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REVIEW

Role of TLRs as signaling cascades to combat infectious diseases: a review

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Abstract

Investigating innate immunity and its signaling transduction is essential to understand inflammation and host defence mechanisms. Toll-like receptors (TLRs), an evolutionarily ancient group of pattern recognition receptors, are crucial for detecting microbial components and initiating immune responses. This review summarizes the mechanisms and outcomes of TLR-mediated signaling, focusing on motifs shared with other immunological pathways, which enhances our understanding of the innate immune system. TLRs recognize molecular patterns in microbial invaders, activate innate immunity and promote antigen-specific adaptive immunity, and each of them triggers unique downstream signaling patterns. Recent advances have highlighted the importance of supramolecular organizing centers (SMOCs) in TLR signaling, ensuring precise cellular responses and pathogen detection. Furthermore, this review illuminates how TLR pathways coordinate metabolism and gene regulation, contributing to adaptive immunity and providing novel insights for next-generation therapeutic strategies. Ongoing studies hold promise for novel treatments against infectious diseases, autoimmune conditions, and cancers.

Keywords TLRs · Infectious diseases · Innate immunity · NF-κB · IRF3 · MyD88 · TRIF · TRAF

Introduction

Microorganisms present in the environment are a constant exposure for all living things, and their bodies must adapt to their invasion. Innate immunity serves as the first line of defence against infections in vertebrates, whereas adaptive immunity acts as the second line of defence. In contrast, adaptive immune responses are more slow processes carried out by T and B cells. These cells express a vast array of antigen receptors that are produced through DNA

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rearrangement, enabling them to react to various potential antigens. This extremely complex antigen detection mechanism is present only in vertebrates, and it has been extensively studied. Since innate immunity is a rather general system whose primary functions are to eliminate infections and present antigens to cells involved in adaptive immunity, it has received far less study than adaptive immunity. Recent studies, however, indicate that the capacity of the innate immune system to distinguish between foreign and self-pathogenic pathogens is highly developed and has a greater specificity than previously believed [1, 2]. Toll-like receptors (TLRs), a class of evolutionarily conserved receptors that play crucial roles in early host defence against invasive infections, are mostly responsible for this discrimination [1–3]. The specificity and accessibility of TLRs have gained prominence in the field of immunotherapy, where they are now investigated as potential targets for modulating immune responses in cancer and autoimmune diseases [4].

Moreover, accumulating data suggest that innate immune system activation is a necessary precondition for triggering adaptive immunity, specifically for triggering T helper 1 (TH1) cell responses [5, 6]. This profound change has altered our understanding of the etiology and management of viral, immunological, and allergy disorders, as well as cancer. Over the past few years, significant advancements have been made in understanding TLR signaling and the immune responses regulated by these receptors. Despite these advances, challenges remain in fully delineating the specific roles of the TLR signaling pathway, such as distinguishing between MyD88-dependent and MyD88-independent responses, which may unlock new therapeutic avenues in immunomodulation [7]. This review focuses on TLRs signaling pathways.

TLR/IL-1R superfamily

The identification of Toll, an insect-expressed receptor that is crucial for establishing dorsoventral polarity during embryogenesis, marked the beginning of the discovery of the TLR family [8, 9]. Further research revealed that Toll plays a crucial role in the innate immune response of insects to fungal infection. Through database searches, homologs of Toll were found, and 11 members of the TLR family have been found in mammals to date. TLRs are type I integral membrane glycoproteins that belong to a broader superfamily that also includes interleukin-1 receptors (IL-1Rs) because of their significant similarities in the cytoplasmic area. However, the extracellular regions of TLRs and IL-1Rs are very different from one another. The extracellular region of TLRs contains leucine-rich repeat (LRR) motifs, whereas the extracellular region of IL-1Rs has three immunoglobulin-like domains.

Toll/IL-1R domain

Following an overview of the TLR/IL-1R superfamily, this section explores the structural and functional roles of the Toll/IL-1R domain in immune signaling. The Toll/IL-1R (TIR) domain [10, 11] is a conserved region of approximately 200 amino acids found in the cytoplasmic tails of TLRs and IL-1Rs. The three conserved boxes that make up the regions of homology within the TIR domain are essential for signaling. TIR domains range in size and often exhibit 20–30% amino acid sequence conservation. After collection and analysis, the crystal structures of the TIR domains of human TLR1 and TLR2 were found to comprise a core five-stranded parallel sheet, which is encircled by five α-helices on each side [12–14]. Loops connect these two secondary structural elements. For instance, the BB loop joins the β-B helix and the α-B strand. The majority of the side chains of the conserved boxes 1 and 2 and the BB loop interact with adaptor molecules and are adjacent to each other. A missense mutation in the Tlr4 gene [15, 16], which modifies the sequence near the tip of the BB loop, farthest from the remainder of the TIR domain, causes C3H/HeJ mice to be less able to respond to lipopolysaccharide (LPS). This finding suggested that the mutation prevents LPS signaling by interfering with direct contact between the mutant and another molecule or molecule, notably those that have TIR domains, rather than by altering the structure of the TIR domain itself.

Leucine-rich repeats

The leucine-rich repeats (LRRs) form another essential structural component that enables ligand recognition. We explore how these domains contribute to the specificity and diversity of TLR-mediated responses. The LRR motif is present in 19–25 tandem copies in the extracellular domain of TLRs. The leucine-rich sequence XLXXLXLXX and another conserved sequence, XØXXØXF_4XXLX [17, 18], are both contained in each repeat and have a length of 24–29 amino acids. X and Ø represent any amino acid and hydrophobic amino acid, respectively. The A-helix and an α-strand joined by loops make up the repetitions. The concave surface of the LRR domains, which are arranged in a horseshoe shape by TLRs, is assumed to play a direct role in pathogen recognition. Table 1 presents the list of the principal ligands identified by various TLRs. Surprisingly, different TLRs can recognize a number of structurally dissimilar ligands [1, 5, 6, 19, 20] even though their LRR domains are conserved. There is a certain degree of correlation between the molecular patterns of ligands and the subcellular localization of distinct TLRs. On the cell surface, TLR1, TLR2, and TLR4 are attracted to phagosomes

Table 1 Receptors and ligands identified by various TLRs

Receptor	Ligand	Origin of ligand	References
TLR1	Triacyl lipopeptides	Bacteria and Mycobacteria	[2]
	Soluble factors	*Neisseria meningitidis*	[162]
TLR2	Lipoprotein/lipopeptides	Various pathogens	[163]
	Peptidoglycan	Gram-positive bacteria	[158, 164]
	Lipoteichoic acid	Gram-positive bacteria	[164]
	Lipoarabinomannan	Mycobacteria	[165]
	Phenol-soluble modulin	*Staphylococcus epidermidis*	[157]
	Glycoinositolphospholipids	*Trypanosoma cruzi*	[166]
	Glycolipids	*Treponema maltophilum*	[167]
	Porins	*Neisseria* spp.	[168]
	Atypical lipopolysaccharide	*Leptospira interrogans*	[169]
	Atypical lipopolysaccharide	*Porphyromonas gingivalis*	[163]
	Zymosan	Fungi	[170]
	Heat-shock protein 70*	Host	[171]
TLR3	Double-stranded RNA	Viruses	[77]
TLR4	Lipopolysaccharide	Gram-negative bacteria	[16]
	Taxol	Plants	[172]
	Fusion protein	Respiratory syncytial virus	[173]
	Envelope protein	Mouse mammary-tumor virus	[124]
	Heat-shock protein 60*	*Chlamydia pneumoniae*	[174]
	Heat-shock protein 70*	Host	[175]
	Type III repeat extra domain A of fibronectin*	Host	[176]
	Oligosaccharides of hyaluronic acid*	Host	[177]
	Polysaccharide fragments of heparan sulphate*	Host	[178]
	Fibrinogen*	Host	[179]
TLR5	Flagellin	Bacteria	[180]
TLR6	Diacyl lipopeptides	Mycoplasma	[181]
	Lipoteichoic acid	Gram-positive bacteria	[164]
	Zymosan	Fungi	[182]
TLR7	Imidazoquinoline	Synthetic compounds	[113]
	Loxoribine	Synthetic compounds	[22, 183]
	Bropirimine	Synthetic compounds	[22, 183]
	Single-stranded RNA	Viruses	[184]
TLR8	Imidazoquinoline	Synthetic compounds	[185]
	Single-stranded RNA	Viruses	[184]
TLR9	CpG-containing DNA	Bacteria and viruses	[27]
TLR10	N.D. (not defined)	N.D	[115]
TLR11	N.D	Uropathogenic bacteria	[186]

following activation by their corresponding ligands. On the other hand, the cell surface does not express TLR3, TLR7, or TLR9, which are involved in the identification of structures resembling nucleic acids [21–25]. For example, it has recently been demonstrated that TLR9 is expressed in the endoplasmic reticulum and that upon activation with DNA containing CpG, it is recruited to endosomal/lysosomal compartments [26, 27].

TLR/IL-1R-superfamily signaling cascade

With a brief understanding of the TLR domain, attention now turns to the signaling cascades initiated upon ligand binding, starting with the activation of key adaptor molecules and the MyD88 pathway. TLRs/IL-1Rs dimerize and undergo the conformational shift needed to attract downstream signaling molecules after ligand engagement. These proteins include the adaptor molecule myeloid differentiation primary response protein 88 (MyD88), IRAKs associated with IL-1R, TAK1-binding protein 1 (TAB1), TAB2,

TGF-κ)-activated kinase (TAK1), and TNF-receptor-associated factor 6 (TRAF6) [5, 28–30].

MyD88

MyD88 is crucial in most TLR-mediated signaling pathways. Initially, MyD88 was identified as a gene that is quickly activated when M1 myeloleukemic cells differentiate into macrophages in response to IL-617. A brief linker sequence divides the carboxy (C)-terminal TIR domain of the encoded protein from its amino (N)-terminal death domain (DD). After being stimulated with IL-1, MyD88 was cloned to serve as an adapter molecule that attracts IRAK to the IL-1R complex [4, 31–35]. The DD-DD interaction mediates the relationship between MyD88 and IRAK. Through DD-DD and TIR-TIR domain interactions, MyD88 forms homodimers. When it is recruited to the receptor complex21, it functions as a dimer. Thus, MyD88 serves as an adapter that connects downstream signaling molecules containing DDs to TLRs/IL-1Rs.

IRAK family

Mammals have revealed four IRAKs with unique gene expression patterns: IRAK1, IRAK2, IRAK4, and IRAK-M. IRAKs have a core serine/threonine kinase domain and an N-terminal DD. Although IRAK2 and IRAK-M do not exhibit detectable kinase activity, IRAK1 and IRAK4 exhibit intrinsic kinase activity. After TLR/IL-1R activation, the kinase activity of IRAK1 increases significantly, and its kinase domain is crucial for signaling through nuclear factor-κB (NF-κB). However, as the overexpression of an IRAK1 kinase-deficient mutant may potently stimulate NF-κB activation in IRAK1-deficient cells, kinase activity by itself is not necessary for signaling [36, 37]. On the other hand, a kinase-inactive mutant of IRAK4 is expressed and prevents IL-1-mediated NF-κB activation, but IRAK4 overexpression does not result in strong NF-κB activation. Moreover, IRAK1 directly substrates IRAK4 but not through other pathways [38, 39]. IRAK4-deficient mice showed almost no response to IL-1, LPS, or other bacterial components, indicating that IRAK4 has a crucial function in IL-1R/TLR signaling [40, 41]. In contrast, cytokine production in response to IL-1 and LPS was reduced but not eliminated in IRAK1-deficient mice [42–47]. Patients with an inherited defect in IRAK4 were recently identified [48, 49]. These individuals did not react to stimulation by any of the five TLRs (TLR2, TLR3, TLR4, TLR5, or TLR9), IL-1, or IL-18. These findings demonstrate that IRAK4 works upstream of IRAK1 and that TLR signaling requires IRAK4 and its kinase activity.

TRAF6

The family of adaptor proteins known as TRAFs has been preserved throughout evolution [50, 51]. To date, the TRAF family has been identified in six different animals. These members are distinguished by having two distinct domains: TRAF-N, which is the N-terminal coiled-coil domain, and TRAF-C, which is the C-terminal domain. While the TRAF-C domain promotes self-association and interacts with upstream receptors and signaling proteins, the N-terminal region of most TRAF proteins has a RING (very intriguing new gene)-finger/zinc-finger region that is critical for downstream signaling processes. Both the TNF-receptor superfamily and the TLR/IL-1R superfamily rely on TRAF6 as a signaling mediator. It interacts directly with members of the TNF receptor superfamily, such as CD40 and the TNF-related activation-induced cytokine receptor TRANCER, or indirectly with members of the TLR/IL-1R superfamily through its association with IRAKs. The consensus sequence P-X-E-X-X-(D/E/F/W/Y) has been found for the TRAF6-binding domain [52–54]. This motif is present in CD40, TRANCER, and IRAKs,IRAK1, IRAK2, and IRAK-M include three of these TRAF6-binding motifs.

TAK1 and TABs

TAK1, two adaptor proteins, TAB1 and TAB2, and the transcription factors NF-B and activator protein 1 (AP1) are activated by TRAF6. TAK1 belongs to the mitogen-activated protein kinase (MAPKKK) 32 family, which has been demonstrated to be necessary for NF-κB activation induced by TNF and IL-1/LPS33. TAB1 and TAB2, two TAK1-binding proteins, have been identified [53, 55–57]. TAB1 functions as an activator of TAK1 because ectopic coexpression of TAK1 [53, 58] increases its kinase activity. TAB2, on the other hand, acts as an adapter by connecting TAK1 to TRAF6 and promoting TAK1 activation [55, 56]. However, NF-κB36 activation by TNF-α or IL-11/LPS is not affected in embryonic fibroblasts derived from TAB2-deficient animals. Moreover, TAB3, a novel molecule that resembles TAB2, was recently discovered. Like TAB2, it has been demonstrated to interact with TAK1 and trigger NF-κB [59, 60]. The activation of TAK1 and NF-κB produced by TAB2 and TAB3 was suppressed by the cotransfection of SMALL-INTERFERING RNA (siRNAs) directed against both TAB2 and TAB3. These findings suggest that TAB2 and TAB3 serve redundantly as mediators of TAK1 activation. It has been demonstrated that TRAF6 acts as an E3 ubiquitin ligase38 and that UBIQUITYLATION is important for TAK1 activation. TRAF6 can interact with ubiquitin-conjugating enzyme 13 (UBC13) through its RING-finger domain. The formation of a lysine-63-linked

polyubiquitin chain by the UBC13-TRAF6 complex then causes TAK1 activation [61, 62].

NF-κB

The five transcription factors that make up the NF-κB family are p65 (REL-A), REL-B, cytoplasmic (c) REL, p50, and p52. These factors can operate as homo- and heterodimers. Inactive NF-κB dimers are typically trapped in the cytoplasm by molecules belonging to the inhibitor of NF-κB (IκB) family. The phosphorylation and proteolysis of IκB proteins, along with the simultaneous release and nuclear translocation of NF-κB factors, are the processes that activate NF-κB. The IKK complex, which consists of the catalytic subunits IKK-α and IKK-β (also known as IKK1 and IKK2) and the regulatory subunit IKK-γ (also known as NF-κB essential modulator, NEMO), mediates this acute activation process [63, 64]. IKK phosphorylates IκBs following upstream signal activation, which causes polyubiquitylation and proteasome-mediated destruction. A different mechanism for activating NF-κB has recently been proposed. It involves the activation of IKK-α by NF-κB-inducing kinase (NIK), which phosphorylates the NF-κB2 precursor protein p100 [65, 66]. An SCF (S-phase kinase-associated protein 1-Cullin1-F box)-family E3 ubiquitin ligase, β-transducin repeat-containing protein (β-TRCP), recognizes phosphorylated p100 and catalyzes its polyubiquitylation, which causes the proteasome to process it into the transcriptionally active p52 form. Sequences in the C-terminal region of p100 strictly regulate this proteolytic event [30, 67]. Despite these discoveries, the nuclear translocation of NFκ-B might not be sufficient to activate NFκ-B-dependent transcription on its own. A number of kinases, including cyclic AMP (cAMP)-dependent protein kinase (PKA), casein kinase II, protein kinase C-ζ (PKC-ζ), and IKK itself, have been implicated in the posttranslational regulation of some NF-κB proteins, most notably p65 [63, 64].

Fitting the TLR/IL-1R signaling pathway together

MyD88 is drawn to the cytoplasmic TIR domain upon TLR/IL-1R stimulation, where it promotes IRAK4 binding to the receptor complex via homophilic DD contact. MyD88 binds to IRAK4 to help phosphorylate one or more essential residues in the kinase-activation loop of IRAK1, which in turn triggers the kinase activity of IRAK1. Following activation, IRAK1 hyperphosphorylates residues in its N-terminus, which allows TRAF6 to attach to this complex. After disengagement from the receptor, the IRAK1-TRAF6 complex interacts with another premade complex composed of TAK1, TAB1, and either TAB2 or TAB3. TAB2/TAB3 and TAK1 become phosphorylated as a result of this interaction, and they then enter the cytoplasm alongside TRAF6 and TAB1. After that, TAK1 is activated in the cytoplasm, which causes IKKs to become active. IKKs then phosphorylate IIIBs. This

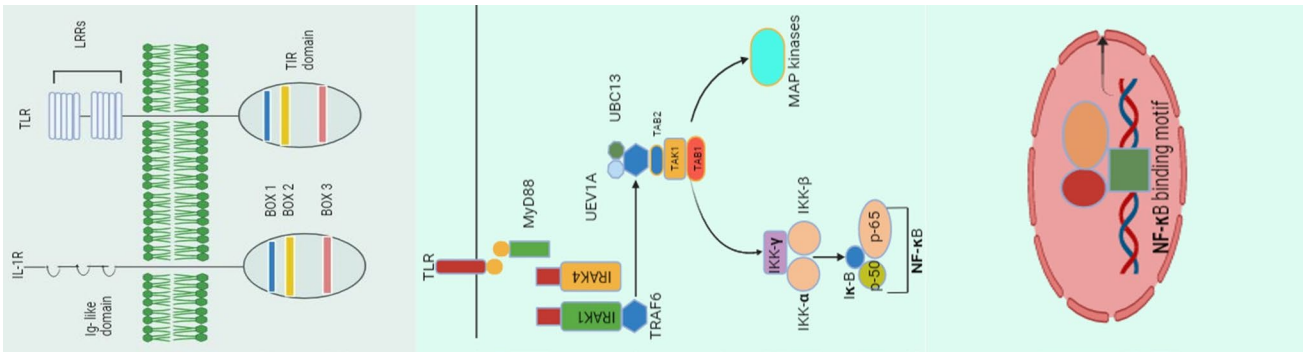

Fig. 1 TLR structure and signaling pathway. The Toll/IL-1R (TIR) domain is a conserved cytoplasmic region shared by interleukin-1 receptors (IL-1Rs) and toll-like receptors (TLRs). There are three highly homologous sections (boxes 1, 2, and 3) that make up the TIR domain. Although the cytoplasmic domains of both molecules are similar, their extracellular sections are very different. TLRs include tandem repeats of leucine-rich regions, leucine-rich repeats, or LRRs, whereas IL-1Rs have three domains that resemble immunoglobulins (Igs). MyD88 (myeloid differentiation primary-response protein 88) is activated by TLR stimulation, which in turn attracts IRAK4 (IL-1R-associated kinase 4), which permits the interaction of IRAK1. IRAK4 subsequently phosphorylates IRAK1. Through its association with phosphorylated IRAK1, TRAF6 (tumor necrosis factor receptor-associated factor 6) is also recruited to the receptor complex. Following their phosphorylation, IRAK1 and TRAF6 separate from the receptor and assemble at the plasma membrane to form a complex with TAK1 (transforming-growth factor-ε-activated kinase), TAB1 (TAK1-binding protein 1), and TAB2 (not shown). This complex triggers the phosphorylation of TAB2 and TAK1. At the plasma membrane, IRAK1 is broken down, resulting in a complex (made up of ubiquitin-conjugating enzyme 13). To associate with the ubiquitin ligases UBC13 and UEV1A (ubiquitin-conjugating enzyme E2 variant 1), TRAF6, TAK1, TAB1, and TAB2 are translocated to the cytoplasm. As a result, TRAF6 becomes ubiquitylated, activating TAK1 during this process. TAK1 phosphorylates the IKK complex (inhibitor of nuclear factor-B)-kinase complex), which is composed of IKK (also known as IKK1, IKK2, and nuclear factor-B (NF-B) essential modulator, NEMO). TAK1 also phosphorylates MAP kinases

phosphorylation causes IIIB to break down, which then allows NF-κB to be released. JUN N-terminal kinase (JNK) and other MAPKs are activated in response to TAK1 activation (Fig. 1).

In summary, Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-I-like receptors (RLRs) are components of the innate immune system and supplement each other, facilitating different types of recognition of pathogens in several locations of the body. TLRs focus on extracellular and endosomal microbes, revolving mainly around MyD88 and Toll/IL-1R domain-containing adaptor-inducing IFN-β (TRIF) pathway integrators to activate NF-κB and IRF factors, which induce the expression of proinflammatory cytokines and type 1 interferons [68]. However, NLRs and RLRs are intracellular pattern recognition receptors; for example, NOD-like receptors (NLRs) are cytoplasmic receptors that sense bacterial pathogens and damage cells, triggering inflammatory cells through classrooms and inducing factors known as inflammasomes, whereas RIG-like receptors (RLRs) detect viral RNA and interact with mitochondrial-associated viral signaling (MAVSs) to activate interferons against the virus. Although these receptors activate distinct signaling programs, they are involved in common inflammatory pathways, enabling the immune system to respond depending on how and where the pathogen is located [6, 68].

MyD88-dependent and -independent pathways

When exposed to IL-1 or microbiological components recognized by TLR2, TLR4, TLR5, TLR7, or TLR9, MyD88-deficient animals do not release TNF-α or IL-6 [31, 69]. Therefore, MyD88 is necessary for defence against a variety

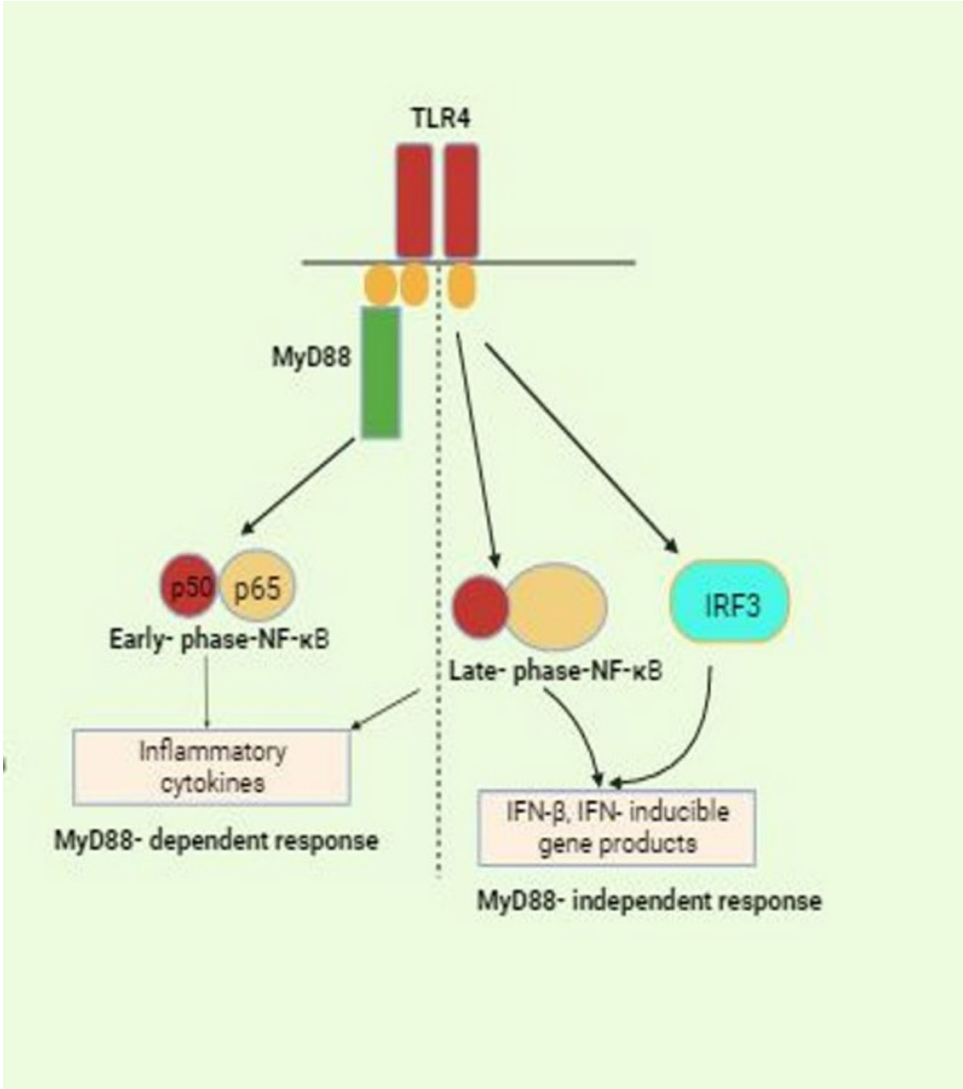

Fig. 2 Structure and mechanism of TLR4, highlighting both MyD88-dependent and MyD88-independent pathways. Two pathways are facilitated by stimulation of Toll-like receptor 4 (TLR4): the MyD88 (myeloid differentiation primary-response protein 88)-dependent pathway and the MyD88-independent pathway. The early stage of nuclear factor-κB (NF-κB) activation, which results in the synthesis of inflammatory cytokines, is involved in the MyD88-dependent pathway. IFN-regulatory factor (IRF3) and the late phase of NF-κB activation are both activated by the MyD88-independent pathway, which results in the synthesis of IFN-β and the expression of IFN-inducible genes

of microbial constituents. However, more investigations into MyD88-deficient cells have revealed the presence of both MyD88-dependent and MyD88-independent signaling pathways that respond to LPS45 (Fig. 2). For example, in myD88-deficient macrophages, the activation of NF-κB in response to a mycoplasmal lipopeptide, a TLR2 ligand, is eliminated; however, NF-κB activation still occurs in response to LPS, a TLR4 ligand, albeit with delayed kinetics. Additionally, LPS-stimulated MyD88-deficient macrophages exhibit delayed MAPK activation.

The genes expressed in MyD88-deficient macrophages after exposure to LPS46 were further used to characterize the MyD88-independent pathway. Glucocorticoid-attenuated response gene 16 (GARG16), immune responsive gene 1 (IRG1), and the gene encoding CXC-chemokine ligand 10 (CXCL10, the product of which is also known as IFN-γ-inducible 10 kDa protein, IP10) are among the genes that have been identified as interferon (IFN)-inducible. Genes encoding inflammatory cytokines, including TNF, IL-6, and IL-1ε, were not expressed, as expected. In TLR4-deficient macrophages, the induction of IFN-inducible genes was eliminated, indicating that TLR4-dependent but MyD88-independent production of CXCL10, GARG-16, and IRG1 occurred. In contrast, TLR2 does not use this MyD88-independent route since activation with TLR2 ligands does not increase the expression of IFN-inducible genes. Research using IFN-α/β-deficient mice revealed that the production of CXCL10 in response to LPS is primarily a secondary effect of IFN-β production [70–73]. The LPS-mediated maturation of dendritic cells (DCs) is facilitated by the MyD88-independent pathway, which also induces the expression of IFN-inducible genes49. MyD88-deficient bone marrow-derived DCs stimulate the proliferation of T lymphocytes and upregulate the cell-surface expression of costimulatory molecules such as CD40, CD80, and CD86 when cultured with LPS. On the other hand, TLR4-deficient DCs do not develop in response to LPS, suggesting that DC maturation occurs independently of MyD88 [74–76]. The induction of costimulatory molecules can be achieved through either the MyD88-dependent or MyD88-independent pathway, although stimulation of wild-type DCs with TLR2 ligands (via the MyD88-dependent pathway) or TLR4 ligands (through the MyD88-dependent and MyD88-independent pathways) was found to increase the cell-surface expression of costimulatory molecules. For TLR4, IFN-β47 production largely outweighs the MyD88-independent synthesis of costimulatory molecules. On the other hand, the MyD88-dependent route regulates the TLR-mediated expression of genes that encode inflammatory cytokines, even though TLR4-mediated production of inflammatory cytokines involves both MyD88-dependent and MyD88-independent pathways [77–80]. Consequently, during TLR signaling, the expression of genes encoding costimulatory molecules and inflammatory cytokines is differentially regulated. Although MyD88 has been linked to TLR3 signaling [81, 82], TLR3 appears to primarily transduce its signals through a MyD88-independent pathway, as stimulation with the TLR3 ligand polyinosinic-polycytidylic acid (poly(I:C)) does not impair the generation of costimulatory molecules and inflammatory cytokines in mice lacking MyD88. The MyD88-dependent pathway mainly stimulates the production of inflammatory cytokines, which are essential for rapid defence against microbial infection. In contrast, the MyD88-independent pathway, particularly in response to TLR4, regulates slower responses, such as the expression of IFN-inducible genes and the maturation of dendritic cells.

Adaptor family

The discovery of the MyD88-independent pathway led researchers to characterize the signaling pathways of various TLRs, the activation of which leads to different patterns of gene expression. As a result, the molecular mechanisms underlying such differences can now be explained, at least in part, by the existence of several adaptors, which are used by different TLRs. These adaptors, which all have TIR domains, include the following (in order of identification): MyD88, TIRAP (TIR-domain-containing adaptor protein; also known as MyD88-adaptor-like protein, MAL), TRIF (TIR-domain-containing adaptor protein inducing IFN-β; also known as TIR-domain-containing molecule 1, TICAM1), and TRAM (TRIF-related adaptor molecule; also known as TIR-domain-containing molecule 2, TICAM2) (Fig. 3).

TIRAP

The discovery of the second TIR domain-containing adaptor, TIRAP, resulted from the identification of the TLR4 signaling pathway, which is independent of MyD88 [75, 76, 83, 84]. TIRAP, which lacks a DD such as MyD88, was once believed to mediate the TLR4 signaling pathway that is independent of MyD88. Nevertheless, the physiological involvement of TIRAP was revealed by the generation of knockout mice; these animals still exhibited delayed activation of NF-κB and expression of IFN-inducible genes, but their ability to produce inflammatory cytokines in response to LPS was compromised [75, 76, 81, 85], showing that TIRAP is necessary for the TLR4-mediated, MyD88-dependent signaling pathway but not the MyD88-independent pathway, as this phenotype resembled that of MyD88-deficient mice. To rule out the possibility that MyD88 and TIRAP perform redundant functions in the MyD88-independent pathway, both genes were deleted in mice. TIRAP most likely functions upstream of MyD88 because forced overexpression of MyD88 in TIRAP-deficient embryonic fibroblasts activates

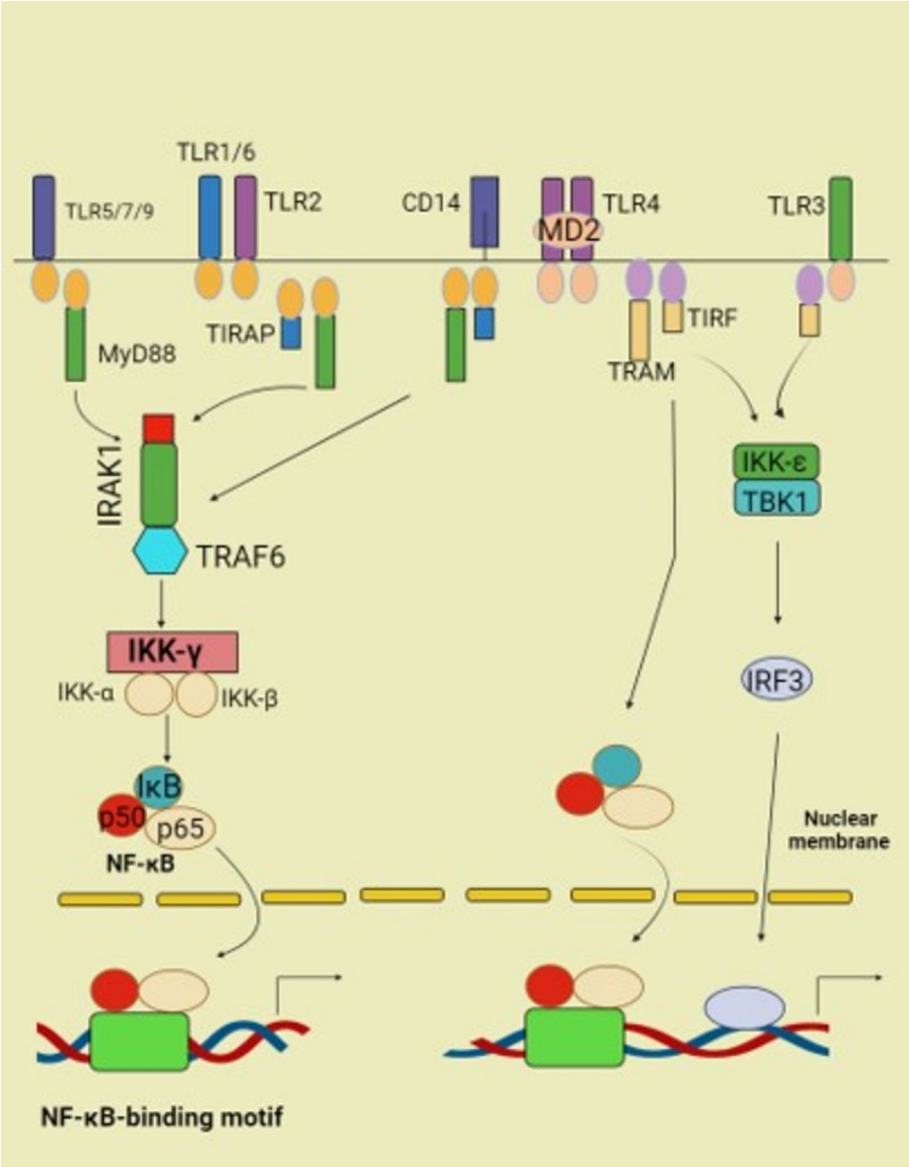

Fig. 3 Involvement of TIR domain-containing adaptors in TLR signaling pathways. MyD88, or myeloid differentiation primary response protein 88, is an adaptor molecule that contains the Toll/interleukin-1 (IL-1) receptor (TIR) domain. This adaptor mediates the Toll-like receptor (TLR)-signaling pathway, which in turn activates IRAKs, IL-1-receptor-associated kinases, and TRAF6, or the tumor necrosis factor receptor (IKK) complex, which is composed of IKK-α, IKK-β, and IKK-γ (also known as IKK1, IKK2, and nuclear factor-κB (NF-κB) essential modulator, NEMO, respectively), is activated upon the activation of related factor 6. TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, and TLR9 all employ this pathway to liberate NF-κB from its inhibitor, allowing it to enter the nucleus and produce inflammatory cytokines. Through TLR2 and TLR4, the MyD88-dependent signaling pathway is mediated by TIR domain-containing adaptor protein (TIRAP), another TIR domain-containing adaptor protein. On the other hand, MyD88 is not required for the TLR3- and TLR4-mediated activation of interferon (IFN)-regulatory factor 3 (IRF3) or the induction of IFN-β. The MyD88-independent route requires TRIF (TIR domain-containing adaptor protein producing IFN-β), a third TIR domain-containing adaptor. IRF3 is activated downstream of TRIF via the nontypical IKKs IKK-ε and TRAF family member-associated NF-κB activator (TANK)-binding kinase 1 (TBK1). TRAM, or TRIF-related adaptor molecule, is a fourth TIR domain-containing adaptor that is unique to the TLR4-mediated, MyD88-independent/TRIF-dependent pathway

the NF-κB-dependent promoter but not the other pathway. Interestingly, while TIRAP-deficient mice respond normally to TLR3, TLR7, and TLR9 ligands, they also exhibit reduced cytokine production in response to TLR2 ligands [29, 75, 76, 81, 86]. TIRAP is, therefore, necessary for MyD88-dependent signaling via TLR2 and TLR4.

Further database searches for TIR domain-containing proteins were carried out in response to the analysis of

TIRAP-deficient mice, which suggested that TIR domain-containing molecules might mediate the specificity of various TLR signaling pathways. These searches resulted in the discovery of TRIF [87, 88], a third TIR domain-containing adaptor. Although it was referred to as TICAM1 by several authors, the same molecule was also shown to bind TLR3 in a yeast two-hybrid screen [89, 90]. In HEK293 (human embryonic kidney 293) cells, forced expression of TRIF activated the IFN-β promoter but not MyD88 or TIRAP. Conversely, a dominant-negative version of TRIF prevents TLR3-dependent activation of the IFN-β promoter. According to these in vitro investigations, TRIF induces IFN-β via a route that is not dependent on MyD88. Targeted deletion of Trif in mice subsequently provided insight into the physiological function of Trif. These TRIF-deficient mice presented reduced expression of IFN-inducible genes50 and poor activation of IFN-regulatory factor 3 (IRF3) in response to TLR3 and TLR4 ligands. In line with this, Trif was found to encode a gene involved in TLR3- and TLR4-mediated responses in an investigation of LPS-hyporesponsive animals produced by random germline mutagenesis [77, 80]. Consequently, research employing two distinct strains of Trif-mutant mice has shown that TRIF is necessary for TLR3- and TLR4-mediated activation of the MyD88-independent pathway, which in turn triggers the generation of IFN-β. Furthermore, inflammatory cytokine production was defective in TRIF-deficient mice in response to TLR4 ligands but not in response to other TLR ligands. On the other hand, the phosphorylation of IRAK1 and early-phase activation of NF-κB indicated that TLR4-mediated activation of the MyD88-dependent pathway was not compromised [77–79]. These results indicate that TLR4 requires signals that are both MyD88-dependent and MyD88-independent/TRIF-dependent to induce the production of inflammatory cytokines. On the other hand, in response to the ligation of TLR2, TLR5, TLR7, or TLR9, none of which activate the MyD88-independent/TRIF-dependent pathway, myD88-dependent pathway activation alone is sufficient to cause the generation of inflammatory cytokines. Why TLR4-mediated signaling pathways stimulate the production of inflammatory cytokines through both MyD88-dependent and MyD88-independent pathways is yet unknown. These results, however, could also suggest that inflammatory cytokine induction requires an unknown molecule or molecules triggered via the MyD88-independent/TRIF-dependent pathway in addition to NF-κB.

TRAM

Sequence homology in database searches has recently revealed the existence of TRAM, a fourth adaptor that contains TIR domains [75, 76, 87, 91–96]. Several in vitro studies revealed that TRAM binds to TRIF and TLR4 but not to TLR3 [87, 93–96] and that TRAM plays a significant role in the activation of IFN-β- and IFN-inducible genes mediated by TLR4 but not by TLR3 when its expression is inhibited by siRNA [93, 95, 96]. Further research using TRAM-deficient animals demonstrated that TRAM is crucial for the MyD88-independent cascade of signals generated by TLR4. Like TRIF-deficient animals, TRAM-deficient mice presented decreased expression of IFN-inducible genes and poor IRF3 activation in response to TLR4 ligands. In contrast, TRAM-deficient animals respond normally to TLR3 stimulation, in contrast to TRIF-deficient mice [91, 92, 97]. Thus, TRAM plays a unique role in activating the TLR4-mediated MyD88-independent/TRIF-dependent signaling pathway. Furthermore, whereas IRAK1 and early-phase NF-κB activation are normal, mice lacking TRAM do not produce inflammatory cytokines in response to LPS, similar to TRIF-deficient mice [27, 91, 92]. These findings suggest that although the exact underlying mechanisms are still unclear, TRAM and TRIF play a role in the TLR4-mediated production of inflammatory cytokines.

Differential use of adaptors in TLR signaling

The crucial roles that TIR domain-containing adaptors play in TLR signaling have been demonstrated by their characterization (Fig. 3). TLR-mediated inflammatory cytokine synthesis requires MyD88. On the other hand, MyD88 is not required for the induction of type I IFNs (IFN-α/β) upon stimulation with TLR3 or TLR4. TRIF is the only factor influencing this MyD88-independent reaction. Furthermore, TRAM is a particular adaptor in the TLR4-mediated MyD88-independent/TRIF-dependent pathway, whereas TIRAP is uniquely implicated in TLR2- and TLR4-mediated MyD88-dependent activation. As a result, TIRAP and TRAM offer specificity for various TLR signaling channels. As demonstrated by in vitro tests, the cytoplasmic part of TLR4 binds directly to TRAM but not to TRIF. This finding suggested that TLR4 may be able to generate type I IFNs by interacting with TRAM, which acts as a bridge between TLR4 and TRIF [93, 94]. Interestingly, each of these adaptors participates in the TLR4 signaling pathway. However, why TLR4 alone needs each adaptor containing the TIR domain to stimulate gene expression is still unknown.

However, the employment of several adaptors and the cooperative stimulation of the MyD88-dependent and MyD88-independent/TRIF-dependent pathways may account for the potent immunostimulatory effects of the TLR4 ligand LPS, which are sufficient to cause ENDOTOXIC SHOCK. In contrast to TLR3 and TLR4, which induce type I IFNs, TLR7 and TLR9 mediate the synthesis of type I IFNs through a MyD88-dependent signaling

cascade. Which molecules or compounds in these signaling pathways provide selectivity is still unknown. Another molecule that has a TIR domain is SARM (sterile α-and armadillo motif-containing protein) [98, 99]. Recently, the *Caenorhabditis elegans* TIR domain-containing protein (TIR1), an ortholog of mammalian SARM, was demonstrated to modulate the expression of genes encoding antimicrobial peptides. This reaction, however, is unrelated to the *C. elegans* TLR [100, 101]. However, clarifying the function of mammalian SARM may advance our knowledge of TLR signaling.

MyD88-independent/TRIF-dependent pathway

IRF3 activation

According to earlier research, IRF3 is necessary for the activation of the IFN-β-encoding gene and IFN-inducible genes [102, 103]. IRFs constitute a transcription factor family that is engaged in the response to IFNs as well as the induction of type I IFNs [89, 102]. Thus far, it has been demonstrated that IRF3, IRF5, and IRF7, three of the nine known members of the IRF family, act as direct transducers of virus-mediated signaling and play critical roles in the development of type I IFNs [103, 104]. Different types of cells constitutively express IRF3, and in response to viral infection, phosphorylation of its C-terminal regulatory domain causes it to become active, enabling the production of IRF3 dimers. Following dimerization, IRF3 quickly moves to the nucleus, where despite the absence of intrinsic transcriptional activity, it recruits the coactivators p300 and cAMP-responsive element-binding protein (CBP) to activate the transcription of type I IFN genes. IRF3 mediates initial type I IFN production during viral infection. Through the JAK (Janus activated kinase)-STAT (signal transducer and activator of transcription) signaling pathway [105, 106], these secreted type I IFNs stimulate the expression of IFN-inducible genes, including CXCL10 and IRG1. Unlike IRF3, most cell types generate the mRNA encoding IRF7 in response to viral infection and IFN. Viral infection induces and activates IRF7 via the JAK-STAT signaling pathway, which is initiated by constitutively produced IRF3 molecules and the resulting generation of type I IFNs. Afterwards, IRF3 and IRF7 contribute to the generation of delayed-type IFNs (IFN-α/β), which in turn increases IFN69 expression. LPS stimulation also activates IRF3. This finding suggested that MyD88 is not necessary for IRF3 activation, as LPS can also increase the production of IFN-β and IFN-inducible genes in a MyD88-independent manner. In fact, MyD88-deficient cells exhibit IRF3 activation, as evidenced by dimer formation and nuclear translocation [72, 73]. According to S.A. and K.T.'s unpublished observations, TLR3 signaling activates IRF3 more quickly and potently than TLR4 signaling does, and this is correlated with greater IFN-β production. When poly(I:C) is used to activate TLR3, C-terminal phosphorylation of IRF3 is detected via a phosphospecific antibody; however, LPS stimulation does not cause any discernible C-terminal phosphorylation [107, 108]. However, rather than qualitative differences, we believe that TLR3- and TLR4-mediated IRF3 activation most likely differ quantitatively since phosphospecific antibodies are more likely not sensitive enough to detect IRF3 phosphorylation after TLR4 activation. The kinases that phosphorylate IRF3 in response to viral infection and stimulate TLR3 have recently been identified as IKK-ε (also known as inducible IKK, IKKi) and TBK1 (TRAF-family-associated NF-κB activator (TANK)-binding kinase 1; also known as NF-κB-activating kinase, NAK) [91, 92, 109–111]. The promoters of IFN-β and IFN-inducible genes are activated by the overexpression of IKK-ε or TBK1, which also causes IRF3 to become phosphorylated and localize to the nucleus. Furthermore, siRNAs targeting IKK-ε and TBK1, but not IKK-β, significantly reduce the degree of viral induction of the IFN-α4 and IFN-β reporter genes as well as the TRIF-dependent activation of a reporter gene containing an IRF DNA-binding motif [91, 91, 92, 92],[27, 109]. Moreover, examination of TBK1-deficient embryonic fibroblasts verified the crucial function of TBK1 in TLR3- and TLR4 signaling-mediated IRF3-dependent gene expression [43, 112].

NF-κB activation

When stimulated with LPS, NF-κB is still activated in mice lacking MyD88, but the kinetics are slower than those in wild-type mice. However, in animals lacking both MyD88 and TRIP, the activation of NF-κB in response to LPS is completely abolished, indicating that TRIP is necessary for NF-κB activation via a MyD88-independent pathway [78, 79]. In contrast to inflammatory cytokine production, which requires both early- and late-phase NF-κB activation mediated by MyD88 and TRIF, respectively, the transcriptional activation of the IFN-β gene requires the activation of both NF-κB and IRF3. However, the production of IFN-β can be induced by TRIF-mediated late-phase NF-κB activation alone (Fig. 4). In vitro investigations revealed that whereas only the N-terminal portion of TRIF is involved in the activation of the IFN-β promoter, both the C- and N-terminal sections of TRIF may independently activate an NF-κB-responsive promoter [88, 95, 96]. Through additional investigations via a yeast two-hybrid screen, the mechanism of NF-κB activation through the N-terminal region of TRIF was discovered, and TRAF6 was shown to interact with TRIF physically. As previously mentioned, the TRAF-C

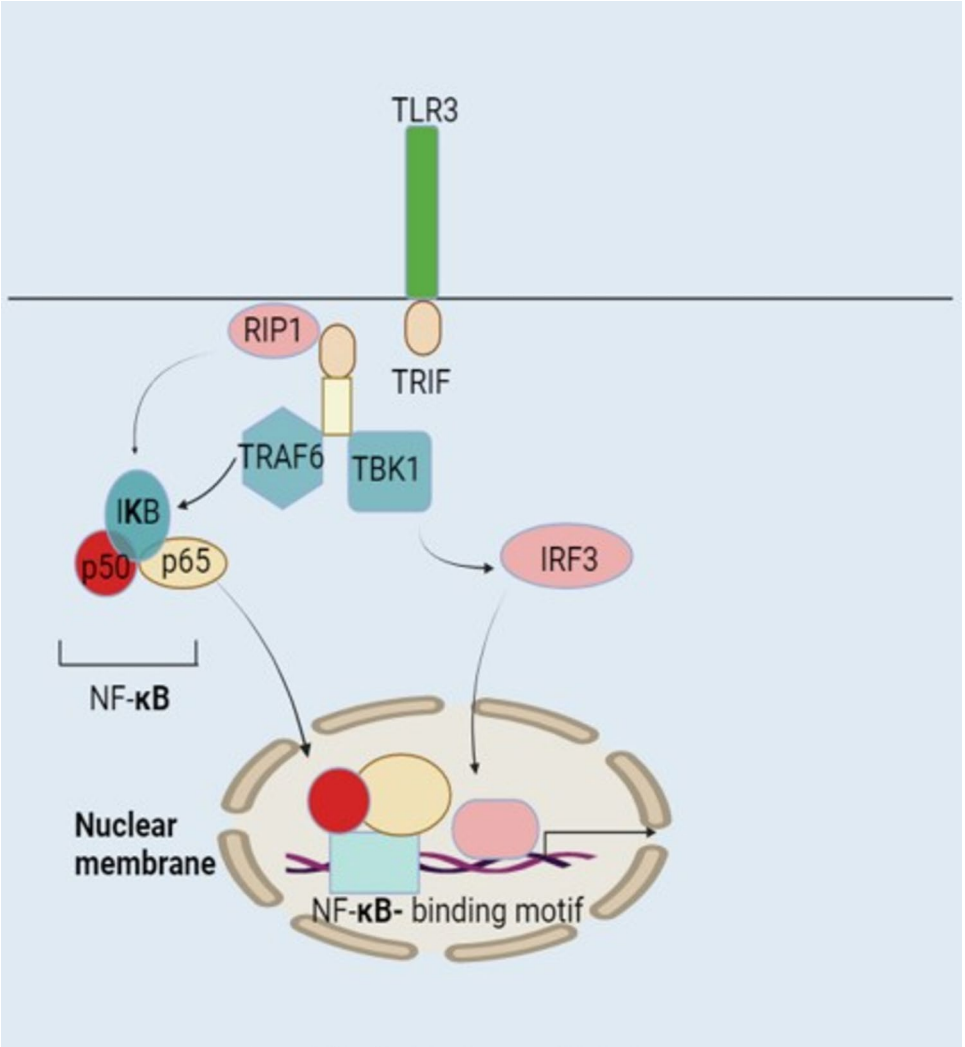

Fig. 4 TRIF-dependent induction of IFN-β. The part of TRIF (Toll/interleukin-1-receptor (TIR)-domain-containing adaptor protein producing interferon (IFN)-β), which is amino-terminally located, interacts with TBK1 (TRAF-family-associated nuclear factor-κB (NF-κB) activator (TANK)-binding kinase 1) as well as TRAF6 (tumor-necrosis factor-receptor associated factor 6). TRAF6 mediates NF-κB activation and TRIF-dependent activation of TBK1 results in the phosphorylation of IRF3 (IFN-regulatory factor 3). The carboxy-terminal region of TRIF triggers NF-κB activation, which is mediated by receptor-interacting protein 1 (RIP1). The IFN-β gene is activated in part by the simultaneous activation of NF-κB and IRF3. NF-κB inhibitor: IκB; Toll-like receptor: TLR

domain of TRAF6 is reported to bind to the consensus motif P-X-E-X-X-(D/E/F/W/Y) [53, 54]. Notably, the N-terminal regions of mouse and human TRIF have three TRAF6-binding motifs. Although the TRAF3A mutant, in which all three TRAF6-binding motifs were mutated, eliminated the interaction of TRIF with TRAF6, its activation of NF-κB was only slightly mitigated.

The C-terminal region of TRIF is responsible for activating NF-κB independently of the N-terminal region, which is likely why activation still occurs. In fact, a TRIF3A mutant lacking the C-terminal region was incapable of activating NF-κB. Furthermore, TBK1 binds to the N-terminal region of TRIF, activating IRF3 and triggering IFN-β [113–115]. As a result, TRAF6 and TBK1 directly interact with the N-terminal region of TRIF, activating NF-κB and the IFN-β gene, respectively (Fig. 4). Moreover, TRIF activates NF-κB via at least two different routes. TRAF6 mediates the first pathway, which involves its N-terminal region, and the second pathway, which involves its C-terminal region. According to a recent study, receptor-interacting protein 1 (RIP1), which binds to the C-terminus of TRIF, is necessary for TRIF-dependent NF-κB activation. A RIP homotypic interaction motif found in this region of TRIF is necessary for its interaction with RIP1 [33, 116]. TLR3-mediated NF-κB activation is defective in embryonic fibroblasts from RIP1-deficient animals, and a dominant-negative variant of RIP1 inhibits TRIF-mediated NF-κB activation. Thus, RIP1 most likely acts as a mediator to activate NF-κB via the C-terminal region of TRIF.

Other molecules involved in TLR signaling

In addition to those previously mentioned, TLRs activate multiple intracellular signaling molecules subsequent to ligand binding. These include the evolutionarily conserved signaling intermediate in Toll pathways (ECSIT), the

SRC-family tyrosine kinases, AKT (also known as protein kinase B, PKB), the pellinos, phosphatidylinositol 3-kinase (PI3K), and Toll-interacting protein (TOLLIP). These compounds are briefly described here because they may be involved in TLR signaling pathways.

TOLLIP

As a protein that interacts with the IL-1R accessory protein, TOLLIP was first cloned. Following the stimulation of these receptors, it has been demonstrated to directly bind with the cytoplasmic TIR domain of IL-1Rs, TLR2 and TLR4 and to impede TLR-mediated cellular responses by inhibiting the phosphorylation and kinase activity of IRAK1 [117–121]. When cells are at rest, TOLLIP binds to IRAK family members to prevent NF-κB activation by preventing IRAK1 phosphorylation.

Following receptor activation, TOLLIP-IRAK1 complexes are attracted to the receptor, causing IRAK1 to autophosphorylate and separate from the receptor rapidly. In addition, TOLLIP is phosphorylated by IRAK1, which may cause TOLLIP to separate from IRAK1 and undergo rapid ubiquitylation and destruction. Therefore, TOLLIP mostly serves to maintain immune cell dormancy and make it easier for TLR/IL-1R-induced cell signaling to end during inflammation and infection.

Pellino

Pellino is a protein that binds to Pelle, the IRAK homolog in Drosophila, and was first discovered in this species [13, 14, 121–124]. Since then, three mammalian homologs of Pellino, pellino-1, pellino-2, and pellino-3, have been found. These findings demonstrate a high level of evolutionary conservation, with human Pellino-2 exhibiting 60% similarity to Drosophila Pellino. Mammalian Pellino-1 and Pellino-2 interact with IRAK1 and are necessary for NF-κB activation in the TLR/IL-1R signaling pathways [125–128]. IRAK1 and Pellino-2 do not cooperate under steady-state conditions, but they unite as a complex when TLR/IL-1R stimulation is applied [52, 126]. It is believed that the pellinos serve as scaffolding proteins that aid in the release of phosphorylated IRAK from the receptor owing to their capacity to interact with IRAKs and the absence of any domain with the potential to perform an enzymatic activity.

PI3K

Because of the direct connection between the receptor and the PI3K p85 regulatory subunit, PI3Ks are activated during TLR/IL-1R signaling [93, 129, 130]. The SRC homology 2 (SH2) domain of the p85 subunit and a receptor domain carrying the Tyr-Xaa-Xaa-Met pattern are involved in this interaction. After the p110 catalytic subunit of PI3Ks is activated, the protein is fully activated, which phosphorylates and activates its downstream target, AKT. Interestingly, only a subset of TLRs, TLR1, TLR2, and TLR6, have the PI3K-binding motif Tyr-Xaa-Xaa-Met, where Xaa represents any amino acid. Nonetheless, MyD88's C-terminus contains a potential PI3K-binding site (Tyr257-Lys258-Ala259-Met260), and it has been demonstrated that LPS stimulation causes MyD88 to undergo tyrosine phosphorylation and form a PI3K-MyD88 complex [131, 132]. Additionally, MyD88 and AKT directly interact; a dominant-negative mutation in AKT results in a disruption in the transcriptional activity of NF-κB, which is dependent on MyD88. Nonetheless, NF-κB attachment to DNA is unaffected when AKT is inhibited, suggesting that NF-κB may play a role in phosphorylating the p65 domain during transactivation. A dominant-negative mutation. The kinase activity of MyD88 AKT was affected by the interaction between IL-1 and LPS, and an NF-κB-inhibiting dominant-negative mutant of p85 was found. LPS- and IL-1-induced activity, but not that induced by TNF [133, 134]. These results suggest that PI3K is a beneficial mediator of the NF-κB activation signaling pathway triggered by IL-1 and LPS. PI3K may play a detrimental role in TLR signaling in DCs, as evidenced by recent research employing animals lacking the p85 regulatory subunit, which revealed an increase in IL-12 production by DCs, perhaps as a result of greater p38MAPK activation [118, 135].

ECSIT

Yeast two-hybrid screening was used to clone ECSIT as a TRAF6-interacting protein, which is not similar to any known protein [66, 136].ECSIT engages in interactions with TRAF6's conserved TRAF domain. There is a homolog of ECSIT in Drosophila, and Drosophila also conserves the connection between ECSIT and TRAF6. Additionally, ECSIT interacts with extracellular signal-regulated kinase 1 (MAPK/ERK) kinase kinase 1, or MEKK1, which can phosphorylate and activate the IKK complex. When a dominant-negative mutant of ECSIT is expressed, TLR4 signaling is blocked, suggesting that ECSIT may transduce TLR signals by connecting the IKK complex and TRAF6.

Furthermore, LPS-induced NF-κB activation but not TNF-α-induced NF-κB activation was hindered when ECSIT expression was inhibited by siRNA in a macrophage line. To establish ECSIT-deficient mice, the physiological role of ECSIT was investigated. These mice died at embryonic day 7.5 [137–139]. Subsequent analysis revealed that ECSIT is a necessary intermediary in bone morphogenetic protein (BMP) signaling, making it a crucial part of the TLR and BMP signaling pathways.

SRC family of tyrosine kinases

A member of the SRC-related TEC family of protein tyrosine kinases, Bruton's tyrosine kinase (BTK) is crucial for B-cell development and B-cell receptor (BCR)-mediated signaling. BTK-deficient macrophages from X-linked immune-deficient mice respond less strongly to LPS, and BTK has also been shown to interact with the TIR domains of TLR4, TLR6, TLR8, and TLR9 [140–142]. Additionally, it has been demonstrated to phosphorylate tyrosine in response to LPS and to interact with MyD88, TIRAP, and IRAK1. In contrast, a dominant-negative version of BTK suppresses the LPS-induced activation of NF-κB, suggesting that BTK is a component of the TLR-mediated signaling cascade. The SRC family of tyrosine kinases, including FYN, LYN, and hemopoietic-cell kinase HCK, interact with and activate BTK during BCR-mediated signaling; however, SRC family kinases play a limited role in LPS signalling [117, 143]. Therefore, more research is needed to determine how BTK functions in TLR signaling.

MAPKKK

Members of the MAPKKK family, TAK1, MEKK1, MEKK2, MEKK3, TPL2 (tumor progression locus 2; sometimes called cancer Osaka thyroid, or COT) and NIK, are connected to the activation of MAPK and IKK-NF-κB. Within this group, MEKK3 has been demonstrated to be involved in TLR4 signaling but not TLR9 signaling [144, 145]. Upon stimulation with a TLR4 ligand but not a TLR9 ligand, embryonic fibroblasts from MEKK3-deficient mice exhibited reduced IL-6 production and faulty activation of NF-κB, JNK, and p38 MAPK. MEKK3 and TRAF6 are also linked by TLR4 stimulation. Thus, MEKK3 participates in the signaling pathway mediated by TLR4. It has been demonstrated that TPL2, another member of the MKKK family, participates in the TLR4-mediated activation of ERK [40, 123, 146]. Mice lacking TPL2 exhibited reduced TNF-α production and aberrant ERK activation in response to a TLR4 ligand. These findings indicate that many MAPKKKs mediate TLR signaling pathways.

Negative regulation of TLR signaling

TLR signaling is tightly regulated to prevent overactivation. This section discusses key mechanisms and molecules that negatively regulate TLR signaling. When excess levels of TLR signaling-related inflammatory cytokines are generated, major systemic illnesses, including endotoxic shock, which can be caused by the TLR4 ligand LPS, can result in a high death rate. Therefore, it is not surprising that organisms have developed TLR-mediated response modulation mechanisms (Fig. 5). The molecules IRAK-M, SOCS1 (suppressor of cytokine signaling 1), MyD88 short (MyD88s), SIGIRR (single immunoglobulin IL-1R-related molecule), and ST2 are considered to affect TLR signaling adversely; they are briefly reviewed here. In contrast to other IRAKs, which are expressed everywhere, IRAK-M expression is specific to monocytes and macrophages and increases in response to TLR ligand stimulation. IRAK-M also has no kinase activity. IRAK-M-deficient animals exhibit poor induction of LPS tolerance and increased production of inflammatory cytokines in response to TLR ligands [147, 148]. According to biochemical studies, IRAK-M stops the IRAK1-IRAK4 complex from dissociating from MyD88, which stops the IRAK1-TRAF6 complex from forming. These results suggest that IRAK-M negatively regulates the TLR signaling pathway. SOCS1 is a protein that belongs to the SOCS family and is activated by cytokines and inhibits cytokine signaling pathways [149, 150]. It has been demonstrated that LPS and CpG-containing DNA can cause macrophages to express SOCS1 [151–153]. Additionally, animals lacking SOCS1 are more susceptible to LPS-induced endotoxic shock (i.e., they produce more inflammatory cytokines) [34, 43, 153, 154]. Moreover, SOCS1-deficient mice did not develop LPS tolerance, and ectopic SOCS1 injection into macrophages prevented LPS-induced NF-κB activation. These results suggest that SOCS1 directly downregulates TLR signaling pathways. Although SOCS1 has been demonstrated to interact with IRAK1 [155, 156], exactly how SOCS1 blocks TLR signaling is still unknown. After LPS stimulation, monocytes produce MyD88s, alternatively spliced versions of MyD88 without the intermediate domain. MyD88s and MyD88 do not bind IRAK4 or cause IRAK1 phosphorylation when overexpressed, in contrast to MyD88. Because MyD88 cannot bind to IRAK4 and increase IRAK1 phosphorylation, it suppresses LPS-induced NF-κB activation. In addition to these cytoplasmic molecules, TLR signaling also has unfavorable effects on membrane-bound compounds containing a TIR. It has recently been demonstrated that domains such as SIGIRR and ST2 are involved in the negative control of TLR signaling. Mice lacking SIGIRR are extremely vulnerable to endotoxic shock caused by LPS100. SIGIRR has also been demonstrated to interact temporarily with TLR4, IRAK1, and TRAF6, which suppresses TLR signaling pathways. In a similar vein, animals lacking ST2 failed to induce LPS tolerance and instead produced increased levels of inflammatory cytokines in response to LPS [113, 157, 158]. The overexpression of ST2 inhibited NF-κB activation because ST2 was linked to and likely sequestered MyD88 and TIPR. As a result, orphan receptors with TIR domains, such as SIGIRR and ST2, are connected to the suppression of TLR signaling.

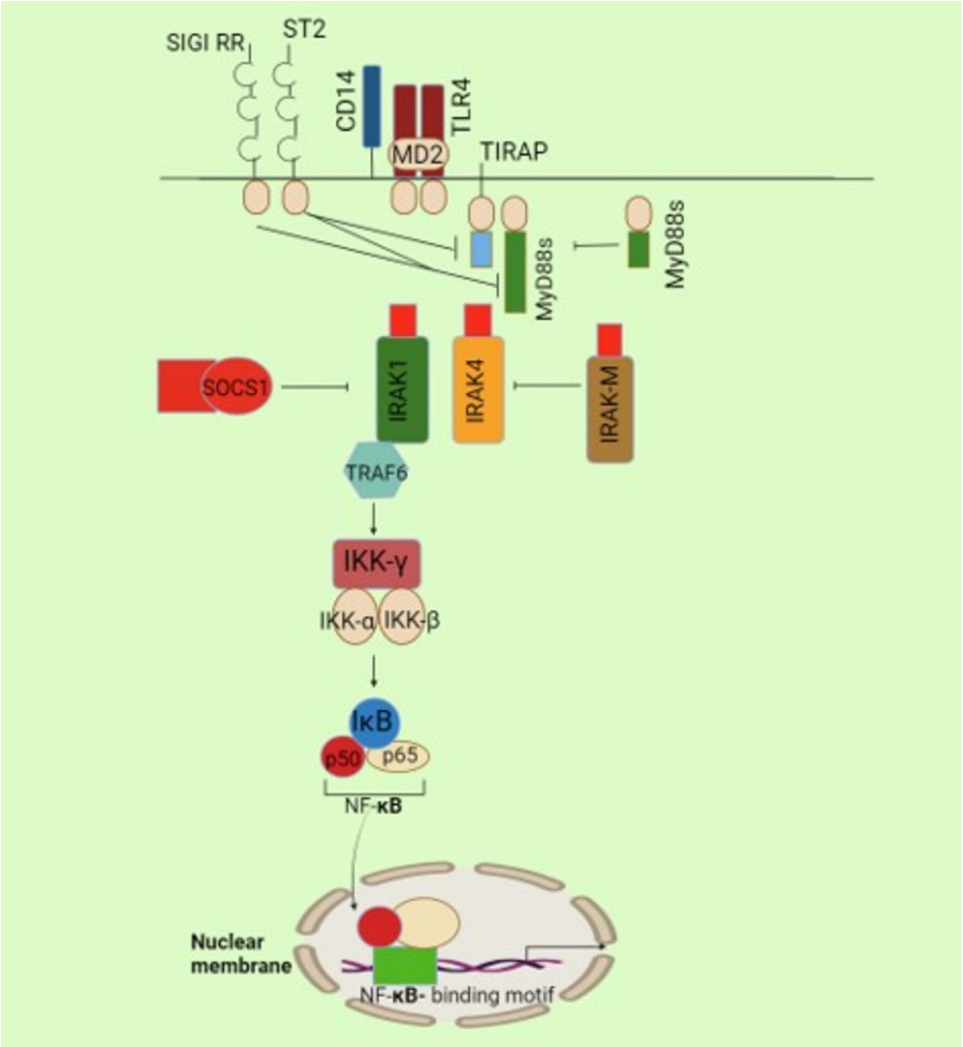

Fig. 5 Downregulation of TLR signaling. Several cellular factors generated by the TLRs stimulation downregulate the TLRs signaling pathways. Interleukin-1 receptor (IL-1R)-associated kinase M, or IRAK-M, prevents the IRAK1-IRAK4 complex from separating from the receptor. It is likely that SOCS1 (suppressor of cytokine signaling 1) binds to IRAK1 and prevents it from functioning. Myeloid differentiation primary-response protein 88 (MyD88s) prevents IRAK4 from attaching itself to MyD88. It has also been demonstrated that SIGIRR (single immunoglobulin IL-1R-related molecule) and ST2, TIR (Toll/IL-1R)-domain-containing receptors, adversely affect TLR signaling. TRAF6, tumor-necrosis-factor-receptor-associated factor 6; IKK, IκB kinase; NF-κB, nuclear factor-κB; TIRAP, TIR-domain containing adaptor protein; IκB, inhibitor of NF-κB

Prospects

By examining mice devoid of certain TLRs or other molecules involved in TLR signaling, the molecular processes by which TLRs trigger innate immunity are being clarified. MyD88, TIRAP, TRIF, and TRAM are examples of TIR domain-containing adaptors that have been shown to play important roles in TLR signaling pathways because they specify each TLR signaling response. However, a few unanswered questions remain. For example, type I IFNs are induced by TLR7 and TLR9 in a MyD88-dependent manner but not by TLR2, suggesting that TLR7 and TLR9 have distinct signaling routes.

This TLR7/TLR9-mediated pathway may involve SARM, an additional adaptor that contains a TIR domain [98, 99]. Elucidating this distinct TLR7/TLR9 pathway could further understand the underlying mechanisms of the TLR-mediated activation of innate immunity. Alternatively, some TLR signaling pathways may be regulated by molecules lacking a TIR domain. Furthermore, we can now investigate animals lacking MyD88 and TRIF, which means that they do not have any of the previously identified TLR signaling pathways. It is now imperative that we thoroughly examine the function of TLR signaling in host defence against a range of pathogenic microbes. For example, mice lacking MyD88 have been demonstrated to be susceptible to both gram-positive and gram-negative bacterial infections; nevertheless, they continue to mount an immunological defence against intracellular microorganisms (*Listeria monocytogenes* and mycobacteria) and viruses [13, 14, 19, 112, 153, 157, 159–161].

Conclusion

TLR-independent mechanisms recognize intracellular viruses and bacteria and broaden our understanding of immune responses. Analyzing animals lacking both MyD88 and TRIF would elucidate the contributions of MyD88-dependent and TRIF-dependent/MyD88-independent TLR signaling pathways to host defence. TLR signaling pathway-deficient mutant mice are promising models for in vivo studies of immune responses, host defences against viral infections, and anticancer responses.

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