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Editor

Encyclopedia of Signaling Molecules

With 686 Figures and 86 Tables

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Panse VG, Johnson AW. Maturation of eukaryotic ribosomes: acquisition of functionality. Trends Biochem Sci. 2010;35:260–6.
Ray P, Basu U, Ray A, Majumdar R, Deng H, Maitra U. The *Saccharomyces cerevisiae* 60S ribosome biogenesis factor Tif6p is regulated by Hrr25p-mediated phosphorylation. J Biol Chem. 2008;283:9681–91.
Russel DW, Spremulli LL. Purification and characterization of a ribosome dissociation factor (eukaryotic initiation factor 6) from wheat germ. J Biol Chem. 1979;254:8796–800.
Sanvito F, Piatti S, Villa A, Bossi M, Lucchini G, Marchisio PC, Biffo S. The beta 4 integrin interactor p27 (BBP/eIF6) is an essential nuclear matrix protein involved in 60S ribosomal subunit assembly. J Cell Biol. 1999;144:823–37.
Senger B, Lafontaine DLJ, Graindorge J-S, Gadal O, Camasses A, Sanni A, Garnier J-M, Breitenbach M, Hurt E, Fasiolo F. The nucle(ol)ar Tif6p and Efl1p are required for a late cytolasmic step of ribosome synthesis. Mol Cell. 2001;8:1363–73.
Si K, Maitra U. The *Saccharomyces cerevisiae* homologue of mammalian translation initiation factor 6 does not function as a translation initiation factor. Mol Cell Biol. 1999;19:1416–26.
Si K, Chaudhuri J, Chevesich J, Maitra U. Molecular cloning and functional expression of a human cDNA encoding translation initiation factor 6. Proc Natl Acad Sci USA. 1997;94:14285–90.
Valenzuela DM, Chaudhuri A, Maitra U. Eukaryotic ribosomal subunit anti-association activity of calf liver is contained in a single polypeptide chain protein of M_r=25,500 (eukaryotic initiation factor 6). J Biol Chem. 1982;257:7712–19.
Venema J, Tollervey D. Ribosome synthesis in *Saccharomyces cerevisiae*. Annu Rev Genet. 1999;33:261–311.

Tif6p

► TIF6 (eIF6)

TIP3 (Tec Interacting Protein-3 = SOCS1)

► SOCS

TIRAP

► Toll-like Receptor Adaptor Protein Family Members

TIR-Domain-Containing Adapter Molecule 1

► Toll-like Receptor Adaptor Protein Family Members

TIR-Domain-Containing Adapter Molecule 2

► Toll-like Receptor Adaptor Protein Family Members

TIR-Domain-Containing Adapter Protein Inducing IFN-Beta

► Toll-like Receptor Adaptor Protein Family Members

TIRP

► Toll-like Receptor Adaptor Protein Family Members

Tlr4

► TLR4, Toll-Like Receptor 4

TLR4, Toll-Like Receptor 4

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Synonyms

ARMD10; CD284; hToll; Lps; Ly87; Ran/M1; Rasl2-8; Tlr4; Toll; Toll-like receptor 4; Toll-like receptor, type 4

Historical Background

Overproduction of the cytokine profile (cytokine tsunami or storm) can be caused by both infectious and non-infectious diseases. During this process, inflammatory responses are activated either to provide a protective mechanism or to damage tissues when excessively produced cytokines attempt to overwhelm the cause of their production. This system is activated whenever there is a foreign invasion of the body. Foreign invasion is attributed to microbial associated molecules known as Pathogen Associated Molecular Patterns (PAMPS), which activate our immune system through Pattern Recognition Receptors (PRRs) present in the body fluids, cell membranes, and cytoplasm. PRRs not only identify PAMPs, but also molecules released from damaged cells known as Damage Associated Molecular Patterns (DAMPs). There is a great deal of evidence that DAMPs such as products of necrosis or breakdown components of extracellular matrix are originated or produced by the host species (Yang et al. 2010; Schaefer 2010), even though this would seem to contradict the original belief that the immune system only responded to non-self compounds.

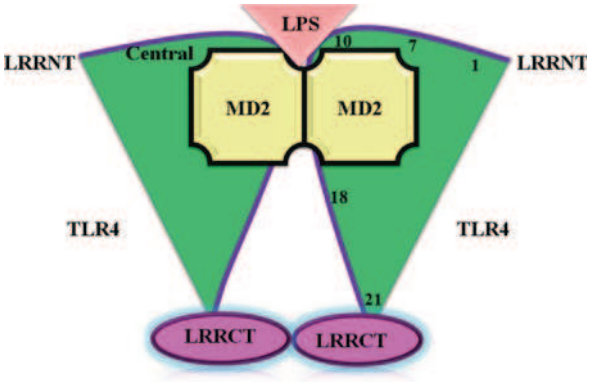
One group of transmembrane PRRs located on the cell membranes and endosomes are known as Toll-like Receptors (TLRs). TLRs are involved in the primary response against invaders, connecting the innate and adaptive immune responses. Recently, three major classes of PRRs were identified in addition to TLRs, C-type lectin receptors (CLR), retinoic acid-inducible gene (RIG)-1-like receptors (RLR), and nucleotide-binding oligomerization domain protein-1- like receptors (NLR). These systems augment the response of TLRs to invading agents. Originally, the activation of any of the aforementioned receptors was thought to be associated with the production of pleotrophic inflammatory cytokines such as TNF, IL-1b, and IL-6. However, type I IFNs are also produced by TLR activation and these compounds enable the cells to resist viral infection, leading to acquired immune responses as well as hematopoietic stem cell production and turnover (Beutler 2009; Kawai and Akira 2009).

Prima facie TLRs were reported in *Drosophila melanogaster* as Toll1 receptor in 1985 (Hansson and Edfeldt 2005). In this fly, Toll gene performs nonimmune functions such as establishing dorsoventral polarity, synaptogenesis, and axon path finding during embryogenesis (Anderson et al. 1985; Halfon et al. 1995; Rose et al. 1997). TLRs are Type I transmembrane glycoproteins that are characterized by the presence of Leucine Rich Repeats (LRRs) and TIR (Toll/Interleukin 1 Receptor) domains. To date, 10 TLRs have been identified in humans, while 13 have been identified in mice. Various TLRs bind with different PAMPs located in the outer wall of microbes or within their cytoplasm. Among the PAMPs, bacterial lipopolysaccharide (LPS) binds with TLR4, while lipopeptides are recognized by TLR2 in conjunction with TLR1 and TLR6, triacylated lipopeptides are bound by TLR1/2 heterodimers, diacylated lipopeptides interact with TLR2 and TLR6, ▶ TLR5 interacts with flagellin, dsRNA binds with TLR3, ssRNA interacts with TLR7 and TLR8, and ▶ TLR9 binds with unmethylated CpG islands. Innate immunity through the TLR system is known to be very important for vaccine design (Hashiguchi et al. 2010). In this entry, we discuss TLR4, its structure and associated receptors, signaling pathways, and the mechanism by which it is activated.

TLR4 Structural Features with MD2

The TLR4 gene, which is also known as Lps; Ly87; Ran/M1; and Rasl2-8, is located in chromosome 4 in mice and chromosome 9 in humans. TLR4 belongs to the LRR family and is composed of an N-terminal, central, and C-terminal domain. TLRs are characterized by two conserved regions, the extracellular leucine rich region (LRR) and the cytoplasmic Toll/IL-1 receptor (TIR) domain. The LRR, which is involved in recognition of the ligand, is composed of 19–25 tandem repeats of 24–29 amino acids folded in strands and helices that are linked by loops. TLR4 is composed of a 608 residue extracellular domain (Medzhitov et al. 1997) and is highly polymorphic when compared with the transmembrane and cytosolic domains (Schmitt et al. 2002; Gay and Gangloff 2007). The TIR domain, which shares homology with the interleukin 1 receptor (IL-1R), modulates protein–protein interactions between the TLRs and the adaptor proteins involved in the signal transduction cascade (O'Neill and Bowie 2007). The TIR domain, which provides a scaffold for the recruitment of MyD88 (myeloid differentiation primary response gene 88), is composed of three highly conserved motifs known as Box-1, Box-2, and Box-3 (Xu et al. 2000). In these three boxes, Box-2

forms loops known as BB loops by connecting β-strands and α-helixes. These loops are indispensable for signaling, as a single point mutation in proline residue (P712H) render C3H/HeJ mice hyporesponsive to LPS (Poltorak et al. 1998). Ligand induced dimerization is one of the hallmarks of TLR4 activation as it is the first line of regulation. CD14, MD2, and TLR4, which are receptosomes, are very important for ligand (LPS) recognition by TLR4. The role of CD14 is to enhance LPS binding to MD2, whereas MD2 is indispensable in the formation of stable receptor-ligand complexes. Interaction between TLR4 and MD2 is mediated in the concave surface at the amino-terminal region of TLR4, especially Glu (24) and Pro (34) and central domains that are critical for MD2 binding (Nishitani et al. 2005) (Fig. 1). Following ligand binding, the TIR domain initiates downstream signaling events starting with adaptor proteins. After recognition of LPS complexes and signaling, the TLR4, MD2, CD14, and LPS complex is ubiquitinated and sorted to late endosomes/lysosomes (dynamin and clathrin dependent mechanism) for degradation, after which the antigens are presented to $CD4^{+}$T cells and signaling is terminated.



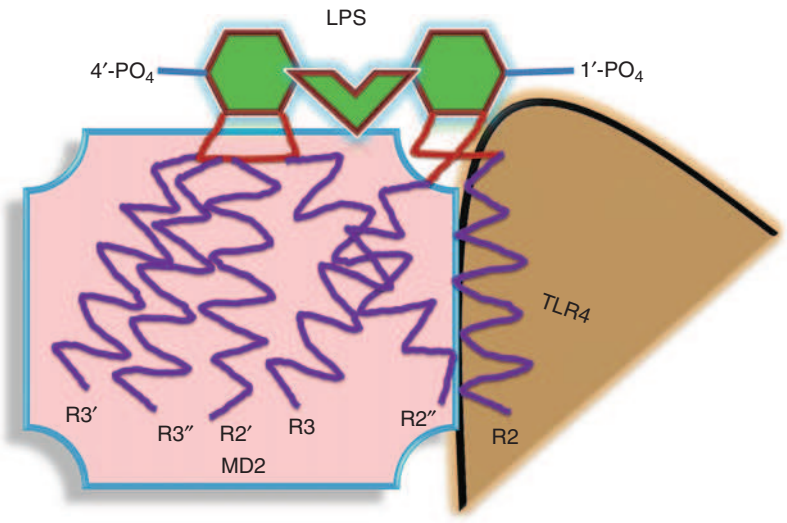
TLR4, Toll-Like Receptor 4, Fig. 1 Diagrammatic representation of the structure of the TLR4-MD2-LPS complex. TLR4 is composed of outer extracellular domain and intracellular domain known as TIR domain. Outer structure is formed of leucine rich repeats and divided as N-terminal, central, and C-terminal domains. MD2 binds with the amino and central domains. After ligand binding and dimerization of TLR4, carboxy terminals fuse together to conduct signaling

Single Nucleotide Polymorphisms of TLR4

Several studies of mice and humans have demonstrated an increased susceptibility to various infections when TLR genes display mutations. Single nucleotide polymorphisms (SNPs) have been reported in various TLR genes, such as TLR2, ▶ TLR4, TLR5, TLR6, TLR9, and TLR10, which are associated with increased disease susceptibility (Schroder and Schumann 2005). TLR4 is unable to function when it shows single nucleotide polymorphisms in its expression on Asp299Gly and Thr399Ile, and is unresponsive to LPS (Douville et al. 2010). TLR4 SNPs (single nucleotide polymorphisms) have been investigated in an extent way. It shows two nonsynonymous SNPs, an A/G transition at SNP rs4986790 that causes the substitution of aspartic acid by glycine at amino acid position 299 (Asp299Gly), and a C/T transition at SNP rs4986791 that causes the substitution of threonine by isoleucine at amino acid position 399 (Thr399Ile). These two SNPs occur in serious infections in HIV-1 infected patients with a history of nadir CD4 cell count of <100 cells/cubic mm (Papadopoulos et al. 2010). Such SNPs also display extensive damage to the system during infections with various diseases and in response to various conditions such as Gram-negative bacteria, septic shock, endotoxemia, disseminated candidiasis, invasive aspergillosis, respiratory syncytial virus infection, meningeal disease, bacterial vaginosis, premature birth, brucellosis, coronary heart disease, asthma, hematogenous osteomyelitis, and *Plasmodium falciparum* infections.

Binding of LPS with MD2 and TLR4

LPS, a TLR4 agonist, is used in several adjuvants that have been shown to be highly effective in both experimental and clinical settings (Thompson et al. 2005; Przetak et al. 2003; Kundi 2007; Baldridge et al. 2004). When LPS interacts with MD2, five of the six lipid chains of LPS are buried deeply in the hydrophobic pocket in MD2, whereas the remaining chain interacts with the conserved phenylalanine in the TLR4 receptor through a hydrophobic interaction (Fig. 2) (Park et al. 2009; Liu et al. 2008; Jin et al. 2007). Park et al. (2009) determined the crystal structure of TLR4-MD2, which helped explain its binding mechanisms. When this complex binds, it provides an "M"- shaped architecture. TLR4 consists of LRRNT (leucine rich repeats N-terminal), central, and LRRCT (leucine rich repeat

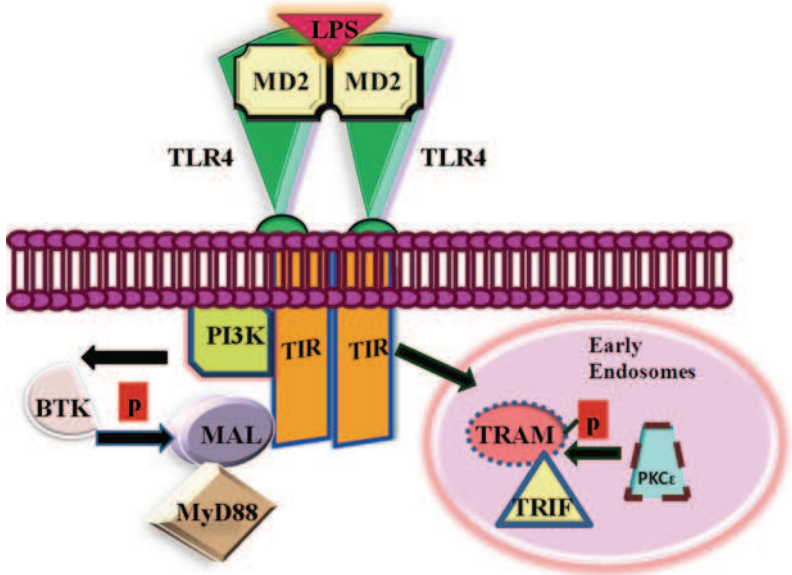


TLR4, Toll-Like Receptor 4, Fig. 2 Binding region of LPS with TLR4 and MD2. Schematically shown is the structure of TLR4-MD2-LPS complex. The receptosomes form an "M" shaped structure. Five acyl chains of LPS named R3′, R3″, R2′, R3, and R2″ form hydrophobic interaction with MD2 and bound in deep interior pockets whereas one chain R2 is exposed to bind with TLR4

C-terminal) domains. MD2 binds with the amino and central domains through their two antiparallel beta-sheets, which forms hydrophobic interactions with LPS. LPS is composed of lipids as well as carbohydrates, the former being referred as endotoxin and the latter being composed of O-antigens. For LPS, it is necessary to have six lipid acyl chains (R3′, R3″, R2′, R3, R2″, and R2) and two phosphate groups to elicit its endotoxic effects. Five of the six acyl chains (R3′, R3″, R2′, R3, R2″) are buried deeply in MD2, whereas the remaining chain (R2) is exposed to the surface to bind with TLR4. The two phosphate groups, 4′-PO_4 and 1′-PO_4, are critical for LPS induced dimer formation, where they form ionic interactions with positively charged amino acids such as lysine and arginine. In addition, the binding of LPS to MD2 causes notable changes in Phe^{126}, which forms the hydrophilic interaction that is critical for MD2 and TLR4 receptor stabilization as well as activation. Eritoran (or E5564, a lipid A antagonist) is a synthetic molecule produced from non-pathogenic *Rhodobactor sphaeroids* (Mullarkey et al. 2003). The crystal structure of TLR4-MD2 complex bound with Eritoran has recently been described, which has shed light into how Eritoran makes its connection with the large hydrophobic internal pocket in MD2 without binding to TLR4 (Kim et al. 2007). Further, in vitro studies have shown that Eritoran blocks the production of cytokines and downregulates intracellular generation of proinflammatory cytokines (Mullarkey et al. 2003; Czeslick et al. 2006). Moreover, there is pharmacological evidence that the drug must be administered every 12 h for its optimum response (Rossignol et al. 2008). Due to its action as an antagonist, Eritoran blocks signaling and subsequent ▶ NF-κB activation. Elucidation of the action of agonists or antagonists on TLR receptors permits design of new therapeutic interventions.

Signaling Components Associated with TLR4

Bruton's tyrosine kinase (BTK) is a TIR-domain-binding protein that participates in NF-κB activation during TLR4 signaling (Fig. 3). BTK binds with Box-1 and Box-2 of the TIR domain and phosphorylates ▶ MAL (MyD88-adaptor-like) at its tyrosine residue. TLR4 interacts directly with Nox4 (NAD(P)H oxidase 4), which is a protein related to gp91phox (Nox2) of phagocytic cells. This binding is involved in the production of ROS and NF-κB activation during LPS induced activation. TLR4 also binds with high mobility group box 1 (HMG1). This binding is mediated by a B-box within one of the two DNA-binding regions in the HMG1 protein. Specifically, HMG1-induced cytokine production through TLR4 stimulation requires the presence of cysteine in position 106 within the HMG1 (Yang et al. 2010). Another key kinase involved in TLR signaling is the p85 subunit of phosphoinositide 3-kinase (PI3K), which binds through its SHC (Src homology 2 domain containing) domain to the phosphotyrosine residue. There are two putative ▶ PI3K binding sites, Tyr674 and Tyr630 in TLR4, and PI3K is



TLR4, Toll-Like Receptor 4, Fig. 3 Signaling components associated with TLR4. TLR4-mediated signaling is identified as MyD88-dependent and independent. MyD88-independent signaling is mediated by TRAM and TRIF. TRIF signaling occurs in early endosomes where it gets phosphorylated by PKCε. PI3K and BTK are also involved in MAL and MyD88-dependent signaling

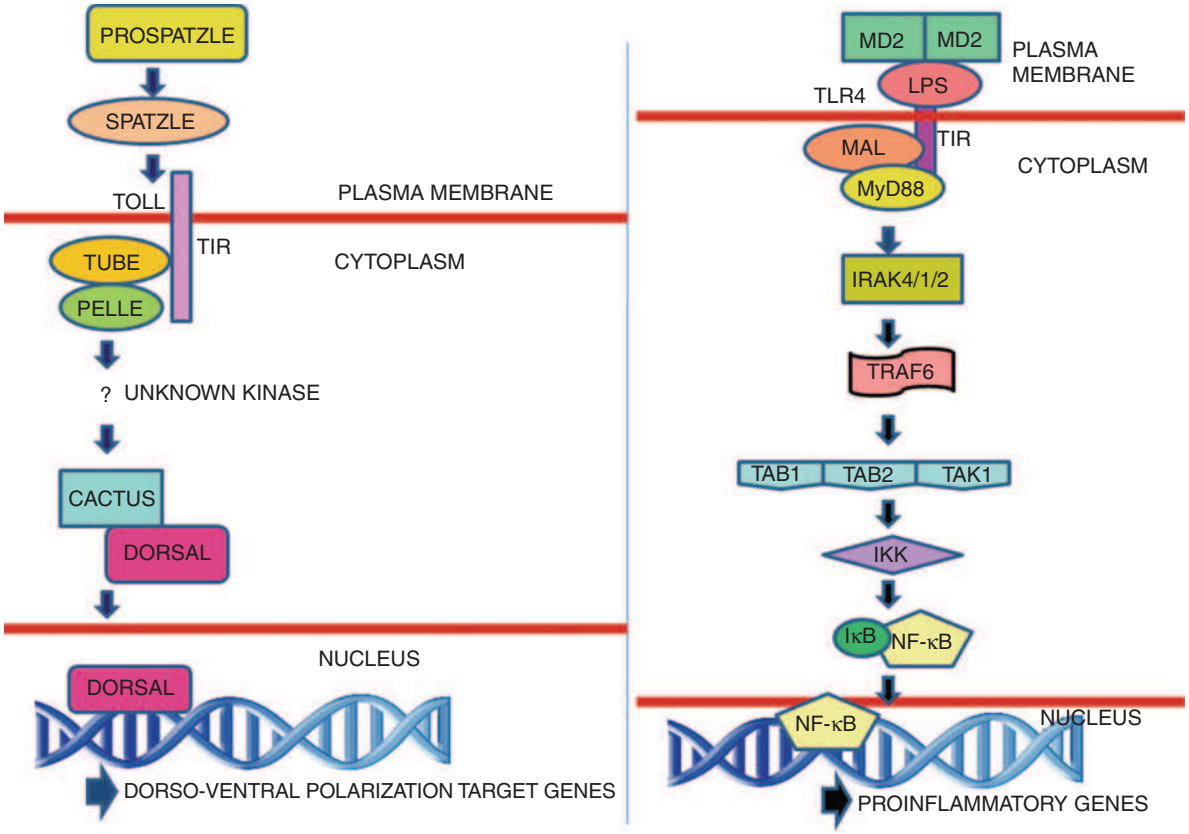
involved in the phosphorylation of TLR2 and TLR3 phosphotyrosine residues as well. ▶ TRAM (TRIF-related adaptor molecule) is phosphorylated by PKCε at its serine-16 residue when stimulated with LPS, which attenuates the response of *Irf3* (Interferon regulatory factor 3) and *Ccl5* (Chemokine (C-C) motif ligand 5). TRAM is trafficked to early endosomes, where it binds with ▶ TRIF (TIR-domain-containing adapter-inducing interferon-β).

Comparison of Toll (*Drosophila*) Signaling with TLR4 Signaling

As discussed earlier, Toll was first identified in *Drosophila* as a protein that mediates dorsoventral polarity during embryonic development (Fig. 4). However, in 1996, Jules A. Hoffman showed that Toll is involved in defense against fungal infection in *Drosophila* (Lemaitre et al. 1996). At the same time, another group searching for LPS-binding protein found a similar structural sequence of Toll in the human genome that resembled the IL1 receptor extracellular domain (Gay and Keith 1991). Thereafter, Janeway group showed that a receptor similar to TLR induced the expression of certain genes involved in adaptive immunity when stimulated with artificial antibodies (Medzhitov et al. 1997). Finally, Poltorak et al. (1998) discovered the current TLR4 and its association with LPS for immune responses. The *Drosophila* genome is composed of nine TLR genes. In *Drosophila*, Toll-1 is maternally transmitted. Spatzle, which is generated from prospatzle during positional signaling in the ventral region of embryo, binds with Toll-1. In turn, Toll-1 recruits and activates ▶ MyD88 homologue Pelle and Tube. Pelle, which is a serine/threonine kinase, phosphorylates itself and Tube as well. The subsequent signaling by an unknown kinase activates cactus (a homologue of IκB proteins) and releases Dorsal (a homologue of NF-κB), which in turn translocates to the nucleus to turn on genes associated with dorsoventral polarity (similar to TLR signaling leading to proinflammatory responses).

Signaling Pathways of TLR4

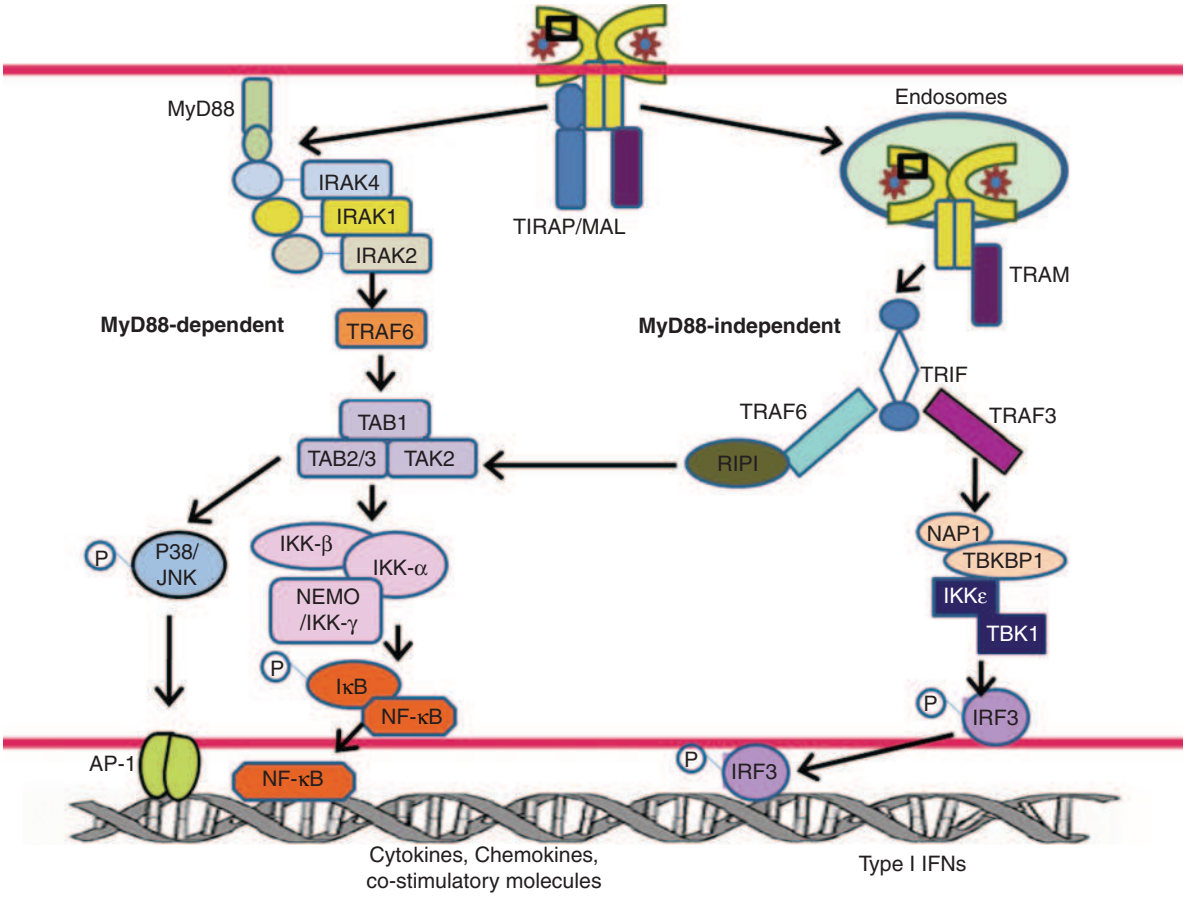
After ligation of the TLR ligands, TLRs dimerize and transmit signals throughout the cell, which leads to the activation of various signaling mechanisms and causes the transcriptional upregulation of distinct genes depending on the cell type and the nature of the stimuli. TLRs use five TIR-domain-containing adaptors, MyD88, Toll/IL-1 receptor (TIR)-domain-containing adaptor inducing interferon-β signal adaptor protein (TRIF also known as TICAM-1), ▶ TIRAP (TIR-domain-containing adaptor protein)/Mal, TRIF-related adaptor molecule (TRAM), and Sterile-alpha and Armadillo motif-containing protein (SARM). These adaptors brace the lacunae between the receptor and



TLR4, Toll-Like Receptor 4, Fig. 4 Comparison of Toll of *Drosophila* with human TLR4. Toll4 resembles Toll of *Drosophila melanogaster*. Both the receptors look alike in their extracellular domains as well as intracellular domains. Toll mediated signaling through cactus and dorsal gives rise to the genes involved in dorsoventral polarization. TLR4 mediated signaling through IκB and NF-κB leads to the robust induction of the genes involved in proinflammatory responses, which connects between innate and adaptive immunity

signaling molecules to elicit biological responses, which are essential for innate immunity.

TLR signaling is classified into two distinct pathways based on the use of MyD88 (widely used receptor) and TRIF. Among various TLR receptors, TLR4 signaling is unique in that it signals via two different pathways, the MyD88-dependent pathway, which is dependent on the MyD88 adaptor, and the MyD88-independent pathway, which depends on TRIF (Fig. 5). The first pathway is crucial for the rapid production of the proinflammatory cytokines IL-6 and ▶ TNF-α. Even though the MyD88-dependent signaling is very important for most infections, it is not involved in resisting the secondary lethal challenge caused by influenza virus (Seo et al. 2010). MyD88 deficient mice display increased risk for obesity associated diabetes (Hosoi et al. 2010). MyD88 is composed of an N-terminal death domain (DD) separated from the C-terminal TIR domain by a short linker region. MyD88-TIR is composed of three sites that are related to conserved boxes 1–3 of the domain and is important for the LPS/TLR4 pathway (Ohnishi et al. 2009). Two of these sites are located at opposite surfaces of the molecule and mediate direct interaction with Mal-TIR. Several knockout studies have provided evidence that MyD88 is essential for various TLR-responses, with the exception of TLR3 (Kawai et al. 1999; Adachi et al. 1998; Schnare et al. 2000). In general, MyD88 serves as a signaling adaptor by communicating signals to downstream kinases. In contrast, MAL acts as a sorting or connecting adaptor that only promotes interaction between MyD88 and TLR4. MyD88 interacts with IL-1R associated kinase (IRAK4) through its N-terminal DD, after which IRAK4 activates other family members such as IRAK1 and IRAK2. Recent studies have identified



TLR4, Toll-Like Receptor 4, Fig. 5 Signaling of TLR4. TLR4 uses four adaptor proteins such as MyD88, TRIF, TRAM, and MAL. Based on the usage of MyD88, TLR4 signaling is classified as MyD88-dependent or independent pathway. Both the pathways use TRAF6. MyD88-dependent pathway activates MAP kinases and IKK leading to NF-κB and AP1 nuclear translocation, thus causing proinflammatory cytokine production. PKCε phosphorylates TRAM, which in turn activates TRIF. TRIF activates both TRAF6 and TRAF3. TRAF6 leads to MAPK and IKK activation through RIP1. TRAF3 leads to IRF3/7 nuclear translocation through TBK1 and IKKε. The translocated IRF complex causes the expression of type I IFNs

critical residues in MyD88 DD as being important for its interaction with IRAK family members, as the substitution of these residues impaired propagation of downstream signaling responses (Loiarro et al. 2009). The activated IRAKs dissociate from MyD88 and interact with TRAF6, an E3 ubiquitin protein ligase. TRAF6 recruits another E2 ubiquitin-conjugating enzyme complex composed of Ubc13 and Uev1A, which catalyze the formation of conjugated lysine 63-linked polyubiquitin chain on TRAF6 itself as well as the formation of unconjugated free polyubiquitin chain (Xia et al. 2009). This macromolecular complex ultimately impinges on TGF-β-activated kinase 1 (TAK1), TAK1-binding protein 1 (TAB1), and TAB2/3. TAB3 is activated by free polyubiquitin chain and phosphorylates IKK complex composed of IKK-α, IKK-β, and NF-κB essential modulator (NEMO). These activated complexes phosphorylate IκB-α, an NF-κB inhibitory protein, followed by freeing NF-κB in the cytoplasm. This freed NF-κB translocates to the nucleus, where it binds with κB sequences to activate various cytokines, chemokines, and costimulatory molecules. Other notable kinases activated by this signaling mechanism and p38 and JNK, which belong to the MAP kinase family and lead to activation of the AP-1 family of transcription factors that further add to the expression of cytokine genes (Kawai and Akira 2006). The ultimate goal of this pathway is to activate the underlying signaling mechanisms/cascades that use NF-κB as a central

vigilance controller and concomitant activation of AP-1 to turn the pleiotropic genes involved in many physiological (cell process and cell proliferation) and inflammatory response on and off.

In the MyD88-independent pathway, TLR4 makes use of TRIF. The presence of well conserved TIR domain and several TRAF6-binding regions makes TRIF the unique adaptor for signal transduction (Yamamoto et al. 2003) as it activates both IRF3/7 and NF-κB (Fitzgerald et al. 2003). However, because TLR4-TRIF is unable to act alone, this recruits TRAM, and it is myristoylated at N-terminus to be associated with plasma membrane and requires PKCε phosphorylation for its activation (Rowe et al. 2006; McGettrick et al. 2006). TRIF-dependent pathway activates TRAF3, and it is important to activate two IKK related kinases, IKKε and TBK1. Various proteins interact with IKKε and TBK1, such as TBK-binding protein 1 (TBKBP1) and NAK-associated protein 1 (NAP1). This complex phosphorylates IRF3/7 at its C-terminus initiating type I IFN production. In addition, TLR4 has recently been shown to recognize not only PAMPS, but also the viral molecules that bind indirectly with viral activated DAMPs following infection with H5N1 avian influenza virus, thus modulating its effects (Imai et al. 2008).

Summary

Toll was first identified in *Drosophila melanogaster* as a gene that controls dorsoventral polarity. Further, continuous investigations by various research groups to identify the receptor responsible for binding of LPS in mammalian cells revealed that a gene very similar to the IL1 receptor extracellular domain had a sequence similar to that of *Drosophila melanogaster* Toll-1, which was named TLR. LPS is captured by LPS-binding protein and presented to the MD2-CD14 and TLR4 complex. Since MD2 lacks an intracellular signaling domain, it presents LPS to TLR4 for further action. Of the six acyl chains of LPS, five chains bind in the deep pocket of MD2 through hydrophobic interactions, while the remaining chain is exposed to the surface to bind with TLR4. The two phosphate groups of LPS aid in the formation of receptor dimer. The signaling responses of TLR4 to LPS are divided into MyD88-dependent and independent events, in which the former uses MyD88 as adaptor and occurs in the cytoplasm and the latter uses TRIF as the major adaptor for signaling mechanisms and occurs in the early endosomes. The gene expression mediated by TLR4 connects innate and adaptive immune responses, which form the first line of defense against microbes. The binding of LPS to TLR4 is determined by the crystal structure, but little research has been conducted to investigate the binding mechanism of various other ligands, such as taxol and heat shock proteins. Dimerization and underlying signaling mechanisms have been elucidated by the identification of crystal structures of TLR1-TLR2, TLR3, and TLR4. Such identification is indispensable for all other TLR families; hence, additional research should be conducted in that area. Overall, TLR4 is a key elicitor of immune responses that is also involved in various types of physiological and pathological events. Post-translational modifications, ubiquitination mechanisms such as mono and linear ubiquitinations, and phosphorylation events make it more arduous to delineate the signaling networks; however, elucidation of the TLR4 structure, extracellular and intracellular binding mechanisms, and signaling events indicate that it is a promising candidate for therapeutics related to various immune-related disorders.

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References

Adachi O, Kawai T, Takeda K, Matsumoto M, Tsutsui H, et al. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity. 1998;9:143–50.

Anderson KV, Jurgens G, Nusslein-Volhard C. Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the Toll gene product. Cell. 1985;42:779–89.

Baldridge JR, McGowan P, Evans JT, Cluff C, Mossman S, et al. Taking a Toll on human disease: Toll-like receptor 4 agonists as vaccine adjuvants and monotherapeutic agents. Expert Opin Biol Ther. 2004;4:1129–38.

Beutler B. Microbe sensing, positive feedback loops, and the pathogenesis of inflammatory diseases. Immunol Rev. 2009;227:248–63.

Czeslick E, Struppert A, Simm A, Sablotzki A. E5564 (Eritoran) inhibits lipopolysaccharide-induced cytokine production in human blood monocytes. Inflamm Res. 2006;55:511–5.

Douville RN, Lissitsyn Y, Hirschfeld AF, Becker AB, Kozyrskyj AL, et al. TLR4 Asp299Gly and Thr399Ile polymorphisms: no impact on human immune responsiveness to

LPS or respiratory syncytial virus. PLoS One. 2010;5: e12087.
Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, et al. LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. J Exp Med. 2003;198:1043–55.
Gay NJ, Gangloff M. Structure and function of Toll receptors and their ligands. Annu Rev Biochem. 2007;76:141–65.
Gay NJ, Keith FJ. Drosophila Toll and IL-1 receptor. Nature. 1991;351:355–6.
Halfon MS, Hashimoto C, Keshishian H. The Drosophila toll gene functions zygotically and is necessary for proper motoneuron and muscle development. Dev Biol. 1995;169:151–67.
Hansson GK, Edfeldt K. Toll to be paid at the gateway to the vessel wall. Arterioscler Thromb Vasc Biol. 2005;25:1085–7.
Hashiguchi S, Yamaguchi Y, Takeuchi O, Akira S, Sugimura K. Immunological basis of M13 phage vaccine: Regulation under MyD88 and TLR9 signaling. Biochem Biophys Res Commun. 2010;402:19–22.
Hosoi T, Yokoyama S, Matsuo S, Akira S, Ozawa K. Myeloid differentiation factor 88 (MyD88)-deficiency increases risk of diabetes in mice. PLoS One. 2010;5:e12537.
Imai Y, Kuba K, Neely GG, Yaghubian-Malhami R, Perkmann T, et al. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell. 2008;133:235–49.
Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, et al. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell. 2007;130:1071–82.
Kawai T, Adachi O, Ogawa T, Takeda K, Akira S. Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity. 1999;11:115–22.
Kawai T, Akira S. TLR signaling. Cell Death Differ. 2006;13:816–25.
Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol. 2009;21:317–37.
Kim HM, Park BS, Kim JI, Kim SE, Lee J, et al. Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran. Cell. 2007;130:906–17.
Kundi M. New hepatitis B vaccine formulated with an improved adjuvant system. Expert Rev Vaccines. 2007;6:133–40.
Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell. 1996;86:973–83.
Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, et al. Structural basis of toll-like receptor 3 signaling with double-stranded RNA. Science. 2008;320:379–81.
Loiarro M, Gallo G, Fanto N, De Santis R, Carminati P, et al. Identification of critical residues of the MyD88 death domain involved in the recruitment of downstream kinases. J Biol Chem. 2009;284:28093–103.
McGettrick AF, Brint EK, Palsson-McDermott EM, Rowe DC, Golenbock DT, et al. Trif-related adapter molecule is phosphorylated by PKC{epsilon} during Toll-like receptor 4 signaling. Proc Natl Acad Sci USA. 2006;103:9196–201.
Medzhitov R, Preston-Hurlburt P, Janeway Jr CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 1997;388:394–7.
Mullarkey M, Rose JR, Bristol J, Kawata T, Kimura A, et al. Inhibition of endotoxin response by e5564, a novel Toll-like receptor 4-directed endotoxin antagonist. J Pharmacol Exp Ther. 2003;304:1093–102.
Nishitani C, Mitsuzawa H, Hyakushima N, Sano H, Matsushima N, et al. The Toll-like receptor 4 region Glu24-Pro34 is critical for interaction with MD-2. Biochem Biophys Res Commun. 2005;328:586–90.
Ohnishi H, Tochio H, Kato Z, Orii KE, Li A, et al. Structural basis for the multiple interactions of the MyD88 TIR domain in TLR4 signaling. Proc Natl Acad Sci USA. 2009;106:10260–5.
O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol. 2007;7:353–64.
Papadopoulos AI, Ferwerda B, Antoniadou A, Sakka V, Galani L, et al. Association of toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms with increased infection risk in patients with advanced HIV-1 infection. Clin Infect Dis. 2010;51:242–7.
Park BS, Song DH, Kim HM, Choi BS, Lee H, et al. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. Nature. 2009;458:1191–5.
Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science. 1998;282:2085–8.
Przetak M, Chow J, Cheng H, Rose J, Hawkins LD, et al. Novel synthetic LPS receptor agonists boost systemic and mucosal antibody responses in mice. Vaccine. 2003;21:961–70.
Rose D, Zhu X, Kose H, Hoang B, Cho J, et al. Toll, a muscle cell surface molecule, locally inhibits synaptic initiation of the RP3 motoneuron growth cone in Drosophila. Development. 1997;124:1561–71.
Rossignol DP, Wong N, Noveck R, Lynn M. Continuous pharmacodynamic activity of eritoran tetrasodium, a TLR4 antagonist, during intermittent intravenous infusion into normal volunteers. Innate Immun. 2008;14:383–94.
Rowe DC, McGettrick AF, Latz E, Monks BG, Gay NJ, et al. The myristoylation of TRIF-related adaptor molecule is essential for Toll-like receptor 4 signal transduction. Proc Natl Acad Sci USA. 2006;103:6299–304.
Schaefer L. Extracellular matrix molecules: endogenous danger signals as new drug targets in kidney diseases. Curr Opin Pharmacol. 2010;10:185–90.
Schmitt C, Humeny A, Becker CM, Brune K, Pahl A. Polymorphisms of TLR4: rapid genotyping and reduced response to lipopolysaccharide of TLR4 mutant alleles. Clin Chem. 2002;48:1661–7.
Schnare M, Holt AC, Takeda K, Akira S, Medzhitov R. Recognition of CpG DNA is mediated by signaling pathways dependent on the adaptor protein MyD88. Curr Biol. 2000;10:1139–42.
Schroder NW, Schumann RR. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. Lancet Infect Dis. 2005;5:156–64.
Seo SU, Kwon HJ, Song JH, Byun YH, Seong BL, et al. MyD88 signaling is indispensable for primary influenza A virus infection but dispensable for secondary infection. J Virol. 2010;84:12713–22.
Thompson BS, Chilton PM, Ward JR, Evans JT, Mitchell TC. The low-toxicity versions of LPS, MPL adjuvant and RC529, are efficient adjuvants for CD4+ T cells. J Leukoc Biol. 2005;78:1273–80.

Xia ZP, Sun L, Chen X, Pineda G, Jiang X, et al. Direct activation of protein kinases by unanchored polyubiquitin chains. Nature. 2009;461:114–9.
Xu Y, Tao X, Shen B, Horng T, Medzhitov R, et al. Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. Nature. 2000;408:111–5.
Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science. 2003;301:640–3.
Yang H, Hreggvidsdottir HS, Palmblad K, Wang H, Ochani M, et al. A critical cysteine is required for HMGB1 binding to Toll-like receptor 4 and activation of macrophage cytokine release. Proc Natl Acad Sci USA. 2010;107:11942–7.

TLR4AP

▶ Toll-like Receptor Adaptor Protein Family Members

TLR5

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Synonyms

Myd88: Myeloid differentiation primary response gene (88); Toll-like receptor 5; TRIF: TIR domain–containing adaptor-inducing interferon β

Introduction

An infected host can quickly recognize an invading pathogen via germ-line-encoded pattern recognition receptors (PRRs) that detect microbe-associated molecular patterns (MAMPs) commonly expressed by many pathogens. Recognition of MAMPs by their corresponding receptor allows the host to initiate a rapid inflammatory response that can limit initial pathogen replication (Janeway and Medzhitov 2002). At least four different groups of PRRs have been described with varying cellular location and ligand specificity. Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are membrane-associated, while RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs) are found in the cytosol. Among the 13 known TLRs, only TLR5 and TLR11 exclusively recognize protein microbial ligands (Kawai and Akira 2010). TLR5 detects flagellin, a protein MAMP that is highly conserved and expressed by both gram-negative and gram-positive bacteria (Hayashi et al. 2001).

Monomeric flagellin is the major subunit of bacterial flagella, which is a whiplike structure that helps bacteria to resist intestinal peristalsis and establish productive infection. Polymerized flagellin forms the majority of the flagellar structure and is therefore one of the most abundant proteins expressed by flagellated bacteria. Furthermore, *Salmonella sp*. is known to secrete monomeric flagellin in response to lyso phosphatidylcholine, a product of intestinal epithelial cells (Subramanian and Qadri 2006). Thus, flagellin represents a major bacterial product, especially within the intestine. After recognition of flagellin, TLR5 forms a homodimer and induces proinflammatory signaling through the recruitment of adaptor molecules. Induction of proinflammatory genes by TLR5 detection of flagellin leads to recruitment of immune cells to the site of infection, thereby allowing the host to contain initial infection.

Background

In 1997, Rock et al. cloned and characterized human TLR5 and mapped this gene to chromosome 1. The corresponding TLR5 gene in mouse was reported 2 years later by Sebastiani et al. (Rock et al. 1998; Sebastiani et al. 2000). Murine TLR5 gene bears 81% homology to human TLR5 gene and around 40% homology to other TLR gene sequences (Sebastiani et al. 2000). In 2001, Hayashi et al. reported that bacterial flagellin is a ligand for TLR5 and requires ▶ Myd88 to produce inflammatory cytokines in response to flagellin stimulation (Hayashi et al. 2001). Further studies on TLR5 demonstrated that it is able to modulate adaptive immune response by inducing the expression of co-stimulatory molecules on antigen-presenting cells and secretion of immunomodulatory cytokines such as IL-12 (Iwasaki and Medzhitov 2004). Because of these stimulatory properties, TLR5 has been shown to function as an effective adjuvant (Mcsorley et al. 2002).