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Editor

Encyclopedia of Signaling Molecules

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TK

▶ Kallikrein-K1

TLCN

▶ Intercellular Adhesion Molecule-5

TLN

▶ Intercellular Adhesion Molecule-5

TLR4

▶ TLR4 (Toll-Like Receptor 4)

TLR4 (Toll-Like Receptor 4)

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Synonyms

ARMD10; CD284; Cluster of Differentiation 284; Homolog of Drosophila toll; HToll; hToll; TLR4; TOLL

Historical Background

Immune responses are necessary to fight the infections incurred by various pathogens such as viruses, bacteria, and parasites in mammals (Kawai and Akira 2006). To sustain a regulated defense response, innate and adaptive immunity must harmonize and overcome the bacterial or viral challenge in which innate immunity precedes adaptive immunity. The innate and adaptive immune responses drastically differ in the type of cells involved, mode and time span of elimination, and in memorizing the specific signatures of the pathogens.

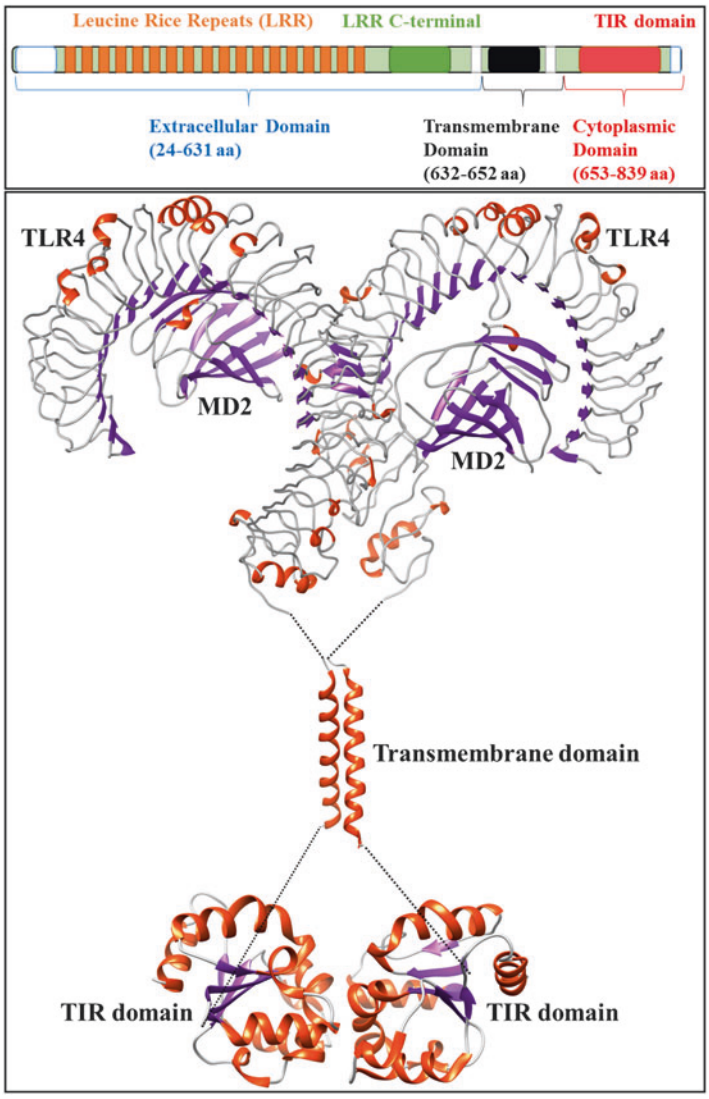
In 1989, Charles Janeway reported that a class of receptors, known as pattern recognition receptors (PRRs), recognize very specific patterns of chemical structures present on the invading pathogens, known as pathogen-associated molecular patterns (Janeway 1989). In 1996, the Hoffman group identified Toll genes, finding that Toll mutants are defective in antifungal responses in *Drosophila* (Lemaitre et al. 1996). Meanwhile, computational tools became available to further identify the Toll homologs and their roles in humans. Beyond doubt, this discovery has generated new avenues in the field of innate immunity, leading to the discovery of more genes in humans, and an understanding of the roles of these genes in inflammation and combating infectious agents (Kawai and Akira 2006).

The search for human homologs of *Drosophila* Toll genes led to the discovery of many human counterparts, such as homologs for Dorsal, Cactus, and Pelle (Hoffmann 2003). Initially, it was thought that *Drosophila* Toll was a homolog of Interleukin-1 Receptor (IL-1R). However, after the discovery of lipopolysaccharide (LPS) sensing receptor, later named Toll-like Receptor 4 (TLR4), TLR4 was recognized as the homolog of *Drosophila* Toll (Gay and Keith 1991). Interestingly, Toll-like receptors (TLRs) and IL-1R have very similar intracellular domains. In addition, *Drosophila* Toll signaling, IL-1R signaling, and Toll signaling all result in proinflammatory cytokine and chemokine production; however, TLRs also produce antiviral responses by production of Type I interferons (IFN) (Kawai and Akira 2006). In this chapter of signaling molecules, we will review the recent reports on the TLR4 structure and signaling; its role in various immune, nonimmune, and cancerous cells; its influence on cellular physiology; and its regulation.

TLR4 Structure and Function

TLR4 belongs to the type-I transmembrane receptor family and is an evolutionarily conserved protein (Medzhitov et al. 1997). Structurally, TLR4 can be divided into three distinct domains: (1) extracellular domain (1–624 aa), (2) transmembrane domain (625–658 aa), and (3) Toll/interleukin-1 receptor (TIR) domain (659–838 aa). The extracellular domain of TLR4 is rich in leucine-rich repeats (LRR) and is divided into the N-terminal region (LRRNT and LRR1–6), central region (LRR7–LRR12), and C-terminal region (LRR13–22 and LRRCT) (Kim et al. 2007) (Fig. 1). Human TLR4, located on chromosome 9, has three exons and it is transcribed to an 11,467 base-pair long pre-mRNA (Smirnova et al. 2000). Human TLR4 is structurally and functionally very similar to TLR4 in other species.

For the proper sensing of ligands and signaling initiation, TLR4 requires a coreceptor, myeloid differentiation protein 2 (MD2). The N-terminal and central domains of TLR4 clearly provide charge complementarity for binding of MD-2, forming a stable 1:1 heterodimer of TLR4 and MD2. Owing to the long and narrow shape of



TLR4 (Toll-Like Receptor 4), Fig. 1 Domain organization and structural features of TLR4. The domain organization of TLR4 is shown, depicting its various domains and motifs. The respective domain length is indicated in parentheses. In the *lower* section, the crystal structure of TLR4 is shown. There is no complete crystal structure of TLR4 defining all of its domains and motifs; therefore, to depict the relative length and position, the TM domain and TIR domain have been adopted from other crystal structures. The ectodomain, TM domain, and TIR domain have been adopted from (3FXI), (2KPF), and TLR6-TIR domain (4OM7) crystal structures, respectively. *LRR* leucine rich repeats; *MD2* myeloid differentiation protein 2; *TIR* Toll/interleukin-1 receptor homology domain; *TLR4* Toll-like receptor 4

TLR4, its surface can be divided into two regions: A and B patches. The A patch is predominantly negative and interacts with the positively charged surface of MD2, whereas the B patch is positively charged, interacting with the negatively charged surface of MD2. Any amino acid substitution that alters charge distribution may impede the LPS-mediated TLR4 signaling pathway. In addition, two polymorphic forms of TLR4, D299G, and T399I are associated with its reduced signaling intensity. The crystal structure of mutated TLR4 has been resolved; the authors reported a localized structural variation at the D299G site (Ohto et al. 2012). The second mutation T399I does not influence the structure. The proposed mechanism by which these two mutations affect TLR4 signaling might not be relevant to conformational disruption of the TLR4/MD2-LPS complex or the disruption in binding affinity of LPS. Rather, these mutations may affect folding efficiency, stability, and cell surface expression of TLR4.

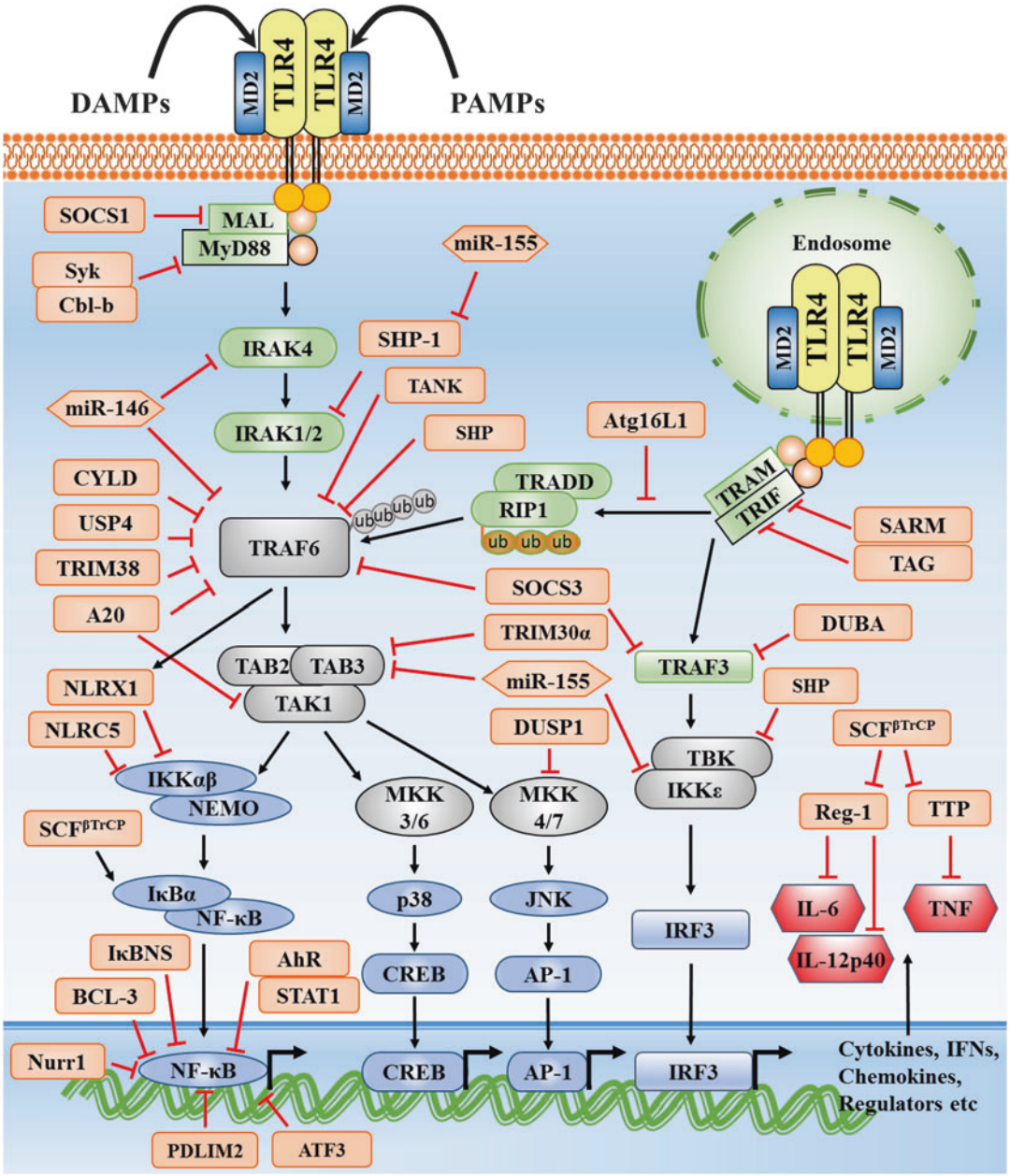
TLR4 is a type of PRR, able to detect LPS and other relevant compounds. The activation of TLR4 always produces inflammation, which is a protective physiological response to any injury or bacterial insult. Moreover, inflammation is essential to repair and integrate the damaged tissues. However, inflammation should be strictly monitored to avoid any devastating consequences due to over- or under-activation of inflammatory cells (Anwar et al. 2013). An abnormal situation arises when various diseases of microbial or nonmicrobial origin cause hyperactivation of TLR4, leading to uncontrolled inflammation. Hyperactivation of TLR4 leads to neurodegenerative diseases, atherosclerosis, diabetes, sepsis, and cancers. Hence, the inhibition of TLR4 has great clinical value. Furthermore, the expression pattern of TLR4 is quite ubiquitous and can be found on multiple cell types such as central nervous system, hematopoietic and nonhematopoietic cells, endothelial cells, and cardiac myocytes (Vaure and Liu 2014).

TLR4 Signaling

TLR4 activation is a complex process: the ligand, LPS, first binds to MD2, followed by binding of the MD2-LPS complex to TLR4, resulting in the partial complex TLR4-MD2/LPS. To form a fully functional complex, two such partial complexes dimerize, forming a hexameric complex [TLR4-MD2/LPS]2. After dimerization, TIR domains of TLR4 reorient and allow the other adaptor molecules to bind and initiate signaling (Nagai et al. 2002). Before being recognized by MD2, LPS is first detected by the LPS binding protein that transfers it to CD14. Next, CD14 delivers LPS to MD2, which noncovalently binds to TLR4. TLR4 is capable of signaling via two distinct pathways: MyD88-dependent signaling from the cell surface or the TRIF-dependent pathway from the endoplasmic compartment. These two pathways engage different adaptor molecules, producing different results (Fig. 2) (Akira and Takeda 2004).

In the MyD88-dependent pathway, MyD88 adaptor like (MAL) facilitates the recruitment of MyD88 onto the TIR domain of TLR4. Once recruited, MyD88 recruits IRAK4 (IL-1 receptor associated kinase) via their respective death domains. Next, the signal is transmitted to TRAF6 (tumor necrosis factor (TNF) receptor-associated factor 6) through IRAK1 and IRAK2. TRAF6 activates TAK1 (transforming growth factor-β-activated kinase 1), which later activates IKK (Inhibitor of κB kinase) and MAPK (mitogen-activated protein kinases) (Fitzgerald et al. 2003; Akira and Takeda 2004). IKK phosphorylates IκBα, leading to its degradation and the subsequent translocation of NF-κB, which induces transcription of proinflammatory mediators and immune-related genes. In addition, MAPK promotes activation of AP-1, which also has a role in the expression of proinflammatory cytokines.

In the MyD88-independent pathway, TRIF plays a critical role in signal transduction that ultimately activates interferon regulatory factor 3 (IRF3) and results in the late-phase activation of NF-κB and MAPK. TRIF contains an RHIM (RIP homotypic interaction motif) that facilitates its interaction with receptor interacting protein 1 (RIP1), enhancing NF-κB activation in the TRIF pathway. RIP1 deletion hampers NF-κB activation; however, it is dispensable for LPS-induced IRF3 activation (Akira and Takeda 2004).



TLR4 (Toll-Like Receptor 4), Fig. 2 TLR4 signaling pathway and its regulation. TLR4 recognizes PAMPs and/or DAMPs at the cell surface or in the endosomes, resulting in the activation of this pathway. Activated TLR4 recruits Mal (TIR-TIR interaction); MyD88 then interacts via death domains with IRAKs (that self- and cross-phosphorylate each other). This results in the activation of TRAF6 through ubiquitination. TRAF6 then releases NF-κB by first activating TAK/TABs and then IKKs that later phosphorylate and induce the destruction of IκBα via ubiquitin-mediated proteosomal degradation. NF-κB then induces proinflammatory mediators, regulators of cell physiology, and aids in negative regulation of TLR4. From TAK/TABs, TAK also triggers MAPKs that further promote inflammation by activating CREB and AP1. TLR4 later translocates into endosomes, where it triggers the TRIF-dependent pathway. TRIF interacts with RIP1 and TRADD and through a complex ubiquitination process, RIP1/TRADD complex activates TRAF6 that leads to the activation of NF-κB, which overlaps MyD88-dependent pathway and causes the second phase of inflammation. Simultaneously, TRIF also activates TRAF3,

There are various mediators in the TRIF-dependent pathway; for instance, TRIF recruits TRAF3 to activate IRF3. TRAF3 can also be associated with TANK (TRAF-associated NF-κB activator), TBK1 (TANK binding kinase 1), and IKKi (inducible inhibitor of IκB kinase). In this pathway, TBK1 and IKKi are vital for IRF3 translocation into the nucleus, where IRF3, together with NF-κB, activates the transcription of target genes, such as Type I IFN. The induction of Type I IFN and IFN-inducible genes is important for antiviral responses.

TLR4 Ligands

Various ligands have been reported to trigger TLR4 signaling. These ligands can be either exogenous, originating from microorganisms, or endogenous, cellular proteins that act as TLR4 activators (Erridge 2010). In either case, these molecules bind to TLR4, culminating in the inflammatory reaction. These ligands can be used in various vaccines as adjuvants and have been tested for various therapeutic purposes (Table 1).

TLR4 Signaling and Human Physiology

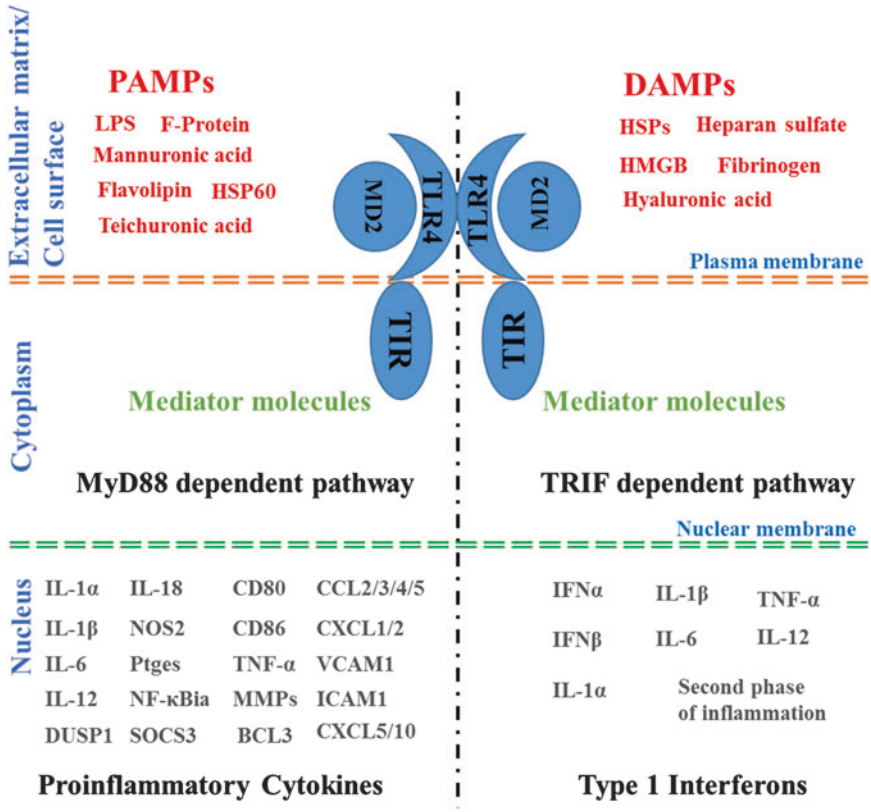
The primary purpose of TLR4 signaling is to defend the host against invading pathogens by activating the innate and adaptive immune systems. For this purpose, TLR4 is highly specific for LPS sensing. By employing various adaptor molecules, it ultimately induces the expression of inflammatory cytokines, interleukins, IFNs, and other molecules that not only activate innate immunity but also bridge innate and adaptive immune responses (Fig. 3). In addition to taking part in immune responses, TLR4 signaling can influence other pathways and physiological responses. TLR4-mediated signal transduction pathways inhibit bone osteoblast differentiation (Liu et al. 2016b). TLR4 inhibition protects neural tissue from inflammation (Gaikwad et al. 2016). LPS via TLR4 increases the sensitivity of fibroblasts to IL-32 to further enhance the TLR4/c-Jun N-terminal kinase (JNK)/AKT/cAMP response element binding protein (CREB) signaling pathway, thus leading to further inflammation (Cho et al. 2016). TLR4 knockout mice had significantly attenuated paraquat-induced cardiac contractile and intracellular Ca^{2+} derangement, as

TLR4 (Toll-Like Receptor 4), Fig. 2 (continued) which triggers IRF3 and facilitates the antiviral response via the TBK/IKK complex. There are several regulatory mechanisms acting on various proteins at different levels of the pathway. The translocation of TLR4 from the cell surface is also a regulatory measure in endosomes, where TLR4 has been disintegrated or dissociated from the ligand and recycled back to the cell surface. The negative regulators of the TLR4 pathway are displayed in the *red* box. *AP-1* activated protein 1, *ATF3* cyclic AMP-dependent transcription factor, *Atg16L1* autophagy related 16 Like 1, *BCL-3* B-cell CLL/lymphoma, *Cbl-b* Casitas B-lineage lymphoma, *CREB* cAMP responsive element binding protein 1, *CYLD* cylindromatosis, *DAMP* danger associated molecular pattern, *DUBA* deubiquitinating enzyme A, *Reg-1* Regnase-1, *DUSP1* dual specificity phosphatase 1, *IFN* interferon, *IκBα* inhibitor of κ-light polypeptide gene enhancer in B-cells, alpha, *IκBNS* inhibitor of κ-light polypeptide gene enhancer in B-cells, delta, *IKK* inhibitor of κ-light polypeptide gene enhancer in B-cells, kinases, *IL* interleukin, *IRAK* IL-1R-associated kinases, *JNK* c-Jun N-terminal Kinase, *MAL* MyD88 adaptor like, *MD2* myeloid differentiation protein 2, *miR* microRNAs, *MKK* mitogen activated protein kinase kinase, *MyD88* myeloid differentiation primary response 88, *NEMO* NF-κB essential modulator, *NF-κB* nuclear factor κB, *NLRC5* NOD-like receptor family CARD domain containing 5, *NLRX1* NOD-like receptor family member X1, *Nurr1* nuclear receptor related 1 protein, *p38* protein 38, *PAMP* pattern associated molecular pattern, *PDLIM2* PDZ and LIM domain protein 2, *RIP* receptor interacting protein 1, *SARM* sterile alpha- and armadillo-motif-containing protein, $SCF^{\beta TrCP}$ Skp1–Cul1–F-box (SCF)-type ubiquitin ligase, *SHP* small heterodimer partner, *SOCS* suppressors of cytokine signaling, *SyK* spleen tyrosine kinase, *TAB* TAK-binding protein, *TAG* TRAM adaptor with GOLD domain, *TAK* transforming growth factor-β-activated kinase 1, *TANK* TRAF associated NF-κB activator, *TLR4* toll-like receptor 4, *TNF* tumor necrosis factor, *TRADD* TNFRSF1A-associated via death domain, *TRAF* TNFR-associated factor 6, *TRAM* TRIF-related adaptor molecule, *TRIF* TIR-domain-containing adaptor protein-inducing IFN-β, *TRIM* tripartite-motif containing protein, *TTP* tristetraprolin, *USP4* ubiquitin specific peptidase 4

TLR4 (Toll-Like Receptor 4), Table 1 Ligands of TLR4. A list of exogenous (PAMP) and endogenous (DAMP) ligands, originating species/subcellular location, and their mode of action

Name	Origin/subcellular location	Mode of action	Reference
Exogenous ligands			
Lipopolysaccharide	Gram-negative bacteria	Activator	(Takeuchi et al. 1999)
Monophosphoryl lipid A	*Salmonella minnesota* R595	Weak activator	(Casella and Mitchell 2013)
Lipopolysaccharide-RS	*Rhodobacter sphaeroides*	Antagonist	(Lohmann et al. 2007)
F-protein	Respiratory syncytial virus	Activator	(Rallabhandi et al. 2012)
Mannuronic acid polymers	*Pseudomonas aeruginosa*	Activator	(Flo et al. 2002)
Teichuronic acid	*Micrococcus luteus*	Activator	(Yang et al. 2001)
Heat shock protein 60	*Chlamydia pneumoniae*	Activator	(Da Costa et al. 2004)
Flavolipin	*Flavobacterium meningosepticum*	Activator	(Kawasaki et al. 2003)
Mannan	*Saccharomyces cerevisiae* and *Candida albicans*	Activator	(Tada et al. 2002)
NS1 protein	Dengue virus	Activator	(Modhiran et al. 2015)
Mutated cholera toxin	*Vibrio cholerae*	Activator	(Liu et al. 2016a)
Endogenous ligands			
Heat shock proteins (22, 60, 70, 72)	Cytosol	Activator	(Zhou et al. 2005)
High mobility group proteins	Nucleus	Activator	(Kim et al. 2013)
Proteoglycans (versican, heparin sulfate, hyaluronic acid)	Extracellular matrix	Activator	(O'Callaghan et al. 2015)
Fibronectin	Extracellular matrix	Activator	(Okamura et al. 2001)
Tenascin-C	Extracellular matrix	Activator	(Midwood et al. 2009)
Fetuin-A	Extracellular space	Activator	(Pal et al. 2012)
Mannan-binding lectin	Extracellular space	Inhibitor	(Wang et al. 2011)
Angiotensin	Extracellular space	Activator	(Ji et al. 2009)
Amyloids	Membrane bound/secreted	Activator	(Walter et al. 2007)
Fibrinogen	Secreted/extracellular space	Activator	(Hodgkinson et al. 2008b)
β-defensin	Secreted	Activator	(Biragyn et al. 2008)
Surfactant protein	Extracellular space	Activator	(Guillot et al. 2002)
AGE-LAL (advanced glycation end-product of low-density-lipoprotein)	Extracellular space	Activator	(Hodgkinson et al. 2008a)
Ox-PAPC (oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatidylcholine)	Extracellular space	Activator	(Walton et al. 2003)
Biglycan	Extracellular matrix	Activator	(Schaefer et al. 2005)
Extra domain A	Extracellular matrix	Activator	(Okamura et al. 2001)
MRP (Myeloid-related protein)-8,14	Secreted/cytoplasm	Activator	(Vogl et al. 2007)
mm/ox-Low density lipoprotein	Extracellular space	Activator	(Miller et al. 2003)
Serum amyloid A	Secreted	Activator	(Sandri et al. 2008)
Heme	Blood/extracellular space	Activator	(Belcher et al. 2014)

well as alterations of autophagy markers (Wang et al. 2016). In human aortic endothelial cells, TNF activates high-mobility group box 1/TLR4 pathways (Yang et al. 2016). TLR4 siRNA inhibits cell proliferation, migration, and invasion by suppressing Acyl coenzyme A cholesterol acyltransferase 1 expression, suggesting that TLR4 may be a potential therapeutic target for



TLR4 (Toll-Like Receptor 4), Fig. 3 The representative TLR4 pathway and its consequences. The TLR4 pathway can be activated through various signaling stimuli that include pathogen associated molecular patterns (PAMPs, e.g., LPS, F-protein, and HSP60 of viral origin) and danger associated molecular patterns (DAMPs, e.g., HSPs and HMBG1). This pathway utilized a multitude of adaptor molecules that all converge to NF-κB in the MyD88-dependent pathway or to IRFs and NF-κB in the TRIF-dependent pathway in the cytoplasm. These transcription factors then initiate the induction of various effector molecules that not only create inflammation but also activate adaptive immunity. These effector molecules are also involved in the reshaping of cellular physiology. *BCL3* B-cell CLL/lymphoma 3, *CCL* C-C chemokine ligand, *CD* cluster of differentiation, *CXCL* C-X-C chemokine ligand, *DAMP* danger associated molecular pattern, *DUSP1* dual specificity phosphatase 1, *HMGB* high-mobility group box protein, *HSP* heat shock protein, *ICAM* intercellular adhesion molecule, *IFN* interferon, *LPS* lipopolysaccharide, *MD2* myeloid differentiation protein 2, *MMP* matrix metalloproteinases, *MyD88* myeloid differentiation primary response protein, *NF-κBia* NF-κB inhibitor-α, *NOS* nitric oxide synthase, *PAMP* pathogen associated molecular pattern, *Ptges* prostaglandin E synthase, *SOCS* suppressor of cytokine signaling, *TIR* Toll-IL-1 receptor, *IL* interleukin, *TLR4* Toll-like receptor 4, *TNF* tumor necrosis factor, *TRIF* TIR-domain-containing adaptor protein-inducing IFN-β, *VCAM* vascular cell adhesion molecule

the treatment of colorectal cancer (Ye et al. 2016). Tenascin C (TNC) upregulates IL-6 expression in human cardiac myofibroblasts, an effect mediated through the fibrinogen-like globe domain of TNC and via TLR4 (Maqbool et al. 2016). Synthesized high-density lipoprotein-like nanoparticles act as powerful endotoxin scavengers and significantly reduce LPS-mediated inflammation (Foit and Thaxton 2016). Severe bacterial infections promote malignant tumor growth through TLR4-dependent signaling (Llitjos et al. 2016). TLR4 enhances visceral pain in high-fat diet-induced obesity (Tramullas et al. 2016). In certain human pathologies, the presence of TLR4 signaling triggers ventricular dysfunction in patients undergoing bypass surgery (Avlas et al. 2015). In cancer cells, the expression of TLR4 regulates glycogen synthase kinase 3β and extracellular-regulated

kinase (ERK) phosphorylation after chemotherapy, enhancing cancer cell survival (Chung and Kim 2016). In contrast, the positive effects of TLR4 stimulation have been reported in multiple studies. TLR4 combined with nucleotide-binding oligomerization domain-containing protein 2 (NOD2) can synergistically enhance the humoral and cellular branches of adaptive immunity. Agonists of these receptors can be used in vaccinations as adjuvants (Tukhvatulin et al. 2016).

Modulation of TLR4 with Other Receptors

Recently, CD300b and its adaptor protein DAP12 have been shown to enhance LPS-induced TLR4-mediated signaling through both MyD88-dependent and MyD88-independent pathways, resulting in an exaggerated cytokine response (Voss et al. 2016). CD300b engagement also activates phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and spleen tyrosine kinase. During TLR4 activation, histamine can differentially modify actin cytoskeleton organization by upregulating IL4 production, decreasing IFNγ production, which influences T-cell priming (Aldinucci et al. 2016). TLR4 may also be involved in the expression of TLR2 in endothelial cells in a MyD88-dependent manner. In this, NADPH oxidase is critical (Fan et al. 2003). The expression of TLR2 can augment the innate immune response in endothelial cells.

Multiple complement system pathways act in coordination with TLR4 to regulate the host immune system and to provide a coordinated and balanced response against a variety of pathogenic challenges (Hajishengallis and Lambris 2010). When these complement system pathways are activated with their cognate receptors, they suppress TLR-induced mRNA expression of IL-12p35, IL-12, IL-23p40, IL-23p19, and IL-27p28, as well as production of bioactive IL-12, -23, and -27. In these pathways, the activation of PI3K and ERK1/2 assists in suppressing the transcription of crucial factors in TLRs (IRF-1 and IRF-8). Other mechanisms may also include modulation at the posttranslational level; CD46 may be involved at this level.

TLR4 may also interact with other TLRs to modulate their responses. For instance, it has been reported that the pretreatment of bone marrow macrophages with LPS can prime the inflammatory response in cases when TLR9 has been activated with 5′-cytosine-phosphate-guanine-3′ (De Nardo et al. 2009). This effect is correlated with enhanced ERK1/2, p38, and JNK activation, and it can possibly involve c-Fms-dependent and -independent mechanisms.

The cross-regulation of TLR4 through NOD2 is well defined. Recently, gene expression profiling demonstrated the global nature of this cross-regulation. In particular, NOD2 can sense the TLR4 signaling intensity. This can lead to either stimulation of NOD2 when TLR4 signaling intensity is lower or suppression of IL-12 production when TLR4 signaling is more intense. This dual behavior is centered on RIP2 and transcriptional regulator CCAAT/enhancer-binding protein α (C/EBPα), when C/EBPα is phosphorylated at 248th amino acid by protein kinase C (Kim et al. 2015).

B-cell responses during inflammation are vital and have been influenced at multiple levels by several TLRs and their ligands. B-cells showed a specific response depending on the cytokine and available ligand. In B-cells, TLR4 predominantly regulates IL-1β and IL-10, whereas TLR2 regulates IL-8 and TNF-α. The difference is due to TLR-influenced alteration in transcription factor and promoter association (Jagannathan et al. 2009). There is evidence that TLR2 and TLR9 influenced TLR4 expression in B-cells suggesting that the crosstalk between these TLRs directs B-cell responses at various levels.

Negative Regulation of TLR4 Pathway

When LPS binds, TLR4 is known to generate a severe response that often leads to inflammatory disorder and sepsis. However, upon stimulation of TLR4, regulatory mechanisms also come into play to balance the inflammatory response.

TLR4 also induces multiple proteins that provide negative feedback to restrict the overactivation of TLR4. Many proteins and mechanisms have been reported and proposed to account for such regulatory measures (Anwar et al. 2013). Here, examples of recently reported negative regulators have been provided to address the fine-tuned regulatory mechanism in the TLR4 signaling pathway.

Recently, it was reported that CD33 might hinder LPS presentation to TLR4 from CD14 in monocyte-derived immature dendritic cells. To prove this, the authors employed various strategies such as a proximity-ligation assay, analysis of the kinetics of LPS uptake by TLR4, and a plate assay (Ishida et al. 2014). The level of LPS binding on the cell surface was similar; however, when CD33 was intact, a higher level of CD14-bound LPS was observed, while the presentation of LPS from CD14 to TLR4 was reduced due to the hindrance imparted by CD33. This results in a reduced level of TLR4-bound LPS, implying defective LPS presentation from CD14 to TLR4 when CD33 is functional (Ishida et al. 2014).

Programmed cell death protein 4 (PDCD4) is recognized as an antitumor protein that enhances inflammation by activating NF-κB and suppressing IL-10. However, it has been observed that mice lacking this protein are protected from LPS-induced lethality. Moreover, in human peripheral blood mononuclear cells, when TLR4 was activated, PDCD4 was also expressed in lower quantities due to the TLR4-induced MyD88-dependent expression of miR-21, which disintegrates *PDCD4* mRNA. Thus, this microRNA has a regulatory effect on PDCD4 and negatively regulates the TLR4 pathway (Sheedy et al. 2010). By protecting the *PDCD4* mRNA from being disintegrated by miR-21, the negative effects can be abolished.

TLR4 degradation is one mechanism to negatively regulate the TLR4 signaling pathway. Wang et al. (2007) reported that Rab7b (a lysosome-associated small guanosine triphosphatase) negatively regulates LPS-induced TNF-α, IL-6, and nitric oxide production by promoting TLR4 degradation. Rab7b, which is localized in lysosome-associated membrane protein 1-positive subcellular compartments, colocalizes with TLR4 after LPS treatment and can decrease TLR4 protein levels (Wang et al. 2007).

miRs are being found to regulate numerous pathways and play important roles in TLR4 signaling. It has been reported that miR-146b was upregulated in monocytes in an IL-10-mediated STAT3-dependent loop. Once upregulated, miR-146b targets TLR4, MyD88, IRAK1, and TRAF6. Additionally, the overexpression of miR-146b can significantly downregulate several cytokines and chemokines, including IL-6 and -8; TNF-α; C-C motif chemokine ligand 2, 3, and 7; and C-X-C motif chemokine ligand 10 (Curtale et al. 2013).

Summary

TLR4 is a widely studied innate immune receptor with a well-acknowledged role in inflammation. As the sole detector of LPS in humans, it mounts the inflammatory response when bacteria manage to penetrate the physical barriers. After its activation, it floods the cells and surrounding environment with antipathogenic components, and if not regulated, this can lead to inflammatory diseases. Other than bacterial LPS, various other chemical compounds, cellular proteins, and glycoprotein components activate this signaling pathway. Activation of TLR4 has many physiological consequences; its crosstalk with other pathways not only modifies the outcome but also regulates cellular response. The role of TLR4 in several cancers has also been studied and various strategies have been devised to employ this pathway in the treatment of different inflammatory diseases and neoplasia.

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TLR4AP

▶ Toll-Like Receptor Adaptor Protein Family Members

TLR5

▶ TLR5 (Toll-Like Receptor 5)