



Toll like receptors: an overview

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Abstract

Toll like receptors (tlrs) are first identified in drosophila and recognise pathogen associated molecular patterns (pamps) expressed by various micro-organisms and are also known as pattern recognition receptors (prrs). pamps are important for both innate and adaptive response. Among all tlrs, tlr1, tlr2, tlr3 and tlr4 are expressed on plasma membrane while tlr3, tlr7, tlr8 and tlr9 are expressed intracellularly. Downstream signalling of tlr activation involves expression of numerous cytokines, chemokines and co-stimulatory molecules such as irak, traf, tirap and sarm via myd88 dependant and independent pathway. This is a key step for both innate and adaptive immunity. tlrs play an important role in pathogenesis of various diseases including infectious diseases, autoimmune diseases, neurodegenerative diseases, lung disease, skin disease and cancer. Most studied tlrs are tlr2, tlr4, tlr3 and tlr9. In brief, this review emphasizes on types of tlr, their ligands, location, signalling pathway and role in various diseases.

Keywords: Toll like Receptors, Pathogen Molecular Patterns, Myd88 Pathway, Adaptor Proteins.

1. Introduction

The Toll like receptors are closely related to Toll is first identified in Drosophila and it recognizes specific structural motifs expressed by microbes which is essential for the establishment of the dorso-ventral pattern in developing embryos (Hashimoto C et al., 1988). The cornerstone of the innate immune system is composed of germline-encoded receptors which are known as pattern-recognition receptors (PRRs), to which the Toll-like receptors belong (Takeda K et al., 2004). Upon recognition of “Pathogen-Associated Molecular Patterns” or PAMPs, the TLRs are get activated. The cell surface Toll-like receptors (TLRs) recognize not only viral, bacterial, fungal and protozoan surface components, but also a plethora of endogenous molecules arising from host cell and tissue damage as well as the inflammatory response itself (Takeda K et al., 2004, Heil F et al., 2003, Gregory et al., 2003). TLRs recognize PAMPs that is enables the innate immune system to distinguish self from non-self and is important for triggering innate immunity against microbial infection and also for priming adaptive immune response. All TLRs activate MyD88-dependent pathways to induce a core set of stereotyped responses, such as inflammation. Individual TLRs can also induce immune responses that are tailored to a given microbial infection. Thus, these receptors are involved in both innate and adaptive immune responses. TLRs also detect endogenous molecules, such as damage-associated molecular patterns (DAMPs), which are produced by cells, in response to injury or infection (Prince LR et al., 2011). The study of Toll-like receptor (TLR) signalling pathways has proven to be a very fruitful area for investigators interested in signal transduction during innate immunity and inflammation (Akira S et al., 2011).

2. Structure of toll like receptors

TLRs are type 1 transmembrane proteins consist of an extracellular leucine-rich (LR) domain and a cytoplasmic tail that contains the resembles Toll/IL-1R gene homology (TIR) domain (Medzhitov R et al., 1997). The IL-1 receptors possess an Ig-like domain essential for signal transduction, whereas TLRs bear leucine-rich repeats (LRRs) in the extracellular domain involved in the ligand recognition (Medzhitov R et al., 1997). The proline residue in the TIR domains is present in all TLRs, except for TLR3, and its substitution leads to change in TLR signalling eg. Change in to histidine causes a dominant negative effect on TLR-mediated signalling (Hoshino K et al., 1999, Underhill DM et al., 1999). To understand the molecular mechanism of TLRs it is necessary to know the molecular structures of the interfaces between proteins in signalling complexes and of the conformational changes that occur during signalling in TLRs (Lemaitre B 2004).

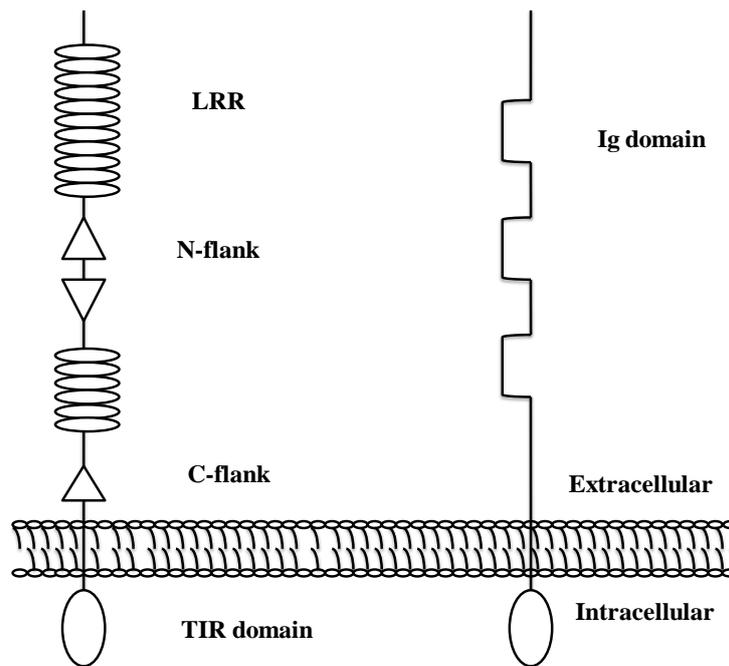


Fig. 1: Structure of Toll like Receptors (Lemaitre B 2004)

2.2. Leucine rich-repeat regions

The ectodomain of TLRs is responsible for recognition of ligands. The ectodomain structure provides a starting point for the understanding of its mode of signalling at the molecular level. The structure of the leucine rich repeat region is present at 2.3 Å in TLR (Li C et al., 2005, Khan JA et al., 2004). It is a large horse-shoe-shaped solenoid, which is stabilized by asparagines conserved in each of the 23 leucine-rich repeat motifs that form hydrogen bonding networks. Eg. In TLR3 is heavily glycosylated apart from one face, which is glycosylation free and therefore contribute to homodimerization by creating a binding site for dsRNA. The binding of dsRNA might cause a conformational change in the dimer which would trigger changes in the disposition of the intracellular TIR domains to bring them closer for signal transduction.

3. Toll like receptors and their ligands

TLRs can be grouped into families according to the types of ligands they recognize. Recently 13 mammalian TLRs (10 in humans and 12 in mice) have been identified (Choe J et al., 2005). TLR10 is expressed in humans but not in mice; TLR11, TLR12 and TLR13 are expressed in mice but not in humans (Bell JK et al., 2005). With these TLRs the innate immune system discriminates different PAMPs from different microbial classes.

Human TLRs can be structurally divided into 5 subfamilies there is only 1 member in TLR3, TLR4 and TLR5 subfamilies; TLR2 subfamily consists of TLR1, TLR2, TLR6 and TLR10; and TLR9 subfamily consists of TLR7, TLR8 and TLR9 (Seki E & Brenner 2008). TLR1/TLR2 and TLR2/TLR6 form heterodimers, and TLR4 and TLR9 form homodimerize. TLR1, TLR2, TLR4, TLR5 and TLR6 on cellular membrane mainly recognize bacterial products, which are not made by the host; so they can discriminate between self and non-self (Akira S & Takeda K 2004). In contrast, TLR3, TLR7, TLR8 and TLR9 are localized to intracellular compartments, and specialize in the detection of viral nucleic acids, which are not unique to the pathogens. Nucleic acids cannot trigger the intracellular TLRs, but when the host derived nucleic acids become available for intracellular

TLRs as their corresponding ligands, they would break immune tolerance and lead to autoimmunity (Beutler B 2005 & Schwabe RF 2006).

4. Types of toll like receptors based on their cellular location

The expression pattern of TLRs differs both inter-and intracellularly. Positive staining of the cell surface with antibodies to specific TLRs reveals that TLR1, TLR2, TLR4, TLR5 and TLR6 are all localized to the plasma membrane whereas TLR3, TLR7, TLR8 and TLR9 are preferentially expressed in intracellular compartments such as endosomes. The nature of the ligand recognized by individual TLRs seems to determine their expression pattern. For example, TLR3, TLR7, TLR8 and TLR9 all recognize nucleic acid structures whereas TLR1, TLR2, TLR4, TLR5 and TLR6 generally recognize cell wall components. It should be noted however that surface exposed TLRs are not completely restricted in their expression pattern, for example, TLR2 can localize to phagosomes following exposure to certain ligands (Schwabe RF et al., 2006).

4.1. Plasma membrane associated toll like receptors

4.1.1. TLR4

The recognition of lipopolysaccharide (LPS) or endotoxin by TLR4 remains one of the most extensively studied aspects of TLR signalling. LPS acts through TLR4 come from studies on a strain of mice called the C3H/HeJ mice. These mice have a point mutation in the gene encoding TLR4 that renders them hyporesponsive to LPS challenge (Poltorak A et al., 1998, Rifkin IR et al., 2005, Takeda K & Akira S 2005). TLR4 is also a receptor for endogenous ligands such as fibrinogen, heat shock proteins (HSP 60 and HSP 70), fibronectins and hyaluronic acid (Takeuchi O et al., 2001). However, the doses at which these compounds were found to activate TLR4 far exceeded those required for the activation of TLR4 by LPS and it is also suspected that these preparations may have been contaminated with traces of LPS.

Table 1: Toll like Receptors and Their Ligands

Receptor	Ligands
	Bacteria :Triacyl lipopeptides lipoarabinomannan (LAM) from mycobacterium Virus: Not found
TLR1/TLR2	Fungi: Yeasts and Zymogens Parasites: Glycosylphosphotidyl inositol linked proteins, T.cruzi Synthetic molecule : Not found Endogenous ligands: Not found
TLR2/TLR2	Published literature suggests the existence of TLR2 homo-dimers, through specific ligands have yet to be identified Bacteria: Diacyllipopeptides, Lipotechoic acid(LTA), Peptidoglycan Virus: Not found
TLR2/TLR	Fungi: Yeasts and Zymogens Parasites: Glycosylphosphotidyl inositol linked proteins, T.cruzi Synthetic Molecule: Not found Endogenous Liganda: Not found Bacteria: Not found Virus: dsRNA
TLR3	Fungi : Not found Parasites: Not found Synthetic molecule: Not found Endogenous Ligands: Not found Bacteria: Lipopolysaccharide (LPS) Virus: Not found Fungi : Not found
TLR4	Parasites: Not found Synthetic molecule: (mouse TLR4) Taxol Endogenous ligands: Hsp60, Hsp70, Hyaluronic Acid, Heparan Sulphate, Fibrinogen Bacteria: Flegellin Virus: Not found Fungi : Not found
TLR5	Parasites: Not found Synthetic molecule: Not found Endogenous ligands: Not found Bacteria: Not found Virus: ssRNA
TLR6	Fungi : Not found Parasites: Imidazoquinolines, (Mouse TLR7), Loxoribine Synthetic molecule: Not found Endogenous ligands: Not found Bacteria: ssRNA Virus: Not found
TLR8	Fungi : Not found Parasites: Imidazoquinolines, (Human TLR7) Synthetic molecule: Not found Endogenous ligands: Not found Bacteria: Bacterial CpG DNA Virus: Viral CpG DNA
TLR9	Fungi : Not found Parasites: Not found Synthetic molecule: Not found Endogenous ligands: Not found
TLR10	Unknown ligands Bacteria: (Mouse TLR11),Uropathogenic Bacteria Virus: Not found Fungi : Not found
TLR11	Parasites: (Mouse TLR11), T.gonadii profiling, apicomplexan profilin Synthetic molecule: Not found Endogenous ligands: Not found
TLR12	Unknown ligands
TLR13	Unknown ligands

4.1.2. TLR1, TLR2, TLR5 and TLR6

TLR2 recognizes a vast array of microbial components including lipoproteins from various pathogens, peptidoglycan from gram positive bacteria, glycosylphosphatidylinositol (GPI) anchors from malaria causing parasites, zymosan from fungi and forms of LPS that are structurally distinct from those recognized by TLR4 (Takeuchi O et al., 2002). The ability of TLR2 to recognize such a wide range of compounds has been attributed to the ability of TLR2 to heterodimerize with other TLRs, namely TLR1 and TLR6. The association of TLR2 with TLR1 permits the recogni-

tion of triacyl lipopeptides whereas TLR2/TLR6 heterodimers recognize diacyl lipopeptides (Alexopoulou L et al., 2001, Jurk M et al., 2002).

4.2. Intracellular TLRs

TLR3, TLR7 and TLR8 are nucleic acid recognizing TLRs in the context of viral infection whereas TLR9 is involved in the recognition of both bacterial and viral DNA (Hemmi, H et al., 2000, Lund J et al., 2003, Sen GC et al., 2005, Sarkar SN et al., 2003).TLR3 is highly expressed in macrophages, dendritic cells and epithelial cells (Sarkar, S.N et al., 2004) and induces the expression of IFN- β following exposure to double stranded (d/s) RNA. TLRs 7 and 9 are expressed in a subset of DCs called plasmacytoid DCs or pre-DC2 cells. Exposure of these cells to ssRNA or DNA from viral genomes results in the secretion of large amounts of IFN- γ . These receptors also differ in their signaling profiles.

4.2.1. TLR3

TLR3 functions independently of MyD88 requiring only the adaptor TRIF to transmit signals to the nucleus whereas TLR7, TLR8 and TLR9 all signal via MyD88. TLR3 can activate NF- κ B and this is thought to be mediated by the interaction of RIP1 with TRIF. The phosphorylation of TLR3 on Tyr759 facilitates the recruitment of PI3K. Mutation of this residue to phenylalanine not only prevented TLR3/PI3K complex formation but also resulted in only a partial phosphorylation of IRF3 and a failure to induce target genes. Phosphorylation on Tyr858 was also found to be critical for full IRF-3 activation. In a simplified scenario, phosphorylated TLR3 recruits both TRIF and PI3K (Kawai T et al., 2004).

4.2.2. TLR7, TLR8, TLR9

MyD88 and TRAF6 directly are directly associate with IRF7 to induce IFN- γ following stimulation of cells with ligands for TLR7 and TLR9 (Uematsu et al., 2005). Silencing of the ubiquitin-conjugating enzyme, UBC13, reduces the activation of IFN- γ promoters indicating that the ubiquitin ligase activity of TRAF6 is required for the activation of IRF-7. It has been reported that IRAK-1 participates in the activation of IRF-7 possibly through direct phosphorylation as suggested by in vitro kinase assays (Hornef MW et al., 2004).

5. Toll like receptors and its distribution

Table 2: Toll like Receptor and Its Distribution (Hornef MW Et Al., 2003)

Receptor	Expression
TLR1	Ubiquitous
TLR2	Monocyte and Dendritic cells
TLR3	Dendritic cells and NK cell
TLR4	Macrophage,Dendritic and Endothelial cells
TLR5	Monocyte, Immature dendriatic cells and NK cells
TLR6	B cells, Monocytes and NK cells
TLR7	B cells , Plasmacytoid precursor dendriatic cells
TLR8	Monocytes, NK and T cells
TLR9	Plasmacytoid precursor dendriatic cells, B cells, Macrophage, Microglial cells
TLR10	Plasmacytoid precursor dendriatic cells
TLR11	Not found

Distribution of TLRs within a particular tissue is vary in such a way that superficial epithelial cells, which are in constant contact with PAMPs having lack TLR expression whereas cells lining deeper areas of the tissue may express these receptors as they may be required to react to PAMP stimulation. For example, the epithelial cells of the intestinal crypt, which is normally a relatively sterile environment, express TLRs and are responsive to microbial PAMPs (Guillot L et al., 2003). TLR4 in crypt epithelial cells is expressed in the Golgi apparatus and it has been shown that LPS

is internalized by these cells, trafficked to this organelle where it interacts with TLR4, which thereby initiates the signaling cascade downstream of this receptor (Backhed F et al., 2003). Similarly, TLR4 presents an intracellular localization in lung epithelia and plays a key role in response of pulmonary cells to Gram-negative bacteria derived ligands (Abreu MT et al., 2001).

6. Toll like receptors signaling pathway

The downstream cellular response of TLR activation involves the expression of numerous cytokines and chemokines as well as co-stimulatory molecules necessary for the activation of the immune response. Stimulation of distinct TLRs can give overlapping responses while in certain cases; a specific response appears to be attributed to a particular TLR.

On interaction with their ligands, TLRs mediate the activation of cell signalling cascades that ultimately result in the induction of

the immune response and clearance of the infection from the host. Engagement of these Type-1 transmembrane receptors with their respective foreign agonist induces dimerization, bringing together two signaling domains which subsequently serve as a platform for the recruitment of various intracellular adaptor molecules (Luke AJ & O'Neill 2003, Xinyan LI 2009).

After ligand binding, the cytoplasmic TIR domain of TLRs activates downstream signaling via intracellular adapters. Five adapter proteins have been discovered, including myeloid differentiation primary response protein 88 (MyD88), Toll/IL-1 receptor domain-containing adapter protein (TIRAP) (MyD88-adaptor-like, also called Mal), Toll/IL-1 receptor domain-containing adapter protein inducing IFN- β (TRIF) (also known as TICAM1), TRIF-related adapter molecule (TRAM) (also known as TICAM2), and sterile α - and armadillo-motif containing protein (SARM). SARM functions as a specific inhibitor of TRIF-dependent pathway.

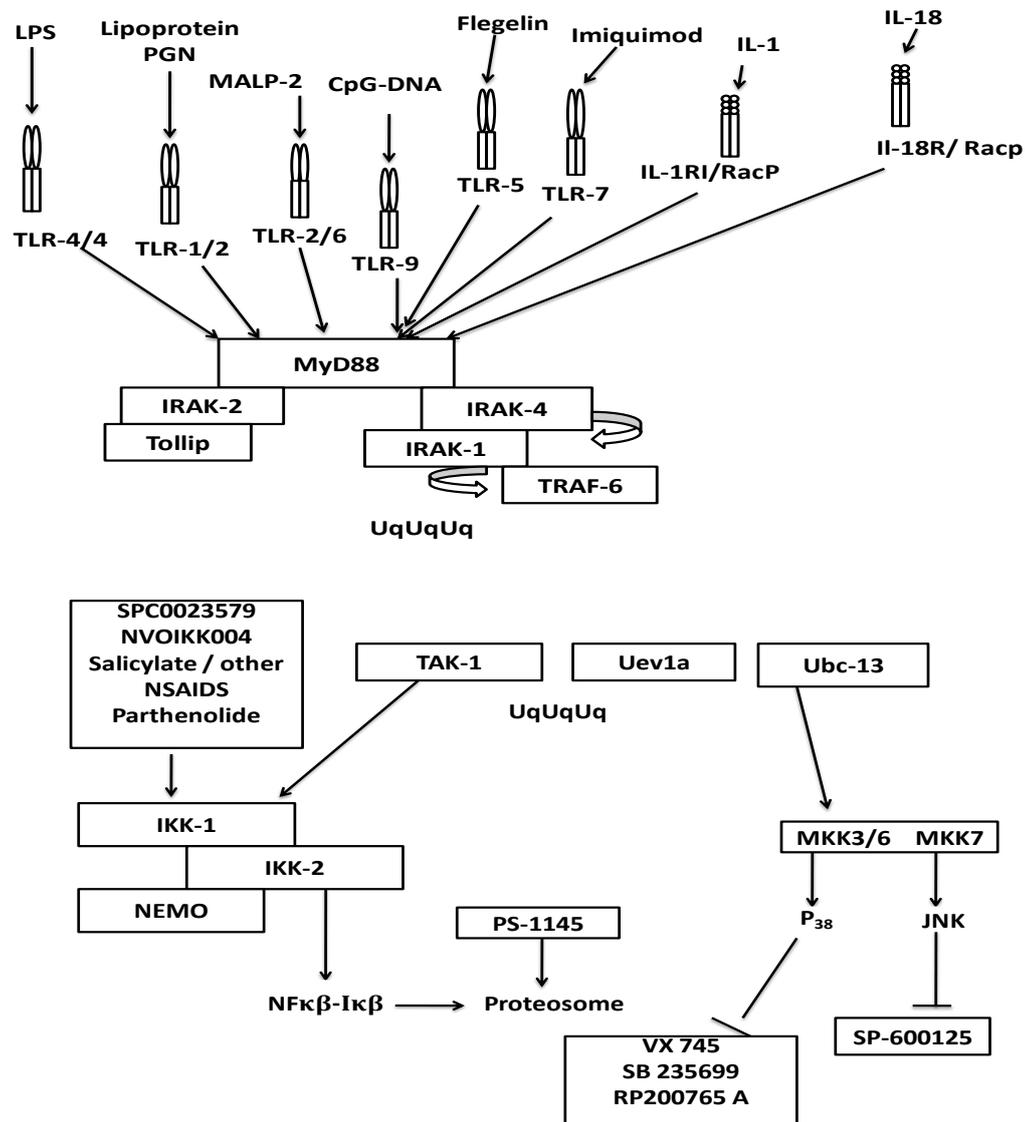
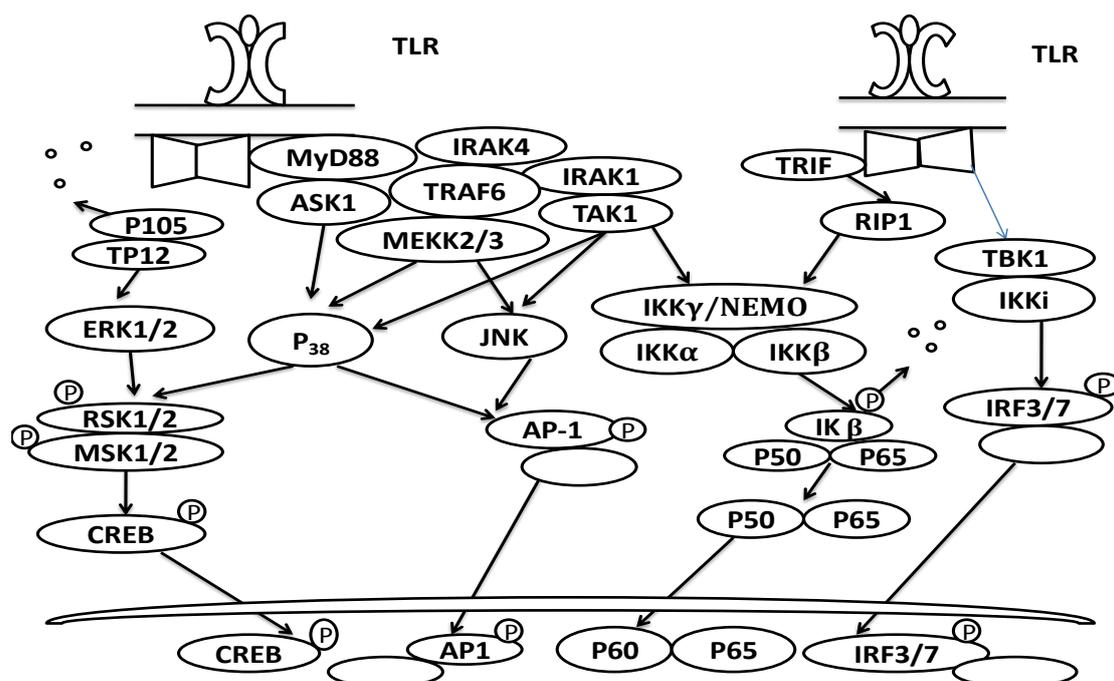


Fig. 2: Toll Like Receptor Signaling (X LI Et Al., 2010)

MyD88 is required for signaling by all TLRs except TLR3. TRIAP is a placement of TLR4 and TLR2 adaptor for mediation of MyD88-independent signaling. TRIF is the adaptor for TLR4 and TLR3 in the MyD88-independent pathway. TRAM functions 'upstream' of TRIF. The downstream signalling molecules include the transcription factors NF- κ B, mitogen-activated protein kinases (MAPKs), interferon regulatory factor-1 (IRF-1) and IRF-7, which

lead to the activation of type I IFNs, proinflammatory cytokines or co-stimulatory molecules.

Recent accumulating evidence indicates that TLR signaling pathways consist, at least, of a MyD88-dependent pathway that is common to all TLRs, and a MyD88-independent pathway that is peculiar to the TLR3- and TLR4 signaling pathways.



Immune Response cell proliferation and survival
 Fig. 3: Adaptor Protein Involved in Signaling Pathway (Takeuchi O Et Al., 2000)

6.1. MyD88-dependent pathway

The adaptor proteins involved in MyD88 pathway are as follows:

6.1.1. MyD88

MyD88 possesses the TIR domain in the C-terminal portion, and a death domain in the N-terminal portion. MyD88 associates with the TIR domain of TLRs. Upon stimulation, (Fig-3) MyD88 recruits IL-1 receptor-associated kinase (IRAK) to TLRs through interaction of the death domains of both molecules. IRAK is activated by phosphorylation and then associates with TRAF6, leading to the activation of two distinct signaling pathways, and finally to the activation of JNK and NFκB.

Four new insights into MyD88 have been made, which has important role in inflammation and host defence. MyD88 knockout mice has showed no responses to the TLR4 ligand LPS in terms of macrophage production of inflammatory mediators, B cell proliferation, or endotoxin shock (Takeuchi O et al., 2000). The cellular responses to the TLR2 ligands peptidoglycan and lipoproteins were abolished in MyD88 knockout mice (Hacker H et al., 2000, Schnare M et al., 2000). Cells from MyD88 knockout mice showed no responses to the TLR9 ligand CpG DNA and the TLR7 ligand imidazoquinoline (Hemmi H et al., 2000, Hayashi F et al., 2001, Burns K et al., 2003). MyD88 knockout mice did not produce any IL-6 in response to the TLR5 ligand flagellin (Cao Z et al., 1996). These findings demonstrated that the TIR domain-containing adaptor MyD88 is essential for the inflammatory responses mediated by all the TLR family members. MyD88s, which lacks the intermediate domain, has been shown to be induced by LPS stimulation and to inhibit LPS-induced NF-κB activation through inhibition of IRAK activity (Janssens S & Beyaert R 2003, Swantek JL et al., 2000). Thus, MyD88s may negatively regulate the inflammatory responses triggered by LPS.

6.1.2. IRAK

IRAK was identified as a serine/threonine kinase associated with the IL-1 receptor. Four members of the IRAK family have been identified these are: IRAK-1, IRAK-2, IRAK-M, and IRAK-4. IRAK proteins consist of an N-terminal death domain, which is responsible for interaction with MyD88, and a central kinase domain. IRAK-1 and IRAK-4 consist of Aspartate residue in the

kinase domain, but this residue is not conserved in IRAK-2 or IRAK-M, which causes them to be catalytically inactive (Suzuki N et al., 2002). The importance of the IRAK family members in TLR-mediated signaling pathways was first demonstrated in IRAK-1 knockout mice (Li S et al., 2002). A biochemical study shown that IRAK-4 acts upstream of, and phosphorylates, IRAK-1 upon stimulation (Kobayashi K et al., 2002). Thus IRAK-4 is a central mediator of TLR signalling by activating IRAK-1.

6.1.3. TRAF6 and downstream molecules

TRAF6 is a member of the tumor necrosis factor receptor (TNFR)-associated factor (TRAF) family that mediates cytokine signaling pathways (Arch RH, Gedrich RW, Thompson CB 1998). TRAF proteins consist of two C-terminal TRAF domains (TRAF-N and TRAF-C), which are responsible for interaction with TRAF proteins and other signaling molecules, N-terminal RING finger, and zinc finger domains. Among the TRAF family members, TRAF6 has been shown to be involved in the TLR signalling pathway in addition to signaling pathways via the OPLD receptor and CD40 (Lomaga MA 1999, Naito A et al., 1999). Upon stimulation of TLRs, TRAF6 is recruited to the receptor complex, and activated by IRAK-1 that binds to the TRAF domain of TRAF6. Then, the IRAK-1/TRAF6 complex dissociates from the receptor and associates with TGF activated kinase 1 (TAK1) and TAK1-binding proteins, TAB1 and TAB2, at the membrane portion. IRAK-1 stays in the membrane and is degraded, whereas the complex of TRAF6, TAK1, TAB1, and TAB2 moves into the cytoplasm, where it forms a large complex with other proteins, such as the E2 ligases Ubc13 and Uev1A (Deng L et al., 2000). The Ubc13 and Uev1A complex has been shown to catalyze the synthesis of a Lys 63-linked polyubiquitin chain of TRAF6 and thereby induce TRAF6-mediated activation of TAK1 and finally of NF-κB (Wang C et al., 2001).

6.1.4. Other molecules

Several other molecules have been implicated in the TLR-mediated signaling pathway. Toll-interacting protein (Tollip) was first identified in an analysis of IL-1 signaling (Burns K et al., 2000). Tollip is present in a complex with IRAK-1. Upon stimulation with IL-1, the Tollip-IRAK-1 complex is recruited to the IL-1 receptor complex. IRAK-1 is then phosphorylated, which leads to the rapid dissociation of IRAK-1 from Tollip, thereby inducing

activation of TRAF6. Subsequently, Tollip has been shown to negatively regulate the TLR-mediated signaling pathway (Bulut Y et al., 2002, Zhang G & Ghosh S et al., 2002). Overexpression of Tollip inhibited activation of NF- κ B in response to IL-1, the TLR2 and TLR4 ligands. Pellino was originally identified in *Drosophila* as a molecule that associates with Pelle, a *Drosophila* homologue of IRAK. In mammals, two Pellino homologues, Pellino-1 and Pellino-2, have been identified. Both Pellino-1 and Pellino-2 have been shown to interact with IRAK-1 in response to IL-1 stimulation (Takeda K & Akira S 2004, Kawai T et al., 2001). Ectopic expression of the Pellino-2 antisense construct inhibited IL-1- or LPS-induced activation of the NF- κ B-dependent promoter, indicating that Pellino-2 is involved in the IL-1 and TLR4 signaling pathways.

6.2. MyD88-independent pathway

In the MyD88-independent pathway, LPS stimulation leads to activation of the transcription factor IRF-3, and thereby induces IFN- α IFN- γ , in turn, activates Stat1, leading to the induction of several IFN-inducible genes (Doyle SE et al., 2002, Toshchakov V et al., 2002, Hoshino K et al., 2002). In addition to the TLR4 ligand, the TLR3 ligand dsRNA has been shown to induce activation of NF- κ B in MyD88 knockout cells (Alexopoulou L et al., 2001). Virus and viral-derived dsRNA are potent activators of IRF-3, which leads to the initial phase of IFN- α induction (Yu KY et al., 2002, Jiang Z et al., 2003, Weaver BK, Kumar KP, Reich NC 1998). Thus, the TLR3 ligand dsRNA also activates the MyD88-independent signaling pathway.

Two independent groups identified kinases responsible for the activation of IRF-3. IRF-3 was associated with I κ B kinases (IKKs) (Yoneyama M et al., 1998). IKKs are composed of IKK- α and IKK- β , both of which phosphorylate Ser32 and Ser36 of I κ B, thereby inducing NF- κ B activation. Two noncanonical IKKs, TANK-binding kinase 1 (TBK1) and IKK ϵ /IKK ι , which have distinct kinase activities compared with the canonical IKK- β and IKK- α .

RNAi-mediated ablation of TBK1 and IKK ϵ /IKK ι resulted in inhibition of virus-induced phosphorylation of IRF-3. Overexpression of TBK1 and IKK ϵ /IKK ι led to activation of IRF-3 and induction of IFN- β (Sato M et al., 2000). And reduced expression of TBK1 and IKK ϵ /IKK ι by RNAi led to impaired induction of IFN- β in response to virus and dsRNA. Thus, TBK1 and IKK ϵ /IKK ι have been shown to be critical regulators of IRF-3 activation, leading to the induction of IFN- γ in response to the TLR3 ligand.

7. TIR domain-containing adaptors

TLR signaling is mediated by an intracellular Toll/IL-1 receptor (TIR) domain, so named because it shares homology with that of IL-1 receptor (Sharma S et al., 2003). Two TIR domain-containing adaptors, TIR domain-containing adaptor protein (TIRAP)/MyD88-adaptor-like (Mal) and TIR domain-containing adaptor inducing IFN- γ (TRIF)/TIR domain-containing adaptor molecule (TICAM-1), were identified (Fitzgerald KA et al., 2003, Bonnard M et al., 2000 & Horng T et al., 2001). Analysis of these two adaptors indicated that TIR domain-containing adaptors regulate the TLR-mediated signaling pathways.

7.1. TIRAP/Mal

Database search analyses led to the identification of TIR domain-containing molecule, which was named TIRAP or Mal (Fitzgerald KA et al., 2001 & Yamamoto M et al., 2002). TIRAP/Mal consists of TIR domain in the C-terminus. MyD88 knockout macrophages, TIRAP/Mal knockout macrophages showed impaired inflammatory cytokine production and delayed activation of JNK and NF- κ B in response to the TLR4 ligand. However, TLR4 ligand-induced activation of IRF-3 and expression of IFN- γ inducible genes was normally observed in TIRAP/Mal knockout macrophages.

TIRAP/Mal is critically involved in the MyD88-dependent pathway, but not in the MyD88-independent pathway, via TLR4. TIRAP/Mal knockout mice showed normal responses to the TLR3, TLR5, TLR7, and TLR9 ligands, but were defective in TLR2 ligands-induced inflammatory cytokine production. TIRAP/Mal is essential for the MyD88-dependent signalling pathway via TLR2 and TLR4, but not for MyD88-independent signalling.

7.2. TRIF

TRIF/TICAM-1 was identified by a database search and as a TLR3-associated molecule by two-hybrid screening (Oshiumi H et al., 2003 & Horng T et al., 2002). Unlike MyD88 and TIRAP/Mal, TRIF is a large protein consisting of 712 amino acids in humans. Over expression of TRIF as well as MyD88 and TIRAP caused activation of the NF- κ B dependent promoter in 293 cells. Over expression of TRIF, but not MyD88 or TIRAP, induced activation of the IFN- β promoter. Dominant negative TRIF inhibited the TLR3 ligand-induced activation of the IFN- β promoter, and RNAi-mediated knockdown of TRIF caused impairment in the TLR3 ligand-induced IFN- β expression. In TRIF knockout mice, TLR3-mediated expression of IFN- β and IFN- γ inducible genes was impaired (Yamamoto M et al., 2002). TRIF knockout mice displayed defective expression of IFN- γ inducible genes in response to the TLR4 ligand. A study of random germline mutagenesis in mice, using the alkylating agent N-ethyl-N-nitrosourea (ENU), also revealed that TRIF-mutant mice were defective in the TLR3- and TLR4- mediated responses (Yamamoto M et al., 2003). Thus, TRIF has been demonstrated to be essential for the TLR3- and TLR4-mediated MyD88 independent pathway. These studies clearly established that TIR domain-containing adaptors provide specificity for individual TLR-mediated signaling pathways. In addition to the impaired MyD88 independent pathway, TRIF knockout mice displayed defective TLR4-mediated inflammatory cytokine production, although activation of the MyD88-dependent pathway, such as IRAK-1 phosphorylation and early phase of NF- κ B activation, was not impaired. Therefore, the TLR4 signaling pathway is likely to require activation of both the MyD88-dependent and -independent pathways to induce inflammatory cytokines.

7.3. SARM

SARM (sterile and Heat-armor motifs) was the last TIR containing adaptor protein. SARM was originally identified as an orthologue of a *Drosophila* protein CG7915 (Belinda LW et al., 2008) and is evolutionarily conserved with orthologues of the protein described in zebra fish (O'Neill LA et al., 2003), *Caenorhabditis elegans* and the horseshoe crab. Recently the horseshoe crab orthologue of SARM (CrSARM) was characterized. As in humans, CrSARM was found to down regulate TRIF dependent TLR signalling in response to infection (Liberati NT et al., 2004). CrSARM was up regulated within 3 h of infection and strongly repressed at 6 h coinciding with the timing of bacterial clearance. This study suggests that the TRIF specific negative regulating role of SARM is evolutionary conserved from horseshoe crab to human (Kim Y et al., 2002).

SARM is a 690 a protein, which contains two sterile motifs (SAMs) domains, a C-terminal TIR domain and N-terminal heat Armadillo repeat motif (ARM) (Kim Y et al., 2002). Of the TLR adaptors, SARM is the least well characterized. A study of the *C. elegans* SARM homologue, TIR1 showed it to be important in the efficient immune response against infections, with knockdown of TIR1 by RNA interference resulting in decreased worm survival in response to fungal and bacterial infection (Persing DH et al., 2002 & , Ohashi K et al., 2000). A study by Carty et al., found that human SARM was a negative regulator of TRIF-dependent TLR signalling. They found that expression of SARM blocked gene induction downstream of TRIF but not MyD88 and knockdown of endogenous SARM led to enhanced TRIF-dependent cytokine

production (Okamura Y et al., 2001). Interaction with SARM is preventing TRIF interacting with upstream or downstream signaling molecules. They found that SARM is primarily expressed in neurons where it associates with mitochondria, microtubules and JNK3 and regulates neuronal death during deprivation of oxygen and glucose (Frantz S et al., 1999) human and mouse myeloid cells expressed little SARM.

8. Targeting toll like receptors

Evidence is emerging that certain TLRs play a role in the pathogenesis of infectious and/or inflammatory diseases. The most studied TLRs in this regard are TLR4, anti-viral TLRs like TLR3 and TLR9.

8.1. TLR4

TLR4 is probably the key driver of tumor necrosis factor (TNF) during sepsis. During infection, conditions such as chronic obstructive pulmonary disease and asthma are exacerbated, and it is possible that TLRs drive the inflammation that gives rise to worsening symptoms. Blocking TLR4 (or indeed TLR2 or TLR5) might therefore be of use in the prevention of exacerbations. Finally, agonistic lipid analogues are also being tested as adjuvants, largely in the context of anti-tumor immunotherapy (Frantz S et al., 1999).

TLR4 has also been shown to be a possible receptor for endogenous factors released during tissue injury and inflammation, such as hsp60 (Alexopoulou et al., 2001) and fibronectin fragments (Alexopoulou et al., 2001 & Hemmi H et al., 2002). In non-infectious inflammation, TLR4 might sense such products and provoke TNF production. TLR4 is expressed in the heart, especially during ischemia (Jurk M et al., 2002), and either in response to microbial products or endogenous factors might provoke inflammation and promote heart disease.

8.2. TLR3

A 'proof-of-principal' that inhibition of anti-viral TLRs is not only possible but also useful has come from a study with Vaccinia virus (VV). VV is a pox-virus containing several genes that encode decoys to block host defences but are not required for replication. It contains two proteins that appear to block TLR signalling, A46R and A52R (Frantz S et al., 1999 & Alexopoulou L et al., 2001). A52R is similar to A46R, but acts to disrupt the downstream signalling components IL-1 receptor-associated kinase (IRAK)-2 and TNF receptor-associated factor-6 (TRAF-6). A52R is a potent inhibitor of TLR3 signalling, which is consistent with the role of TLR3 in anti-viral host defence. Importantly, a version of VV lacking A52R is attenuated in terms of causing illness during infection compared with wild-type virus, indicating that the virus uses A52R to limit the host defence against itself (Alexopoulou L et al., 2001). This implies that it might be possible to develop inhibitors based on these viral proteins to limit TLR signalling.

TLR3 has been identified as an important receptor for polyI:C, a mimic of viral double-stranded RNA (Syed TA 2001). Stimulating TLR3 would be predicted to have anti-viral adjuvant effects, whereas blocking TLR3 might be useful in limiting viral virulence. TLR7 and TLR8 appear to be receptors for a family of anti-viral compounds based on imidazo quinolones that are potent inducers of type I interferon (Bowie A et al., 2000 & Harte MT et al., 2003). These compounds are currently in trial for genital herpes (Leadbetter EA et al., 2002).

8.3. TLR9

TLR9 is the receptor for CpG motifs, which occur in both bacterial and viral DNA. Apart from having potential as a target for adjuvants, a recent study suggests that TLR9 might have a role in

autoantibody production (Alexopoulou L et al., 2001). In a mouse model of systemic lupus erythematosus, it was demonstrated that immune complexes containing self DNA activate self-IgG-specific B cells as a result of co-stimulation of these B cells through TLR9 and the B cell receptor. This raises the intriguing possibility that TLRs, by sensing the products of damaged tissues, might provide the stimulus that gives rise to auto reactive B cell proliferation and autoantibody production. TLR9 could be a useful therapeutic target for both autoimmunity and asthma.

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