5531 was a first-generation lipid A analogue synthesized as part of a program to develop therapeutic agents for septic shock [82], while eritoran (E5564) is a second-generation lipid A analogue designed for the same purpose but was found to be more potent in its anti-endotoxin effects, longer lasting and easier to manufacture

Table 1 Summary of the effect of natural TLR4 antagonists in previous pre-clinical *in vitro, in vivo* and *in silico* studies

Study	Study model	TLR4 antagonist	Outcome
Qureshi et al. [61]	Bacterial sepsis *In vivo*: BDF1 mice injected with LPS (1 μg)	*Rhodobacter sphaeroides* lipid A (RsDPLA)	RsDPLA (100 μg, i.p.) pre-treatment was associated with 91% inhibition of LPS-induced response as measured by serum TNF-α concentration (246±95 pg/mL vs. 2653±286 pg/mL vehicle control).
Kirikae et al. [66]	*In vitro* inflammation model *In vitro*: mouse macrophage-like J774.1 cell line challenged with LPS		RsDPLA treatment decreased LPS response in a dose-dependent fashion as measured by TNF and IL-6 secretion (65% inhibition at 1:3 and 100% at 1:62 LPS:RsDPLA ratio). Mechanism proposed to be through binding of CD14 receptor.
Anwar et al. [58]	*In silico* Molecular dynamics simulation		Simulation predicted inhibitory behavior of RsDPLA on the TLR4/MD2 complex in rodents and humans.
Malgorzata-Miller et al. [62]	Septic shock *In vitro*: Human PBMC challenged with LPS *In vivo*: C57Bl/6 mice injected with LPS	Lipooligosaccharide (LOS) from *Bartonella quintana* (BqLOS)	Human PBMCs pre-incubated with BqLOS (100 ng/mL) was associated with inhibition of LPS-induced response measured by supernatant concentration of IL-1β, TNF-α, IL-6, IL-8 ($p<0.001$). Mice pre-treated with BqLOS (100 μg) had improved survival rates.
De Paola et al. [67]	Amyotrophic lateral sclerosis *In vitro*: Motor neuron/glia co-cultures *In vivo*: Wobbler mice	LPS from *Oscillatoria Planktothrix FP1* (Cyp/VB3323)	*In vitro:* Cells exposed to LPS (1 μg/mL) reduced viability by 30.8±11.9% ($p<0.001$ vs. control). This toxic effect was reduced by VB3323 (20 μg/mL) which almost completely restored motor neuron viability in the cells (91.3±9.9% with $p<0.001$ vs. LPS). *In vivo:* Wobbler mouse with spontaneous motor neuron degeneration chronically treated with VB3323 (5 mg/kg/d i.p., final concentration 0.5 mg/mL) displayed decreased microglial activation and morphological alterations of spinal cord neurons; and better performance in the paw abnormality and grip-strength tests.
Balducci et al. [64]	Alzheimer's disease *In vivo*: C57Bl/6 mice		Amyloid-β oligomers (AβO) injection (7.5 μL at 1 μM) rapidly activated glial cells and induced a memory establishment deficit. When treated with CyP (10 μg, ICV) before AβO, the memory deficit was prevented ($p=0.0055$).
Iori et al. [68]	Seizures *In vivo*: C57Bl/6 mice		Carbamazepine (CBZ) is an anticonvulsant to treat neuropsychiatric disorders. Mice treated with CyP (1 mg/mouse, i.p.) + CBZ (20 mg/mouse, in food) during disease onset. CBZ-treated mice displayed a three-fold higher seizure frequency compared to CyP-treated mice ($p<0.01$). TLR4 antagonism by CyP was effective in delaying seizure onset and reduced recurrence in the established murine model of acquired epilepsy.
Yao et al. [69]	Inflammatory bowel disease *In vitro*: Sprague-Dawley rats injected with 2,4,6-trinitro-benzene sulfonic acid	Probiotics, Golden bifid	Rats treated with the probiotics had a significantly lower disease activity ($p<0.05$), histopathological score ($p<0.05$) and

Table 1 (continued)

Study	Study model	TLR4 antagonist	Outcome
			inflammatory cytokine levels (TNF-α and IL-1β, $p<0.05$) compared to control groups.
Chu et al. [70]	*In silico* Docking analysis	Berberine, extracted from the herb Huang Lian (*Rhizoma coptidis*)	Docking analysis suggested that 3 berberine molecules were able to bind to MD2 and block TLR4/NF-κB downstream signalling. Binding free energies of the 3 berberine molecules was 7.70, −7.33 and −6.75 kcal/mol, respectively.
	Bacterial infection *In vivo*: Balb/c mice challenged with *Salmonella typhimurium* and bacterial endotoxin		Mice treated with 2 EU/mL endotoxin solution (i.p.) had a lethal rate of 80%. When treated with berberine at different doses (0.13, 0.16 and 0.20 g/kg) after endotoxin administration, mice had survival rate of 50%, 50% and 60% respectively. Average death time of each mouse group treated with berberine was significantly better compared to mice only exposed to LPS ($p<0.05$).
Liang et al. [71]	*In vitro* inflammation model *In vitro*: THP-1 human monocyte cells challenged with LPS Sepsis *In vivo*: C57Bl/6 mice administered with LPS	Sparstolonin B (SsnB) extracted from a Chinese herb (*Sparganium stoloniferum*)	*In vitro:* SsnB (100 μM) inhibited LPS-induced (50 ng/mL) response as measured by an 18-fold decrease in TNF-α and 10-fold decrease in IL-6 expression levels vs. LPS-treated cells only. Mechanism proposed to be through binding of the CD14/TLR4 receptor. *In vivo:* Mice co-treated with LPS (100 μg/mouse) and SsnB (100 μg/mouse) displayed lower expression of TNF-α ($p=0.0075$), IL-6 ($p=0.1077$) and IL-1β ($p<0.0001$) vs. LPS-treated mice. SsnB was able to suppress inflammation induced by LPS by attenuating the TLR4-mediated activation of NF-κB.
Li et al. [72]	Leukemia *In vitro*: THP-1 cells treated with LPS	Parthenolide (PTL), extracted from the plant feverfew (*Tanacetum parthenium*)	3 and 12 μM PTL significantly decreased pro-inflammatory cytokine expression and diminished LPS-induced (1 μg/mL) TLR4 expression compared to LPS-treated group ($p<0.01$). PTL was able to inhibit the expression of these cytokines by blocking the TLR4 which in turn blocks the subsequent downstream signalling cascade.
Saadane et al. [73]	Cystic fibrosis *In vitro*: 16 HBE (human bronchial epithelial cell line) transfected with AS oligonucleotide that inhibits expression of CFTR. Stimulated using IL-1β/TNF (100 ng/mL each). *In vivo*: Cystic fibrosis transmembrane conductance regulator (CFTR)–knockout mice challenged with LPS		*In vitro*: At 3 h and 6 h, AS cells pre-treated with PTL (40 μM) had decreased IL-8 secretion vs non-treated cells ($p=0.02$ and 0.03, respectively). *In vivo*: LPS (25 ng, intratracheally)–treated mice had increased polymorphonuclear leukocytes (PMN) (9±1.54% at 1 h, 38.8±7.23% at 3 h and 63±6.0% at 8 h). When co-treated with PTL (3 μg/g), a decrease in PMN % at 8 h was observed ($p=0.006$). Proposed mechanism of action for PTL was NF-κB-dependent inhibition of cellular responses.
Gradišar et al. [74]	*In vitro* inflammation model *In vitro*: Human embryonic kidney (HEK) 293 cells stimulated with LPS.	Curcumin, extracted from the turmeric plant (*Curcuma longa*)	40% inhibition of TLR4/MD2 complex observed at approximately equimolar concentration of curcumin and MD2 in presence of

Table 1 (continued)

Study	Study model	TLR4 antagonist	Outcome
			LPS. Cells co-treated with LPS (100 ng/mL) and higher doses of curcumin (0–20 μM) showed no difference in NF-κB activity.
Zhu et al. [75]	Traumatic brain injury (TBI) *In vivo*: Feeney weight-drop contusion model on C57Bl/6 mice		Injured brain tissue had a significant increase in TLR4 expression vs. sham control brains 24 h post-trauma ($p<0.01$). Curcumin (100 and 200 mg/kg) administered post-trauma reduced TLR4 expression and had lower neurological deficit scores and brain water content vs, vehicle-treated mice with $p<0.01$ and $p<0.05$, respectively. A decrease in concentrations of inflammatory mediators (IL-1β, IL-6, TNF-α, MCP-1) vs. vehicle-treated mice ($p<0.01$) was also observed.
Zhang et al. [76]	Acute lung injury *In vivo*: BALB/c mice with injury induced by LPS	Atractylenolide I (AO-I/ AT-I) extracted from the Chinese herb Cang Zhu (*Rhizoma Atractylodis macrocephalae*)	LPS (10 μg)–treated mice displayed pathological changes: inflammatory cells infiltration, interalveolar septal thickening and edema which were attenuated in co-treated mice (LPS + AO-I at 5, 10 and 20 mg/kg). MPO activity and inflammatory cell infiltrate were reduced in co-treated mice (5 mg/kg: $p<0.01$, 10 mg/kg: $p<0.01$ and 20 mg/kg: $p<0.01$) and (5 mg/kg: $p<0.05$, 10 mg/kg: $p<0.01$ and 20 mg/kg: $p<0.01$) vs. LPS-treated mice, respectively.
Wang et al. [63]	Sepsis *In vivo*: Cecal ligation and puncture (CLP) model of mice		Survival of mice increased with AT-I dose at 10, 20 and 40 mg/kg (i.p.) ($p<0.05$, $p<0.01$, $p<0.01$, respectively) vs. control, respectively. AT-I-treated mice took a shorter time to return to normal temperature ($p<0.05$) and displayed dose-dependent decrease in pro-inflammatory cytokines TNF-α and IL-6 ($p<0.05$, $p<0.01$, $p<0.01$, respectively). Decrease in white blood cells ($p<0.05$) and IL-1β ($p<0.05$ and $p<0.01$, respectively) was observed at 20 and 40 mg/kg doses.
Li et al. [77]	Acute respiratory distress syndrome *In vivo*: BALB/c mice with LPS administered intranasally to induce lung injury	Asiatic acid (AA) extracted from the plant Gotu Kola/Pennywort (*Centella asiatica*)	LPS-treated mice displayed increased lung wet/dry weight ratio, inflammatory cell infiltrate and MPO activity. Co-treated mice (LPS + AA at 25, 50 and 100 mg/kg) displayed decreased lung wet/dry weight ratio ($p<0.05$, $p<0.01$, $p<0.01$, respectively) inflammatory cell infiltrate ($p<0.05$, $p<0.01$, $p<0.01$, respectively) and MPO activity ($p<0.01$, $p<0.01$, $p<0.01$, respectively) vs. LPS group.
Lee et al. [78]	*In vitro* inflammation model *In vitro*: Bone marrow cells isolated from C57Bl/6 mice challenged with LPS	Celastrol extracted from the plant Thunder God Vine (*Tripterygium wilfordii*)	Celastrol (0.1, 0.5 and 1 μM) inhibited LPS-induced (10 ng/mL) responses measured by TNF-α, IL-6, IL-12 and IL-1β at mRNA and protein levels ($p<0.05$). Confocal imaging analysis of celastrol demonstrated decreased co-localisation of fluorescent LPS with MD2.
Yuan et al. [65]	Arthritis *In vivo*: C57Bl/6 mice with induced adjuvant arthritis		Celastrol (0.5 mg/kg) improved clinical outcome via clinical and histopathological scoring vs. non-treated mice ($p<0.01$). Decreased expression of TNF-α (1.9-fold)

Table 1 (continued)

Study	Study model	TLR4 antagonist	Outcome
			and IL-6 (3.1-fold) in celastrol treated mice vs. non-treated mice.
Cho et al. [79]	*In vitro* inflammation model *In vitro*: RAW264.7 cells challenged with LPS	Xanthohumol (XN) extracted from the plant Hops (*Humulus lupulus*)	Cells co-treated with LPS (0.1–0.5 μg/mL) and XN (0.5, 1, 2.5 and 5 μg/mL) displayed a dose-dependent decrease in NO levels (2.5 and 5 μg/mL, $p<0.01$ vs. LPS group), TNF-α (2.5 and 5 μg/mL, $p<0.01$ vs. LPS group) and IL-1β (1, 2.5 and 5 μg/mL, $p<0.01$ vs. LPS group).
Ungaro et al. [35]	Inflammatory bowel disease C57Bl/6J mice with dextran sulphate sodium (DSS) administered in drinking water.	IgG2b monoclonal antibody	Mice co-treated with DSS (2.5%) and IgG2b (20 mg/kg) displayed decrease in expression of TNF-α (141.5±16.3 pg/mL vs. 336±53.8 pg/mL, $p<0.01$), IL-6 (4816±145.5 pg/mL vs. 5850.4±144.4 pg/mL, $p<0.01$) and % dendritic cells in the lamina propria (3.4±0.7 vs. 8.7±0.4%, $p<0.05$) vs. control. No difference in DAI scoring vs. control (1.28±0.19 vs. 1.33±0.23, $p=0.42$, maximum score: 4).
Zhang et al. [32]	Inflammatory bowel disease C57Bl/6 mice with dextran sulphate sodium (DSS) administered in drinking water.	Paeoniflorin extracted from peony root	Mice pre- or co-treated with paeoniflorin (50 mg/kg) and DSS (4%) suppressed weight loss: Pre-paeoniflorin at day 7 ($p<0.05$), co-paeoniflorin at days 5–6 ($p<0.05$), day 7 ($p<0.001$); Diarrhea/bloody diarrhea: Pre-paeoniflorin at days 5 ($p<0.05$), 6 ($p<0.01$), 7 ($p<0.001$), co-paeoniflorin at days 6–7 ($p<0.05$); Shortening of colon length: Pre- and co-paeoniflorin ($p<0.05$); Histological score: Pre- and co-paeoniflorin ($p<0.01$) vs. vehicle control. Paeoniflorin-treated mice had lower expression of TLR4 protein and mRNA vs. DSS only mice ($p<0.001$).

RsDPLA Rhodobacter sphaeroides lipid A, *i.p.* intraperitoneal injection, *LPS* lipopolysaccharide, *TNF-α* tumor necrosis factor alpha, *IL-6* interleukin 6, *CD14* cluster of differentiation 14, *TLR4* toll-like receptor 4, *MD2* myeloid differentiation factor 2, *LOS* lipooligosaccharide, *BqLOS Bartonella quintana, PBMC* peripheral blood mononuclear cell, *IL-1β* interleukin 1 beta, *IL-8* interleukin 8, *AβO* amyloid β oligomers, *ICV* intracerebroventricular injection, *CBZ* carbamazepine, *NF-κB* nuclear factor kappaB, *SsnB* Sparstolonin B, *PTL* parthenolide, *HBE* human bronchial epithelial cell line, *AS* allele specific, *CFTR* cystic fibrosis transmembrane conductance regulator, *PMN* polymorphonuclear leukocytes, *HEK* human embryonic kidney, *MCP-1* monocyte chemoattractant protein 1, *AO-I/AT-I* atractylenolide I, *MPO* myeloperoxidase, *CLP* cecal ligation and puncture, *AA* asiatic acid, *mRNA* messenger RNA, *XN* xanthohumol, *NO* nitric oxide, *DSS* dextran sulphate sodium, *DAI* disease activity index

compared to E5531 [83]. Studies on eritoran have shown a positive effect against sepsis [83] and other inflammatory conditions [84, 85]. This led to the development of other synthetic analogues such as TAK-242 and FP7 with antagonistic effects on the TLR4/MD2 complex to treat various inflammatory diseases such as neuroinflammation and influenza infections. Although the main focus of this study is on the therapeutic potentials of TLR4 antagonists on intestinal inflammation, there are a limited number of studies, which used intestinal inflammation as a disease model. Therefore, studies which encompass different inflammatory diseases have been included and summarized in Table 2 in order to show the potential broader anti-inflammatory effects of TLR4 antagonism.

The most well-known TLR4 antagonist to enter the clinical phase was eritoran, followed by TAK-242; and although many synthetic TLR4 antagonists have been developed and studied, very few have actually made it into clinical trials, due to the limited evidence currently available. Table 3 summarises only the TLR4 antagonists that have been, or are, undergoing clinical trials in different inflammatory disease models. However, this will allow for a broader view of using TLR4

Table 2 Summary of the effect of synthetic TLR4 antagonists in pre-clinical *in vitro* and *in vivo* research studies

Study	Study model	TLR4 antagonist	Outcome
Mullarkey et al. [83]	Sepsis *In vivo*: C57Bl/6 mice, Hartley guinea pigs, Fischer rats challenged with i.v. LPS	Eritoran (E5564)	Mice: E5564 (100, 300, 1000 μg/kg) co-treatment was associated with 37%, 81% and 93% inhibition of LPS-induced (100 μg/kg) response as measured by serum TNF-α levels, respectively ($p<0.05$ vs. control). Guinea pigs: E5564 (30, 100, 300 μg/kg) co-treatment was associated with 29%, 57% and 94% inhibition of LPS-induced (1000 μg/kg) response as measured by serum TNF-α levels, respectively ($p<0.05$ vs. control). Rats: E5564 (10, 100, 1000 μg/kg) co-treatment was associated with 84%, 97% and 100% inhibition of LPS-induced (3 μg/kg) response as measured by serum TNF-α levels, respectively ($p<0.05$ vs. control).
Kitazawa et al. [84]	Acute liver failure (ALF) *In vivo*: Wistar rats challenged with D-galactosamine (GalN) and LPS		Rats treated with E5564 after ALF (500 mg/kg GalN + 50 μg/kg LPS) displayed a decrease in serum TNF-α levels and had an improved survival rate of 42.9% compared to untreated rats ($p<0.05$).
Liu et al. [85]	Inflammatory effects of ischemia-reperfusion in kidneys *In vivo*: Fisher rats with kidney nephrectomy and ischemia performed.		Rats treated with E5564 displayed a significant improvement in renal function as measured by serum creatinine levels ($p<0.05$) and higher survival rates ($p<0.05$) vs. vehicle controls.
Sha et al. [86]	Endotoxin shock *In vivo*: BALB/c mice treated with LPS i.p.	TAK-242 (Resatorvid)	Pre-treatment of TAK-242 (0.1, 0.3, 1 and 3 mg/kg) was associated with a decrease in LPS-induced (10 mg/kg) responses as measured by IL-6, IL-10, MIP-2, IL-1β and NO serum levels vs. vehicle control ($p<0.025$). A 40% increase in survival rate of mice was also observed vs vehicle control ($p\leq0.05$). Post-treatment of TAK-242 (1 mg/kg) was associated with a decrease in LPS-induced (5 mg/kg) response as measured by IL-6 and MIP-2 serum levels vs. vehicle control ($p\leq0.01$). A survival rate of 45% was also observed vs. vehicle control ($p\leq0.01$).
Kuno et al. [87]	Endotoxemia *In vivo*: Hartley guinea pigs treated with LPS i.v.		TAK-242 (3 and 10 mg/kg) pre-treatment was associated with a dose-dependent decline in colonic muscle tension ($p=0.001$ and $p<0.001$, respectively) and mean arterial pressure ($p=0.036$ and $p=0.004$, respectively) caused by LPS (10 mg/kg, i.v.). A 50% survival rate was observed when pre-treated with TAK-242 at 10 mg/kg vs. the 10% observed in the control group.
Garate et al. [88]	Neuroinflammation *In vivo*: Wistar Hannover rats restrained to induce stress.		Pre-treatment of TAK-242 (0.5 mg/kg, i.v.) decreased expression of the pro-inflammatory enzymes: IL-1β, COX-2 and iNOS expression levels, $p<0.05$ vs control, $p<0.05$ vs stress only group.
Hua et al. [89]	Cerebral ischemia *In vivo*: C57Bl/6 mice induced with focal cerebral ischemia/reperfusion		Treatment with TAK-242 (3 mg/kg) was associated with reduce levels of serum TNF receptor II, monocyte chemotactic protein-1, macrophage inflammatory protein-1γ and tissue inhibitor of metalloproteinases-1 ($p<0.05$ vs. untreated mice). An 8.8% reduction in brain infarct size and improved neurologic function score (6.73) were also observed ($p<0.05$ vs. untreated mice).
Perrin-Cocon et al. [90]	Lethal influenza infection *In vitro*: monocyte-derived dendritic cells (DCs) challenged with influenza virus, strain A/PR/8/34 *In vivo*: C57Bl/6 infected with mouse-adapted influenza virus, strain A/PR/8/34	FP7	*In vitro*: FP7 (1 and 10 μM) treatment was associated with decreased levels of LPS-induced (10 ng/mL) responses as measured by supernatant levels of IL-8, IL-6, MIP-1β, TNF-α, IL-12 and IL-10 in both monocytes and DCs ($p<0.05$ vs. LPS). *In vivo*: Mice treated with FP7 (200μg/mouse, i.v.) after influenza infection displayed reduced gene production of TNF-α,

Table 2 (continued)

Study	Study model	TLR4 antagonist	Outcome
			IL-1β, IFN-β, murine IL-8 ($p<0.01$) and IL-6 ($p<0.05$) in the lungs. FP7-treated mice had decreased viral load (log FP7-treated titre=4.1±0.39) vs. vehicle-treated mice (log vehicle-treated titre=5.27±0.15) as measured by a virus titration assay ($p=0.0225$).
Palmer et al. [91]	Cardiovascular inflammatory-based diseases *In vitro*: Human umbilical vein endothelial cells (HUVEC), THP-1 and mouse RAW-264.7 macrophages challenged with LPS *In vivo*: Angiotensin II-infused apolipoprotein E-deficient mice		*In vitro*: FP7 (0–10 μM) negatively regulated LPS-induced production (100 ng/mL) of pro-inflammatory cytokines in a dose-dependent manner: THP-1: IL-8 ($p<0.001$), IL-6 ($p<0.01$), MIP-1α ($p<0.001$) at 5 μM and IL-1β ($p<0.001$) at 0.1, 1, 5 μM vs. LPS. RAW-264.7: p65 NF-κB at 1, 5, 10 μM ($p<0.001$), IL-6 at 5 μM ($p<0.05$), 10 μM ($p<0.001$) and p38 MAPK at 0.1 ($p<0.05$), 1 ($p<0.01$), 5 and 10 μM ($p<0.001$) vs. LPS. HUVEC: p38 MAPK and p65 NF-κB at 0.1, 0.5 and 1 μM ($p<0.01$, $p<0.05$, $p<0.01$, respectively), MCP-1 at 1 μM ($p<0.05$) vs. LPS. *In vivo*: FP7 (3 mg/kg/day) inhibited angiotensin II-driven production of pro-inflammatory proteins, and MIP-1γ and JNK phosphorylation ($p<0.05$ vs. angiotensin II group).
Facchini et al. [92]	Inflammatory bowel disease *In vitro:* Peripheral blood mononuclear cells and lamina propria mononuclear cells collected from patients with IBD *In vivo*: BALB/c mice with DSS administered in their water.		*In vitro*: FP7 at 10 μM negatively regulated LPS-induced production (100 ng/mL) of pro-inflammatory cytokines: mRNA relative expression: TNF-α ($p<0.001$); IL-1β ($p<0.05$); IL-6 ($p<0.05$). ELISA: TNF-α, IL-1β and IL-6 ($p<0.05$). *In vivo*: FP7 (250 μg/kg) treatment was associated with a lower histological score ($p<0.01$ vs. DSS) and significantly reduced the release of inflammatory cytokines TNF-α ($p<0.05$), IL-1β ($p<0.001$) and IL-6 ($p<0.05$).
Huggins et al. [93]	Abdominal aortic aneurysm (AAA) *In vitro*: HUVEC challenged with LPS *In vivo*: C57Bl/6 mice induced with AAA	IAXO-102	*In vitro*: IAXO-102 (10 μM) blocked LPS-stimulated (100 ng/mL) production of JNK, ERK, p65 NF-κB ($p<0.05$) and p38, MCP-1, IL-8 ($p<0.01$) vs. LPS. *In vivo*: IAXO-102 (3 mg/kg/day) blocked angiotensin II-induced response as measured by protein expression of JNK, ERK, p65, NF-κB ($p<0.05$) vs. angiotensin II only group. IAXO-102 also downregulated expression of MIP-1γ and TLR4 ($p<0.05$ vs. angiotensin II group) and reduced incidence of AAA (30% IAXO-102-treated vs. 86% angiotensin II group).
Zhang et al. [94]	Acute lung injury (ALI) *In vitro*: Mouse RAW 264.7 macrophages challenged with LPS *In vivo*: Sprague-Dawley rats with ALI induced by intratracheal LPS instillation	Chalcone derivatives - Compound 20	*In vitro*: Fluorescent probe determined compound 20 is a specific inhibitor of MD2 (KD=189 μM). Addition of compound 20 (10 μM) inhibited LPS-induced (0.5 μg/mL) secretion of TNF-α, IL-1β, COX-2 ($p<0.01$) and IL-6 ($p<0.05$) vs. LPS. *In vivo*: Compound 20 (20 mg/kg) reduced LPS-induced (5 mg/kg) pulmonary edema as measure by the decrease in lung wet/dry weight ratio ($p<0.01$) vs. LPS. Compound 20 also inhibited IL-1β secretion ($p<0.01$) and MPO activity ($p<0.05$) vs. LPS.

Table 2 (continued)

Study	Study model	TLR4 antagonist	Outcome
Wang et al. [95]	Septic shock and lung injury *In vitro*: Mouse primary peritoneal macrophages challenged with LPS *In vivo*: C57Bl/6 mice injected with LPS	Curcumin analogues - L48H37	*In vitro*: Fluorescent probe determined L48H37 is a specific inhibitor of MD2 (KD=11.3 μM). L48H37 (1, 2.5, 5 or 10 μM) inhibited LPS-induced (0.5 μg/mL) phosphorylation in a dose-dependent manner: ERK at 1, 2.5, 5 and 10 μM ($p<0.01$), p38 at 2.5 μM ($p<0.05$), 5 and 10 μM ($p<0.01$), and JNK at 5 and 10 μM ($p<0.01$) vs. LPS. L48H37 (10 μM) inhibited secretion of TNF-α, IL-6, IL-1β and iNOS ($p<0.01$ vs. LPS-treated group); IL-10 and COX-2 ($p<0.05$ vs. LPS-treated group). *In vivo*: L48H37-treated (10 mg/kg) mice had higher survival rates vs. LPS (20 mg/kg, i.v.) ($p<0.01$). Pulmonary damage and LPS-injured tissue structure of lungs was amended.
Hodgkinson and Ye. [96]	*In vitro* inflammation model *In vitro*: Human embryonic kidney (HEK) 293-CD14- MD2 cells challenged with LPS	Statins - Simvastatin - Pravastatin	Both simvastatin and pravastatin (2 μM) pre-treatment was associated with the inhibition of LPS-induced (5 ng/mL) response as measured by supernatant concentrations of NF-κB, IL-6 and TNF-α ($p<0.05$ vs. LPS).
Katsargyris et al. [97]	Carotid atherosclerotic plaques *Ex vivo*: atherosclerotic plaques from patients		Patients who used statins had lower TLR4 expression in their endothelial cells and atherosclerotic plaques vs. non-statin patients ($p=0.02$ and $p=0.03$, respectively). Prevalence cerebrovascular accident was 18.6% in statin group vs. 61.4% of non-statin group (odds ratio [95% CI] 0.14 [0.07–0.31] $p<0.001$).
Fort et al. [98]	Inflammatory bowel disease BALB/c mice with DSS administered in their water.	Lipid A-mimetic - CRX-526	CRX-526 (2, 10, 50 μg) treatment was associated with a lower DAI ($p=0.421$, 0.056, 0.016, respectively) and histological score ($p=0.032$, 0.008, 0.008, respectively) vs. DSS in a dose-dependent manner.

E5564 eritoran, *LPS* lipopolysaccharide, *IV* intravenous, *TNF-α* tumor necrosis factor alpha, *ALF* acute liver failure, *GalN* D-galactosamine, *i.p.* intraperitoneal, *Resatorvid* TAK-242, *IL-6* interleukin 6, *IL-10* interleukin 10, *MIP-2* macrophage inflammatory protein 2, *IL-1β* interleukin 1 beta, *NO* nitric oxide, *COX-2* cyclooxygenase 2, *iNOS* nitric oxide synthase, *DCs* dendritic cells, *IL-8* interleukin 8, *MIP-1β* macrophage inflammatory protein 1 beta, *IL-12* interleukin 12, *IFN-β* interferon beta, *HUVEC* human umbilical vein endothelial cells, *THP-1* human acute monocytic leukemia cell, *MAPK* mitogen-activated protein kinase, *MIP-1α* macrophage inflammatory protein 1 alpha, *MAPK* mitogen-activated protein kinase, *MIP-1γ* macrophage inflammatory protein 1 gamma, *JNK* c-Jun N-terminal kinase, *IBD* inflammatory bowel disease, *DSS* dextran sulphate sodium, *mRNA* messenger RNA, *AAA* abdominal aortic aneurysm, *HUVEC* human umbilical vein endothelial cell, *ERK* extracellular signal-regulated kinase, *ALI* acute lung injury, *MD2* myeloid differentiation factor 2, *KD* equilibrium dissociation constant, *HEK* human embryonic kidney, *CD14* cluster of differentiation 14, *DAI* disease activity index

antagonists in inflammatory diseases to support its use in intestinal inflammation.

Conclusions

Both IBD and IM have significant effects on a patient's quality of life as well as economic and social burdens [51, 99, 100]. While the pathophysiology for chronic intestinal inflammation remains unknown, previous research has identified that TLR4 signalling in the intestinal tract is a critical regulator of intestinal immune homeostasis. The use of a TLR4 antagonist has potential as a novel therapeutic for IBD and IM patients whose disease pathogenesis relies heavily on TLR4 signalling. Previous studies have shown that inhibiting LPS-induced TLR4 stimulation with antagonists can reduce intestinal inflammation in animal models [101]. Regardless of how promising TLR4 antagonists are in the treatment of intestinal inflammation, there are still challenges in bioavailability and delivery. Nonetheless, anti-TLR4 therapies present a promising alternative for future innovative treatments for both IBD and IM. In the future, there is a need for tissue specific studies looking into these anti-TLR4 therapies in order to mimic the therapeutic setting of IBD and IM.

Table 3 Summary of TLR4 antagonists used in clinical trials

TLR4 antagonist	Condition/disease	Mechanism of action	Clinical trial design and aim	Trial status and outcome	Reference/ clinical trial number
Eritoran (E5564)	Sepsis/severe sepsis/septic shock	Lipid A mimic, binds to MD2	Phase 2, a safety and efficacy study of intravenous E5564 in patients with severe sepsis	Completed. Eritoran appeared well tolerated and showed a lower mortality rate (105 mg dose) in patients with severe sepsis and high predicted risk of mortality.	NCT00046072
			Phase 3, a controlled comparison of eritoran tetrasodium and placebo in patients with severe sepsis	Completed. Patients with severe sepsis did not have reduced 28-day mortality when administered with eritoran, compared with placebo.	NCT00334828
Resatorvid (TAK-242)	Severe sepsis	Binds covalently to Cys747 of TLR4-TIR domain and blocks TLR4/TIRAP and TLR4/TRAM interactions	Phase 3, a pivotal, multicentre, multinational, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of TAK-242 in adults with severe sepsis	Completed. TAK-242 did not suppress cytokine levels in patients with sepsis. TAK-242 was well tolerated but patients developed mild increases in serum methemoglobin levels.	NCT00143611
			Phase 3, randomized, double-blind, placebo-controlled study of the efficacy and safety of TAK-242 vs. placebo in subjects with sepsis-induced cardiovascular and respiratory failure	Terminated. Business decision; no safety or efficacy concerns.	NCT00633477
NI-0101	Healthy volunteers	Monoclonal antibody blocking TLR4 signalling	Phase 1, randomized double-blind study to determine the safety, tolerability and distribution and elimination of a novel therapeutic drug (NI-0101) when administered to healthy volunteers.	Completed. NI-0101 showed good tolerability, favorable safety and PK profile, and durable anti-inflammatory effect in healthy volunteers.	NCT01808469
	Rheumatoid arthritis		Phase 2, randomized, placebo-controlled, double-blind study to explore the effect of a new antibody to treat patients with rheumatoid arthritis.	Completed. Results unavailable.	NCT03241108

E5564 eritoran, *MD2* myeloid differentiation factor 2, *TAK-242* resatorvid, *TLR4* toll-like receptor 4, *TIR* toll-interleukin receptor domain, *TIRAP* TIR domain–containing adaptor protein, *TRAM* TRIF-related adaptor molecule, *PK* pharmacokinetics

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Compliance with ethical standards

Conflict of interest JSYT, JKC, PAH, CAP, and JMB declare that they have no conflict of interest.

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