

Toll-like Receptors and their Adaptors in Innate Immunity

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Abstract: Toll-like receptors (TLRs) recognize specific molecular patterns of pathogenic microorganisms, including bacteria, fungi, protozoa, and virus. Stimulation of TLRs triggers gene expression involved in innate immune response and further instructs development of antigen-specific adaptive immunity. Molecular mechanisms by which TLRs activate innate immunity are now being elucidated through analysis of TLR-mediated signaling pathways. TLR signaling originates from the cytoplasmic Toll/IL-1 receptor (TIR) domain, which is conserved among all TLRs. In addition, recent evidence indicates that TIR domain-containing adaptors, such as MyD88, TRIF, TIRAP, and TRAM, play essential roles in TLR signaling. MyD88 is essential for inflammatory cytokine production *via* all TLRs, whereas TRIF mediates a MyD88-independent induction of type I IFNs *via* TLR3 and TLR4. TIRAP is specifically involved in TLR2-, and TLR4-mediated MyD88-dependent pathway, and TRAM acts in the TLR4-mediated TRIF-dependent pathway. Therefore, the specific functions of individual TLRs can be elicited by utilizing different combinations of TIR domain-containing adaptors. These recent progresses have made us aware of the fact that innate immunity possesses a skillful system to detect microbial invasion in the host and trigger appropriate immune responses.

Keywords: Toll-like receptor, signaling pathway, TIR domain, adaptor, MyD88, TRIF, knockout mice.

INTRODUCTION

Invasion of a host by microbial pathogens triggers activation of the immune system, which eliminates them. The immune system consists of two components: innate immunity and adaptive immunity, both of which sense invading microorganisms by virtue of receptors able to recognize non-self. In adaptive immunity, lymphocytes utilize immunoglobulins and T cell receptors as non-self recognition receptors. However, these receptors are present only in vertebrates, and accordingly we could not fully understand the mechanism for non-self recognition in less evolved organisms. In innate immunity, macrophages and dendritic cells have long been known to be activated by microbial components (non-self), such as lipopolysaccharide (LPS) from Gram-negative bacteria. However, a receptor that recognizes these microbial components remained unknown. At the end of 20th century, a study in *Drosophila*, which only has an innate immune system, demonstrated that Toll is an essential receptor for host defense against fungal infection [1]. One year later, a mammalian homologue of Toll receptor (now termed TLR4) was shown to induce expression of genes involved in inflammatory responses [2]. In addition, a point mutation in the *Tlr4* gene has been identified in a mouse strain that is unresponsive to LPS [3]. Subsequent studies in the past several years have revealed that mammalian Toll-like receptors (TLRs) recognize specific molecular patterns of microbial components [4]. These studies have made immunologists aware that the innate immune system possesses a skillful system that senses

invasion of microbial pathogens by TLRs, which act as non-self recognition receptors in this arm of the immune system. Further progress has been made in elucidating the molecular mechanisms of activation of innate immunity. In this review, I will focus on recent progress on TLR-mediated signaling pathways, which regulate not only activation of the innate immunity, but also development of antigen-specific adaptive immunity.

MICROBIAL RECOGNITION BY TLRs

Mammalian TLRs comprise a large family consisting of at least 11 members. Involvement of TLRs in the recognition of microbial components was first demonstrated in TLR4, as mentioned above [3]. Subsequently, individual TLRs have been shown to recognize specific microbial components derived from pathogens including bacteria, fungi, protozoa, and virus (Fig. 1).

(1) TLR1, TLR2, TLR6

TLR2 is involved in the recognition of components from a variety of pathogens. These include lipoproteins from various pathogens, peptidoglycan and lipoteichoic acid from Gram-positive bacteria, lipoarabinomannan from mycobacteria, glycosylphosphatidylinositol anchors from *Trypanosoma Cruzi*, a phenol-soluble modulin from *Staphylococcus epidermis*, zymoan from fungi, and glycolipids from *Treponema maltophilum* [4]. In addition, TLR2 presumably recognizes atypical LPS from *Leptospira interrogans* or *Porphyromonas gingivalis*, both of which differ from the typical LPS of Gram-negative bacteria in several biochemical and physical properties [5, 6].

An interesting aspect of TLR2-mediated recognition is that TLR2 forms heterofilic dimers with other TLRs such as

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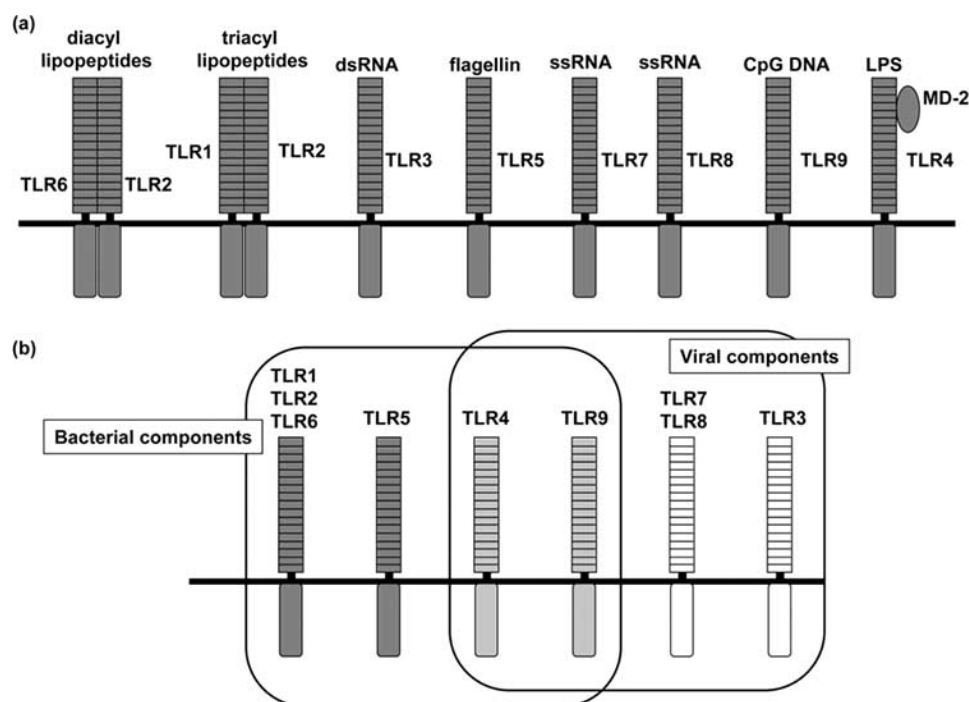


Fig. (1). TLRs and their ligands

TLR2 is essential in the recognition of microbial lipopeptides. TLR1 and TLR6 functionally cooperate with TLR2 to discriminate subtle differences between triacyl and diacyl lipopeptides, respectively. TLR4 is the receptor for LPS. TLR5 recognizes bacterial flagellin. TLR9 is essential in CpG DNA recognition, whereas TLR3 is implicated in the recognition of viral dsRNA. TLR7 and TLR8 are involved in the recognition of single-stranded RNA (ssRNA) of virus. Thus, the TLR family members recognize specific patterns of microbial components. TLR1/TLR2/TLR6 subfamily and TLR5 are specific to the recognition of bacterial and fungal components, whereas TLR3 and TLR7/TLR8 subfamily are responsible for viral recognition. TLR4 and TLR9 are involved in the recognition of both bacterial and viral components.

TLR1 and TLR6. This may partially explain the ability of TLR2 to respond to the wide spectrum of microbial components. TLR1 and TLR6 are structurally related to TLR2 and both associate with TLR2. Macrophages from TLR6-deficient mice did not show any production of inflammatory cytokines in response to mycoplasma-derived diacyl lipopeptides. However, these cells showed normal production of inflammatory cytokines in response to triacyl lipopeptides derived from Gram-negative bacteria [7]. On the contrary, macrophages from TLR1-deficient mice showed a normal response to mycoplasma-derived diacyl lipopeptides, but an impaired response to triacyl lipopeptides and lipoproteins from mycobacteria [8]. Thus, TLR1 and TLR6 functionally associate with TLR2 and discriminate between diacyl or triacyl lipopeptides. Moreover, the involvement of TLR1 in the recognition of the outer surface lipoprotein of *Borrelia burgdorferi* has also been shown [9].

(2) TLR3

TLR3-deficient mice are impaired in the response to double-stranded RNA (dsRNA) [10]. dsRNA is produced by most viruses during their replication and induces the synthesis of type I interferons (IFN- α/β), which exert antiviral and immunostimulatory activities. Thus, TLR3 is implicated in the recognition of dsRNA and viruses. However, the impairment in the dsRNA response observed in TLR3-deficient mice is only partial [10, 11]. In addition, introduction of dsRNA into the cytoplasm of dendritic cells

leads to the induction of type I IFNs via a mechanism partially dependent on dsRNA-dependent protein kinase (PKR), but independent of TLR3 [12]. These findings indicate the presence of TLR3-independent recognition of dsRNA and virus.

(3) TLR4

TLR4 is an essential receptor for LPS-mediated responses, as demonstrated by positional cloning of the gene responsible for hypo-responsiveness to LPS in the C3H/HeJ mouse strain and generation of TLR4-deficient mice [3, 13]. In addition, TLR4 is implicated in the recognition of several other ligands, such as Taxol, a diterpene purified from the bark of the Western yew (*Taxus brevifolia*) [14, 15]. Furthermore, recent reports indicate that TLR4 recognizes several endogenous ligands. These include heat shock proteins such as HSP60 and HSP70, the extra domain A (EDA) of fibronectins, oligosaccharides of hyaluronic acid, heparan sulfate, and fibrinogen [4]. Thus, TLR4 is seemingly implicated in the recognition of several endogenous ligands involved in the inflammatory response even in the absence of infection. However, all of these endogenous TLR4 ligands activate immune cells only when stimulated at very high concentrations. In addition, the ability of HSP70 to activate macrophages has been attributed to contaminating LPS in the HSP70 preparation [16]. LPS is the most powerful immunostimulator among microbial components, and even small amount of LPS contamination will result in TLR4-dependent

immune activation. Therefore, more careful experiments are required before we can conclude that TLR4 recognizes these endogenous ligands.

(4) TLR5

Expression of human TLR5 in CHO cells conferred response to flagellin, a monomeric constituent of bacterial flagella [17]. A subsequent study revealed that TLR5 recognizes an evolutionarily conserved domain of flagellin and physically interacts with flagellin [18]. Exposure of the basolateral, but not apical, surface of intestinal epithelial cell lines to flagellin induces IL-8 production, which is correlated with the exclusive expression of TLR5 on the basolateral side of the intestinal epithelial cells [19]. Flagellin also activates lung epithelial cell to induce inflammatory cytokine production [20]. These findings indicate the important role of TLR5 in epithelial microbial recognition. A common stop codon polymorphism in the ligand-binding domain of TLR5 has been shown to be associated with susceptibility to pneumonia caused by the flagellated bacterium *Legionella pneumophila* [20].

(5) TLR7, TLR8

TLR7-deficient mice are impaired in the response to synthetic compounds, imidazoquinolines, which are approved for treatment of diseases associated with viral infection [21]. Expression of human TLR7 or TLR8, but not mouse TLR8, confers response to imidazoquinoline compounds [22]. Another compound, loxoribine, which has anti-viral and anti-tumor activities, has also been shown to activate the immune cells via TLR7 [23]. Both imidazoquinoline and loxoribine are structurally related to guanosine nucleoside, therefore TLR7 and human TLR8 were predicted to recognize a nucleic acid-like structure of virus. This hypothesis was soon proved by findings showing TLR7 and human TLR8 to be receptors for guanosine- and uridine-rich single-stranded RNA (ssRNA) from viruses such as human immunodeficiency virus, vesicular stomatitis virus, and influenza virus [24-26]. Thus, TLR7 and TLR8 sense viral infection by recognizing ssRNA.

(6) TLR9

TLR9-deficient mice do not show any response to bacterial CpG DNA, indicating that TLR9 is a receptor for CpG DNA [27]. Bacterial DNA contains unmethylated CpG motifs, which confer its immunostimulatory activity. In vertebrates, the frequency of CpG motifs is severely reduced and the cysteine residues of CpG motifs are highly methylated, leading to abrogation of the immunostimulatory activity. In addition, recent evidence indicates that TLR9 mediates recognition of viral-derived CpG DNA in plasmacytoid dendritic cells [28, 29]. In support of these findings, TLR9-mutant mice have been shown to be susceptible to mouse cytomegalovirus infection [30].

(7) TLR11

The most recently identified TLR, TLR11, has been shown to be expressed in bladder epithelial cells and mediate resistance to infection by uropathogenic bacteria [31]. TLR11-deficient mice are highly susceptible to

uropathogenic bacterial infection. In human, TLR11 protein is seemingly inactive since genomic sequence corresponding to full-length TLR11 is not present. This may correlate with high incidence of urinary tract infections in human.

SUBCELLULAR LOCALIZATION OF TLRs

Individual TLRs show distinct patterns of subcellular distribution. Flow cytometric analysis using monoclonal antibodies against TLR1, TLR2 or TLR4 clearly demonstrated that these TLRs are expressed on the cell surface. In contrast, expression of TLR3, TLR7, TLR8, and TLR9 has been shown in intracellular compartments such as endosomes [23, 32-34]. The TLR9 ligand CpG DNA has been shown to be recognized in endosomes after non-specific uptake into the cells, which correlates well with the endosomal expression of TLR9 [35]. Thus, CpG DNA may be exposed after degradation of bacteria in endosomes/lysosomes. In addition, TLR3, TLR7, TLR8, and TLR9 are all implicated in viral recognition. Virus-induced production of type I IFNs requires endosomal maturation [25, 26]. Therefore, during viral infection, the receptor-mediated virus entry into the endosomal compartment may occasionally result in degradation of viral particles, leading to subsequent exposure of TLR ligands such as dsRNA, ssRNA, and CpG DNA. Other TLRs, which are expressed on the cell surface, may sense bacterial or fungal invasion by recognizing microbial components at the extracellular compartment. Even these TLRs, especially TLR2, are recruited to the phagosomal compartment of macrophages after stimulation with fungal components [36]. Thus, phagosomal/lysosomal and endosomal/lysosomal compartments may be the main sites for TLRs to sense microbial invasion.

TLR SIGNALING PATHWAYS

Stimulation of TLRs by microbial components triggers expression of several genes that are involved in immune responses. The molecular mechanisms by which TLRs induce these gene expressions are now rapidly being elucidated through the analyses of TLR-mediated signaling pathways. All TLR signaling pathways originate from a cytoplasmic Toll/IL-1 receptor (TIR) domain, which is conserved among all TLRs. A crucial role for the TIR domain was first revealed in the C3H/HeJ mouse strain, which has a point mutation within the TIR domain resulting in a dominant negative effect on TLR-mediated signaling [3, 13]. In the signaling pathways downstream of the TIR domain, the first characterized TIR domain-containing adaptor, MyD88, was shown to be a common adaptor for all TLRs [37]. However, activation of specific TLRs induces distinct profiles of gene expression. For example, activation of TLR3 and TLR4, but not TLR2, signaling pathways results in induction of type I interferons (IFNs) [38-40]. TLR7 and TLR9 signaling pathways also induce type I IFNs through mechanisms distinct from TLR3/4-mediated induction [21, 41, 42]. These findings indicate the presence of specificity in individual TLR signaling pathways. Indeed, subsequent studies revealed that there are MyD88-dependent and MyD88-independent pathways, and additional TIR domain-containing adaptors play crucial roles in TLR signaling. In the following section, TLR-mediated signaling

pathways consisting of MyD88-dependent and MyD88-independent cascades are discussed by focusing on TIR domain-containing adaptors, such as MyD88, TIRAP/Mal, TRIF, and TRAM.

MYD88-DEPENDENT PATHWAY

MyD88, harboring a C-terminal TIR domain and an N-terminal death domain, associates with the TIR domain of TLRs. Upon stimulation; MyD88 recruits IRAK-4 to TLRs through interaction of the death domains of both molecules, and facilitates IRAK-4-mediated phosphorylation of IRAK-1. Activated IRAK-1 then associates with TRAF6, leading to the activation of two distinct signaling pathways, and finally results in the activation of transcription factors such as AP-1 and NF- κ B (Fig. 2).

(1) MyD88

MyD88 is the first-characterized TIR domain-containing adaptor, which was originally identified as a gene that is rapidly induced during IL-6-mediated myeloid differentiation in M1 myeloblastic leukemia cell lines [43]. MyD88 was subsequently shown to play an essential role in signaling pathways involving IL-1 receptor family members, all of which also possess a TIR domain [44]. MyD88-deficient mice further showed no production of inflammatory

cytokines such as TNF- α , IL-6 and IL-12p40 in response to all TLR ligands [17, 21, 37, 45-47]. These findings demonstrated that the TIR domain-containing adaptor MyD88 is essential for the induction of inflammatory cytokine mediated by all TLR family members.

However, MyD88-deficient macrophages showed activation of NF- κ B and JNK with delayed kinetics in response to the TLR4 ligand LPS in spite the absence of inflammatory cytokine production [45]. This indicates that although LPS-induced production of inflammatory cytokines completely depends on the MyD88-dependent pathway, there exists a MyD88-independent component in TLR4 signaling.

(2) TIRAP/Mal

A database search for molecules that are structurally related to MyD88 led to identification of the second TIR domain-containing molecule TIRAP (TIR domain-containing adaptor protein) [48]. This protein is also called Mal (MyD88-adaptor-like) [49]. Similar to MyD88-deficient macrophages, TIRAP/Mal-deficient macrophages showed impaired inflammatory cytokine production and delayed activation of JNK and NF- κ B in response to the TLR4 ligand LPS [50, 51]. TIRAP/Mal-deficient mice were not impaired in their response to TLR3, TLR5, TLR7, and TLR9 ligands,

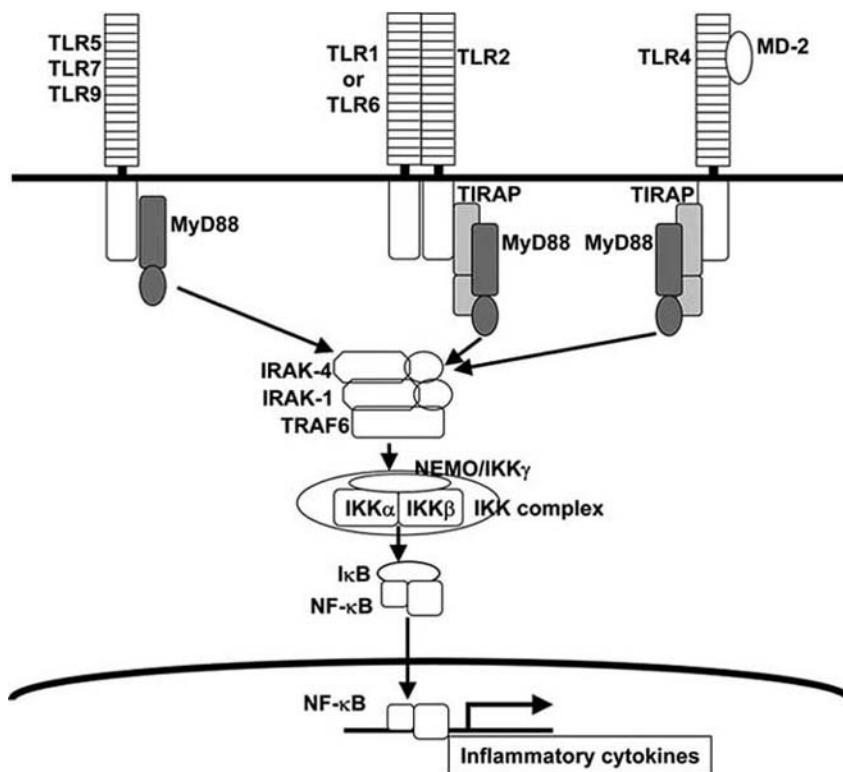


Fig. (2). MyD88-dependent pathway

A TIR domain-containing adaptor, MyD88, associates with the cytoplasmic TIR domain of TLRs, and recruits IRAKs to the receptor upon ligand binding. IRAKs then activate TRAF6, leading to the activation of the I κ B kinase (IKK) complex consisting of IKK α , IKK β and NEMO/IKK γ . The IKK complex phosphorylates I κ B, resulting in nuclear translocation of NF- κ B that induces expression of inflammatory cytokines. TRAF6 also mediates activation of JNK leading to activation of AP-1 transcription factors. TIRAP is involved in the MyD88-dependent pathway *via* TLR2 and TLR4.

but were defective in TLR2 ligand-induced inflammatory cytokine production. Thus, analyses of TIRAP/Mal-deficient mice revealed that TIRAP/Mal is essential for the MyD88-dependent signaling pathway via TLR2 and TLR4.

MYD88-INDEPENDENT/TRIF-DEPENDENT PATHWAY

As described above, TLR4 ligand stimulation leads to activation of NF- κ B and JNK with delayed kinetics. In addition, MyD88-deficient, but not TLR4-deficient, dendritic cells showed functional maturation in response to the TLR4 ligand LPS [52]. MyD88-deficient macrophages showed LPS-induced expression of several IFN-inducible genes, such as those encoding IP-10 and GARG16 [53]. Subsequent studies clearly demonstrated that there is a MyD88-independent pathway, as well as a MyD88-dependent pathway in TLR4 signaling. In the MyD88-independent pathway, LPS stimulation leads to activation of the transcription factor IRF-3, and thereby induces IFN- β . IFN- β in turn activates Stat1, leading to the induction of several IFN-inducible genes [38-40]. IRF-3 was originally demonstrated to be activated by viral infection or dsRNA [54]. Accordingly, the TLR3 ligand dsRNA activates IRF-3 and thereby induces IFN- β in a MyD88-independent manner. Hence, TLR3 and TLR4 utilize the MyD88-independent component to induce IFN- β .

A search for molecules that associate with IRF-3 by two-hybrid screening has led to identification of non-canonical

I κ B kinases (IKKs) [55]. Canonical IKKs, IKK α and IKK β , phosphorylate Ser32 and Ser36 of I κ B, thereby inducing NF- κ B activation. IKK γ is involved in the MyD88-dependent pathway, since IKK γ -deficient cells failed to produce any inflammatory cytokines in response to TLR ligands [56]. The non-canonical kinases that associate with IRF-3 are structurally related to IKK α and IKK β , but have distinct kinase activity. These non-canonical IKKs are called TRAF family member-associated NF- κ B activator (TANK)-binding kinase 1 (TBK1) and IKK α /IKK β i, both of which phosphorylate Ser36, but not Ser32, of I κ B [57-59]. Introduction of TBK1 or IKK α /IKK β i, but not IKK γ , resulted in phosphorylation and nuclear translocation of IRF-3 [55]. RNAi-mediated inhibition of TBK1 or IKK α /IKK β i expression led to impaired induction of IFN- β in response to virus and dsRNA [55, 60]. Furthermore, embryonic fibroblast cells obtained from TBK1-deficient mice showed impaired expression of IFN- β and IFN-inducible genes in response to TLR3 and TLR4 ligands [61]. Thus, TBK1 and IKK α /IKK β i are critical regulators of IRF-3 activation, which leads to the induction of IFN- β in the MyD88-independent pathway. In the course of analyzing the MyD88-independent pathway, two TIR domain-containing adaptors, TRIF and TRAM, were identified (Fig. 3).

(1) TRIF

Characterization of TIRAP/Mal indicated that TIR domain-containing adaptors provide specificity for

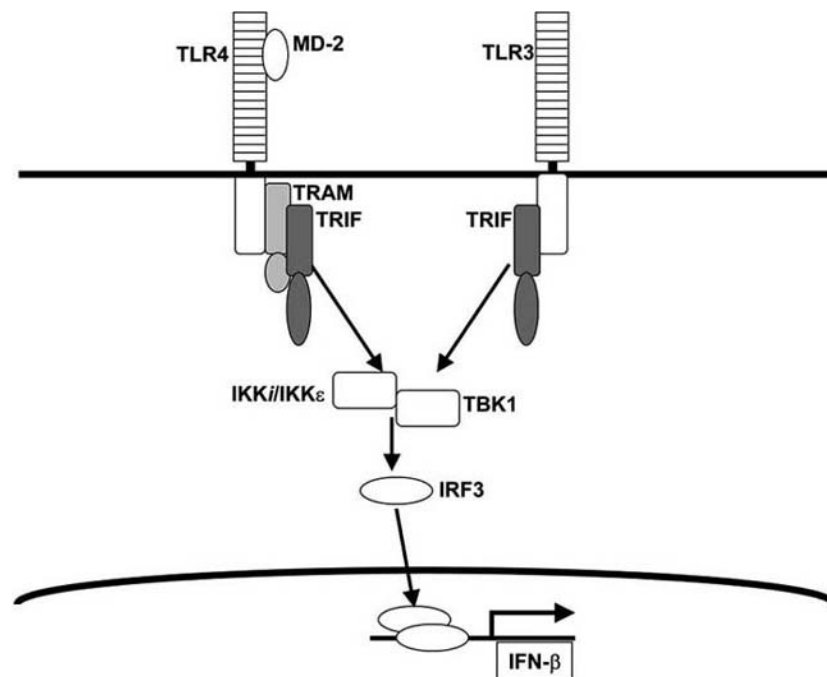


Fig. (3). MyD88-independent pathway/TRIF-dependent pathway

In TLR3- and TLR4-mediated signaling pathways, activation of IRF-3 and induction of IFN- β are observed in a MyD88-independent manner. TRIF is essential for the MyD88-independent pathway. IKK-related kinases, IKK α /IKK β and TBK1, mediate activation of IRF-3 downstream of TRIF. TRAM is specific to the TLR4-mediated MyD88-independent/TRIF-dependent pathway.

individual TLR signaling pathways, and prompted us to search similar adaptor molecules. As a result, a third TIR domain-containing adaptor, TIR domain-containing adaptor inducing IFN- γ (TRIF) was identified [62]. The same molecule was also identified as a TLR3-associated molecule by two-hybrid screening and named TIR domain-containing adaptor molecule (TICAM-1) [63]. Unlike MyD88 and TIRAP/Mal, which have relative low molecular weight, TRIF is a large protein consisting of 712 amino acids in humans. Introduction of TRIF, MyD88, or TIRAP all led to activation of the NF- κ B-dependent promoter in 293 cells. In addition, introduction of TRIF, but not MyD88 or TIRAP, induced activation of the IFN- γ promoter. A dominant negative form of TRIF inhibited TLR3 ligand-induced activation of the IFN- γ promoter, and RNAi-mediated knockdown of TRIF caused impairment in TLR3 ligand-induced IFN- γ expression. Thus, *in vitro* studies suggested that TRIF is involved in TLR3-mediated induction of the IFN- γ gene, which is mediated by the MyD88-independent pathway. This hypothesis was soon revealed to be true by generation of mice lacking TRIF. TRIF-deficient mice generated by targeted gene disruption showed impaired TLR3-mediated expression of IFN- γ and IFN-inducible genes [64]. Furthermore, TRIF-deficient mice displayed defective expression of IFN-inducible genes in response to the TLR4 ligand. Another mouse strain mutated in the *Trif* gene generated by random germline mutagenesis also revealed that TRIF-mutant mice were defective in the TLR3- and TLR4-mediated induction of IFN- γ and IFN-inducible genes [65]. TRIF-deficient mice showed no activation of IRF-3 in response to TLR3 and TLR4 ligands. Thus, TRIF has been demonstrated to be essential for TLR3- and TLR4-mediated MyD88-independent pathway. In TRIF-deficient mice, TLR4 ligand-induced phosphorylation of IRAK-1 was not impaired, indicating that TLR4-mediated activation of the MyD88-dependent pathway is normally induced. However, inflammatory cytokine production induced by the TLR4 ligand was not observed in TRIF-deficient mice, in contrast to normal responses to other TLR ligands. Therefore, the TLR4 signaling pathway requires activation of both the MyD88-dependent and -independent pathways to induce inflammatory cytokines, although the mechanisms remain to be elucidated.

Signaling pathway downstream of TRIF has become a focus of attention. The TIR domain of TRIF is located in the middle portion of the molecule, flanked by the N-terminal and C-terminal portions having no homology with known conserved motifs. Both N-terminal and C-terminal portions of TRIF activate the NF- κ B-dependent promoter, whereas only the N-terminal portion activates the IFN- γ promoter [62]. The N-terminal portion of TRIF was shown to associate with IKK α /IKK β and TBK1, which activate IRF-3 and thereby induce IFN- γ [60, 66]. The N-terminal portion of TRIF was also shown to associate with TRAF6 [66, 67]. Indeed, three TRAF6 binding motifs (PxExxZ, Z; acidic or aromatic residue) are present in the N-terminal portion of TRIF. Failure to activate the NF- κ B-dependent promoter results when these TRAF6 binding motifs are mutated [66, 68]. Since TRAF6 is critically involved in TLR-mediated NF- κ B activation, TRAF6 may regulate TRIF-mediated NF- κ B activation. However, TLR4 ligand-induced NF- κ B

activation was still observed in MyD88/TRAF6 double deficient cells [53], indicating that NF- κ B activation can be induced independent of the TRIF-TRAF6 pathway. In this regard, it is of note that the C-terminal portion of TRIF is also involved in the NF- κ B activation, as mentioned above. Sequence analysis of TRIF has recently showed that approximately 35 amino acids at the C-terminal portion are homologous to a RIP homotypic interaction motif (RHIM) of receptor interacting protein 1 (RIP1) and RIP3 [69]. Furthermore, TRIF associates with RIP1 and RIP3 through homophilic interaction of RHIM domains. A dominant negative form of RIP1 inhibits TRIF-mediated NF- κ B activation. Embryonic fibroblasts from RIP1-deficient mice showed impaired NF- κ B activation in response to TLR3 ligand. Therefore, RIP1 is responsible for TRIF-mediated NF- κ B activation. More precise analysis will probably be required for full understanding of the mechanisms for TRIF-dependent activation of NF- κ B.

(2) TRAM

Database searches further led to identification of a fourth TIR domain-containing adaptor, TRIF-related adaptor molecules (TRAM) [70, 71]. The same molecule was independently identified as TICAM-2 or TIRP [72, 73]. *In vitro* studies indicated that TRAM bridges TLR4 and TRIF by associating with both molecules. SiRNA-mediated knockdown of TRAM expression showed that TRAM mediates TLR4-, but not TLR3-mediated induction of IFN- γ and IFN-inducible genes [70, 72]. TRAM-deficient mice showed impaired activation of IRF-3 and expression of IFN-inducible genes in response to TLR4 ligands. TRAM-deficient mice further showed defective TLR4-mediated production of inflammatory cytokines, although TLR4-mediated IRAK-1 activation was not impaired [71]. These phenotypes are reminiscent of those found in TRIF-deficient mice, demonstrating that TRAM is essential for the TLR4-mediated MyD88-independent/TRIF-dependent pathway. However, unlike TRIF-deficient mice, TRAM-deficient mice were not impaired in the response to the TLR3 ligand. Thus, TRAM is specifically involved in TLR4-mediated activation of the MyD88-independent/TRIF-dependent pathway.

REGULATION OF TLR SIGNALING BY TIR DOMAIN-CONTAINING ADAPTORS

As described above, TIR domain-containing adaptors have been established to play essential roles in TLR signaling (Figs. 2,3). MyD88 is essential for all TLR-mediated inflammatory cytokine production, whereas TRIF is essential for TLR3- and TLR4-mediated induction of IFN- γ . Since MyD88/TRIF double deficient cells showed no activation of signaling molecules such as NF- κ B, MAP kinases, and IRF-3 in response to TLR ligands, both MyD88 and TRIF are main adaptors in the currently known TLR signaling pathways. In addition, TIRAP and TRAM provide specificity for individual TLR signaling. TLRs exhibit their specific gene induction through differential utilization of these adaptors.

TLR3- and TLR4-mediated activation of the MyD88-independent/TRIF-dependent pathway leads to induction of type I IFNs. In contrast, stimulation of TLR7, TLR8, or

TLR9, but not TLR2, results in the MyD88-dependent induction of type I IFNs. TLR7, TLR8 and TLR9 exhibit high structural homology. Functionally, they all recognize nucleic acid-like patterns of microorganisms. Therefore, it would not be surprising if there is a unique pathway for TLR7, TLR8, and TLR9, which all mediate the MyD88-dependent type I IFN induction. There is one additional TIR domain-containing molecule designated SARM, whose function remains to be elucidated [74]. It is possible that SARM is involved in TLR7, TLR8, and TLR9-mediated MyD88-dependent type I IFN induction. Alternatively, molecules that do not harbor the TIR domain may regulate this pathway. Elucidation of this unique pathway will lead to better understanding the molecular mechanisms of TLR signaling pathways.

FUTURE PROSPECTS

It is now well established that TLRs trigger activation of innate immunity through recognition of specific patterns of microbial components. In addition, the molecular mechanisms by which TLRs activate the innate immunity are being rapidly understood, mainly by the analyses of mice lacking TIR domain-containing adaptors, such as MyD88, TIRAP/Mal, TRIF, and TRAM. We now know that innate immunity plays an important role in the initiation of immune response that follows the activation of antigen-specific adaptive immunity. Moreover, TLRs have already been implicated in several disease models. For example, constitutive activation of innate immune cells such as macrophages and dendritic cells caused by the deficiency of Stat3, which is essential for IL-10 signaling, led to development of chronic enterocolitis [75]. In these mutant mice, additional ablation of TLR4 prevented development of bowel inflammation, indicating that TLR-mediated microbial recognition in the intestine triggers activation of innate immunity, and thereby leads to chronic enterocolitis [76]. Thus, there is a possibility that TLRs are involved in triggering development of several inflammatory diseases. Some autoimmune diseases have also been proposed to be associated with infection and dysregulation of innate immune activation [77]. Indeed, autoreactive B cells specific for self-IgG have been shown to proliferate by sequential engagement of the B cell receptor (BCR) and TLR9 recognizing hypomethylated CpG motifs in the IgG/chromatin immune complex [78, 79]. Progression of atherosclerosis is dependent on chronic inflammation in the artery wall probably caused by infection [80]. Ablation of MyD88 resulted in a reduction of aortic atherosclerotic lesions, indicating that TLR-mediated pathway is responsible for the development of atherosclerosis [81]. Utilizing mice lacking both MyD88 and TRIF, in which all of the TLR signaling pathways characterized so far are abolished, we can now precisely analyze the involvement of TLR-mediated activation of the innate immunity in the development of several models of inflammatory and autoimmune diseases.

Involvement of the innate immunity in host defense against infectious diseases should also be analyzed using MyD88/TRIF double deficient mice. Although MyD88-deficient mice showed high susceptibility to Gram-positive bacterial infection, they mounted significant adaptive

immune response to infection by viruses and intracellular bacteria such as mycobacteria and *Listeria monocytogenes* [82-86]. Thus, the MyD88-independent responses are also involved in host defense against these microorganisms. At present, we do not know whether these responses are elicited through activation of the TRIF-dependent pathway or a TLR-independent pathway. Analysis of MyD88/TRIF double deficient mice will highlight the importance of TLR-mediated innate immune responses in infectious diseases caused by viruses and mycobacteria.

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