Toll-like receptors and intestinal defence: molecular basis and therapeutic implications

Elke Cario

Toll-like receptors (TLRs) play a principle role in distinct pathogen recognition and in the initiation of innate immune responses of the intestinal mucosa. Activated innate immunity interconnects downstream with adaptive immunity in complex feedback regulatory loops. Intestinal disease might result from inappropriate activation of the mucosal immune system driven by TLRs in response to normal luminal flora.

A broad variety of immune cells of the intestinal mucosa express so-called pattern recognition receptors (PRRs) that specifically discriminate between 'self' and microbial 'nonself' based on the recognition of conserved pathogen-specific molecular patterns (PAMPs) (Ref. 1). Toll-like receptors (TLRs), which comprise one class of PRR, play a key role in microbial recognition, in the induction of antimicrobial genes and in the control of adaptive immune responses (Ref. 2). Immune reactions in the intestinal mucosa are complicated by the need to recognise pathogens specifically and to mount defensive responses rapidly, yet remain quiescent to harmless, commensal bacteria that normally inhabit the gut. Activation of the host defence system of the intestinal mucosa appears to be substantially regulated by TLRs in order to maintain a protective equilibrium.

TLR definition and structure

Following the discovery of a role for Toll in *Drosophila* host defence, a human homologue of the *Drosophila* Toll protein was cloned in 1997 (Ref. 3). A constitutively active mutant of a human Toll homologue transfected into human cell lines could induce: (1) activation of the transcription factor NF-κB; (2) expression of the inflammatory cytokines interleukin 1 (IL-1), IL-6 and IL-8; and (3) expression of the costimulatory molecule B7.1, which is required for the activation of naive T cells (Ref. 3). Signalling through Toll therefore appears to parallel the signalling pathway induced by the IL-1 receptor (IL-1R) in mammalian cells. In 1998, five Toll-like molecules in humans were characterised and named the 'Toll-like receptors' (Ref. 4) and, to date, a total of ten TLRs (1–10) have now been identified (reviewed in Ref. 5). The TLRs therefore constitute a class of putative human receptors with a protein architecture that is similar to the *Drosophila* Toll protein.

Mammalian TLRs are type I transmembrane proteins and contain several common structural features, including multiple leucine-rich repeats (LRRs) and one or two cysteine-rich regions in the large and divergent ligand-binding ectodomain,

Elke Cario
Instructor, Division of Gastroenterology and Hepatology, University of Essen, Institutsgruppe I /C/8, Virchowstr. 171, D-45147 Essen, Germany. Tel: +49 201 723 4527; Fax: +49 211 495 7035; E-mail: elke.cario@uni-essen.de

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

as well as a short transmembrane region and a highly conserved cytoplasmic domain (Ref. 5) (Fig. 1a). The intracellular domain is highly homologous among the individual TLRs and contains a Toll/IL-1R (TIR) domain similar to the cytoplasmic domain of the IL-1R. Mutagenesis and functional studies have shown that human TLRs interact via TIR with downstream signalling partners such as the MyD88 adaptor molecule (Ref. 6), as described below.

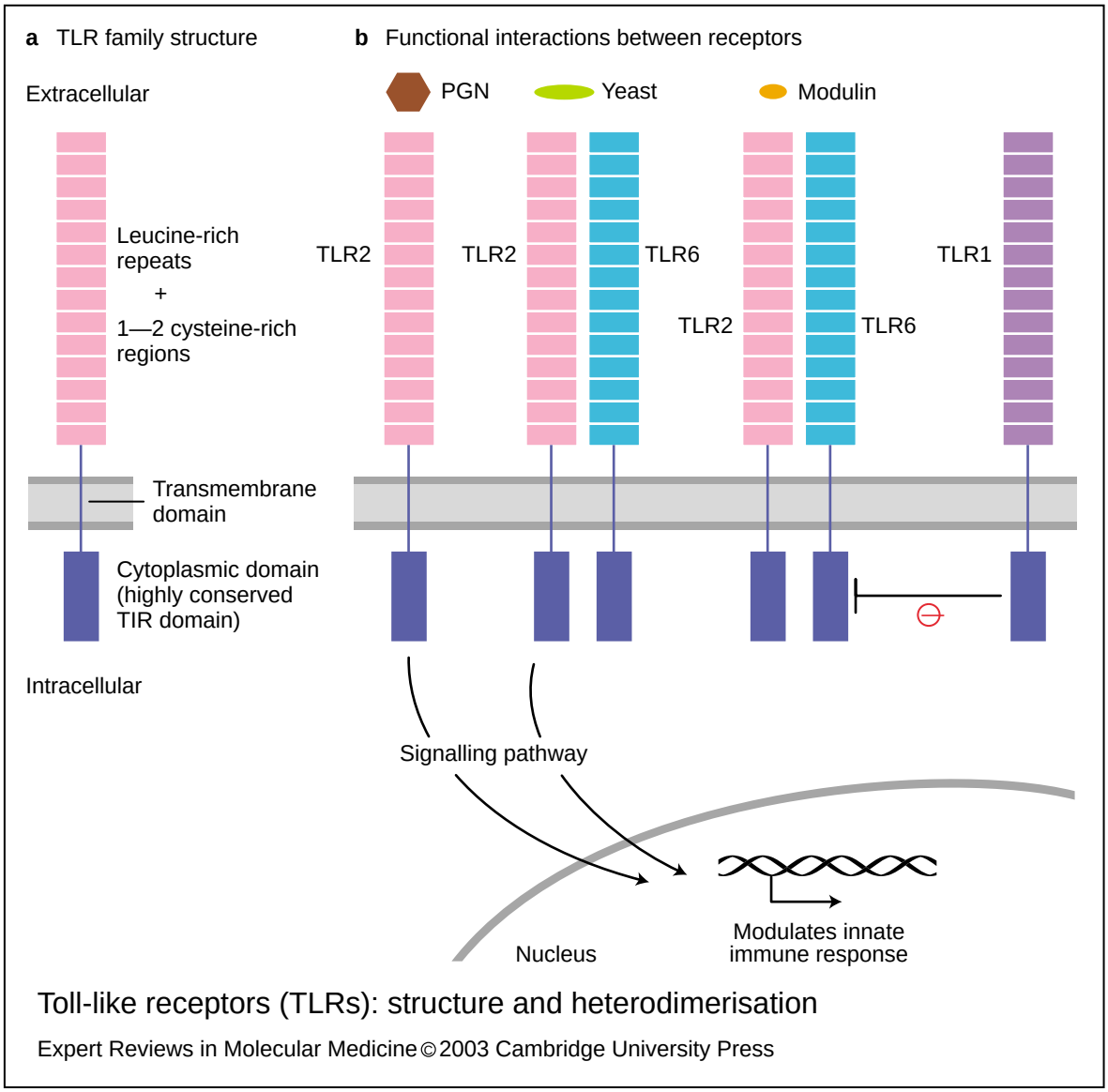


Figure 1. Toll-like receptors (TLRs): structure and heterodimerisation. (a) The mammalian TLR family are type I transmembrane proteins with the following common structural features: multiple leucine-rich repeats and one or two cysteine-rich regions in the large and divergent ligand-binding ectodomain; a short transmembrane region; and a conserved cytoplasmic domain that is highly homologous among the individual TLRs and contains a Toll/IL-1R (TIR) domain similar to the cytoplasmic domain of the interleukin 1 receptor (IL-1R). (b) TLRs function in activating innate immunity by recognising conserved molecular patterns carried by microorganisms. Pattern recognition can be achieved by individual TLRs [e.g. recognition of peptidoglycan (PGN), which is a bacterial cell wall component, by TLR2], or by heterodimerised TLRs (e.g. recognition of yeast components or bacterial modulin by TLR2–TLR6). Furthermore, functional interactions between TLRs can lead to inhibition of responses, as shown here by the interference of TLR1 in the TLR2–TLR6 response **(dfig001ece)**.

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

Diversity of TLRs and their ligands

TLRs are variably expressed in a wide variety of intestinal cell types, including intestinal epithelial cells (IECs; Refs 7, 8, 9, 10, 11), gastric pit cells (Ref. 12), foetal intestinal cells (Ref. 13) and intestinal macrophages of the lamina propria (Refs 14, 15, 16). TLRs are also expressed in Kupffer cells (Ref. 17) and hepatocytes (Refs 18, 19) of the liver.

Specific TLRs bind different 'molecular signatures' of different classes of microorganisms or individual features present on different microbes. 'Nonself' but also 'self' ligands might distinctly activate TLRs to mediate either pro- or anti-inflammatory responses in different cell types and organs. This complex ligand discrimination and specificity might be accounted for by diverse TLR dimerisation that is dynamically modulated by different PAMPs, thereby expanding the plasticity of this family of receptors to maximise innate pattern recognition (Fig. 1b). It is not yet clear whether modulatory cross-recognition of additional as-yet-unknown co-receptors also contributes either to the specificity of activated TLR complexes or to the diversity of differential immune responses in cells activated with certain TLR stimuli. In addition, endogenous TLR antagonists have not yet been identified.

TLR1, TLR2 and TLR6

TLR2 recognises a broad spectrum of molecular patterns from Gram-positive bacteria and mycobacterial species, including peptidoglycan (PGN), bacterial lipoprotein/lipopeptides, glycosylphosphatidylinositol (GPI) anchors and lipoteichoic acid, as well as yeast and fungal cell wall components (Refs 20, 21, 22, 23, 24, 25, 26, 27). TLR2 might synergistically cooperate with TLR6 and TLR1 in the recognition of certain PAMPs in order to expand the repertoire of TLR-mediated responses (Refs 20, 28, 29) (Fig. 1b). However, cooperation is not necessarily needed for all TLR2-mediated immune responses; for instance, $TLR6^{-/-}$ and $TLR1^{-/-}$ mice respond to PGN, whereas $TLR2^{-/-}$ mice are unresponsive to PGN, implying that TLR1 and TLR6 are not essential for responses to PGN via TLR2 (Ref. 30). Furthermore, TLR1 appears to interact with TLR2 only in response to certain lipid configurations of lipoproteins, implying rigid specificity in the differential recognition of PAMPs by individual TLR combinations (Ref. 31). Conversely, cotransfection studies have recently shown that TLR1 might block TLR4-induced downstream signalling effects (Ref. 32), suggesting that TLRs might also negatively regulate the activity of each other through heterodimerisations.

TLR3

Although TLR3 is mostly expressed by mature human dendritic cells (DCs) (Ref. 33), IECs also constitutively express TLR3 (Ref. 14). Mammalian TLR3 recognises polyinosine–polycytidylic acid [poly(I:C)], which is a synthetic analogue of double-stranded RNA (dsRNA), leading to NF-κB activation and production of type I interferons (IFNs) (Refs 34, 35). However, so far, it remains to be shown whether viral dsRNA is actually a ligand of TLR3 (Ref. 36).

TLR4

The first in vivo evidence that mammalian TLR4 primarily subserves the pattern recognition of lipopolysaccharide (LPS) was achieved by Poltorak et al. (Ref. 37), and has been confirmed by subsequent genetic and biochemical evidence (Ref. 38). Although early studies have suggested that TLR2 might also mediate responses to LPS, Hirschfeld et al. (Ref. 39) provided convincing evidence that contaminants in commercially available LPS preparations were responsible for TLR2-mediated signalling and not purified LPS itself. MD-2, an accessory protein to TLR4, is essential for the correct intracellular distribution of TLR4 (Ref. 40) in order to initiate LPS signalling (Refs 41, 42, 43). CD11, CD18, CD14 and LPS-binding protein (LBP) might also cooperatively increase TLR4-mediated cellular activation by LPS (Refs 44, 45). Of note, IECs lack constitutive expression of membrane-bound CD14 in vitro (Ref. 7), but expression might be induced under inflammatory conditions in vivo (Ref. 46).

TLR4 does not mediate responses to all LPS serotypes derived from different species (Ref. 47), and this might be dependent on conformations of the lipid A component of LPS (Ref. 48). Furthermore, bacteria can alter the acylation state of their LPS in response to environmental changes during bacterial–host adaptation (Ref. 49). For instance, *Pseudomonas aeruginosa* synthesises more highly acylated (hexa-acylated) LPS structures when found in the airway of individuals with cystic fibrosis compared with bacteria in normal airways, which synthesise penta-acylated LPS. The TLR4–MD-2 complex recognises

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

this adaptation and transmits pro-inflammatory signals only in response to hexa-acylated but not penta-acylated LPS (Ref. 49).

TLR2 and TLR4 are present at the apical pole of differentiated IECs in vitro and in vivo and thus are well positioned to monitor the lumenal milieu of bacterial products (Ref. 50). TLR4 is present in lipid rafts at the cellular membrane, and these are enriched in glycosphingolipids and cholesterol (Ref. 51). In response to LPS stimulation, TLR4 is redistributed from its apical location to intracytoplasmic compartments near the basolateral membrane in differentiated IECs (Ref. 50). Conversely, Hornef et al. have recently demonstrated that TLR4 preferentially resides in the Golgi apparatus, co-localising with endocytosed LPS in immature murine IECs (Ref. 10).

TLR5

Pathogenic and commensal bacteria secrete flagellin, the structural component of bacterial flagella, and this protein has recently been identified as a ligand for TLR5 (Ref. 52). In contrast to TLR2 and TLR4 (Refs 14, 50), TLR5 on IECs appears to be preferentially expressed at the basolateral pole (Refs 9, 53). Pathogenic enteric *Salmonella* translocate flagellin across IECs, and it has been demonstrated that detection of flagellin by basolateral TLR5 activates pro-inflammatory gene expression, driving epithelial inflammatory responses to *Salmonella* (Ref. 9).

TLR7 and TLR8

Plasmacytoid DCs and B cells express high levels of TLR7 (Ref. 54). The antiviral imidazoquinoline resiquimod (R-848) activates immune cells via both TLR7 (Ref. 55) and TLR8 (Ref. 56), inducing potent antiviral immune responses through NF-κB activation, suggesting a possible redundancy among these two TLRs. Of note, murine TLR8 appears to be nonfunctional (Ref. 56). The natural ligands for TLR7 and TLR8 have not yet been identified.

TLR9

CpG DNA (rich in cytosine-phosphatediester-guanosine) interacts with two independent receptor molecules, DNA-dependent protein kinase (DNA-PKcs) (Ref. 57) and TLR9 (Refs 58, 59), stimulating immune responses characterised by the production of various cytokines, chemokines and antibodies in B cells, natural killer (NK) cells, macrophages and DCs (Refs 54, 60, 61, 62, 63), as well as in IECs (Ref. 64). TLR9 is localised to intracellular endosomal compartments (Ref. 65). Cellular uptake via endocytosis and subsequent endosomal maturation is therefore essential for signalling induced by CpG DNA (Ref. 66).

TLR10

TLR10 mRNA is expressed in plasmacytoid DCs and B cells (Ref. 54), and also in IECs (Ref. 67). Its ligands and function have not yet been identified.

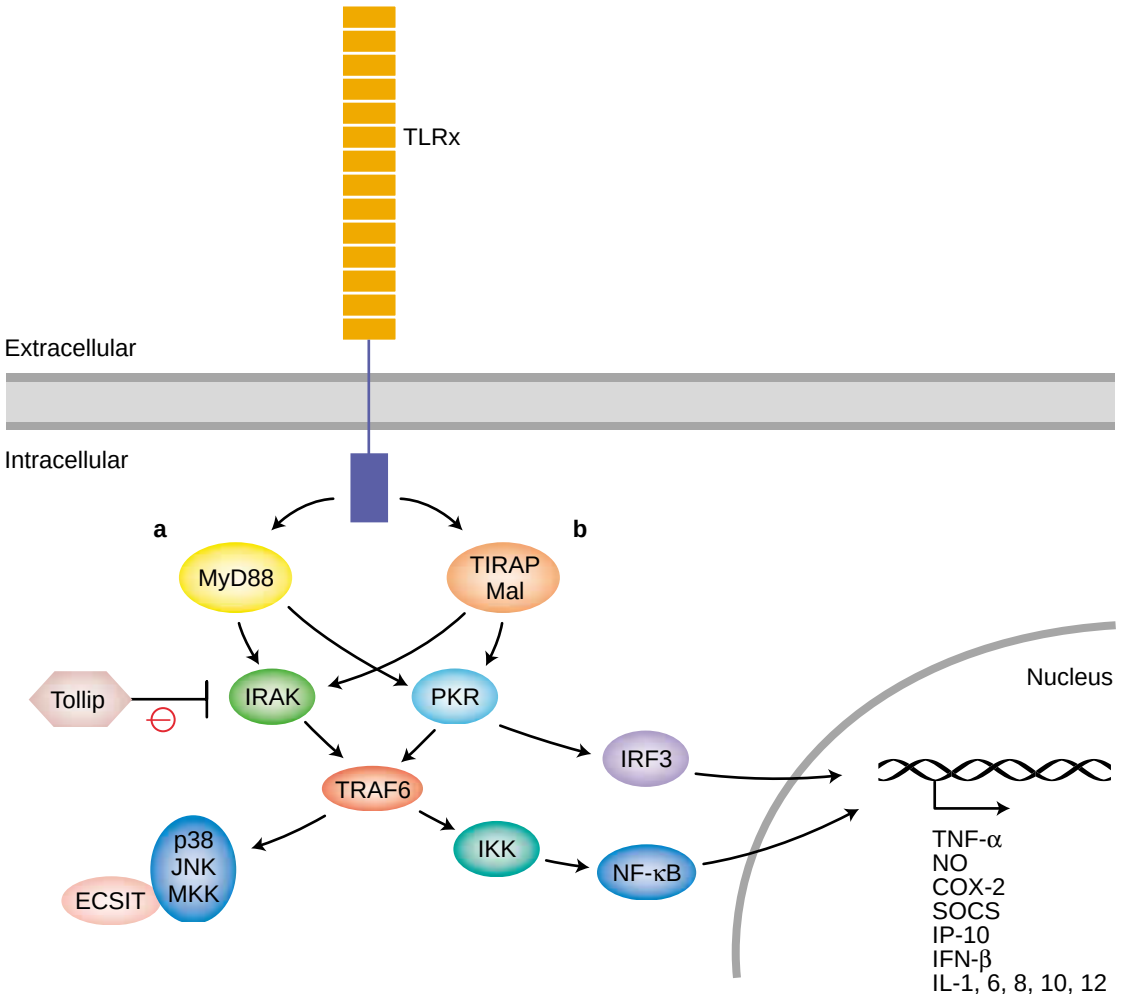
TLR signalling and downstream effects

MyD88-dependent and -independent pathways

TLRs might differentially activate distinct downstream signalling events via different cofactors and adaptor proteins mediating diverse immune responses. The conserved TIR cytoplasmic domain in TLRs is required for activation of the 'classical' TLR signalling pathway by providing a scaffold for recruitment of the adaptor molecule MyD88 and serine/threonine kinases of the IL-1R-associated kinase (IRAK) family (Fig. 2a). IRAK becomes autophosphorylated and another adaptor, TRAF6, then interacts with IRAK. In addition, TLRs bridge the signalling pathway via ECSIT (for 'evolutionarily conserved signalling intermediate in Toll pathways') to TRAF6 for p42/p44 mitogen-activated protein kinase (MAPK), p38 and JNK in response to specific bacterial products (Refs 68, 69). This signalling module results in the translocation of the transcription factor NF-κB to the nucleus, and the transcriptional activation of genes encoding cytokines and chemokines, as well as the induction of costimulatory molecules (Refs 3, 6). Toll-interacting protein (Tollip) plays an inhibitory role in TLR2/4-mediated cell activation (Ref. 29) by suppressing the activity of IRAK (Ref. 70).

MyD88-deficient mice have profound defects in the activation of antigen-specific T helper 1 (Th1) but not Th2 immune responses, suggesting distinct pathways for activation of the two effector arms of adaptive immunity (Ref. 71). LPS-induced activation of NF-κB and MAPK is not abolished in the absence of MyD88 (Ref. 72). MyD88-deficient cells respond to LPS by activating IFN-regulatory factor 3 (IRF3), and inducing IP-10 gene expression and DC maturation, suggesting MyD88-independent signal propagation via TLR4

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003



Toll-like receptor (TLR) signalling is mediated by at least two distinct pathways

Expert Reviews in Molecular Medicine ©2003 Cambridge University Press

Figure 2. Toll-like receptor (TLR) signalling is mediated by at least two distinct pathways. After recognition of a pathogen-specific molecular pattern, TLRs are capable of differentially activating distinct downstream signalling events via different cofactors and adaptor proteins mediating diverse immune responses. (a) The 'classical' MyD88-dependent TLR signalling pathway is activated via the conserved, cytoplasmic TIR domain [for 'Toll/interleukin 1 receptor (IL-1R)], which provides a scaffold for recruitment of the adaptor molecule MyD88 and serine/threonine kinases of the IL-1R-associated kinase (IRAK) family. Following IRAK autophosphorylation, the TRAF6 adaptor protein interacts and induces translocation of the transcription factor NF-κB to the nucleus, resulting in transcriptional activation of genes encoding cytokines and chemokines [e.g. tumour necrosis factor α (TNF-α), nitric oxide (NO), cyclooxygenase 2 (COX-2), SOCS (for 'suppressor of cytokine signalling'), IP-10, interferon β (IFN-β) and IL-1, 6, 8, 10, 12]. In addition, TLRs bridge the signalling pathway via ECSIT (for 'evolutionarily conserved signalling intermediate in Toll pathways') to TRAF6 for p42/p44 mitogen-activated protein kinase (MAPK) kinase (MKK), p38 and JNK in response to specific bacterial products. Toll-interacting protein (Tollip) plays an inhibitory role in TLR2/4-mediated cell activation by suppressing the activity of IRAK. (b) The MyD88-independent TLR signalling pathway is activated via the TIR-domain-containing adaptor protein (TIRAP; also designated Mal for 'MyD88-adapter-like') and results in activation of the dsRNA-binding protein kinase PKR. This protein has been proposed to be a central downstream component of both the TIRAP- and MyD88-dependent signalling pathways and could mediate potential crosstalk between them. The MyD88-independent pathway appears to utilise both IFN-regulatory factor 3 (IRF3) and NF-κB, and results in the expression of IFN-γ-inducible genes including IP-10 **(dfig002ece)**.

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

to LPS (Ref. 73). The TIR-domain-containing adaptor protein (TIRAP; also designated Mal for 'MyD88-adapter-like') (Ref. 74) has recently been identified as controlling activation of these MyD88-independent signalling pathways downstream of TLR4 (Ref. 75) (Fig. 2b). The dsRNA-binding protein kinase PKR has been proposed to be a central downstream component of both the TIRAP/Mal- and MyD88-dependent signalling pathways and could mediate potential crosstalk (Ref. 75). Receptor-interacting protein (RIP) has also recently been identified as acting downstream of TLRs as a common checkpoint (Refs 76, 77), thus possibly allowing potential regulatory crosstalks to the Nod pathway (Ref. 78) and tumour necrosis factor receptor (TNFR) pathway (Ref. 77).

IFN–STAT and JAK–STAT pathways

The IFN–STAT pathway might mediate differential patterns of gene expression in response to activation of certain TLRs. The TLR4 ligand LPS, but not any of the TLR2 ligands, induces TIRAP-dependent IFN-β mRNA expression, leading to phosphorylation of signal transducer and activator of transcription 1 (STAT1) (Ref. 79). Furthermore, IFN-γ upregulates expression of the crucial TLR4 co-receptor MD-2 through Janus kinase (JAK)–STAT in IECs, thus conferring mucosal responsiveness in inflammatory conditions of the intestine (Ref. 80). By contrast, the TLR9 agonist, CpG DNA, triggers synthesis of SOCS (for 'suppressor of cytokine signalling') proteins that act as negative regulators of the JAK–STAT pathway (Ref. 81).

Downstream effects

Activation of individual TLRs induces gene transcription of distinct cytokines in different cell types (Ref. 60), including IL-1, IL-6, IL-8, IL-10, IL-12 and TNF-α (Refs 82, 83, 84). TLR-regulated transcription of cytokine genes is coordinated by complex nucleosome remodelling, apparently independently from NF-κB (Ref. 85). This suggests that other, as-yet-unknown, TLR-inducible factors stimulate remodelling, perhaps leading to NF-κB-mediated transcription of specific cytokine genes such as IL-12. Furthermore, following stimulation with TLR2 ligands, chromatin remodelling also occurs at the TLR2 promoter region itself, possibly allowing the access of transcription factors to initiate the transcription of TLRs (Ref. 86). These observations suggest that TLR-regulated transcription is regulated by at least two independent pathways: one that activates certain transcription factors and one that leads to chromatin remodelling that allows access of the transcription factors to certain promoter regions. However, the signalling mediator that forms and controls the remodelling complex has yet to be identified.

Cytokines produced in response to pathogen-induced TLR activation might essentially drive adaptive immune responses. For instance, IL-12 secretion by DCs and phagocytes (Ref. 82) induces activation of Th1 cells and IFN-γ, which in turn enhances IL-12 production in positive-feedback loops. In response to TLR activation, released TNF-α and IL-1 might act as secondary auto/paracrine mediators through their cognate receptors TNFR-1 and IL-1R, respectively, to further activate and recruit various immune cells, thus contributing to exaggerated immune responses that contribute to inflammatory diseases.

However, TLR activation might also lead to tissue repair: necrosis-induced inflammation and tissue damage provide nonself danger signals that might function as major inducers of tissue repair gene responses via TLRs (Ref. 83). The actual TLR ligands released from necrotic cells have not yet been identified. In addition, numerous pro-inflammatory genes encoding chemokines (Ref. 86), complement proteins and adhesion molecules (Ref. 87) might also be activated in response to different TLR ligands. Nitric oxide (NO) secretion and cyclooxygenase 2 (COX-2) gene expression, which play pathophysiological roles in intestinal inflammation, might be induced via TLRs (Refs 88, 89). All of these various downstream effects are critically involved in the control of pathogen elimination and the transition to adaptive immune responses.

Tolerance and prolonged survival as keys to effective mucosal host defence

In the intestine, tolerance represents an essential mucosal defence mechanism that maintains hyporesponsiveness to harmless lumenal bacterial antigens. However, it is unclear how the intestinal epithelium discriminates between the numerous, nonharmful, indigenous microbial commensals and invasive pathogens. The molecular mechanisms underlying how IECs maintain microbial tolerance, yet efficiently react with immediate immune responses to harmful

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

lumenal bacteria during infection, are poorly defined but might involve altered TLR function combined with blockage of central downstream signalling events through IRAK.

Induction of tolerance has been shown to be associated with suppression of TLR expression (Refs 90, 91). TLR2 and TLR4 are normally only present in small amounts on IECs in vivo, thus minimising recognition of lumenal bacterial in the healthy intestine (Ref. 14). As shown by Abreu et al. (Ref. 8), decreased expression of TLR4 surface protein and the absence of cofactor MD-2 correlate with inhibition of downstream cytokine production in the tolerance of IECs to LPS. Continuous exposure to LPS leads to suppression of downstream immune responses in IECs in vitro (Refs 10, 92, 93). IL-1β, but not TNF-α, induces a LPS-refractory state in vivo (Ref. 94), suggesting that common downstream signalling molecules of both the IL-1R and TLR pathways might be targeted in tolerance. IRAK has recently been identified as an important negative regulator of TLR signalling (Refs 95, 96), with microbial tolerance correlating with downregulation of IRAK expression (Ref. 97) and inhibition of the release of IRAK from TLRs (Ref. 98) in host cells. Disruption of the IRAK response might also lead to 'cross-tolerance' (Ref. 99) between a variety of microbial components differentially affecting MyD88-dependent pathways (Ref. 100).

Conversely, a cytokine-induced imbalance in inflammation might lead to intestinal 'intolerance' by altering TLR expression and downstream signalling events that could play a role in promoting disease (Fig. 3). For instance, pro-inflammatory cytokines, such as IFN-γ, could counteract LPS-induced downregulation of TLR4 (Ref. 101) and modulate IRAK function by upregulating its expression, inhibiting its degradation and promoting its association with MyD88. This would effectively prevent the induction of tolerance (Ref. 102) and instead prime hyper-reactivity to LPS (Ref. 101).

Pathogenic bacteria have evolved various strategies to overcome recognition and develop resistance against the host. Enteric bacteria can directly block antibacterial peptide synthesis (Ref. 103). Conversely, nonpathogenic bacteria can help the host to maintain mucosal homeostasis by directly suppressing inflammatory responses and inhibiting specific intracellular signal transduction pathways. It was recently shown that nonpathogenic *Salmonella pullorum* is capable of attenuating IL-8 secretion by blocking NF-κB activation elicited by pathogenic *Salmonella typhimurium* (Ref. 104).

Commensal-associated bacterial cell wall components might even enhance survival of the host against deleterious threats of the lumen by activating TLR signalling pathways that convey signals to transcription factors that orchestrate the inflammatory response. Indeed, it has recently been demonstrated that exposure to PGN results in significant phosphorylation of Akt, an essential factor in the phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway via TLR2 and involves downstream substrates such as forkhead transcription factor (FKHR) and glycogen synthase kinase 3β (GSK3β) (Ref. 105). Furthermore, LPS phosphorylates Akt, which in turn reduces the ubiquitination of β-catenin, resulting in its nuclear accumulation and transcriptional activity (Ref. 106). Inhibition of this pathway by wortmannin (a PI3K inhibitor) leads to the predominance of LPS-induced stress responses via MAPKs and NF-κB (Ref. 107). Rac1/Akt controls NF-κB activation via TLR2 (Ref. 108). Similarly, it was recently shown that *Bacteroides vulgatus* induces NF-κB transcriptional activity via TLR4 in a PI3K/Akt-dependent pathway in IECs (Ref. 11). PI3K also facilitates the interaction of CpG DNA with TLR9 in endocytic vesicles (Ref. 109).

Apoptosis of IECs has been implicated in the generation and resolution of inflammation in response to bacterial pathogens, and TLR activation might transiently promote caspase-dependent apoptosis through MyD88 (Refs 110, 111), possibly interconnecting with Fas-associated death domain (FADD) protein via their death domains (Ref. 112). The TLR–PI3K/AKT–β-catenin innate survival mechanism might enable IECs to resist induction of apoptosis by pathogenic bacteria or other pro-inflammatory challenges and thereby maintain mucosal homeostasis in vivo. In this context, it will be essential to define how an imbalance in signalling events induced by pathogenic versus nonpathogenic bacteria could lead to impaired survival and exaggerated inflammatory responses of the mucosal immune system in intestinal disease.

Clinical and therapeutic implications

Chronic recurrent intestinal inflammation in inflammatory bowel disease (IBD) might result from stimulation of the mucosal immune system

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

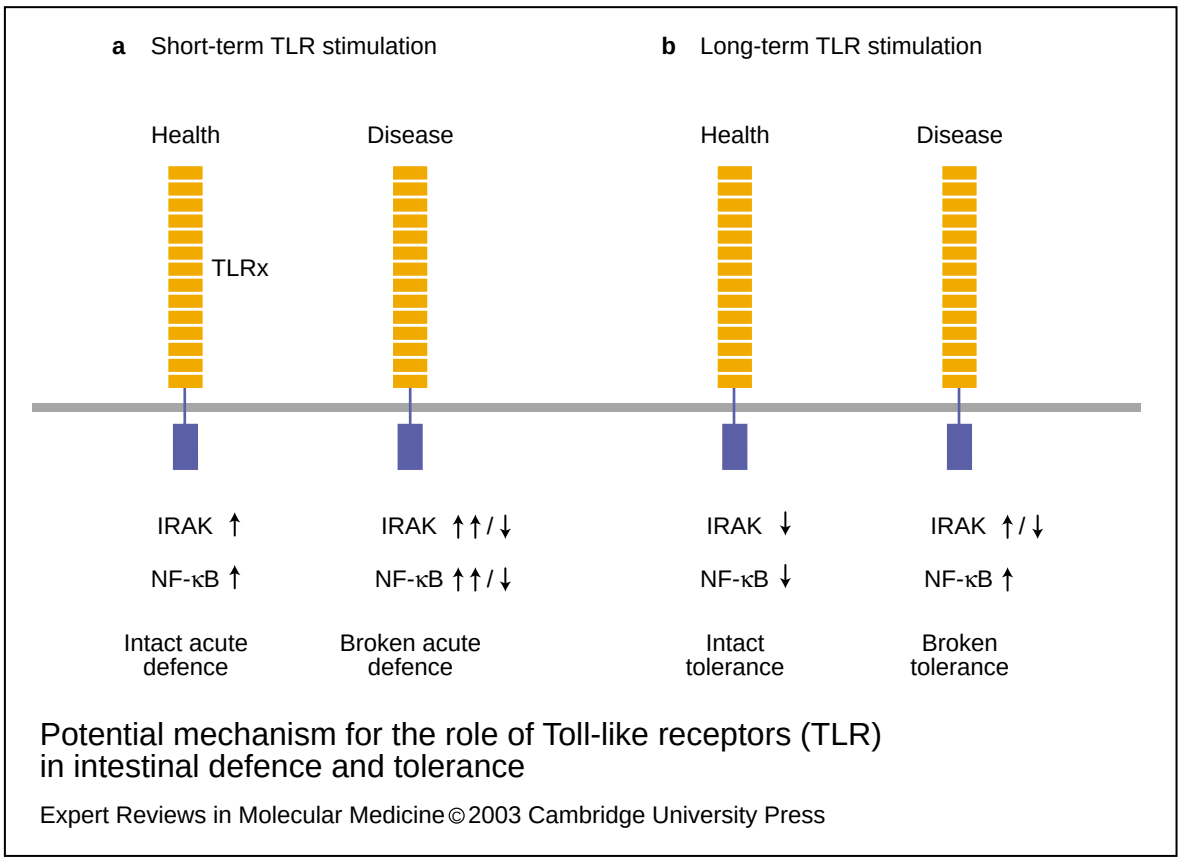


Figure 3. Potential mechanism for the role of Toll-like receptors (TLRs) in intestinal defence and tolerance. Interleukin 1 receptor (IL-1R)-associated kinase (IRAK) appears to be an important negative regulator of TLR signalling. Thus, following short-term TLR stimulation (a), expression of IRAK and NF-κB might be appropriately upregulated, thereby rapidly and efficiently defending the host against an acute threat; this acute defence mechanism might be dysregulated in disease, leading to exaggerated immune responses. Following long-term TLR stimulation (b), expression of IRAK and NF-κB might be appropriately downregulated, maintaining hyporesponsiveness in health; again, this might be dysregulated in disease **(dfig003ece)**.

by products of commensal bacteria in the lumen. Bacteria might penetrate through the impaired mucosal barrier, leading to direct interaction with immune cells of the lamina propria, such as lymphocytes, monocytes and DCs (Ref. 113). Normal and IBD tissues exhibit low significant TLR2 expression in IECs, and TLR5 is not significantly modulated in acute intestinal inflammation in IBD (Ref. 14). However, TLR2 and TLR4 are highly upregulated in inflammatory cells of the lamina propria in active IBD (Refs 14, 16), including monocytes, macrophages and lymphocytes, suggesting that these immune cells are primarily involved.

'Healthy' intestinal mucosa expresses a low concentration of TLR2 or TLR4 protein in vivo (Refs 14, 16, 80). However, there is emerging evidence that intestinal TLR expression is selectively altered in patients with IBD (Refs 14, 16). It has recently been demonstrated that TLR4 is significantly increased in IECs throughout the lower gastrointestinal tract in active disease of both Crohn's disease (CD) and ulcerative colitis (UC) (Ref. 14). However, others have failed to detect any TLR4 expression in IECs during inflammation (Ref. 16). These contrasting results might be partially due to different technical conditions used [e.g. cell-permeabilising fixation with Triton X-100 (Ref. 14) versus fixation without Triton X-100 (Ref. 16)]. In addition, it remains to be shown whether upregulated TLR4 expression simply reflects a loss of the appropiate immune response in host defence.

In acute dextran sulfate sodium (DSS)-induced colitis, bacteria and/or bacterial products play a major role in the initiation of inflammation

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

(Ref. 114). C3H/HeJ mice (which possess a spontaneous TLR4 mutation corresponding to Pro712His) do not develop spontaneous colitis but show increased susceptibility to DSS- and trinitrobenzenesulfonic acid (TNBS)-induced colitis in comparison with C3H/SnJ mice (no TLR4 mutation) (E. Cario and D.K. Podolsky, unpublished), suggesting that homeostasis between indigenous intestinal flora and the host response might be broken in TLR4 dysfunction. It remains to be determined whether TLR polymorphisms might occur more frequently in patients with IBD and could be linked with differences in the inflammatory response of the intestinal mucosa elicited by PAMPs, and thereby could potentially play a role in the pathogenesis of IBD.

Characterisation of the regulation of TLR expression in different cell types of the intestinal mucosa will be of considerable interest in order to understand tissue distribution and function in health and disease. Of note, both IL-4 and IFN-γ, which play key roles in mediating colonic inflammation in IBD, have already been shown to regulate TLR activation. IL-4 downregulates TLR4 expression in monocytes (Ref. 115) and IECs (Ref. 116), suggesting that Th2-type adaptive immune responses might inhibit TLR activation. Conversely, IFN-γ might upregulate intestinal epithelial TLR4 expression (Ref. 116), priming cells to LPS-dependent IL-8 secretion (Ref. 80).

The factors and mechanisms regulating TLR expression in IBD remain to be elucidated further. In active IBD, variant alleles in the gene encoding TLRx (where x indicates any TLR) and downstream signalling partners could induce functional dysregulation of responses to its/their ligands. Impaired function could exacerbate intestinal inflammation by uncontrolled clonal expansion and activation of mucosal immune cells to resident antigens in IBD and other inflammatory disorders of the gut. TLR dysfunction could also derive from secondary effects of nonbacterial ligands, either endogenously or exogenously induced, which have not yet been identified.

There is emerging evidence that dysregulation of the innate immune response via TLRs might contribute to many different immunological disorders, such as autoimmune diseases or chronic inflammatory responses. Targeting the TLR signalling pathways to blunt harmful cellular responses during such diseases such as IBD could provide a new therapeutic avenue. So far, neutralising antibodies against specific TLRs have mainly been used in assay systems in vitro, preventing ligand-induced activation of downstream signalling (Refs 24, 35); unfortunately, large-scale production of such antibodies for in vivo approaches would be enormously costly. Ligand-induced TLR activation might also have a beneficial effect in cancer treatment by triggering a desired antitumor immune response (Ref. 117). Soluble TLRs that bind the respective class of ligands, thus preventing recognition through the host, could also be an alternative to modulate sensing.

At present, not enough is known about the functional roles of TLRs and their downstream cascades under physiological and pathophysiological conditions to be able to define an optimal therapeutic strategy for the treatment of human disease caused by dysregulation of the innate immune response. However, initial research has started to exploit the potential adjuvant properties of TLR signalling in the modulation of colonic inflammation. It has recently been demonstrated that pretreatment with the TLR9 ligand CpG DNA ameliorates both chemically induced and spontaneous colitis in mice by inhibiting the induction of various pro-inflammatory cytokines and chemokines (Ref. 118). IECs express functional TLR9 (Ref. 64), but it remains to be verified whether CpG DNA-induced anti-inflammatory immune responses are indeed mediated via TLR9 in the intestinal mucosa. An improved understanding of the precise mechanisms leading to protective cellular immunity in the intestinal mucosa following DNA vaccination could help in the design of novel DNA constructs containing immunostimulatory features that target one or more of these signalling mechanisms via TLRs, potentially preventing exaggerated immune responses in IBD.

Conclusions and outstanding questions

In the five years that have followed the discovery of human Toll, enormous progress has been made in characterising TLR regulation and function in different cell types and animal models, mainly in identifying new TLR ligands and downstream signalling pathways. There is an emerging understanding of the complex mechanisms through which dysregulated innate immune responses might lead to disease. Definitive understanding of TLR dysfunction in active IBD and other intestinal disorders will require

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

elucidation of the structural diversity of numerous lumenal bacteria and their specific differential immunomodulatory activities via the innate immune system. Some pathogenic bacteria seem to have developed elaborate ways to escape TLR recognition. Furthermore, it is likely that others might directly limit TLR activation, effectively desensitising the mucosal immune system towards constant exposure to lumenal commensals. Thus, in disease, tolerance might essentially be broken. Other pathogenic bacteria might acutely induce TLR hyper-responsiveness, possibly in self-sustaining cycles via feedback regulators of the adaptive immune system, leading to exaggerated inflammatory immune responses. There is increasing evidence that TLRs not only recognise 'nonself' but also abnormalities of mammalian 'self'. Further in-depth evaluation of inter-dependent host–microbial, as well as host–host, cross-talks via the innate immune system could provide insight into disease pathophysiology, potentially leading to new therapeutic targets to fight microbe-associated gastrointestinal disorders such as IBD.

Acknowledgements and funding

My current research is supported by the Deutsche Forschungsgemeinschaft (Ca226/4-1 and Ca226/5-1, Priority Program Innate Immunity) and the University of Essen (IFORES). I thank Drs Bruce Beutler (Scripps Research Institute, La Jolla, CA, USA), Andre J. Ouellette (University of California, Irvine, CA, USA) and Daniel K. Podolsky (Harvard Medical School, Boston, MA, USA) for critically reviewing the manuscript and for helpful suggestions.

References

1 Janeway, C.A., Jr. (1992) The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol Today 13, 11-16, PubMed: 92153261
2 Janeway, C.A., Jr. and Medzhitov, R. (2002) Innate immune recognition. Annu Rev Immunol 20, 197-216, PubMed: 21849632
3 Medzhitov, R., Preston-Hurlburt, P. and Janeway, C.A., Jr. (1997) A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 388, 394-397, PubMed: 97379437
4 Rock, F.L. et al. (1998) A family of human receptors structurally related to Drosophila Toll. Proc Natl Acad Sci U S A 95, 588-593, PubMed: 98118556
5 Medzhitov, R. (2001) Toll-like receptors and innate immunity. Nat Rev Immunol 1, 135-145, PubMed: 21902584
6 Xu, Y. et al. (2000) Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. Nature 408, 111-115, PubMed: 20531768
7 Cario, E. et al. (2000) Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. J Immunol 164, 966-972, PubMed: 20090890
8 Abreu, M.T. et al. (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. J Immunol 167, 1609-1616, PubMed: 21359552
9 Gewirtz, A.T. et al. (2001) Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. J Immunol 167, 1882-1885, PubMed: 21382422
10 Hornef, M.W. et al. (2002) Toll-like receptor 4 resides in the Golgi apparatus and colocalizes with internalized lipopolysaccharide in intestinal epithelial cells. J Exp Med 195, 559-570, PubMed: 21866503
11 Haller, D. et al. (2002) IKK beta and phosphatidylinositol 3-kinase/Akt participate in non-pathogenic Gram-negative enteric bacteria-induced RelA phosphorylation and NF-kappa B activation in both primary and intestinal epithelial cell lines. J Biol Chem 277, 38168-38178, PubMed: 22254825
12 Kawahara, T. et al. (2001) Type I Helicobacter pylori lipopolysaccharide stimulates toll-like receptor 4 and activates mitogen oxidase 1 in gastric pit cells. Infect Immun 69, 4382-4389, PubMed: 21295088
13 Fusunyan, R.D. et al. (2001) Evidence for an innate immune response in the immature human intestine: toll-like receptors on fetal enterocytes. Pediatr Res 49, 589-593, PubMed: 21165366
14 Cario, E. and Podolsky, D.K. (2000) Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 68, 7010-7017, PubMed: 20536451
15 Smith, P.D. et al. (2001) Intestinal macrophages lack CD14 and CD89 and consequently are down-regulated for LPS- and IgA-mediated activities. J Immunol 167, 2651-2656, PubMed: 21400838
16 Hausmann, M. et al. (2002) Toll-like receptors 2

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

and 4 are up-regulated during intestinal inflammation. Gastroenterology 122, 1987-2000, PubMed: 22050398
17 Su, G.L. (2002) Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. Am J Physiol Gastrointest Liver Physiol 283, G256-265, PubMed: 22116809
18 Liu, S. et al. (2002) Role of toll-like receptors in changes in gene expression and NF-kappa B activation in mouse hepatocytes stimulated with lipopolysaccharide. Infect Immun 70, 3433-3442, PubMed: 22060659
19 Matsumura, T. et al. (2000) Endotoxin and cytokine regulation of toll-like receptor (TLR) 2 and TLR4 gene expression in murine liver and hepatocytes. J Interferon Cytokine Res 20, 915-921, PubMed: 20508579
20 Ozinsky, A. et al. (2000) The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. Proc Natl Acad Sci U S A 97, 13766-13771, PubMed: 20558581
21 Coelho, P.S. et al. (2002) Glycosylphosphatidylinositol-anchored mucin-like glycoproteins isolated from Trypanosoma cruzi trypomastigotes induce in vivo leukocyte recruitment dependent on MCP-1 production by IFN-gamma-primed-macrophages. J Leukoc Biol 71, 837-844, PubMed: 21990367
22 Iwaki, D. et al. (2002) The extracellular toll-like receptor 2 domain directly binds peptidoglycan derived from Staphylococcus aureus. J Biol Chem 277, 24315-24320, PubMed: 22086205
23 Into, T. et al. (2002) Mycoplasmal lipoproteins induce toll-like receptor 2- and caspases-mediated cell death in lymphocytes and monocytes. Microbiol Immunol 46, 265-276, PubMed: 22056500
24 Flo, T.H. et al. (2002) Involvement of toll-like receptor (TLR) 2 and TLR4 in cell activation by mannuronic acid polymers. J Biol Chem 277, 35489-35495, PubMed: 22217970
25 Werts, C. et al. (2001) Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. Nat Immunol 2, 346-352, PubMed: 21174329
26 Wang, Q. et al. (2001) Micrococci and peptidoglycan activate TLR2-MyD88-IRAK-TRAF-NIK-IKK-NF-kappaB signal transduction pathway that induces transcription of interleukin-8. Infect Immun 69, 2270-2276, PubMed: 21153588
27 Mambula, S.S. et al. (2002) Toll-like receptor (TLR) signaling in response to Aspergillus fumigatus. J Biol Chem 277, 39320-39326, PubMed: 22267279
28 Hajjar, A.M. et al. (2001) Cutting edge: functional interactions between toll-like receptor (TLR) 2 and TLR1 or TLR6 in response to phenol-soluble modulin. J Immunol 166, 15-19, PubMed: 20571875
29 Bulut, Y. et al. (2001) Cooperation of Toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and Borrelia burgdorferi outer surface protein A lipoprotein: role of Toll-interacting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling. J Immunol 167, 987-994, PubMed: 21334385
30 Alexopoulou, L. et al. (2002) Hyporesponsiveness to vaccination with Borrelia burgdorferi OspA in humans and in TLR1- and TLR2-deficient mice. Nat Med 8, 878-884, PubMed: 22146600
31 Takeuchi, O. et al. (2002) Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. J Immunol 169, 10-14, PubMed: 22072166
32 Spitzer, J.H. et al. (2002) Toll-like receptor 1 inhibits Toll-like receptor 4 signaling in endothelial cells. Eur J Immunol 32, 1182-1187, PubMed: 21929704
33 Muzio, M. et al. (2000) Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. J Immunol 164, 5998-6004, PubMed: 20281693
34 Alexopoulou, L. et al. (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413, 732-738, PubMed: 21519099
35 Matsumoto, M. et al. (2002) Establishment of a monoclonal antibody against human Toll-like receptor 3 that blocks double-stranded RNA-mediated signaling. Biochem Biophys Res Commun 293, 1364-1369, PubMed: 22050141
36 Jefferies, C.A. and O'Neill, L. (2002) Signal transduction pathway activated by Toll-like receptors. Mod Asp Immunobiol 2, 169
37 Poltorak, A. et al. (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 282, 2085-2088, PubMed: 99069627
38 Beutler, B. and Poltorak, A. (2001) The sole gateway to endotoxin response: how LPS was identified as Tlr4, and its role in innate immunity. Drug Metab Dispos 29, 474-478,

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

PubMed: 21159729
39 Hirschfeld, M. et al. (2000) Cutting edge: repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. J Immunol 165, 618-622, PubMed: 20341659
40 Nagai, Y. et al. (2002) Essential role of MD-2 in LPS responsiveness and TLR4 distribution. Nat Immunol 3, 667-672, PubMed: 22082220
41 Re, F. and Strominger, J.L. (2002) Monomeric recombinant MD-2 binds toll-like receptor 4 tightly and confers lipopolysaccharide responsiveness. J Biol Chem 277, 23427-23432, PubMed: 22075185
42 Visintin, A. et al. (2001) Secreted MD-2 is a large polymeric protein that efficiently confers lipopolysaccharide sensitivity to Toll-like receptor 4. Proc Natl Acad Sci U S A 98, 12156-12161, PubMed: 21477444
43 Mancek, M., Pristovsek, P. and Jerala, R. (2002) Identification of LPS-binding peptide fragment of MD-2, a toll-receptor accessory protein. Biochem Biophys Res Commun 292, 880-885, PubMed: 21942118
44 Triantafilou, M. and Triantafilou, K. (2002) Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. Trends Immunol 23, 301-304, PubMed: 22068377
45 Monick, M.M. et al. (2002) Interaction of matrix with integrin receptors is required for optimal LPS-induced MAP kinase activation. Am J Physiol Lung Cell Mol Physiol 283, L390-402, PubMed: 22108936
46 Meijssen, M.A. et al. (1998) Alteration of gene expression by intestinal epithelial cells precedes colitis in interleukin-2-deficient mice. Am J Physiol 274, G472-479, PubMed: 98191178
47 Bainbridge, B.W. and Darveau, R.P. (2001) Porphyromonas gingivalis lipopolysaccharide: an unusual pattern recognition receptor ligand for the innate host defense system. Acta Odontol Scand 59, 131-138, PubMed: 21392791
48 Netea, M.G. et al. (2002) Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? Trends Immunol 23, 135-139, PubMed: 21854530
49 Hajjar, A.M. et al. (2002) Human Toll-like receptor 4 recognizes host-specific LPS modifications. Nat Immunol 3, 354-359, PubMed: 21918606
50 Cario, E. et al. (2002) Commensal-associated molecular patterns induce selective toll-like receptor-trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. Am J Pathol 160, 165-173, PubMed: 21645765
51 Triantafilou, M. et al. (2002) Mediators of innate immune recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide-induced cell activation. J Cell Sci 115, 2603-2611, PubMed: 22040501
52 Hayashi, F. et al. (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410, 1099-1103, PubMed: 21223351
53 Reed, K.A. et al. (2002) The Salmonella typhimurium flagellar basal body protein FliE is required for flagellin production and to induce a proinflammatory response in epithelial cells. J Biol Chem 277, 13346-13353, PubMed: 21935392
54 Hornung, V. et al. (2002) Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 168, 4531-4537, PubMed: 21966251
55 Hemmi, H. et al. (2002) Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol 3, 196-200, PubMed: 21671738
56 Jurk, M. et al. (2002) Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. Nat Immunol 3, 499, PubMed: 22028556
57 Chu, W. et al. (2000) DNA-PKcs is required for activation of innate immunity by immunostimulatory DNA. Cell 103, 909-918, PubMed: 20578808
58 Hemmi, H. et al. (2000) A Toll-like receptor recognizes bacterial DNA. Nature 408, 740-745, PubMed: 21012018
59 Bauer, S. et al. (2001) Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. Proc Natl Acad Sci U S A 98, 9237-9242, PubMed: 21374405
60 Kadowaki, N. et al. (2001) Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. J Exp Med 194, 863-869, PubMed: 21445136
61 Krug, A. et al. (2001) Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. Eur J Immunol 31, 3026-3037, PubMed: 21475558

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

62 Leadbetter, E.A. et al. (2002) Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 416, 603-607, PubMed: 21945910
63 Takeshita, F. et al. (2001) Cutting edge: Role of Toll-like receptor 9 in CpG DNA-induced activation of human cells. J Immunol 167, 3555-3558, PubMed: 21448678
64 Akthar, M. et al. (2002) Human-derived colonic epithelial cells express the Toll-like receptor 9 (TLR9) and respond to E. coli DNA by up-regulation of IL-8 and COX-2 messenger RNA. Gastroenterology 122, A281
65 Ahmad-Nejad, P. et al. (2002) Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. Eur J Immunol 32, 1958-1968, PubMed: 22110091
66 Hacker, H. et al. (1998) CpG-DNA-specific activation of antigen-presenting cells requires stress kinase activity and is preceded by non-specific endocytosis and endosomal maturation. Embo J 17, 6230-6240, PubMed: 99016044
67 Chuang, T. and Ulevitch, R.J. (2001) Identification of hTLR10: a novel human Toll-like receptor preferentially expressed in immune cells. Biochim Biophys Acta 1518, 157-161, PubMed: 21167384
68 Vasselon, T. et al. (2002) Toll-like receptor 2 (TLR2) mediates activation of stress-activated MAP kinase p38. J Leukoc Biol 71, 503-510, PubMed: 21856744
69 Kopp, E. et al. (1999) ECSIT is an evolutionarily conserved intermediate in the Toll/IL-1 signal transduction pathway. Genes Dev 13, 2059-2071, PubMed: 99396712
70 Zhang, G. and Ghosh, S. (2002) Negative regulation of toll-like receptor-mediated signaling by Tollip. J Biol Chem 277, 7059-7065, PubMed: 21850678
71 Schnare, M. et al. (2001) Toll-like receptors control activation of adaptive immune responses. Nat Immunol 2, 947-950, PubMed: 21461142
72 Kawai, T. et al. (1999) Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity 11, 115-122, PubMed: 99361937
73 Kawai, T. et al. (2001) Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol 167, 5887-5894, PubMed: 21555199
74 Fitzgerald, K.A. et al. (2001) Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. Nature 413, 78-83, PubMed: 21429433
75 Horng, T., Barton, G.M. and Medzhitov, R. (2001) TIRAP: an adapter molecule in the Toll signaling pathway. Nat Immunol 2, 835-841, PubMed: 21417617
76 Kobayashi, K. et al. (2002) RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. Nature 416, 194-199, PubMed: 21891094
77 McCarthy, J.V., Ni, J. and Dixit, V.M. (1998) RIP2 is a novel NF-kappaB-activating and cell death-inducing kinase. J Biol Chem 273, 16968-16975, PubMed: 98307936
78 Inohara, N. et al. (2000) An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways. J Biol Chem 275, 27823-27831, PubMed: 20428692
79 Toshchakov, V. et al. (2002) TLR4, but not TLR2, mediates IFN-beta-induced STAT1alpha/beta-dependent gene expression in macrophages. Nat Immunol 3, 392-398, PubMed: 21918603
80 Abreu, M.T. et al. (2002) TLR4 and MD-2 expression is regulated by immune-mediated signals in human intestinal epithelial cells. J Biol Chem 277, 20431-20437, PubMed: 22037906
81 Dalpke, A.H. et al. (2001) Suppressors of cytokine signaling (SOCS)-1 and SOCS-3 are induced by CpG-DNA and modulate cytokine responses in APCs. J Immunol 166, 7082-7089, PubMed: 21286451
82 Thoma-Uszynski, S. et al. (2000) Activation of toll-like receptor 2 on human dendritic cells triggers induction of IL-12, but not IL-10. J Immunol 165, 3804-3810, PubMed: 20487199
83 Li, M. et al. (2001) An essential role of the NF-kappa B/Toll-like receptor pathway in induction of inflammatory and tissue-repair gene expression by necrotic cells. J Immunol 166, 7128-7135, PubMed: 21286457
84 Netea, M.G. et al. (2002) Non-LPS components of Chlamydia pneumoniae stimulate cytokine production through Toll-like receptor 2-dependent pathways. Eur J Immunol 32, 1188-1195, PubMed: 21929705
85 Weinmann, A.S. et al. (2001) Nucleosome remodeling at the IL-12 p40 promoter is a TLR-dependent, Rel-independent event. Nat Immunol 2, 51-57, PubMed: 21170165
86 Wang, T. et al. (2002) Rapid chromatin remodeling of Toll-like receptor 2 promoter during infection of macrophages with

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

Mycobacterium avium. J Immunol 169, 795-801, PubMed: 22092271
87 Khatri, S. et al. (2002) Regulation of endotoxin-induced keratitis by PECAM-1, MIP-2, and toll-like receptor 4. Invest Ophthalmol Vis Sci 43, 2278-2284, PubMed: 22086078
88 Byrd-Leifer, C.A. et al. (2001) The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. Eur J Immunol 31, 2448-2457, PubMed: 21391735
89 Perera, P.Y. et al. (2001) CD11b/CD18 acts in concert with CD14 and Toll-like receptor (TLR) 4 to elicit full lipopolysaccharide and taxol-inducible gene expression. J Immunol 166, 574-581, PubMed: 20571943
90 Hajishengallis, G. et al. (2002) Dependence of bacterial protein adhesins on toll-like receptors for proinflammatory cytokine induction. Clin Diagn Lab Immunol 9, 403-411, PubMed: 21863802
91 Wang, J.H. et al. (2002) Induction of bacterial lipoprotein tolerance is associated with suppression of toll-like receptor 2 expression. J Biol Chem 277, 36068-36075, PubMed: 22229387
92 Cario, E. and Podolsky, D.K. (2001) Prolonged exposure of lipopolysaccharide induces intestinal epithelial tolerance to endotoxin via downregulation of the Toll-like receptor signaling pathway. Gastroenterology 120, A327
93 Otte, J.M., Cario, E. and Podolsky, D.K. (2002) Cross-tolerance of TLR ligands in intestinal epithelial cells. Gastroenterology 122, A142
94 Alves-Rosa, F. et al. (2002) Interleukin-1beta induces in vivo tolerance to lipopolysaccharide in mice. Clin Exp Immunol 128, 221-228, PubMed: 22036451
95 Hu, J. et al. (2002) Regulation of IL-1 receptor-associated kinases by lipopolysaccharide. J Immunol 168, 3910-3914, PubMed: 21935499
96 Kobayashi, K. et al. (2002) IRAK-M is a negative regulator of Toll-like receptor signaling. Cell 110, 191-202, PubMed: 22145925
97 Li, L. et al. (2000) Characterization of interleukin-1 receptor-associated kinase in normal and endotoxin-tolerant cells. J Biol Chem 275, 23340-23345, PubMed: 20390101
98 Mizel, S.B. and Snipes, J.A. (2002) Gram-negative flagellin-induced self-tolerance is associated with a block in interleukin-1 receptor-associated kinase release from toll-like receptor 5. J Biol Chem 277, 22414-22420, PubMed: 22063326
99 Jacinto, R. et al. (2002) Lipopolysaccharide- and lipoteichoic acid-induced tolerance and cross-tolerance: distinct alterations in IL-1 receptor-associated kinase. J Immunol 168, 6136-6141, PubMed: 22050748
100 Sato, S. et al. (2002) A variety of microbial components induce tolerance to lipopolysaccharide by differentially affecting MyD88-dependent and -independent pathways. Int Immunol 14, 783-791, PubMed: 22090611
101 Bosisio, D. et al. (2002) Stimulation of toll-like receptor 4 expression in human mononuclear phagocytes by interferon-gamma: a molecular basis for priming and synergism with bacterial lipopolysaccharide. Blood 99, 3427-3431, PubMed: 21961565
102 Adib-Conquy, M. and Cavaillon, J.M. (2002) Gamma interferon and granulocyte/monocyte colony-stimulating factor prevent endotoxin tolerance in human monocytes by promoting interleukin-1 receptor-associated kinase expression and its association to MyD88 and not by modulating TLR4 expression. J Biol Chem 277, 27927-27934, PubMed: 22140360
103 Lindmark, H. et al. (2001) Enteric bacteria counteract lipopolysaccharide induction of antimicrobial peptide genes. J Immunol 167, 6920-6923, PubMed: 21602075
104 Neish, A.S. et al. (2000) Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. Science 289, 1560-1563, PubMed: 20425274
105 Cario, E. et al. (2002) Commensal-associated peptidoglycan activates distinct intestinal epithelial cell survival mechanisms via Toll-like receptor 2. Gastroenterology 122, A65
106 Monick, M.M. et al. (2001) Lipopolysaccharide activates Akt in human alveolar macrophages resulting in nuclear accumulation and transcriptional activity of beta-catenin. J Immunol 166, 4713-4720, PubMed: 21154076
107 Guha, M. and Mackman, N. (2002) The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. J Biol Chem 277, 32124-32132, PubMed: 22191270
108 Arbibe, L. et al. (2000) Toll-like receptor 2-mediated NF-kappa B activation requires a Rac1-dependent pathway. Nat Immunol 1, 533-540, PubMed: 21170025
109 Ishii, K.J. et al. (2002) Potential role of phosphatidylinositol 3 kinase, rather than DNA-dependent protein kinase, in CpG DNA-induced immune activation. J Exp Med 196, 269-274, PubMed: 22115074
110 Aliprantis, A.O. et al. (1999) Cell activation and

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003

apoptosis by bacterial lipoproteins through toll-like receptor-2. Science 285, 736-739, PubMed: 99357867
111 Aliprantis, A.O. et al. (2000) The apoptotic signaling pathway activated by Toll-like receptor-2. Embo J 19, 3325-3336, PubMed: 20341088
112 Choi, K.B. et al. (1998) Lipopolysaccharide mediates endothelial apoptosis by a FADD-dependent pathway. J Biol Chem 273, 20185-20188, PubMed: 98352052
113 Podolsky, D.K. (2002) Inflammatory bowel disease. N Engl J Med 347, 417-429, PubMed: 22157922
114 Hans, W. et al. (2000) The role of the resident intestinal flora in acute and chronic dextran sulfate sodium-induced colitis in mice. Eur J Gastroenterol Hepatol 12, 267-273, PubMed: 20212683
115 Mita, Y. et al. (2002) Toll-like receptor 4 surface expression on human monocytes and B cells is modulated by IL-2 and IL-4. Immunol Lett 81, 71-75, PubMed: 21830653
116 Suzuki, M. and Podolsky, D.K. (2001) Differential regulation of Toll-like receptors by pro- and anti-inflammatory cytokines between human intestinal epithelial and monocytic cell lines. Gastroenterology 120, A327
117 Hasegawa, T. et al. (2002) Toll-like receptor 2 is at least partly involved in the antitumor activity of glycoprotein from Chlorella vulgaris. Int Immunopharmacol 2, 579-589, PubMed: 21957828
118 Rachmilewitz, D. et al. (2002) Immunostimulatory DNA ameliorates experimental and spontaneous murine colitis. Gastroenterology 122, 1428-1441, PubMed: 21979342

Further reading, resources and contacts

A useful list of links (some of which do not provide free access) to websites related to various clinical aspects of immunology, including inflammatory bowel disease, is provided by the Clinical Digital Libraries Project of the University of Alabama at Birmingham:
http://uasom-dl.slis.ua.edu/clinical/immunology/general.htm

Every February, *Current Opinion in Immunology* provides a special issue of reviews on 'hot' topics in innnate immunity, including Toll-like receptors (see issues 2000, 2001 and 2002):
http://www.current-opinion.com/jimm/about.htm?jcode=jimm

A collection of excellent reviews on various topics on innate immunity can be found in the 2002 edition of *Annual Reviews in Immunology*, Volume 20:
http://immunol.annualreviews.org/

A helpful table on the essentials of the family of TLRs has been created by Naohiro Inohara, Dept of Pathology, University of Michigan Medical School:
http://www-personal.umich.edu/~ino/List/TOLLRE.htm

Medzhitov, R. (2001) Toll-like receptors and innate immunity. Nat Rev Immunol. 1, 135-145, PubMed:
Figures from this article are provided online as slides at:
http://www.nature.com/nri/journal/v1/n2/slideshow/nri1101-135a_F5.html

Features associated with this article

Figure 1. Toll-like receptors (TLRs): structure and heterodimerisation (dfig001ece).
Figure 2. Toll-like receptor (TLR) signalling is mediated by at least two distinct pathways (dfig002ece).
Figure 3. Potential mechanism for the role of Toll-like receptors (TLRs) in intestinal defence and tolerance (dfig003ece).

Citation details for this article

Elke Cario (2003) Toll-like receptors and intestinal defence: molecular basis and therapeutic implications. Exp. Rev. Mol. Med. Vol. 5, 7 July, DOI: 10.1017/S1462399403006501

Accession information: DOI: 10.1017/S1462399403006501; Vol. 5; 7 July 2003