University of Groningen

Site-specific contribution of Toll-like receptor 4 to intestinal homeostasis and inflammatory disease

Bruning, Elise E.; Collier, Janet K.; Wardill, Hannah R.; Bowen, Joanne M.

Published in:
Journal of Cellular Physiology

DOI:
[10.1002/jcp.29976](https://doi.org/10.1002/jcp.29976)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2021

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Bruning, E. E., Collier, J. K., Wardill, H. R., & Bowen, J. M. (2021). Site-specific contribution of Toll-like receptor 4 to intestinal homeostasis and inflammatory disease. *Journal of Cellular Physiology*, (2), 877-888. <https://doi.org/10.1002/jcp.29976>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

REVIEW ARTICLE

Site-specific contribution of Toll-like receptor 4 to intestinal homeostasis and inflammatory disease

Elise E. Bruning¹ | Janet K. Collier² | Hannah R. Wardill^{1,3} | Joanne M. Bowen¹ ¹Discipline of Physiology, Adelaide Medical School, The University of Adelaide, Adelaide, Australia²Discipline of Pharmacology, Adelaide Medical School, The University of Adelaide, Adelaide, Australia³Department of Paediatric Oncology/Haematology, The University of Groningen (University Medical Centre Groningen), Groningen, The Netherlands**Correspondence**Elise E. Bruning, Discipline of Physiology, Adelaide Medical School, The University of Adelaide, Level 2 Helen Mayo South Building, Frome Road, Adelaide 5005, South Australia.
Email: elise.bruning@adelaide.edu.au**Funding information**

National Health and Medical Research Council CJ Martin Biomedical Research Fellowship, Grant/Award Number: (2018–2022); Australian Government Research Training Program (RTP) Scholarship, Grant/Award Number: (2019–2022)

Abstract

Toll-like receptor 4 (TLR4) is a highly conserved protein of innate immunity, responsible for the regulation and maintenance of homeostasis, as well as immune recognition of external and internal ligands. TLR4 is expressed on a variety of cell types throughout the gastrointestinal tract, including on epithelial and immune cell populations. In a healthy state, epithelial cell expression of TLR4 greatly assists in homeostasis by shaping the host microbiome, promoting immunoglobulin A production, and regulating follicle-associated epithelium permeability. In contrast, immune cell expression of TLR4 in healthy states is primarily centred on the maturation of dendritic cells in response to stimuli, as well as adequately priming the adaptive immune system to fight infection and promote immune memory. Hence, in a healthy state, there is a clear distinction in the site-specific roles of TLR4 expression. Similarly, recent research has indicated the importance of site-specific TLR4 expression in inflammation and disease, particularly the impact of epithelial-specific TLR4 on disease progression. However, the majority of evidence still remains ambiguous for cell-specific observations, with many studies failing to provide the distinction of epithelial versus immune cell expression of TLR4, preventing specific mechanistic insight and greatly impacting the translation of results. The following review provides a critical overview of the current understanding of site-specific TLR4 activity and its contribution to intestinal/immune homeostasis and inflammatory diseases.

KEYWORDS

immunity, inflammation, intestines, mucous membrane, Toll-like receptor 4

1 | INTRODUCTION

The human body processes trillions of microbiological signals daily, from both external non-self-pathogens to internal self-derived signs of danger (Comalada & Xaus, 2013; Pott & Hornef, 2012). Initial avoidance of infection and tissue damage is highly dependent on the evolutionary conserved innate immune system (Gribar, Richardson, Sodhi, & Hackam, 2008; Pardo-Camacho, Gonzalez-Castro, Rodino-Janeiro, Pigrau, & Vicario, 2018). Unlike the adaptive immune system, the innate immune system is not specific to particular pathogens. Rather, innate immunity utilises a variety of

barriers, protein receptors, and phagocytic cells which recognise threats and activate potent inflammatory responses (Pott & Hornef, 2012). Although each component of innate immunity plays a crucial role in maintaining homeostasis, the relationship between pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) is of great interest. It is this connection that allows for the initial recognition of danger, stimulating signalling cascades which leads to inflammation and elimination of the invading pathogen (Kishore, 2009). A widely researched class of PRRs are Toll-like receptors (TLRs)—due to their expansive range of

complimentary ligands, complex signalling pathways, and significance to disease. Although there are 10 human TLR subtypes and 13 mouse TLR subtypes (Nie, Cai, Shao, & Chen, 2018), TLR4 has received significant attention due to its interaction with bacterial products and relevance to multiple disease states.

Although TLR4 expression and signalling are vital for the maintenance of homeostasis and immune tolerance (Chung et al., 2012), this receptor has also been implicated in the development of many inflammatory pathologies, particularly those of gastrointestinal origin, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), alcoholic-induced barrier injury, and chemotherapy-induced gastrointestinal toxicity (CIGT; Belmonte et al., 2012; Wardill, Gibson et al., 2016). In both diarrhoea-dominant (IBS-D) and CIGT, genetic mutations in the TLR4 pathway have been associated with increased disease risk. Similarly, upregulated expression of TLR4 and its downstream targets have been reported in both clinical studies and preclinical models emphasising the detrimental effects of TLR4 hyperactivation. These findings have prompted an enthusiastic investigation of interventions aimed at inhibiting the proinflammatory effects of TLR4 with the goal of controlling chronic inflammatory diseases or preventing intestinal injury. However, reports are highly variable with conflicting evidence regarding the efficacy of TLR4 inhibition, with some reports suggesting detrimental effects on chronic inflammation and tumour growth. These findings clearly highlight an underappreciated level of complexity in TLR4-dependent mechanisms and a significant gap in our fundamental understanding of how to modify TLR4 signalling in the context of disease prevention.

A critical and often overlooked aspect of TLR4 signalling is its site specificity. TLR4 is expressed primarily on immune cell subsets; however, it is also expressed on intestinal epithelial cells acting as the first point of immunosurveillance. Despite the intensity of interest in TLR4, few studies acknowledge or address the site-specific implications of TLR4 signalling. This common oversight is likely to impact the true nature and translational impact of results and is a possible reason for the high degree of variability seen in the efficacy of TLR4-targeted interventions and the growing number of adverse events reported following TLR4 inhibition. As such, the following review will provide a critical overview of the site-specific actions of TLR4, with a specific focus on the unique contribution of epithelial- and immune-cell TLR4 signalling in the maintenance of healthy states and contribution to gastrointestinal diseases. IBD and IBS are chronic inflammatory conditions that primarily affect the intestines, resulting in diarrhoea, constipation, and pain (Belmonte et al., 2012). Genetic studies on IBD susceptibility indicate that the TLR4 signalling molecule, Toll/interleukin-1 (IL-1) receptor domain-containing adapter protein, plays a significant role in increased susceptibility to combined IBD risk and Crohn's disease (CD) risk (De Jager et al., 2007). Further clinical research using human colon samples showed a significant increase in colonic TLR4 expression in IBS-D, suggesting that the proinflammatory nature of TLR4 may exaggerate disease severity and progression (Kocak, Akbal, Koklu, Ergul, & Can, 2016). In the context of alcoholic steatohepatitis and alcohol-induced barrier

injury, X. Li et al. (2013) study found that chronic exposure to ethanol significantly increases intestinal permeability of mice via TLR4-dependant downregulation of phosphorylated occludin and increased protein kinase C (PKC) activity. This is reflected in the setting of CIGT, a severe inflammatory complication of cancer therapy characterised by upregulation of proinflammatory cytokines including IL-6, IL-1 β , and tumour necrosis factor- α (TNF- α), and all downstream targets of TLR4 (Bossi et al., 2016; Logan et al., 2008). Further research has shown that TNF- α and IL-6 induce tight junction protein dysfunction and the breakdown of actin filaments (Wardill, Bowen, & Gibson, 2012), with global TLR4 knockout (KO) shown to mitigate the clinical severity of CIGT in a mouse model via its regulation of claudin-1 internalisation (Wardill, Bowen et al., 2016). In fact, TLR4-related genetic mutations have been shown to elevate the risk of CIGT in patients undergoing standard-dose chemotherapy, echoing genetic susceptibility results from IBD cohorts (De Jager et al., 2007). Therefore, TLR4-mediated intestinal barrier dysfunction is now considered critical in the initiation and potentiation of gastrointestinal damage (Wardill, Gibson et al., 2016).

Although TLR4 has been the subject of many studies regarding the inflammatory gastrointestinal disease, the distinction between site-specific expression of TLR4 is rarely addressed or acknowledged. This common oversight is likely to impact the true nature and translational impact of results, as site-specific expression may significantly impact the contextual role of TLR4. The following review will, therefore, contrast intestinal epithelial and immune cell-expressed TLR4, dissecting their unique contribution to the maintenance of healthy states and in gastrointestinal diseases highlighting methods of improving mechanistic insight of TLR4-based signalling and translation of TLR4-targeted therapeutics.

2 | THE IMPORTANCE OF DISSECTING SITE-SPECIFIC TLR4-DEPENDANT MECHANISMS

The current level of understanding regarding cell-specific involvement of TLR4 in healthy and diseased states is vague, with the majority of research relying on global KO models to demonstrate TLR4-dependant mechanisms or failing to identify target cell populations. The mechanisms of epithelial and immune TLR4 differ and as such, there are likely to be site-specific mechanisms that govern disease initiation and progression. Failure to acknowledge and specifically investigate or target these pathways is a critical oversight in translational research efforts. This is particularly pertinent when considering the dichotomous role of TLR4, which exerts both beneficial and detrimental effects on host physiology depending on its cellular location and degree of activation. When considering this, it is unsurprising to see the degree of conflicting data regarding TLR4 manipulation in an attempt to prevent or control the disease. For example, TLR4 activation has been widely reported to promote the development of experimental dextran sodium sulfate (DSS)-mediated colitis (Fukata et al., 2007), yet TLR4 deficiency has paradoxically

been reported to aggravate symptoms (Shi et al., 2019). Similarly, global TLR4 deletion was robustly demonstrated to mitigate chemotherapy-induced mucosal injury; however, inhibition with naloxone was unable to control symptoms and reduced the efficacy of chemotherapy. Therefore, it is critical that we appreciate the contextual roles of TLR4-dependent inflammation to appropriately modulate its activity without inhibiting restorative/healing processes that are critical in the resolution of chronic inflammatory insult, and without influencing extraintestinal TLR4-dependent mechanisms. Achieving this requires a greater insight into the site-specific actions of TLR4 in both the maintenance of homeostasis and initiation of disease.

3 | SITE-SPECIFIC TLR4 EXPRESSION IN HEALTHY STATES

In healthy states, TLR4 contributes to intestinal homeostasis via several distinct and well-defined mechanisms: (a) maintenance of the intestinal barrier; (b) recognition and response to invading pathogens; (c) metabolic regulation; and (d) gastric motility (Anitha, Vijay-Kumar, Sitaraman, Gewirtz, & Srinivasan, 2012; Guo et al., 2015). TLR4 is a key sentinel protein for luminal and subepithelial signals of infection and sterile injury (Akira, Takeda, & Kaisho, 2001). This type 1 transmembrane protein has a distinct tripartite structure, including a leucine-rich extracellular domain, transmembrane domain, and an intercellular signalling domain (De Nardo, 2015). In a healthy condition, TLR4 expression is relatively low, with research showing that in intestinal epithelial cells, the transcription factor, ZNF160, repressed TLR4 expression to maintain

homeostasis of the intestines and allow for commensal microbiome development (Takahashi, Sugi, Hosono, & Kaminogawa, 2009). Situated in either the outer cell membrane, Golgi apparatus, or within the endosome, TLR4 recognises a variety of ligands including, but not limited to lipopolysaccharides (LPS) from the outer membrane of Gram-negative bacteria, heat-shock proteins, hyaluronic acid, and high-mobility group protein 1 (Cheng, Taylor, Ourthiague, & Hoffmann, 2015; Santaolalla, Sussman, & Abreu, 2011). Myeloid differentiation factor 2 (MD2) is a glycoprotein on the extracellular domain of TLR4 which assists in the binding of LPS and other ligands (C. C. Lee, Avalos, & Ploegh, 2012; J. Meng, Lien, & Golenbock, 2010). An example of TLR4 ligand binding and activation is LPS recognition, which is driven by the binding of lipid A, of LPS, to the TLR4/MD2 complex (Steehgs et al., 2008). Upon ligand binding, TLR4 activates the myeloid differentiation primary response 88 (MyD88) and TIR-domain-containing adapter-inducing interferon- β pathways, to increase expression of the nuclear transcription factor κ -light-chain-enhancer of activated B cells (NF- κ B) and interferon regulatory transcription factor 3 activations (Cheng et al., 2015). This cascade results in the production of proinflammatory cytokines including IL-6, IL-8, and interferon- β (IFN- β ; Figure 1).

3.1 | Intestinal epithelial TLR4 expression in the maintenance of healthy states

While TLR4 is expressed on a range of cell types throughout the body, including dendritic cells, myeloid cells, macrophages (Vaure & Liu, 2014), and microglia (Vaure & Liu, 2014), its expression in the gastrointestinal tract is particularly significant due to the high

FIGURE 1 Intracellular signalling pathways of TLR4 activation by LPS in both epithelial and/or immune cells, resulting in inflammatory responses. LPS expressed on Gram-negative bacterial cells binds to TLR4 on immune and/or epithelial cells causing the activation of an inflammatory cytokine cascade mediated by MyD88 or TRAM/TRIF activation, which leads to the upregulation of NF- κ B and IRF3, respectively. This cascade results in the production of proinflammatory cytokines including interleukin 6 (IL-6), IL-1 β , and tumour necrosis factor- α . CXCL10, C-X-C motif chemokine; IFN- β , interferon- β ; I κ B, κ B kinase; IKK, inhibitor of kappa B kinase; IRAK 1, interleukin-1 receptor-associated kinase 1; IRF3, interferon regulatory transcription factor 3; LPS, lipopolysaccharides; MyD88, myeloid differentiation primary response 88; NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; P, phosphorylation; TLR4, Toll-like receptor 4; TRAM, translocating chain-associating membrane; TRIF, TIR-domain-containing adapter-inducing interferon- β

microbial load and interaction with exogenous stimuli (McClure & Massari, 2014). TLR4 is widely expressed along the small and large intestines under normal physiological conditions (Cairo & Podolsky, 2000), with higher rates of epithelial expression found in the distal ileum and colon (Cairo & Podolsky, 2000; Gourbeyre et al., 2015). Epithelial TLR4 is expressed on both the apical and basolateral cell membranes, as well as within endosomes and the Golgi apparatus (Abreu, 2010; Hornef, Frisan, Vandewalle, Normark & Richter-Dahlfors, 2002). This variety of epithelial expression has been shown to change the site-specific role of TLR4, with basolateral TLR4 expression on epithelial cells recognising LPS and stimulating an inflammatory response, whereas apical expression promotes tolerance to LPS, without causing inflammation (Vamadevan et al., 2010).

Lotz et al. (2006) were the first to explain the role of TLR4 in intestinal epithelial cells immediately after birth. Using both TLR4 KO mice and cell culture stimulation assays, this study found that intestinal epithelial TLR4 is the first to respond to exogenous endotoxins, like LPS, rather than immune-expressed TLR4 on macrophages (Lotz et al., 2006). This initial site-specific recognition helps us to establish the microbial colonisation of the intestine and is fundamental in the development of a healthy microbiome and host-immune tolerance (Lotz et al., 2006). Previous evidence shows that the absence of intestinal epithelial TLR4 resulted in a hyporesponsiveness to LPS (Abreu et al., 2001; Naik, Kelly, Meijer, Petterson & Sanderson, 2001), therefore demonstrating a clear link between intestinal homeostasis and epithelial TLR4 expression. Furthermore, Zeuthen, Fink, and Frokiaer (2008) study using Caco-2 cell culture and monocyte-derived dendritic cells found that intestinal epithelial cells ready the immune system response to commensal bacteria via partially TLR4 driven mechanisms, leading to a tolerogenic phenotype to commensals. This study supports the importance of epithelial TLR4 and its role in establishing immune

tolerance to intestinal bacteria and the development of a healthy microbiome.

In addition to immunosurveillance, epithelial TLR4 signalling is involved in a variety of essential functions for the establishment and maintenance of a healthy epithelial function, including inflammation control, regulation of host-microbiome, B cell recruitment, immunoglobulin A (IgA) production, follicle-associated epithelial permeability, and antimicrobial peptide expression (Abreu, 2010; S. Chabot, Wagner, Farrant, & Neutra, 2006). Of particular relevance to intestinal homeostasis are interactions between TLR4 and the mucosal barrier, a highly regulated interface between the luminal environment and mucosal compartment comprised of intestinal epithelial cells and basement membrane.

Mucosal barrier integrity is maintained by the apical junction complex (tight junction, desmosome, and adherens junction), with tight junctions maintaining the semiselective state of the paracellular mucosal route. Careful control of the molecular structure of the tight junction allows for absorption of solutes across their respective electro-osmotic gradients and also serves as an immunosurveillance checkpoint (Anderson, Van Itallie, & Fanning, 2004; Blijevens, 2005; Hooper, Littman & Macpherson, 2012; Wells et al., 2017). The tight junction complex was first identified at the apico-lateral surface of adjacent enterocytes by Farquhar and Palade (1963). This foundational study utilised haemoglobin as a mass tracer in rats and Guinea pig tissue to assess permeability (Farquhar & Palade, 1963). Combined with electron microscopy techniques, this protocol showed that the tight junction is responsible for the diffusion of the tracer along with the intercellular space, providing the first evidence highlighting the importance of tight junctions in regulating intestinal permeability (Farquhar & Palade, 1963). Since this pivotal study, the specific proteins that comprise this complex have been identified, including occludin, zonular occludin 1 (ZO-1), and claudins (Figure 2). The tight junction is indicated in the healthy functioning of the

FIGURE 2 Representative diagram of the intestinal epithelial barrier and tight junction protein complex. The junctional complex, including tight junction proteins (pictured), adherens, and desmosomes, is located at the apical lateral junction of epithelial cells and acts to bind cells together to form a selective barrier. In a healthy condition, physiological solutes and ions are able to cross this barrier; however, pathogenic bacteria are unable to cross, therefore maintaining the sterile subepithelial environment. ZO-2, zonular occludin 2

intestinal barrier, where damage to tight junction proteins often results in increased permeability and progression of the illness (S. H. Lee, 2015; Salim & Soderholm, 2011; Wardill et al., 2012). Previous evidence concluded that TLR4 expression did not influence tight junction protein development (Wardill, Bowen et al., 2016), although tight junctions were primarily assessed after a mucosal challenge of irinotecan treatment. Of note, a recent study found that the colon epithelial cells treated with 5-HT or commensal *Escherichia coli* showed increased expression of TLR-4 which correlates with the downregulation of E-cadherin and claudin-2 (Banskota et al., 2017). Similarly, increased TLR4 signalling (transgenic villin-TLR4 hyperactivity) was reported to increase intestinal permeability as detected by fluorescein isothiocyanate (FITC)-dextran and tight junction protein expression (Dheer et al., 2016). These findings implicate a functional role for epithelial TLR4 in tight junction protein control. These results were supported by Bein, Eventov-Friedman, Arbell, and Schwartz (2018) whose study into necrotising enterocolitis (NEC) in neonates found that the decrease of TLR4 expression seen in NEC is associated with a decrease in tight junction proteins, occludin, cingulin, claudin-4, and ZO-1. Although this may be a by-product of NEC pathophysiology, this could also suggest a homeostatic role of TLR4 in tight junction maintenance and TLR4 impact on intestinal permeability. Furthermore, recent research has shown that increased TLR4 expression negatively influences the function of the adherens junction. The adherens junction is comprised of cadherin transmembrane receptors and their associate binding proteins, forming a junction that allows for cell-to-cell adhesion and maintenance of tissue integrity (Pinheiro & Bellaiche, 2018). Research conducted by Ralls, Demehri, Feng, Woods Ignatoski, and Teitelbaum (2015) using human inflamed small bowel tissue found that in underfed sections of bowel, TLR4 is significantly increased and E-cadherin expression is decreased resulting in loss of epithelial barrier function. This evidence shows the dual impact of TLR4 expression on the intestinal junctional complexes, often leading to exacerbated intestinal disruption.

In addition to its probarrier properties, epithelial TLR4 also influences the structural development of the intestine and the intestinal immune environment (Lu, Sodhi, & Hackam, 2014; D. Meng et al., 2015). A transgenic mouse model, constitutively expressing TLR4 in the intestinal epithelium, found links between epithelial TLR4 signalling and B cell recruitment and IgA production (Shang et al., 2008). In the transgenic model B cells and B cell, tropic chemokines were increased with CCL20 protein being fivefold higher in transgenic mice than wild-type (WT) counterparts, and CCL28 being 1.6-fold higher than WT (Shang et al., 2008). Furthermore, the continuous overexpression of epithelial TLR4 resulted in increased IgA⁺ cells in the small intestine lamina propria and produced higher faecal IgA levels as compared to WT (Shang et al., 2008). Finally, this study also found that TLR4 signalling in intestinal epithelial cells induces immunoglobulin class switching to IgA in the small intestinal lamina propria (Shang et al., 2008). Taken together, this evidence supports the important role intestinal epithelial cells play in response to external signalling and presents a mechanism by which epithelial TLR4

prevents attachment of pathogens via the regulation of luminal IgA production (Shang et al., 2008).

The importance of epithelial TLR4 in intestinal function was convincingly demonstrated using intestinal epithelial conditional KO mice (TLR4^{ΔIEC}) in investigations into the regulatory contribution of epithelial TLR4 to altered metabolic symptoms and disease (Lu et al., 2018). TLR4^{ΔIEC} mice were shown to develop metabolic syndrome faster than their WT counterparts, showing significant weight gain independent of food intake or hormone levels and the presence of macrophage accumulation within adipose tissue via histologic analyses (Lu et al., 2018). Furthermore, intestinal epithelial TLR4 was shown to significantly impact the composition of the intestinal microbiota in mice, where TLR4^{ΔIEC} mice showed both a lower diversity of bacteria and altered bacterial clustering (Lu et al., 2018). In addition, TLR4^{ΔIEC} mice showed an altered expression of metabolic and inflammatory genes in comparison to WT. Metabolic pathway genes associated with peroxisome proliferator-activated receptor signalling were significantly downregulated in the ileum of TLR4^{ΔIEC} mice (Lu et al., 2018). Finally, the conditional KO of intestinal epithelial TLR4 resulted in a proinflammatory phenotype, including the upregulation of macrophage markers (*Cd68*, *F4/80*, and *Mcp1*) and neutrophil markers (*Mpo* and *Elane*). Together, these results show that intestinal epithelial TLR4 expression in healthy states assists with the regulation of metabolic control, microbiota composition and function, metabolic gene expression, and prevention of unnecessary inflammation.

In contrast, the impact of TLR4 on the microbial invasion was elegantly demonstrated using a C57BL/6 villin-TLR4 transgene model which constitutively expressed intestinal epithelial TLR4. Interestingly, where intestinal epithelial TLR4 was consistently overexpressed, an increase in microbial invasion, alterations to luminal and mucosal microbiota, and disruptions to epithelial barrier function were found (Dheer et al., 2016). This evidence emphasises the dichotomous role of TLR4, with its physiological benefits restricted within a threshold of expression/activity (Abreu, 2010). Importantly, it is consistently shown that site-specific overexpression is detrimental to intestinal homeostasis resulting in the breakdown of the mucosal barrier and exaggerated immune activation, which is likely to be mediated by the basolaterally expressed epithelial TLR4. Hyperactivation of TLR4 is, therefore, a key driver of many diseased states affecting gastrointestinal function, many of which will be discussed further in later sections of this review.

3.2 | Immune cell TLR4 expression in healthy states

The majority of our fundamental knowledge regarding TLR4 signalling has been gained from studying its activity in immune cell subsets, likely reflecting the higher expression of TLR4 on immune cells compared to enterocytes. Gourbeyre et al. (2015) state a 2.5-fold increase of TLR4 expression within the mesenteric lymph nodes of pigs compared to intestinal tissue and a 1.5-fold increase of TLR4 expression in the intestinal lumen compared to the jejunal villi.

This increase of TLR4 abundance is due to the high immune cell presence within these organ systems with TLR4 expressed on dendritic cells, macrophages, and myeloid cells (Vaure & Liu, 2014). Although immune-derived TLR4 signalling is crucial to the recognition and response to dangerous PAMPs and DAMPs (Apetoh et al., 2007; Cairo & Podolsky, 2000; Fukata et al., 2005; Hajjar et al., 2012; Leaphart et al., 2007), immune cell TLR4 expression also helps us to regulate the immune and intestinal environment in healthy states. Arguably, one of the most important immune cells to express TLR4 is dendritic cells.

Dendritic cells are a highly specialised form of antigen-presenting cells, which provide an important link between innate immunity and activation of the adaptive immune system (Pufnock et al., 2011). Upon capture and recognition of an antigen, mature dendritic cells migrate from the site of recognition to the lymphoid organs and initiate the development of effector and memory T cells from primary T cells (Michelsen et al., 2001; Pufnock et al., 2011). Maturation of dendritic cells is the process whereby the role of dendritic cells changes from immature phagocytic actions to cytokine production and efficient T-cell antigen-presenting. This process occurs before reaching the lymphoid organs and is highly dependent on the recognition and digestion of pathogens (Michelsen et al., 2001). A foundational study using mice with a mutated TLR4 protein examined the role of immune-expressed TLR4 on dendritic cell maturation (Michelsen et al., 2001). Results showed that both TLR2- and TLR4-related mechanisms were crucial for the normal maturation of dendritic cells in response to bacterial ligands (Michelsen et al., 2001). A further immunological study by Pufnock et al. (2011) found that coactivation of both TLR4 and TLR7/8 provides an increased maturation of dendritic cells and an increased generation of CD8+ memory T cells as well. Together, this evidence supports the importance of immune cell expression of TLR4 for dendritic cell maturation and successful priming of the T-cell response to invading pathogens.

Current research has also indicated a strong relationship between TLR4 expressed on dendritic cells and immunological tolerance via the release of indoleamine-2,3-dioxygenase (IDO; Salazar, Awuah, Negm, Shakib, & Ghaemmahami, 2017). Apart from the role of IDO as an enzyme which catalyses the amino acid tryptophan, IDO also plays an important role in immune regulation (Chen, 2011). IDO produced by dendritic cells promotes immunosuppression and helps us to establish tolerance to commensal microbiota (Harden & Egilmez, 2012). Critically, LPS-primed human dendritic cells produced a higher IDO than unprimed cells, and that the primed cells resulted in a tolerogenic phenotype (Salazar et al., 2017). Furthermore, unprimed cells challenged with LPS also resulted in increased IDO and a tolerogenic phenotype (Salazar et al., 2017). Interestingly, when monocytes were challenged with LPS, there was no increase in IDO production, suggesting that this is a unique trait of dendritic cells (Salazar et al., 2017). This evidence characterises the role of TLR4 expression on dendritic cells and its importance in homeostasis and supports previous research in which the TLR4 ligand, LPS, induced expression of IDO in dendritic cells which contributed to an

immunogenic tolerance (Von Bubnoff, Scheler, Wilms, Fimmers, & Bieber, 2011). Although the links between TLR4-mediated dendritic cell IDO expression and immune tolerance are clearly stated, Albrecht, Hofer, Foxwell, Frankenberger, and Ziegler-Heitbrock (2008) study found a further mechanism whereby dendritic-expressed TLR4 assists in homeostatic tolerance. This study using *in vitro* LPS precultured human dendritic cells found that further stimulation with high-dose LPS produced an immune tolerance phenotype in these cells, marked by the ablation of the IL-1 receptor-associated kinase 1 adaptor protein and decreased NF- κ B activation (Albrecht et al., 2008).

While a well-defined link among dendritic expression of TLR4, immune tolerance, and adaptive immune activation is clear, the role of TLR4 expressed on monocytes remains uncertain. Current evidence shows that monocytic TLR4 expression may not contribute to enhanced immune tolerance (Salazar et al., 2017); however, current evidence is sparse and further research is, therefore, required to dissect monocytic-specific TLR4 signalling and its contribution to healthy and diseased states. With this being said, the monocyte-derived macrophage expression of TLR4 has been well-characterised to engage the NF- κ B pathway, leading to active cytokine release and promotion of inflammation in response to pathogenic bacteria (Q. Li & Cherayil, 2003). The innate immune response to pathogens is highly dependent on ligand binding to TLR4 on macrophages, which enables a maximised release of TNF- α , resulting in beneficial inflammation and elimination of harmful bacteria (Q. Li & Cherayil, 2003). Furthermore, previous research indicates the role of TLR4 in increasing oxidative stress in activated macrophages to aid in removing pathogens from the body (Matsebatlela, Anderson, Gallicchio, Elford, & Rice, 2015). In contrast, this relationship is also implicated in the development and progression of renal hypertension, with research showing that TLR4 deficiency reduces oxidative stress and, therefore, improves the hypertensive condition (Pushpakumar et al., 2017). Therefore, TLR4 on macrophages seem to play both homeostatic roles, as well as contributing roles in disease.

4 | DISEASE-SPECIFIC IMPACT OF TLR4 EXPRESSION

4.1 | Role of TLR4 and LPS response

As outlined, TLR4 signalling is best known for its well-established role in LPS recognition from Gram-negative bacteria and initiation of inflammation (Abreu et al., 2001; Hajjar et al., 2012). Historically, this process was believed to be heavily dependent on immune expression of TLR4. However, research by Abreu et al. (2002) found that TLR4 expression is significantly increased in response to LPS with T-cell-derived cytokines, IFN- γ , and TNF- α , increasing TLR4 expression in intestinal epithelial cells, therefore increasing the inflammatory response to LPS administration. Furthermore, a study conducted by Hornef et al. (2002) produced strong evidence highlighting the importance of intestinal epithelial TLR4 in LPS recognition. Using

isolated murine small intestinal crypt epithelial cells, this study showed proinflammatory mediator secretion and CD14 upregulation in response to LPS administration (Hornef et al., 2002). Furthermore, epithelial cell TLR4 expression was found within the Golgi apparatus (Hornef et al., 2002), identical to the site of internalised LPS, showing that intestinal epithelial cells may be responsible for the initial recognition and signalling in response to LPS. It is this which then attracts the accumulation and stimulation of the more advanced immune response (Hornef et al., 2002). The damaging inflammatory response to LPS from Gram-negative bacteria in the intestinal tract is partially characterised by increased intestinal permeability. Research by Guo, Al-Sadi, Said, and Ma (2013) found a significant decrease in epithelial resistance in response to LPS administration in Caco-2 cell monolayers from 100% relative epithelial resistance in untreated control versus 70% relative epithelial resistance in LPS-treated monolayers. Furthermore, in an in vivo C57BL/6 mouse model, LPS administration was shown to increase permeability flux of the macromolecular Dextran 10K across the intestinal epithelium of WT mice with threefold higher permeability in LPS-treated mice; however, in a global TLR4 KO mouse, permeability and flux did not increase from baseline with LPS treatment (Guo et al., 2013). This study concluded that LPS administration results in an increased intestinal permeability mediated in part by increased TLR4 and CD14 expression and localisation along intestinal enterocytes (Guo et al., 2013).

Although current evidence strongly suggests the role of epithelial TLR4 in recognition and response to LPS, many foundational studies have investigated the relationship between LPS and TLR4, finding that immune TLR4 is essential for the development of inflammation and the elimination of bacteria (Poltorak et al., 1998). A study conducted by Hoshino et al. (1999) investigated the impact of TLR4 elimination on LPS responsiveness. This study utilised an immune-specific TLR4 KO mouse model, which was deemed successful due to the harvested macrophages and B cells showing no response to LPS administration (Hoshino et al., 1999). In the TLR4 KO model, LPS response was greatly decreased compared to WT counterparts (Hoshino et al., 1999). This study is further supported by Qureshi et al. (1999), who found that endotoxin hyporesponsive mice showed unique mutations within the *Tlr4* gene coding. More recent evidence showed that the mechanism of the LPS-derived increased gastrointestinal permeability is caused primarily by the TLR4/MyD88 signalling pathway (Guo et al., 2015). Two strains of KO mice were utilised including a TLR4 KO and MyD88 KO. Results found that permeability was increased as a result of the injection of LPS to mice and that this was strongly dependant on TLR4 expression (Guo et al., 2015). These findings were supported by Nighot et al. (2017) further clarifying the role of the TLR4/MyD88 pathway in increased permeability. This study introduced LPS to Caco-2 monolayers and C57BL/6 mice and found an increase in permeability, both in vitro and in vivo, via the upregulation of myosin light chain kinase. Although these more recent studies do provide evidence of TLR4 involvement in LPS recognition, they did not distinguish between epithelial versus immune cell expression, negatively impacting the translatability of results.

4.2 | Role of TLR4 in altered intestinal permeability, IBD, and IBS

Although the TLR4/LPS-mediated inflammatory pathway has been well-characterised, recent literature has indicated the involvement of epithelial TLR4 in the development of many intestinal diseases and illnesses. The pathogenesis of many IBDs and intestinal toxicities is characterised by increased permeability and disruption to the tight junction complex. A study by X. Li et al. (2013) investigated the role of intestinal epithelial TLR4 on the phosphorylation of the tight junction protein, occludin, via PKC, and its contribution to increased permeability in alcoholic steatohepatitis. Using both Caco-2 monolayer cell cultures and C57BL/6 WT mice, this study found that the neutralisation of TLR4 using monoclonal antibody pretreatment reduced ethanol-induced paracellular permeability. Furthermore, in the presence of ethanol, TLR4 expression was increased and phosphorylated occludin was decreased (X. Li et al., 2013). This decrease in functional occludin mediated by TLR4 may explain why with high concentrations of ethanol, a significant increase of intestinal permeability is seen. This study further supports the involvement of epithelial TLR4 in the development of intestinal barrier dysfunction in several disease states.

Altered intestinal permeability is also a hallmark of IBD, which includes CD and ulcerative colitis (UC), each being a chronic inflammatory disease which significantly impacts wellbeing and an individual's ability to thrive due to symptoms of pain, diarrhoea, and constipation. A large genetic study collated 1,539 DNA samples from human IBD patients and included a pooled analysis of 4,805 cases of IBD (De Jager et al., 2007). Focussing on 23 genes related to TLR4 expression and associated signalling pathways, this study genetically identified TLR4 as a disease risk factor for both CD and overall IBD (De Jager et al., 2007). Due to the large sample sizes and well-tested methodologies used, this study provides strong genetic evidence that TLR4 plays a role in the development of gastrointestinal pathologies. However, due to the genetic nature of this study, it is unable to provide any distinguishing information regarding cell-specific TLR4 involvement as is common in the current literature. An early study by Cairo and Podolsky (2000) investigated the differential expression of different TLRs in the intestinal epithelia in IBD. This patient-based study utilised human tissues of the colon and terminal ileum, from those with active IBD. Western blot and immunohistochemistry analyses revealed that epithelial TLR4 was greatly upregulated in both UC and CD and may contribute to disease progression (Cairo & Podolsky, 2000). Interestingly, a significant difference in the positioning of epithelial cell TLR4 expression was noted between UC and CD. Higher expression of TLR4 on the basolateral cell surface was noted in patients with UC, whereas higher expression of TLR4 on the apical cell surface was observed in patients with CD (Cairo & Podolsky, 2000). As expected, an increase in TLR4 expression was also found on immune cells of the lamina propria due to the inflammatory nature of these diseases. Knowing that intestinal epithelial TLR4 is overexpressed in patients with IBD, a study conducted by Dheer et al. (2016) investigated the impact of constitutive TLR4

expression on epithelial function and the microbiota composition. Increased TLR4 signalling resulted in the invasion of intestinal microbiota and significant alterations to bacterial composition within the intestines (Dheer et al., 2016). This led to an exacerbation of DSS-mediated colitis in animal models (Dheer et al., 2016).

Another common gastrointestinal condition is irritable bowel disease (IBS), which affects one in five Australians (Belmonte et al., 2012). IBS is often characterised by bloating, abdominal pain, diarrhoea, and/or constipation, which significantly impacts the quality of life (Belmonte et al., 2012). A study conducted by Belmonte et al. (2012) investigated the impact and prevalence of TLR4 expression on IBS development. In this clinical study, a total of 48 patients with IBS (including similar rates of IBS-D, constipation-dominant [IBS-C], and mixed symptoms phenotypes [IBS-M]) and 31 control patients were enrolled, and colonic biopsy samples were collected via colonoscopy (Belmonte et al., 2012). A twofold increase of TLR4 expression was found in patients with IBS-M when compared to healthy controls. In addition, TLR4 expression was highest in IBS patients with a disease duration of over 5 years (Belmonte et al., 2012). Furthermore, increased TLR4 expression in patients with IBS was predominantly found in intestinal epithelial cells, with a higher TLR4 presence in the crypts of intestinal tissue compared to surface epithelial layers (Belmonte et al., 2012). These results are supported by a more recent study, which also analysed human colonic tissue from both IBS patients and healthy control patients (Kocak et al., 2016). This study only evaluated two IBS subgroups, being IBS-D and IBS-C, omitting the IBS-M group. However, results showed significantly increased expression of TLR4 in colonic mucosa for both subgroups of patients with IBS when compared to control (Kocak et al., 2016). These findings support the detrimental role of TLR4 hyperexpression in the context of IBD and IBS and help us to shape our understanding of disease pathophysiology and progression. However, due to the nonspecific reporting of TLR4 expression states, it is difficult to distinguish between epithelial and immune TLR4 contextual roles, and thus a translation of these findings is challenging.

4.3 | Role of TLR4 in NEC

NEC is a leading cause of death in premature infants, where intestinal injury permits bacterial translocation into the usually sterile sub-epithelial space (Anand, Leaphart, Mollen, & Hackam, 2007; Leaphart et al., 2007). This disease mechanism results in necrosis of the small intestine leading to sepsis, multiorgan failure, and death (Hackam, Upperman, Grishin, & Ford, 2005). A study by Leaphart et al. (2007) investigated the role of intestinal mucosal TLR4 on NEC development. TLR4 expression within the mucosa is significantly increased in NEC and in TLR4 mutant mice, and the severity of NEC is significantly reduced compared to WT counterparts. However, interestingly, this study also identified a potential role of TLR4 in mucosal repair via increased proliferation and association with intestinal focal adhesion kinases to induce healing (Leaphart et al., 2007). Together,

these findings show that TLR4 expression in the mucosa is important in the context of NEC. A major limitation of this study was the uncertainty of which specific cell types were examined, as mucosal tissue includes both epithelial and immune cell lines. A more recent study conducted by Sodhi et al. (2012) further investigated the role of TLR4 in NEC and found that it is the epithelial expression of TLR4 which contributes to NEC development and goblet cell regulation. This ambiguity is mimicked across many fields of TLR4 and intestinal disorders, including both intestinal disease and CIGT.

4.4 | TLR4 regulates CIGT risk and severity

CIGT is a serious complication of current anticancer treatments, affecting up to 80% of people treated with chemotherapy (Blijevens, 2005; Richardson & Dobish, 2007). Symptoms of CIGT include severe diarrhoea, pain, bleeding, and ulceration often resulting in an increased risk of infection, leading to delayed, or discontinued treatment (Andreyev et al., 2014; Fuchs, Mitchell, & Hoff, 2006). These symptoms are predominantly underpinned by changes to the intestinal barrier, which is comprised of polarised epithelial cells acting as a highly selective barrier between the intestinal lumen and the subepithelial tissue (Pott & Hornef, 2012; Wardill et al., 2012). Irinotecan is a common chemotherapy associated with CIGT development, due to its unique enterohepatic recirculation which causes a double exposure to its toxic metabolite, SN-38, in the intestines (G. Chabot, 1997; Kawato, Aonuma, Hirota, Kuga, & Sato, 1991). Wardill, Gibson et al. (2016) study examined the link between irinotecan-induced CIGT and TLR4 involvement, demonstrating reduced clinical indicators of toxicity in the TLR4 KO mice versus WT. Specifically, TLR4 KO mice showed decreased diarrhoea and lost significantly less body weight compared to their WT counterparts (Wardill, Gibson et al., 2016). Furthermore, Wardill, Gibson et al. (2016) reported less severe intestinal barrier dysfunction, with TLR4 KO mice demonstrating lower serum FITC-dextran. Together with intestinal injury, CIGT is also characterised by an increase of pain and discomfort hypothesised to occur via neuroinflammation and neurotoxicity, controlled via the gut-brain axis (Y. Li et al., 2015). Results from an in vivo mouse study found that the administration of the chemotherapy, paclitaxel, activated TLR4 which sensitised transient receptor potential vanilloid subtype 1 to dorsal root ganglia neurons, creating adverse physiological effects (Y. Li et al., 2015). This evidence emphasises the importance of TLR4-mediated mechanisms in governing intestinal barrier function and its secondary consequences. However, given the ubiquitous expression of TLR4 on epithelial cells, enteric glia, CNS supportive cells, and immune cells, it remains challenging to decipher the exact mechanisms at play, thus hindering the design of suitable interventions. Despite these challenges, inhibition of TLR4 via naloxone has been investigated in the setting of CIGT (Coller et al., 2017). Naloxone was administered orally, with the goal of inhibiting epithelial TLR4 and thus prevent both direct SN-38-dependant TLR4 activation and host-microbiome interactions that govern mucosal injury (Coller

et al., 2017). Although naloxone was unable to attenuate CIGT, it was also shown to impact tumour growth, with adjuvant TLR4 inhibition decreasing the efficacy of irinotecan against adenocarcinoma cells. This parallels findings of Apetoh et al. (2007) and Fukata et al. (2007), with increased basal tumour growth and decreased chemoefficacy both shown in TLR4 KO mice. Together, these findings highlight the potential hazards of broad-spectrum antagonism of TLR4 in conjunction with chemotherapies and emphasise the importance of dissecting immune and epithelial mechanisms before transitioning to interventional studies.

5 | CONCLUDING REMARKS AND FUTURE DIRECTIONS

TLR4 is widely expressed on epithelial and immune cells along the gastrointestinal tract and is responsible for a range of homeostatic control mechanisms including immune system development. Recent evidence indicates the role of TLR4 signalling in the development, and progression, of many gastrointestinal diseases and toxicities. In normal physiology, both epithelial and immune expression of TLR4 is required to maintain host microbiome (Abreu, 2010), B cell recruitment, immune tolerance (Salazar et al., 2017), and maturation of dendritic cells (Michelsen et al., 2001). Although it is crucial to understand the role of TLR4 in a healthy condition, the impact of site-specific TLR4 expression in gastrointestinal diseases is of great importance. New disease mechanisms involving the activation of TLR4, and its associated signalling pathways, are continually emerging in this field. Although TLR4-based research continues to grow, there is a significant lack of specificity in the literature concerning cell-specific TLR4 signalling and its contribution to both health and disease. This common oversight almost certainly introduces translational errors and clouds interpretation of preclinical evidence. The use of novel intestinal-specific KO models will provide much-needed resolution into the site-specific roles of TLR4. These new models aim to better inform the development of new interventions targeting TLR4, providing greater specificity in augmenting immune or epithelial TLR4 without compromising core homeostatic mechanisms.

ACKNOWLEDGEMENTS

Elise Bruning work was supported by an Australian Government Research Training Program Scholarship (2019–2022). Hannah R. Wardill work was supported by a National Health and Medical Research Council CJ Martin Biomedical Research Fellowship (2018–2022).

ORCID

Elise E. Bruning <http://orcid.org/0000-0002-0333-5530>

Janet K. Coller <http://orcid.org/0000-0002-8273-5048>

Hannah R. Wardill <http://orcid.org/0000-0002-6613-3661>

Joanne M. Bowen <http://orcid.org/0000-0003-0876-0031>

REFERENCES

- Abreu, M. T. (2010). Toll-like receptor signalling in the intestinal epithelium: How bacterial recognition shapes intestinal function. *Nature Reviews Immunology*, 10(2), 131–144. <https://doi.org/10.1038/nri2707>
- Abreu, M. T., Arnold, E. T., Thomas, L. S., Gonsky, R., Zhou, Y., Hu, B., & Arditi, M. (2002). TLR4 and MD-2 expression is regulated by immune-mediated signals in human intestinal epithelial cells. *Journal of Biological Chemistry*, 277(23), 20431–20437. <https://doi.org/10.1074/jbc.M110333200>
- Abreu, M. T., Vora, P., Faure, E., Thomas, L. S., Arnold, E. T., & Arditi, M. (2001). Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *Journal of Immunology*, 167(3), 1609–1616. <https://doi.org/10.4049/jimmunol.167.3.1609>
- Akira, S., Takeda, K., & Kaisho, T. (2001). Toll-like receptors: Critical proteins linking innate and acquired immunity. *Nature Immunology*, 2(8), 675–680.
- Albrecht, V., Hofer, T. P., Foxwell, B., Frankenberger, M., & Ziegler-Heitbrock, L. (2008). Tolerance induced via TLR2 and TLR4 in human dendritic cells: Role of IRAK-1. *BMC Immunology*, 9, 69. <https://doi.org/10.1186/1471-2172-9-69>
- Anand, R. J., Leaphart, C. L., Mollen, K. P., & Hackam, D. J. (2007). The role of the intestinal barrier in the pathogenesis of necrotizing enterocolitis. *Shock*, 27(2), 124–133. <https://doi.org/10.1097/01.shk.0000239774.02904.65>
- Anderson, J. M., Van Itallie, C. M., & Fanning, A. S. (2004). Setting up a selective barrier at the apical junction complex. *Current Opinion in Cell Biology*, 16(2), 140–145. <https://doi.org/10.1016/j.ceb.2004.01.005>
- Andreyev, J., Ross, P., Donnellan, C., Lennan, E., Leonard, P., Waters, C., ... Ferry, D. (2014). Guidance on the management of diarrhoea during cancer chemotherapy. *The Lancet Oncology*, 15(10), e447–e460. [https://doi.org/10.1016/s1470-2045\(14\)70006-3](https://doi.org/10.1016/s1470-2045(14)70006-3)
- Anitha, M., Vijay-Kumar, M., Sitaraman, S. V., Gewirtz, A. T., & Srinivasan, S. (2012). Gut microbial products regulate murine gastrointestinal motility via Toll-like receptor 4 signaling. *Gastroenterology*, 143(4), 1006–1016.e4. <https://doi.org/10.1053/j.gastro.2012.06.034>
- Apetoh, L., Ghiringhelli, F., Tesniere, A., Obeid, M., Ortiz, C., Criollo, A., ... Zitvogel, L. (2007). Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature Medicine*, 13(9), 1050–1059. <https://doi.org/10.1038/nm1622>
- Banskota, S., Regmi, S. C., Gautam, J., Gurung, P., Lee, Y. J., Ku, S. K., ... Kim, J. A. (2017). Serotonin disturbs colon epithelial tolerance of commensal *E. coli* by increasing NOX2-derived superoxide. *Free Radical Biology and Medicine*, 106, 196–207. <https://doi.org/10.1016/j.freeradbiomed.2017.02.034>
- Bein, A., Eventov-Friedman, S., Arbell, D., & Schwartz, B. (2018). Intestinal tight junctions are severely altered in NEC preterm neonates. *Pediatric Neonatology*, 59(5), 464–473. <https://doi.org/10.1016/j.pedneo.2017.11.018>
- Belmonte, L., Beutheu Youmba, S., Bertiaux-Vandaele, N., Antonietti, M., Lecleire, S., Zalar, A., ... Ducrotte, P. (2012). Role of toll like receptors in irritable bowel syndrome: Differential mucosal immune activation according to the disease subtype. *PLoS One*, 7(8), e42777. <https://doi.org/10.1371/journal.pone.0042777>
- Blijevens, N. (2005). Implications of treatment induced mucosal injury. *Current Opinion in Oncology*, 17, 605–610.
- Bossi, P., Bergamini, C., Miceli, R., Cova, A., Orlandi, E., Resteghini, C., ... Rivoltini, L. (2016). Salivary cytokine levels and oral mucositis in head and neck cancer patients treated with chemotherapy and radiation therapy. *International Journal of Radiation Oncology, Biology, Physics*, 96(5), 959–966. <https://doi.org/10.1016/j.ijrobp.2016.08.047>

- Cairo, E., & Podolsky, D. (2000). Differential alteration in intestinal epithelial cell expression of Toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infection and Immunity*, 68, 7010–7017.
- Chabot, G. (1997). Clinical pharmacokinetics of irinotecan. *Clinical Pharmacokinetics*, 33, 245–259.
- Chabot, S., Wagner, J. S., Farrant, S., & Neutra, M. R. (2006). TLRs regulate the gatekeeping functions of the intestinal follicle-associated epithelium. *Journal of Immunology*, 176(7), 4275–4283. <https://doi.org/10.4049/jimmunol.176.7.4275>
- Chen, W. (2011). IDO: More than an enzyme. *Nature Immunology*, 18, 809–811.
- Cheng, Z., Taylor, B., Ourthiague, D. R., & Hoffmann, A. (2015). Distinct single-cell signaling characteristics are conferred by the MyD88 and TRIF pathways during TLR4 activation. *Science Signaling*, 8, ra69. <https://doi.org/10.1126/scisignal.aaa5208>
- Chung, H., Pamp, S. J., Hill, J. A., Surana, N. K., Edelman, S. M., Troy, E. B., ... Kasper, D. L. (2012). Gut immune maturation depends on colonization with a host-specific microbiota. *Cell*, 149(7), 1578–1593. <https://doi.org/10.1016/j.cell.2012.04.037>
- Coller, J. K., Bowen, J. M., Ball, I. A., Wardill, H. R., vanSebille, Y. Z., Stansborough, R. L., ... Gibson, R. J. (2017). Potential safety concerns of TLR4 antagonism with irinotecan: A preclinical observational report. *Cancer Chemotherapy and Pharmacology*, 79(2), 431–434. <https://doi.org/10.1007/s00280-016-3223-3>
- Comalada, M., & Xaus, J. (2013). Toll-like receptors and intestinal immune tolerance. *Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease*, 597–609. <https://doi.org/10.1016/B978-0-12-397154-8.00023-3>
- De Nardo, D. (2015). Toll-like receptors: Activation, signalling and transcriptional modulation. *Cytokine*, 74(2), 181–189. <https://doi.org/10.1016/j.cyto.2015.02.025>
- De Jager, P. L., Franchimont, D., Waliszewska, A., Bitton, A., Cohen, A., Langelier, D., ... Consortium, N. I. G. (2007). The role of the Toll receptor pathway in susceptibility to inflammatory bowel diseases. *Genes and Immunity*, 8(5), 387–397. <https://doi.org/10.1038/sj.gene.6364398>
- Dheer, R., Santaolalla, R., Davies, J. M., Lang, J. K., Phillips, M. C., Pastorini, C., ... Abreu, M. T. (2016). Intestinal epithelial toll-like receptor 4 signaling affects epithelial function and colonic microbiota and promotes a risk for transmissible colitis. *Infection and Immunity*, 84(3), 798–810. <https://doi.org/10.1128/IAI.01374-15>
- Farquhar, M., & Palade, G. (1963). Junctional complexes in various epithelia. *Journal of Cell Biology*, 17, 375–412.
- Fuchs, C., Mitchell, E. P., & Hoff, P. M. (2006). Irinotecan in the treatment of colorectal cancer. *Cancer Treatment Reviews*, 32(7), 491–503. <https://doi.org/10.1016/j.ctrv.2006.07.001>
- Fukata, M., Chen, A., Vamadevan, A., Cohen, J., Breglio, K., Krishnareddy, S., ... Abreu, M. (2007). Toll-like receptor (TLR4) promotes the development of colitis-associated colorectal tumors. *Gastroenterology*, 133, 1869–1881.
- Fukata, M., Michelsen, K. S., Eri, R., Thomas, L. S., Hu, B., Lukasek, K., ... Abreu, M. T. (2005). Toll-like receptor-4 is required for intestinal response to epithelial injury and limiting bacterial translocation in a murine model of acute colitis. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 288(5), G1055–G1065. <https://doi.org/10.1152/ajpgi.00328.2004>
- Gourbeyre, P., Berri, M., Lippi, Y., Meurens, F., Vincent-Naulleau, S., Laffitte, J., ... Oswald, I. P. (2015). Pattern recognition receptors in the gut: Analysis of their expression along the intestinal tract and the crypt/villus axis. *Physiological Reports*, 3(2), e12225. <https://doi.org/10.14814/phy2.12225>
- Gribar, S. C., Richardson, W. M., Sodhi, C. P., & Hackam, D. J. (2008). No longer an innocent bystander: Epithelial toll-like receptor signaling in the development of mucosal inflammation. *Molecular Medicine*, 14(9–10), 645–659. <https://doi.org/10.2119/2008-00035.Gribar>
- Guo, S., Al-Sadi, R., Said, H. M., & Ma, T. Y. (2013). Lipopolysaccharide causes an increase in intestinal tight junction permeability in vitro and in vivo by inducing enterocyte membrane expression and localization of TLR-4 and CD14. *American Journal of Pathology*, 182(2), 375–387. <https://doi.org/10.1016/j.ajpath.2012.10.014>
- Guo, S., Nighot, M., Al-Sadi, R., Alhmoud, T., Nighot, P., Ma, T. Y., ... Consortium, N. I. G. (2015). Lipopolysaccharide regulation of intestinal tight junction permeability is mediated by TLR4 signal transduction pathway activation of FAK and MyD88. *Journal of Immunology*, 195(10), 4999–5010. <https://doi.org/10.4049/jimmunol.1402598>
- Hackam, D. J., Upperman, J. S., Grishin, A., & Ford, H. R. (2005). Disordered enterocyte signaling and intestinal barrier dysfunction in the pathogenesis of necrotizing enterocolitis. *Seminars in Pediatric Surgery*, 14(1), 49–57. <https://doi.org/10.1053/j.sempedsurg.2004.10.025>
- Hajjar, A. M., Ernst, R. K., Fortuno, E. S., 3rd, Brasfield, A. S., Yam, C. S., Newlon, L. A., ... Wilson, C. B. (2012). Humanized TLR4/MD-2 mice reveal LPS recognition differentially impacts susceptibility to *Yersinia pestis* and *Salmonella enterica*. *PLoS Pathogens*, 8(10), e1002963. <https://doi.org/10.1371/journal.ppat.1002963>
- Harden, J. L., & Egilmez, N. K. (2012). Indoleamine 2,3-dioxygenase and dendritic cell tolerogenicity. *Immunological Investigations*, 41(6–7), 738–764. <https://doi.org/10.3109/08820139.2012.676122>
- Hooper, L., Littman, D., & Macpherson, A. (2012). Interactions between the microbiota and the immune system. *Science*, 336, 1268–1273.
- Hornef, M., Frisan, T., Vandewalle, A., Normark, S., & Richter-Dahlfors, A. (2002). Toll-like receptor 4 resides in the Golgi apparatus and colocalizes with internalized lipopolysaccharide in intestinal epithelial cells. *Journal of Experimental Medicine*, 195, 559–570.
- Hoshino, K., Takeuchi, O., Kawai, T., Sanjo, H., Ogawa, T., Takeda, Y., ... Akira, S. (1999). Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: Evidence for TLR4 as the Lps gene product. *Journal of Immunology*, 162, 3749–3752.
- Kawato, Y., Aonuma, M., Hirota, Y., Kuga, H., & Sato, K. (1991). Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Research*, 51, 4187–4191.
- Kishore, U. (2009). *Target pattern recognition in innate immunity* (653). New York, NY: Springer.
- Kocak, E., Akbal, E., Koklu, S., Ergul, B., & Can, M. (2016). The colonic tissue levels of TLR2, TLR4 and nitric oxide in patients with irritable bowel syndrome. *Internal Medicine*, 55(9), 1043–1048. <https://doi.org/10.2169/internalmedicine.55.5716>
- Leaphart, C. L., Cavallo, J., Gribar, S. C., Cetin, S., Li, J., Branca, M. F., ... Hackam, D. J. (2007). A critical role for TLR4 in the pathogenesis of necrotizing enterocolitis by modulating intestinal injury and repair. *Journal of Immunology*, 179(7), 4808–4820. <https://doi.org/10.4049/jimmunol.179.7.4808>
- Lee, S. H. (2015). Intestinal permeability regulation by tight junction: Implication on inflammatory bowel diseases. *Intestinal Research*, 13(1), 11–18. <https://doi.org/10.5217/ir.2015.13.1.11>
- Lee, C. C., Avalos, A. M., & Ploegh, H. L. (2012). Accessory molecules for Toll-like receptors and their function. *Nature Reviews Immunology*, 12(3), 168–179. <https://doi.org/10.1038/nri3151>
- Li, Y., Adamek, P., Zhang, H., Tatsui, C. E., Rhines, L. D., Mrozkova, P., ... Dougherty, P. M. (2015). The cancer chemotherapeutic paclitaxel increases human and rodent sensory neuron responses to TRPV1 by activation of TLR4. *Journal of Neuroscience*, 35(39), 13487–13500. <https://doi.org/10.1523/JNEUROSCI.1956-15.2015>
- Li, Q., & Cherayil, B. J. (2003). Role of Toll-like receptor 4 in macrophage activation and tolerance during *Salmonella enterica* serovar Typhimurium infection. *Infection and Immunity*, 71(9), 4873–4882. <https://doi.org/10.1128/iai.71.9.4873-4882.2003>
- Li, X., Wang, C., Nie, J., Lv, D., Wang, T., & Xu, Y. (2013). Toll-like receptor 4 increases intestinal permeability through up-regulation of

- membrane PKC activity in alcoholic steatohepatitis. *Alcohol*, 47(6), 459–465. <https://doi.org/10.1016/j.alcohol.2013.05.004>
- Logan, R. M., Stringer, A. M., Bowen, J. M., Gibson, R. J., Sonis, S. T., & Keefe, D. M. (2008). Serum levels of NF- κ B and pro-inflammatory cytokines following administration of mucotoxic drugs. *Cancer Biology & Therapy*, 7(7), 1139–1145. <https://doi.org/10.4161/cbt.7.7.6207>
- Lotz, M., Gutle, D., Walther, S., Menard, S., Bogdan, C., & Hornef, M. W. (2006). Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. *Journal of Experimental Medicine*, 203(4), 973–984. <https://doi.org/10.1084/jem.20050625>
- Lu, P., Sodhi, C. P., & Hackam, D. J. (2014). Toll-like receptor regulation of intestinal development and inflammation in the pathogenesis of necrotizing enterocolitis. *Pathophysiology*, 21(1), 81–93. <https://doi.org/10.1016/j.pathophys.2013.11.007>
- Lu, P., Sodhi, C. P., Yamaguchi, Y., Jia, H., Prindle, T., Jr., Fulton, W. B., ... Hackam, D. J. (2018). Intestinal epithelial Toll-like receptor 4 prevents metabolic syndrome by regulating interactions between microbes and intestinal epithelial cells in mice. *Mucosal Immunology*, 11(3), 727–740. <https://doi.org/10.1038/mi.2017.114>
- Matsebatlela, T. M., Anderson, A. L., Gallicchio, V. S., Elford, H., & Rice, C. D. (2015). 3,4-Dihydroxy-benzohydroxamic acid (Didox) suppresses pro-inflammatory profiles and oxidative stress in TLR4-activated RAW264.7 murine macrophages. *Chemico-Biological Interactions*, 233, 95–105. <https://doi.org/10.1016/j.cbi.2015.03.027>
- McClure, R., & Massari, P. (2014). TLR-dependent human mucosal epithelial cell responses to microbial pathogens. *Frontiers in Immunology*, 5, 386. <https://doi.org/10.3389/fimmu.2014.00386>
- Meng, J., Lien, E., & Golenbock, D. T. (2010). MD-2-mediated ionic interactions between lipid A and TLR4 are essential for receptor activation. *Journal of Biological Chemistry*, 285(12), 8695–8702. <https://doi.org/10.1074/jbc.M109.075127>
- Meng, D., Zhu, W., Shi, H. N., Lu, L., Wijendran, V., Xu, W., & Walker, W. A. (2015). Toll-like receptor-4 in human and mouse colonic epithelium is developmentally regulated: A possible role in necrotizing enterocolitis. *Pediatric Research*, 77(3), 416–424. <https://doi.org/10.1038/pr.2014.207>
- Michelsen, K. S., Aicher, A., Mohaupt, M., Hartung, T., Dimmeler, S., Kirschning, C. J., & Schumann, R. R. (2001). The role of toll-like receptors (TLRs) in bacteria-induced maturation of murine dendritic cells (DCs). Peptidoglycan and lipoteichoic acid are inducers of DC maturation and require TLR2. *Journal of Biological Chemistry*, 276(28), 25680–25686. <https://doi.org/10.1074/jbc.M011615200>
- Naik, S., Kelly, E., Meijer, L., Petterson, S., & Sanderson, I. (2001). Absence of Toll-Like receptor 4 explains endotoxin hyporesponsiveness in human intestinal epithelium. *Journal of Pediatrics Gastroenterology and Nutrition*, 32, 449–453.
- Nie, L., Cai, S. Y., Shao, J. Z., & Chen, J. (2018). Toll-Like receptors, associated biological roles, and signaling networks in non-mammals. *Frontiers in Immunology*, 9, 1523. <https://doi.org/10.3389/fimmu.2018.01523>
- Nighot, M., Al-Sadi, R., Guo, S., Rawat, M., Nighot, P., Watterson, M. D., & Ma, T. Y. (2017). Lipopolysaccharide-induced increase in intestinal epithelial tight permeability is mediated by toll-like receptor 4/myeloid differentiation primary response 88 (MyD88) activation of myosin light chain kinase expression. *American Journal of Pathology*, 187(12), 2698–2710. <https://doi.org/10.1016/j.ajpath.2017.08.005>
- Pardo-Camacho, C., Gonzalez-Castro, A. M., Rodino-Janeiro, B. K., Pigrau, M., & Vicario, M. (2018). Epithelial immunity: Priming defensive responses in the intestinal mucosa. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 314(2), G247–G255. <https://doi.org/10.1152/ajpgi.00215.2016>
- Pinheiro, D., & Bellaiche, Y. (2018). Mechanical force-driven adherens junction remodeling and epithelial dynamics. *Developmental Cell*, 47(1), 3–19. <https://doi.org/10.1016/j.devcel.2018.09.014>
- Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., ... Beutler, B. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 gene. *Science*, 282, 2085–2088.
- Pott, J., & Hornef, M. (2012). Innate immune signalling at the intestinal epithelium in homeostasis and disease. *EMBO Reports*, 13(8), 684–698. <https://doi.org/10.1038/embor.2012.96>
- Pufnock, J. S., Cigal, M., Rolczynski, L. S., Andersen-Nissen, E., Wolf, M., McElrath, M. J., & Greenberg, P. D. (2011). Priming CD8+ T cells with dendritic cells matured using TLR4 and TLR7/8 ligands together enhances generation of CD8+ T cells retaining CD28. *Blood*, 117(24), 6542–6551. <https://doi.org/10.1182/blood-2010-11-317966>
- Pushpakumar, S., Ren, L., Kundu, S., Gamon, A., Tyagi, S. C., & Sen, U. (2017). Toll-like receptor 4 deficiency reduces oxidative stress and macrophage mediated inflammation in hypertensive kidney. *Scientific Reports*, 7(1), 6349. <https://doi.org/10.1038/s41598-017-06484-6>
- Qureshi, S. T., Lariviere, L., Leveque, G., Clermont, S., Moore, K. J., Gros, P., & Malo, D. (1999). Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (TLR4). *Journal of Experimental Medicine*, 189, 615–625.
- Ralls, M. W., Demehri, F. R., Feng, Y., Woods Ignatoski, K. M., & Teitelbaum, D. H. (2015). Enteral nutrient deprivation in patients leads to a loss of intestinal epithelial barrier function. *Surgery*, 157(4), 732–742. <https://doi.org/10.1016/j.surg.2014.12.004>
- Richardson, G., & Dobish, R. (2007). Chemotherapy induced diarrhea. *J Oncol Pharm Practice*, 13, 181–198.
- Salazar, F., Awuah, D., Negm, O. H., Shakib, F., & Ghaemmaghami, A. M. (2017). The role of indoleamine 2,3-dioxygenase-aryl hydrocarbon receptor pathway in the TLR4-induced tolerogenic phenotype in human DCs. *Scientific Reports*, 7, 43337. <https://doi.org/10.1038/srep43337>
- Salim, S. Y., & Soderholm, J. D. (2011). Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflammatory Bowel Diseases*, 17(1), 362–381. <https://doi.org/10.1002/ibd.21403>
- Santaolalla, R., Sussman, D. A., & Abreu, M. T. (2011). TLR signaling: A link between gut microflora, colorectal inflammation and tumorigenesis. *Drug discovery today. Disease mechanisms*, 8(3–4), e57–e62. <https://doi.org/10.1016/j.ddmec.2012.02.002>
- Shang, L., Fukata, M., Thirunarayanan, N., Martin, A. P., Arnaboldi, P., Maussang, D., ... Lira, S. A. (2008). Toll-like receptor signaling in small intestinal epithelium promotes B-cell recruitment and IgA production in lamina propria. *Gastroenterology*, 135(2), 529–538. <https://doi.org/10.1053/j.gastro.2008.04.020>
- Shi, Y. J., Hu, S. J., Zhao, Q. Q., Liu, X. S., Liu, C., & Wang, H. (2019). Toll-like receptor 4 (TLR4) deficiency aggravates dextran sulfate sodium (DSS)-induced intestinal injury by down-regulating IL6, CCL2 and CSF3. *Annals of Translational Medicine*, 7(23), 713. <https://doi.org/10.21037/atm.2019.12.28>
- Sodhi, C. P., Neal, M. D., Siggers, R., Sho, S., Ma, C., Branca, M. F., ... Hackam, D. J. (2012). Intestinal epithelial Toll-like receptor 4 regulates goblet cell development and is required for necrotizing enterocolitis in mice. *Gastroenterology*, 143(3), 708–718.e5. <https://doi.org/10.1053/j.gastro.2012.05.053>
- Steehls, L., Keestra, A. M., van Mourik, A., Uronen-Hansson, H., van der Ley, P., Callard, R., ... van Putten, J. P. (2008). Differential activation of human and mouse Toll-like receptor 4 by the adjuvant candidate LpxL1 of *Neisseria meningitidis*. *Infection and Immunity*, 76(8), 3801–3807. <https://doi.org/10.1128/IAI.00005-08>
- Takahashi, K., Sugi, Y., Hosono, A., & Kaminogawa, S. (2009). Epigenetic regulation of TLR4 gene expression in intestinal epithelial cells for the maintenance of intestinal homeostasis. *Journal of Immunology*, 183(10), 6522–6529. <https://doi.org/10.4049/jimmunol.0901271>
- Vamadevan, A. S., Fukata, M., Arnold, E. T., Thomas, L. S., Hsu, D., & Abreu, M. T. (2010). Regulation of Toll-like receptor 4-associated MD-2 in intestinal epithelial cells: A comprehensive analysis. *Innate Immunity*, 16(2), 93–103. <https://doi.org/10.1177/1753425909339231>

- Vaure, C., & Liu, Y. (2014). A comparative review of toll-like receptor 4 expression and functionality in different animal species. *Frontiers in Immunology*, 5, 316. <https://doi.org/10.3389/fimmu.2014.00316>
- Von Bubnoff, D., Scheler, M., Wilms, H., Fimmers, R., & Bieber, T. (2011). Identification of IDO-positive and IDO-negative human dendritic cells after activation by various proinflammatory stimuli. *Journal of Immunology*, 186(12), 6701–6709. <https://doi.org/10.4049/jimmunol.1003151>
- Wardill, H. R., Bowen, J. M., & Gibson, R. J. (2012). Chemotherapy-induced gut toxicity: Are alterations to intestinal tight junctions pivotal? *Cancer Chemotherapy and Pharmacology*, 70(5), 627–635. <https://doi.org/10.1007/s00280-012-1989-5>
- Wardill, H. R., Bowen, J. M., Van Seville, Y. Z., Secombe, K. R., Collier, J. K., Ball, I. A., ... Gibson, R. J. (2016). TLR4-dependent claudin-1 internalization and secretagogue-mediated chloride secretion regulate irinotecan-induced diarrhea. *Molecular Cancer Therapeutics*, 15(11), 2767–2779. <https://doi.org/10.1158/1535-7163.MCT-16-0330>
- Wardill, H. R., Gibson, R. J., Van Seville, Y. Z., Secombe, K. R., Collier, J. K., White, I. A., ... Bowen, J. M. (2016). Irinotecan-induced gastrointestinal dysfunction and pain are mediated by common TLR4-dependent mechanisms. *Molecular Cancer Therapeutics*, 15(6), 1376–1386. <https://doi.org/10.1158/1535-7163.MCT-15-0990>
- Wells, J. M., Brummer, R. J., Derrien, M., MacDonald, T. T., Troost, F., Cani, P. D., ... Garcia-Rodenas, C. L. (2017). Homeostasis of the gut barrier and potential biomarkers. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 312(3), G171–G193. <https://doi.org/10.1152/ajpgi.00048.2015>
- Zeuthen, L. H., Fink, L. N., & Frokiaer, H. (2008). Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-beta. *Immunology*, 123(2), 197–208. <https://doi.org/10.1111/j.1365-2567.2007.02687.x>

How to cite this article: Bruning EE, Collier JK, Wardill HR, Bowen JM. Site-specific contribution of Toll-like receptor 4 to intestinal homeostasis and inflammatory disease. *J Cell Physiol*. 2020;1–12. <https://doi.org/10.1002/jcp.29976>