Adaptors in Toll-like Receptor Signalling and their Potential as Therapeutic Targets

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Abstract: To initiate the innate immune response, Toll-like receptors (TLRs) associate with cytoplasmic adaptor proteins through TIR (Toll/interleukin-1 receptor) domain interactions. The four principal signaling adaptor proteins include MyD88, MAL, TRIF and TRAM, and the fifth protein SARM, involved in negative regulation of TLR pathways, is usually considered a part of the TIR domain-containing adaptor protein group. Other TIR domain-containing proteins have also been shown to regulate these signaling pathways, including ST2 and SIGIRR, as well as several bacterial and viral TIR domain-containing proteins that modulate these pathways as virulence factors. TLR pathways and the adaptor proteins are associated with a number of diseases, including infection, sepsis, inflammatory, allergic and autoimmune diseases and cancer. We review our current understanding of the structure and function of adaptor proteins and their regulatory proteins, their association with disease and their potential as therapeutic targets in human disease.

Keywords: Toll-like receptors (TLRs); TIR (Toll/interleukin-1 receptor) domain; adaptor proteins.

INTRODUCTION

Toll-like receptors (TLRs) are germline-encoded pattern recognition receptors (PRRs) that play a fundamental role in the innate immune response of vertebrates. They recognize conserved microbial structures known as pathogen-associated molecular patterns (PAMPs), and activate transcription factors such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and interferon regulatory factor (IRF)-3, as well as the MAP kinase (MAPK) pathways, resulting in the production of pro-inflammatory mediators and maturation of the adaptive immune response [1, 2]. TLRs are single-pass transmembrane proteins consisting of an extracellular leucine-rich repeat (LRR) domain involved in PAMP recognition, a transmembrane helix, and a cytosolic Toll/interleukin-1 receptor (TIR) domain. In humans, 10 TLRs have been identified and they are either located at the cell surface or in intracellular membranes such as the endosomal compartment. Bacterial and fungal PAMPs such as lipopolysaccharides, lipopeptides or bacterial flagellin are recognized by the surface-associated TLRs, while viral or microbial nucleic acids are recognised by TLRs localized to intracellular compartments [3]. PAMP binding to TLRs stabilizes a dimeric conformation of the extracellular LRR domain and places the C-terminal regions of both molecules in close proximity to each other. This allows the transmembrane regions and the intracellular TIR domains of the two receptors to come into close proximity, which creates a new scaffold for recruitment of downstream TIR domain-containing adaptor proteins and subsequent activation of signaling pathways [4]. The TLR-mediated responses are specific to each ligand-receptor interaction, TLR cellular localization and cell type, resulting in a complex response network (Fig. **1**). For recent reviews on TLR downstream signaling the reader is directed to [1, 5-8]. The ability of the adaptors to activate distinct TLR signaling pathways makes them attractive candidates for drug development. We review our current understanding of the structure and function of adaptor proteins and their regulatory proteins, their association to disease and their potential as therapeutic targets in human disease.

OVERVIEW OF THE TIR DOMAIN-CONTAINING ADAPTOR PROTEINS

PAMP recognition by TLRs leads to recruitment of a single, or specific combination of, intracellular TIR domain-containing adaptor proteins comprising MyD88 (myeloid differentiation primary response gene 88), MAL (MyD88 adaptor-like protein), TRIF (TIR domain-containing adaptor protein inducing IFNβ) and TRAM (TRIF-related adaptor molecule)**.** MyD88 was the first adaptor protein to be identified [9, 10], and is utilized by all of the TLRs except TLR3, which only interacts with TRIF [11, 12]. The C-terminal TIR domain of MyD88 (see Fig. **2** for schematic diagrams of the domain structures of the proteins discussed here) interacts with the TIR domains of the activated TLR receptors [9, 10, 13], or the adaptor protein MAL [14], while the N-terminal

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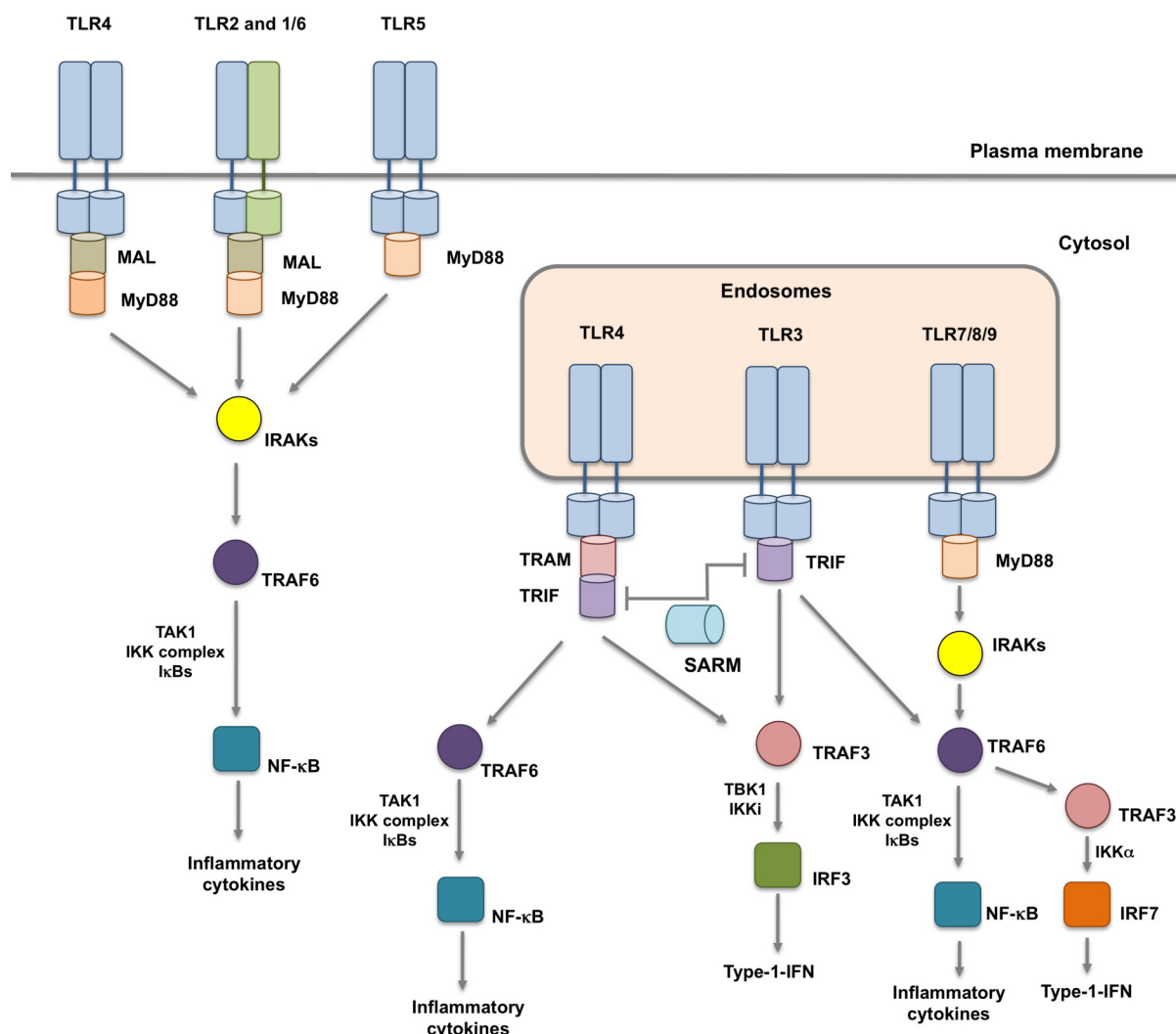
Fig. (1). TLR signaling pathways.
Recognition of PAMPs stabilizes homo- and heterotypic interactions between TLRs, which leads to recruitment of a single or specific combination of the adaptor proteins MyD88, MAL, TRIF, and TRAM. Binding of MyD88 to cell surface-localized TLRs provides a platform for recruitment of IRAKs (interleukin-1 receptor-associated kinases). Activation of IRAK4 by MyD88 leads to auto-phosphorylation of IRAK1 or IRAK2 [183-185], which subsequently associates with the E3 ubiquitin ligase TRAF6 (TNF receptor associated factor 6) [186]. TRAF6 recruits TAK1 (TGF-beta activated kinase 1) and the IκB kinase (IKK) complex, which activate the MAP kinase and NF-κB pathways leading to production of proinflammatory cytokines. The cell surface-localised receptors TLR2 and TLR4 require the bridging adaptor MAL for recruitment of MyD88. Recruitment of MyD88 to intracellular TLRs such as TLR7 and TLR9 leads to activation of NF-κB, but also IRF7 by recruitment of TRAF3 and IKKα. Activation of IRF7 leads to production of type-1-IFN. The adaptor protein TRIF is recruited by the endosome-localised receptors TLR3 and TLR4. TLR3 can interact directly with TRIF, while the TLR4-TRIF interaction requires the bridging adaptor TRAM. TRIF can activate NF-κB by binding of TRAF6 or by recruiting RIP1 through the C-terminal region containing the RHIM motif [187], while IRF3 activation involves recruitment of TRAF3 and the protein kinases TBK1 and IKKi. This complex is responsible for phosphorylation of IRF3, which induces its nuclear translocation (reviewed in [1]), leading to production of type-1-IFN. The adaptor protein SARM can inhibit the signaling pathways induced by TRIF.

death domain recruits IRAK4 (interleukin-1 receptor-associated kinase 4) through death domain interactions [15]. This results in activation of the MAP kinase and NF-κB pathways, leading to production of inflammatory cytokines (Fig. **1**; reviewed in [1]). MyD88 can also induce IRF3/IRF7-dependent type-I-interferon (IFN) production when recruited to the endosomal TLRs such as TLR7 and TLR9 [16-18] (Fig. **1**). Recently, the crystal structure of a ternary death domain complex (Myddosome), comprising MyD88, IRAK4 and IRAK2 death domains revealed a four-

layered left-handed helical arrangement consisting of six MyD88, four IRAK4, and four IRAK2 death domains [19] (structures of TIR domains are discussed in the following section). The structure suggests a sequential assembly process in which a receptor-induced homo-oligomer of MyD88 forms first and provides a platform for recruitment of four IRAK4 and then four IRAK2 molecules. The interdomain (ID) region in MyD88 (Fig. **2**) has an important regulatory role as it has been shown that a splice variant of MyD88 (MyD88s) lacking this region cannot interact with IRAK4,
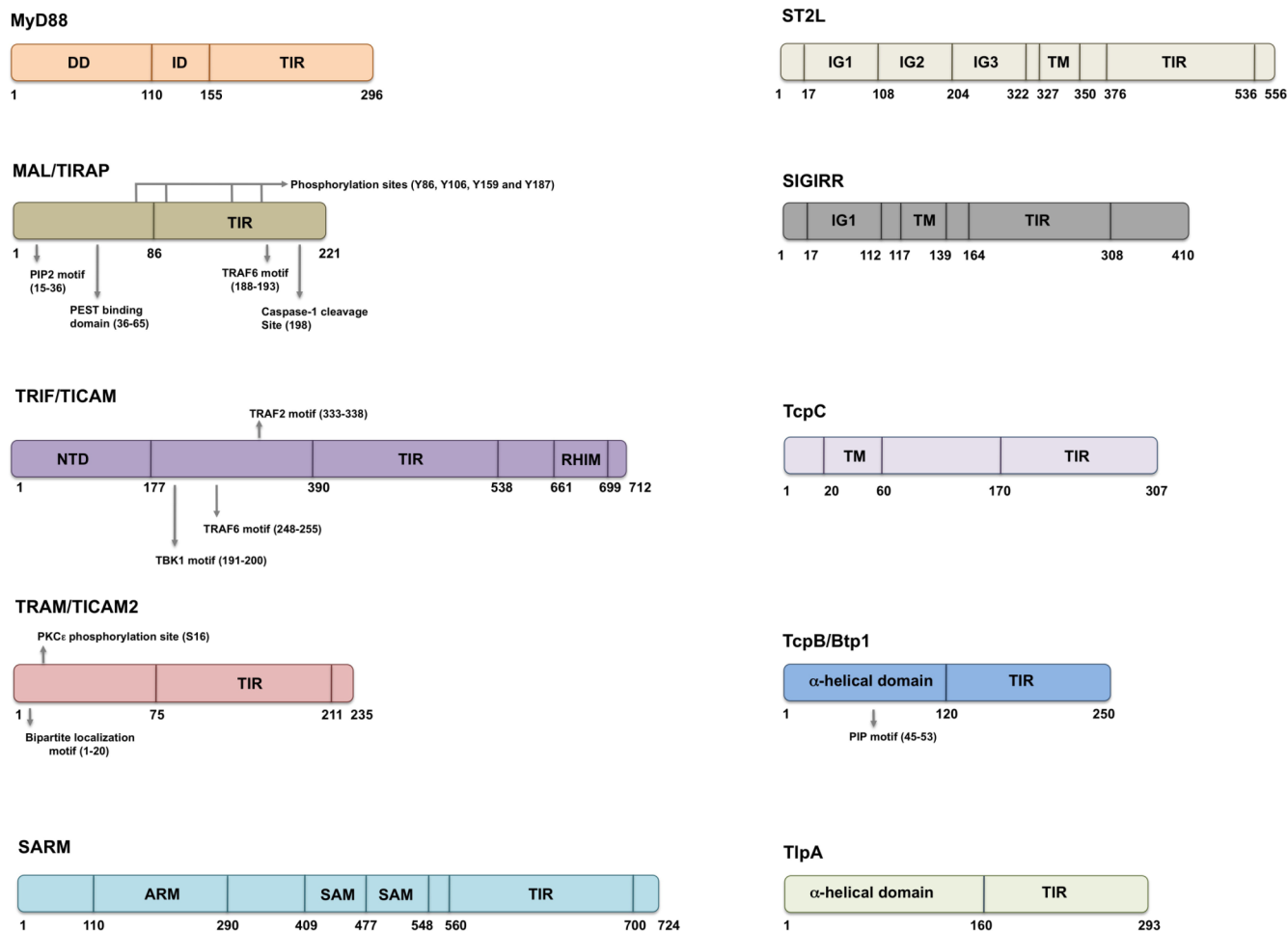
MyD88

DD	ID	TIR

1   110   155   296

MAL/TIRAP

Phosphorylation sites (Y86, Y106, Y159 and Y187)

	TIR

1   86   221

PIP2 motif (15-36)
PEST binding domain (36-65)
TRAF6 motif (188-193)
Caspase-1 cleavage Site (198)

TRIF/TICAM

TRAF2 motif (333-338)

NTD	TIR	RHIM

1   177   390   538   661   699 712

TRAF6 motif (248-255)
TBK1 motif (191-200)

TRAM/TICAM2

PKCε phosphorylation site (S16)

	TIR

1   75   211 235

Bipartite localization motif (1-20)

SARM

ARM	SAM	SAM	TIR

1   110   290   409   477   548 560   700 724

ST2L

IG1	IG2	IG3	TM	TIR

1   17   108   204   322 327   350 376   536 556

SIGIRR

IG1	TM	TIR

1   17   112 117   139 164   308   410

TcpC

TM	TIR

1   20   60   170   307

TcpB/Btp1

α-helical domain	TIR

1   120   250

PIP motif (45-53)

TlpA

α-helical domain	TIR

1   160   293

Fig. (2). Schematic overview of TIR domain-containing proteins involved in TLR signaling.

MyD88 contains an N-terminal death domain, a linker region, and a C-terminal TIR domain [9, 10]. **MAL** contains an N-terminal PIP2-binding motif, a PEST domain, and a C-terminal TIR domain [24, 25, 31]. MAL also contain a TRAF6-binding motif. MAL is phosphorylated by Bruton's tyrosine kinase (BTK) after being recruited to activated TLR2 or TLR4. BTK-dependent phosphorylation increased the interactions with TLR4, which rendered MAL a dominant negative inhibitor of TLR4 signaling [30]. MAL is also regulated by the cysteine protease caspase-1, which cleaves MAL at residue D198 in the αE helical region [32-34]. **TRIF** contains an N terminal α-helical domain, a central TIR domain, and a C-terminal region containing a receptor-interacting protein (RIP) homotypic interaction motif (RHIM). The region between the N-terminal domain and the TIR domain contains binding motifs for TRAF6, TRAF2 and TBK1 (Tank-binding kinase 1) [40, 188-190]. **TRAM** contains a C-terminal TIR domain, which has significant similarity to the TIR domain in TRIF, but is more distantly related to the TIR domains of MyD88 and MAL. The bipartite sorting signal in the N-terminal region of TRAM consists of a myristoylation site followed by a polybasic region [42, 43]. TRAM is also phosphorylated by PKCε on Ser16. **SARM** contains a N-terminal armadillo repeat (ARM) region, two central sterile α-motif (SAM) domains, and a C-terminal TIR domain [191]. Both the SAM domains and the TIR domains are required for inhibition of TRIF mediated signaling.

ST2L contains three extracellular immunoglobulin domains, a transmembrane (TM) region and a C-terminal TIR domain. **SIGIRR** has only one extracellular Ig domain, and a cytoplasmic tail consisting of 95 residues follows the TIR domain [123]. **TlpA** and **TcpB/Btp1** both have an α-helical N-terminal domain followed by a TIR domain [131-133]. TcpB can bind to phosphoinositides, and mutational analysis revealed that a basic motif consisting of residues 45-53 in the N-terminal region is important for phosphoinositide binding [134]. **TcpC** also has a C-terminal TIR domain, but contains a predicted TM helix in the N-terminal region [131]

thereby preventing phosphorylation of IRAK1 and activation of NF-κB [13, 20, 21]. This region is also essential for MyD88 recruitment to TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) in B cells. TACI lacks a TIR domain, but can trigger immunoglobulin class-switch recombination in B cells by activating NF-κB through a TLR/MyD88 dependent-like pathway [22].

MAL, also called TIRAP (Toll/interleukin-1 domain-containing adaptor protein), was the second identified TLR adaptor. PIP2 (phosphatidylinositol-4,5-bisphosphate) binding localizes MAL to the plasma membrane, where it recruits MyD88 to TLR2 and TLR4 through receptor:adaptor and adaptor:adaptor TIR:TIR domain interactions [14, 23-26] (Fig. **1**). MAL can also associate directly with TRAF6 and mutation of a key TRAF6-binding residue (E190A) prevents NF-κB activation mediated by both TLR2 and TLR4, demonstrating that the MAL-TRAF6 interaction is essential for signaling by these receptors [27, 28]. MAL undergoes several types of post-translation modifications important for

function. These include phosphorylation by Bruton's tyrosine kinase (BTK) [29, 30], SOCS-1 (suppressor of cytokine signaling) induced poly-ubiquitination targeting MAL for degradation [31], and proteolytic cleavage by the cysteine protease caspase-1, which presumably attenuates the interaction with MyD88 [32-34].

TRIF, also called TICAM-1 (TIR-containing apdaptor molecule-1), acts as the sole signaling adaptor for TLR3, and can also associate with TLR4 *via* TRAM (Fig. **1**). Recruitment of TRIF by TLR3 and TLR4 activates NF-κB and IRF3 resulting in production of both pro-inflammatory cytokines and type-1-IFN [14, 35-37] (Fig. **1**). Live-cell imaging and confocal immuno-fluorescence analyses have shown that TRIF alters its distribution profile from a diffuse cytoplasmic form to a speckle-like structure in response to TLR3 activation by dsRNA [38]. Yeast two-hybrid and co-immuno-precipitation studies using TRIF deletion mutants revealed that both the TIR domain and the C-terminal region but not the N-terminal domain are required for self-association of TRIF [39] (Fig. **2**). The N-terminal domain of TRIF appears to inhibit self-association and it can interact directly with the TIR domain, suggesting that it folds back onto the TIR domain in the resting state of TRIF, and acts as a negative regulator preventing downstream signaling molecules such as TBK1 and TRAF6 from accessing their binding sites [40].

TRAM, also called TICAM-2, is responsible for recruiting the signaling adaptor TRIF to the activated and endocytosed TLR4 [37, 41]. Unlike MAL, TRAM operates both at the plasma membrane and the endosomes, and it has been shown that TRAM localization to the endosomes is both necessary and sufficient for TRIF-dependent IRF3 activation [42]. Trafficking of TRAM between the plasma membrane and the endosomes is controlled by a bipartite sorting signal in the N-terminal region (Fig. **2**) [42, 43].

SARM (sterile α and armadillo-motif containing protein) was the fifth identified adaptor protein involved in TLR signaling, and it is the most conserved TLR adaptor protein with homologs identified in zebrafish, horseshoe crab, Drosophila and *Caenorhabditis elegans* [44-47]. Multiple roles have been assigned to SARM and the roles appear to differ among species. In humans, SARM can act as a specific inhibitor of TLR3 and TLR4 signaling mediated by TRIF [48] and has also been shown to inhibit both TRIF- and MyD88-mediated AP-1 activation [49]. More recently, a role for SARM in hypoxic neuronal cell death and neuronal morphology has been reported [50-52]. SARM further acts as a suppressor of TLR signaling in horseshoe crab [47], while *C. elegans* SARM is involved in activating host defenses against fungal and bacterial infections, as well as having a role in neuronal development [46, 50, 53]. Phylogenetic analyses have shown that the TIR domain region in SARM is closely related to bacterial TIR domains, which may indicate that this family has a different evolutionary history from the other TIR domain-containing adaptors, and has been recruited *via* lateral gene transfer from bacteria [54].

BCAP (B-cell adaptor for PI3K, also known as PIK3AP1) has recently been suggested as an additional adaptor molecule. While BCAP was originally described as an adaptor molecule linking the B-cell receptor to PI3 kinase signaling [55, 56], Troutman *et al* [57] have recently reported it to be a regulator of TLR signaling. These workers showed that the BCAP TIR domain could associate with MyD88. Furthermore, when expressed in a macrophage cell line, both full-length BCAP and the TIR domain alone repressed TLR4-mediated activation of NF-κB and cytokine production; conversely, when BCAP was silenced, TLR stimulation resulted in elevated cytokine production [57].

ADAPTOR MOLECULE FUNCTION AND ASSOCIATION WITH DISEASE

Along with MAL, MyD88 is the most polymorphic of the adaptor molecules. Despite this, there is only limited information regarding functional SNPs and disease association for MyD88. One study reported that nine children with inherited mutations in MyD88 displayed susceptibility to infections by *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [58]. Interestingly, these patients appeared to have normal resistance to most common bacteria, viruses, fungi and parasites [58], although there was a much greater risk of life-threatening infections, especially at early ages [59]. Lack of MyD88 has been linked to the inability of patients to develop antibody-secreting autoreactive B cells, suggesting that downregulation of MyD88 (and other TLR signaling molecules) may thwart auto-immunity [60]. Six nonsynonymous single nucleotide polymorphisms (SNPs) have been identified in the coding region: S34Y; L35V; L74M; R98C and M178I. Of these, only the S34Y and R98C appear to have significance, as they interfered with Myddosome assembly, while the other mutants appeared to have normal function [61, 62]. The analysis of knockout mice has uncovered a crucial role for MyD88 in TLR signaling, as animals deficient in MyD88 are unresponsive to TLR2, TLR4, TLR5, TLR7 and TLR9 signals [11, 12]. Numerous disease models have been investigated using these knockout animals. As might be expected, given the central role of MyD88 in TLR signaling, animals lacking MyD88 animals are susceptible to a number of microbial infections including *S. aureus* [12], *Plasmodium* [63], *Toxoplasma* [64], *Listeria* [65] and *Leishmania* [66].

More than a hundred SNPs have been identified in MAL, but of these, only seven code for changes in the amino acid sequence of the protein [67]. The most common of these is S180L, and a number of studies have implicated it in resistance/susceptibility to infectious disease or autoimmunity. This amino acid alteration in the TIR domain of MAL appears to confer to heterozygotes an increased risk of sepsis and a failure to respond to *Haemophilus influenzae* type b vaccine [68, 69], and an increased risk of bacterial infection after surgery [70]. This same mutation has been reported to be associated with the inflammatory disorder Bechet's disease in a white Caucasian UK cohort, but not in Middle Eastern patients [71]. However, on the positive side, heterozygotic individuals with the S180L mutation are relatively resistant to malarial, and pneumococcal infections and bacteraemia [72, 73]. There is some controversy over whether the S180L mutation is protective or deleterious in tuberculosis (TB); one study did not find any association of TB protection and this SNP [74], however two studies have found an association with susceptibility to TB [75, 76]. A large meta-analysis concluded that MAL S180L polymorphism is unlikely to substantially contribute to TB suscepti-

bility [77]. Populations from different ethnic backgrounds associate differently with disease [78, 79]. Indeed, the frequencies of occurrence of these SNPs differ substantially in different ethnic groups [67, 78]; for example the frequencies of heterozygotes for the S180L mutation in European, African American, Asian and sub-Saharan African populations are 23.8%, 13.6%, 4.5% and 0%, respectively. It seems that the other SNPs leading to amino acid changes occur at only low levels (<1%) in all populations tested. Thus, both the SNP frequency and disease association appear to be dependent on the genetic background of the individuals. Because a number of studies indicated a potential association with acute inflammation and infection, a number of chronic inflammatory conditions have been investigated and there is an association of this SNP with protection against systemic lupus erythematosus (SLE) [73], but not rheumatoid arthritis [80]. Heterozygotes with this SNP have also been reported to have a lower risk of developing chronic Chagas cardiomyopathy [81]. Other rare SNPs either in noncoding regions or which do not change the coding sequence have variously been reported. In particular, the nucleotide C558T SNP (which does not change the amino acid sequence; amino acid A186A), has been associated either with susceptibility or resistance to meningial TB [82], however the mechanism by which this might occur is unknown.

The functional consequences of the absence of MAL have been examined in a number of disease models using knockout animals. Because the initial data suggested that MAL played a role in susceptibility to bacterial infections, not surprisingly infectious disease models have primarily been investigated using mice in which MAL had been ablated. MAL has been found to be essential for the generation of an effective early immune response against *E. coli* [83]. These responses appear to be pathogen-specific, as MAL is also essential for defense in the lung against *Klebsiella pneumoniae* but not *P. aeruginosa* [84] and in fact MAL appears to be at least nonessential and possibly detrimental to the clearance of circulating *Salmonella* [85]. In an LPS inhalation model of lung disease, cytokine and chemokine secretion and neutrophil recruitment were abrogated in mice deficient in either MAL or MyD88, but not in TRIF-deficient mice [86]. Two groups have reported that MAL is not required for TLR2-mediated macrophage responses at high concentrations of ligand, but is required for responses at low ligand concentrations [87, 88], thus it is possible that some of the discrepancies observed with different bacteria are due to dosage effects, with high and low levels of bacteria differentially activating different pathways. Extremely high infection levels may overcome the MAL requirement for signaling *via* TLR2. As well as mediating the activation of proinflammatory cytokines, MAL is also a crucial mediator in the induction of the anti-inflammatory protein IL-10 *via* its ability to trigger activation of CREB. While TLR2 signalling has been shown to be important in ischemia-mediated heat damage, the role of MAL is less clear, with it possibly having both beneficial [89] and detrimental [89, 90] effects.

Mice in which TRIF has been ablated show defective activation of IFNβ and IRF3 and impaired proinflammatory cytokine production through TLR3 and TLR4, although TLR2, 7 and 9 signalling is unaffected [37, 41]. Furthermore, mice carrying an inactivating mutation of TRIF showed in-

creased susceptibility to viral infection, but were resistant to *E. coli* bacteraemia [35, 91]. TRIF-deficient mice also exhibit enhanced susceptibility to otitis media induced by nontypeable *Haemophilus influenzae* [92]. There is little genetic evidence linking TRIF to human disease, however this adaptor molecule has been targeted by some viruses as a mechanism for evading immune responses. For example, TRIF is degraded by the protease NS3-4A produced by the hepatitis C virus, thus inhibiting TLR3-mediated cytokine activation and abrogating the antiviral response [93]. Vaccinia virus produces a protein called A46 that interacts with TRIF (as well as other TIR domain containing proteins) to inhibit TLR3-mediated antiviral responses [94]. This has formed the basis of a potential TLR inhibitory compound (see below). Immunization of TRIF-deficient mice using LPS as an adjuvant revealed a lack of T-cell differentiation, which could partially be overcome by enforced co-stimulation, suggesting that targeting TRIF may be useful for vaccination [95]. Because T cells are essential for both cell-mediated and humoral immunity, it would be expected that such approaches would be useful for both bacterial and viral pathogens.

As with TRIF, there is a paucity of information on mutations in TRAM associated with human disease. Mice in which TRAM has been ablated exhibit TLR4-specific defects in cytokine production [14, 35-37]. Interestingly, *S. aureus* infection activates PPARgc1α and PPARgc1β gene expression in the liver, however these genes are not expressed in TRAM-deficient mice. Thus *S. aureus* sepsis links TLR signaling to PPARgc1α/β *via* TRAM and TRIF [96]. TRAM-deficient mice also display reduced TNFα production in response to LPS, because TRAM regulates TNFα mRNA translation [97].

There is little data linking SARM specifically to disease, however the development of SARM knockout mice has revealed that these mice are more susceptible to infection by the neurotropic flavivirus West Nile virus [98]. These animals also display reduced TNF production, reduced microglial activation and increased brainstem neuronal cell death. This study concluded that SARM restricts viral infection and neuronal injury in a brain region-specific manner. These findings are consistent with a role for SARM in neuronal functions (see above). Table **1** summarises the main phenotypes of adaptor molecule-deficient animals.

BCAP-deficient mice displayed elevated cytokine production and recruitment of inflammatory cells in response to *Salmonella typhimurium* infection [57]. Thus, an inhibitor of BCAP TIR domain interactions might be predicted to enhance responses to certain bacterial infections. However, experimental colitis was exacerbated in BCAP-deficient mice compared with wild-type animals, and so it is possible that interfering with BCAP TIR domain interactions might increase susceptibility to chronic inflammatory diseases.

STRUCTURAL BASIS OF TIR: TIR DOMAIN INTERACTIONS IN TLR SIGNALING

The TIR domains of the TLRs and the adaptor proteins are responsible for mediating receptor:receptor, receptor:adaptor and adaptor:adaptor interactions, all of which are crucial for TLR signal transduction. The TIR domains of both receptors and adaptors are expected to be able to form

Table 1. Summary of Phenotypes of Mice in which Adaptor Molecule Genes have been Ablated

Adaptor	Phenotype	Reference(s)
MyD88	Abrogation of cytokine production and neutrophil recruitment in LPS lung inhalation model	[86]
	Susceptibility to *S. aureus, Plasmodium, Toxoplasma, Listeria* and *Leishmania*	[12, 63-66]
MAL	Defective immune responses against *E. coli* and *K. pneumoniae*	[83, 84]
	Abrogation of cytokine production and neutrophil recruitment in LPS lung inhalation model	[86]
TRIF	Defective activation of TLR3 and TLR4 pathways	[37, 41]
	Susceptibility to viral infections	[35, 91]
	Susceptibility of otitis media induced by *H. influenzae*	[92]
	Lack of LPS-induced T cell differentiation	[95]
	Resistance to *E. coli* bacteraemia	[35, 91]
TRAM	Defects in TLR4-induced cytokine production	[14, 35-37]
	Reduced TNF production in response to LPS	[97]
SARM	Susceptibility to West Nile virus	[98]

homodimers. The receptor TIR domains interact directly with the TIR domains of the adaptors: TLR2, 5, 7, 8 and 9 can interact directly with MyD88, while TLR3 interacts with TRIF. TLR4 cannot interact directly with MyD88 or TRIF but recruits both MAL and TRAM, which can interact with MyD88, and TRIF, respectively, through TIR domain interactions [25, 36, 39, 41, 87, 99-102]. SARM has only been shown to interact directly with full-length TRIF, presumably also through TIR domain interactions [48].

In solution, all the TIR domains that have been biochemically characterized to date behave as monomers, and attempts to form complexes of receptor and adaptor TIR domains from purified components have been unsuccessful. This suggests that weak interactions are general feature of the TIR domains, and that a high local concentration is required for stabilizing the interaction, which can be achieved either by membrane attachment or by being linked to other more strongly self-associating domains such as the death domain of MyD88.

Structures are available for human (TLR1, TLR2, TLR10, IL-1RAPL (interleukin-1 receptor accessory protein-like), MAL and MyD88), plant and bacterial TIR domains [34, 102-107]. All of the structures have a similar fold consisting of a central five-stranded parallel β-sheet surrounded by α-helices and loop regions (Figs. **3A-C**). A comparative analysis suggests that the TIR domain has a conserved side consisting of the αA and αE helices (the elements of secondary structure nomenclature follows [103]; see Fig. **3**), and a divergent side consisting of the αD helical region and part of the αB and αC helices [52]. Both the BB and DD loops, which have been implicated in TIR:TIR domain interactions in TLR signaling (described in more detail below) are located on the divergent side. The recently solved crystal structure of the adaptor MAL differs significantly from the other human TIR domains as it lacks the helical segment (αB) between the βB and βC strands and instead contains a long disordered loop (AB) connecting the first helix (αA) and the βB strand [34] (Fig. **3B**). The structure is also stabilized by two disulphide bonds, which have not been observed in the other TIR domain structures and is unusual for cytosolic

proteins. It is possible that the disulphide bond between C89 in the βA strand and the C135 in the BB loop may stabilize the structural rearrangements observed in this region of the protein.

Although structures of TIR domain complexes in TLR signaling are not available, analyses of the monomer structures combined with mutagenesis data and computational modeling and docking studies have led to several models for the TIR domain assembly process and the positions of the interacting interfaces.

HOMODIMERISATION INTERFACES

Several of the TIR domain crystal structures reveal TIR:TIR domain interfaces consistent with weak and transient complexes in solution. For example, the crystal structure of the TIR domain of human TLR10 contains a symmetric dimer in the asymmetric unit, consisting of residues from the BB-loop, DD-loop, and the αC-helix (Fig. **3C**). Mutational data from TLR4 are consistent with this interface being physiologically relevant, and models of the TLR4 homodimer has been presented using the asymmetric unit of TLR10 TIR domain as a template [108, 109]. However, a recent study utilizing the decoy peptide approach to map the TLR4 TIR dimerization interface suggests that a different region encompassing the TLR4 BB loop and the αE helix and not the αC helix is involved in mediating TLR4 TIR dimerization [110]. In the crystal structure of the C713S mutant in TLR2, an extensive asymmetric dimeric interface is observed consisting of residues from the αB, αC, and αD helices and CD and DD loops of one symmetry partner, with the αB helix and BB loop of another [111]. Mutations of residues in the interface abolished signaling by TLR2 and the authors argued that the interface may reflect the associations in the natural heterodimeric TLR2:TLR1/TLR6 signaling complex. In MAL, a symmetric homodimeric interface consisting of residues from the αC helix and the region between the βD and βE strands is likely to be physiologically relevant [34]. In this complex, the N-termini of the two molecules are oriented in the same direction, which would allow both monomers to interact with the membrane through PIP2 bind-

ing. Mutations of residues involved in the homodimeric interface of MAL inhibit the interaction with MyD88, suggesting that MAL self-association may be required for facilitating the interaction with MyD88. It is also possible that the homotypic or heterotypic TIR:TIR domain interactions involved in TLR signaling are related events with some functional overlap.
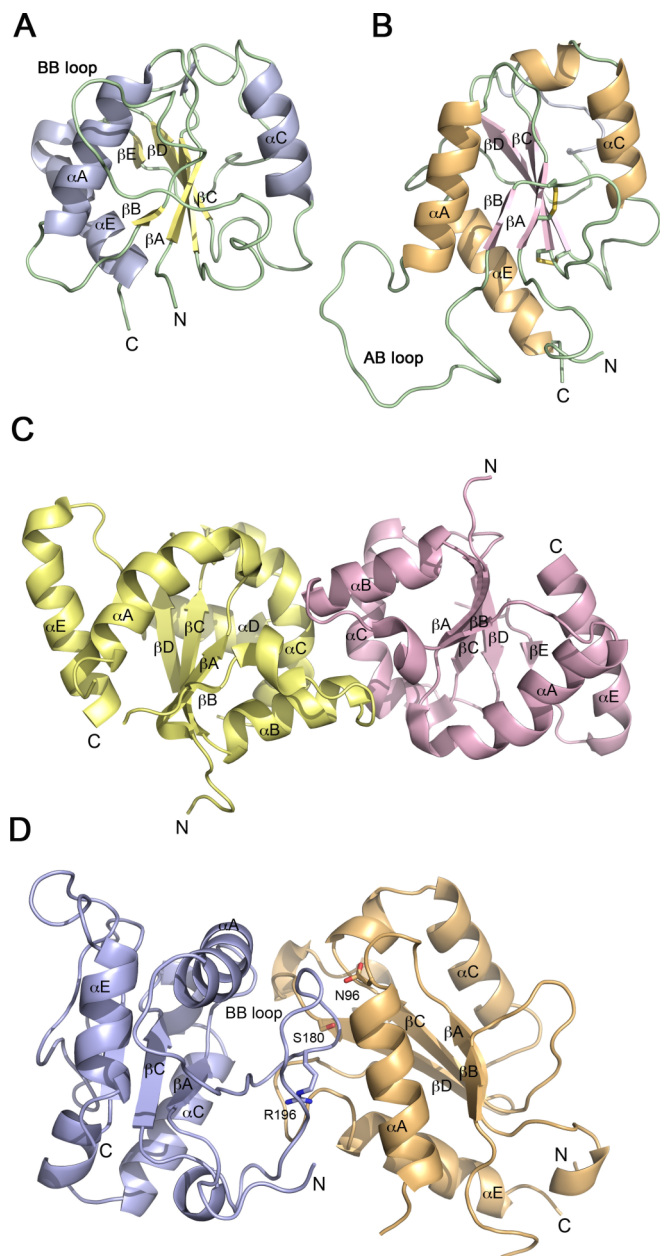

Fig. (3). TIR domain structures.
(A) NMR structure of MyD88 TIR domain [102] (PDB ID 2Z5V). The secondary structure elements and loops are named according to the nomenclature used for TLR1 TIR domain [103]. **(B)** Crystal structure of MAL TIR domain in similar orientation to MyD88 in (A) [34] (PDB ID 2Y92). The long loop between the αA helix and the βB strand is disordered in the crystal. **(C)** Crystal structure of the TLR10 TIR domain homodimer [34, 102-104] (PDB ID 2J67). The interface consists of residues from the BB-loop, DD-loop, and the αC-helix. **(D)** Docking-derived model of the MyD88:MAL TIR domain complex [34]. The R196 residue in MyD88 is in close proximity to the D96 and S180 residues in MAL.

Overall, the reported interfaces in different TIR domains differ significantly from each other, suggesting that there may not be a universal homo-dimerization interface in TIR domains, and that the specific properties of the different interfaces may depend on the function of the TIR domain and its interacting partners.

RECEPTOR-ADAPTOR INTERFACES

Several lines of evidence suggest an important role for the BB-loop region in receptor:adaptor TIR:TIR domain interactions during TLR signaling (Fig. **3**). In TLR4, the BB loop is the site of a naturally occurring mutation, P712H of C3H/HeJ mice, which renders them non-responsive to LPS [112]. Mutation of this and neighboring residues in TLR4 and other TIR domains also affects TLR-dependent signaling. The crystal structures of the TIR domains from TLR1 and TLR2 revealed that the BB-loop region contained several conserved surface-exposed residues and extended away from the rest of the TIR domain, forming a protrusion on the surface of the structures [103]. Mutation of the conserved proline to a histidine did not change the overall fold of the TIR domain of TLR2, suggesting that the effect of the mutation is due to disruption of direct contact with interacting molecules [103].

Computational docking studies combined with site-directed mutagenesis analysis have also been used in attempts to unravel the TIR:TIR domain interfaces in receptor:adaptor and adaptor:adaptor complexes, and they support a role for the BB loop region in heteromeric TIR:TIR domain interactions. Initial studies by Dunne *et al.* [113], using homology models of TLR4, MAL and MyD88 based on the structure of TLR2 TIR domain, suggested that MAL and MyD88 bind to different regions in TLR2 and TLR4. Non-overlapping binding sites were predicted for binding to TLR4, and MAL and MyD88 bind to opposite sides of the BB loop region through their DD/DE loops and AA/DD loops, respectively. An overlapping binding site was predicted for TLR2, with the BB loop of TLR2 forming a possible point of contact with MAL and MyD88. Jiang *et al.* [114] performed docking studies of the TLR2 TIR domain with a homology model of MyD88 and the results indicated that the BB loops and the αA helices of both proteins interacted in an anti-parallel fashion. Both of these studies used monomeric receptors in the modeling process and did not take into account ligand-induced dimerisation of the TIR domain. Nunez *et al.* [109] performed docking studies of homology models of MAL and TRAM to a homology model of the TLR4 homodimer, based on the TLR10 TIR domain crystal structure (Fig. **3C**). The models suggested that both MAL and TRAM bind to the same two symmetry-related sites formed at the center of the interface of the TLR4 dimer. A model where MAL and TRAM compete for the same binding site on the TLR4 dimer is also supported by a recent study that utilized the mammalian protein protein interaction trap (MAPPIT) strategy to identify the TLR4 TIR surface regions involved in TLR4:TLR4, TLR4:MAL and TLR4:TRAM interactions [108]. The MAPPIT data does not support binding of MAL or TRAM to the center of the TLR4 dimer interface as predicted by Nunez-Miguel *et al.* [111] but the study suggests that two MAL or TRAM molecules can bind to a large con-

served surface region that extends across the dimer interface and contains the BB loop.

ADAPTOR-ADAPTOR INTERFACES

Analysis of the NMR structure of MyD88 combined with mutagenesis studies revealed three distinct surface regions important for TLR4 signaling [102]. Site 1 consists of R217 in the BC loop region, site 2 consists of the conserved BB loop residues R196 and D197, while site 3 consists of K282 and R288 located in the αE helix. A role for R196 in TLR signaling is also supported by another recent study, which showed that an R196C mutation is present in 3 out of 9 patients with MyD88 deficiency [58]. Both NMR and glutathione-S-transferase (GST)-pulldown assays using purified proteins indicated that both R196 and R288 are involved in binding to MAL, and as these two residues are located on different sides of the molecule, Ohnishi *et al.* [102] suggested that MyD88 has two MAL binding sites involving the BB loop region and the αE helix. Docking studies performed using the NMR structure of MyD88 and the crystal structure of MAL also support a role for the BB loop region of MyD88 in the interaction with MAL [34]. Furthermore, docking placed MyD88 in close proximity to the surface-exposed D96 and S180 residues, which are located in the AA and DD loops respectively, of MAL, and defined a surface region for MyD88 interaction (Fig. **3D**). Both D96 and S180 have previously been shown to be important for TLR signaling; supporting co-immuno-precipitation experiments showed that D96, but not S180, is critical for the interaction with MyD88, which is consistent with studies indicating that mutation of S180 only leads to a partial loss of function. Valkov *et al.* [34] also demonstrated that increasing concentrations of DTT affected the interaction between MyD88 and MAL in co-immuno-precipitation experiments, but not the interaction between TLR4 and MAL, or MAL homodimerisation, suggesting that formation of the disulphide bridges identified in the MAL crystal structure may be important for facilitating the interaction with MyD88.

The above examples suggest an important role for the BB loop region in heteromeric TIR:TIR domain interactions, but detailed structural analysis of receptor:adaptor or adaptor:adaptor complexes coupled with biochemical data on the affinities, stoichiometries and kinetics of the interactions will be required to elucidate the molecular mechanisms of TIR domain assembly and the exact nature of the interfaces.. The transient nature of the TIR domain interactions makes it difficult to study TIR domain complexes by x-ray crystallography, but mapping of the interfaces using NMR-based approaches should be feasible, in particular for the MyD88-MAL interaction as structures are already available for the TIR domains of both of these proteins.

INHIBITION OF TIR DOMAIN INTERACTIONS IN TLR SIGNALING

ST2 and SIGIRR

Apart from the adaptor protein SARM, the TIR domain-containing proteins SIGIRR (single immunoglobulin IL-1 related protein) and ST2 (suppressor of tumorigenicity 2) have been shown to negatively regulate TLR signaling. ST2, which has been identified as the receptor for the IL-33 cyto-

kine, exists in both a transmembrane and a soluble form [115-117]. The transmembrane variant, ST2L, contains an extracellular region consisting of 3 immunoglobulin (Ig) like domains and an intracellular TIR domain (Fig. **2**), while the soluble form, sST2, consists only of the extracellular region and has nine unique residues at the C-terminus. ST2L is expressed on mast cells and T helper type-2 (Th2) cells and studies using ST2-deficient mice indicate an important role for ST2L in developing Th2-cell responses [118-120]. ST2-deficient mice also have an elevated production of pro-inflammatory cytokines in response to IL-1, LPS, bacterial lipopeptide and CpG, but not polyI:C, and failed to develop LPS-induced endotoxin tolerance [121]. Overexpression of ST2L attenuates IL-1R and TLR4, but not TLR3 mediated NF-κB activation, and suggests that ST2L only regulates the MyD88-dependent TLR pathways [121]. Co-immuno-precipitation assays using GST-ST2L fusion protein demonstrated an interaction with both MyD88 and MAL, but not TRIF or IRAK4. A P431H mutation in the predicted BB loop of the TIR domain failed to inhibit LPS-induced NF-κB activation and also had a reduced ability to bind to MyD88 [121]. Overall, these studies suggest that ST2 can sequester MAL and MyD88 through heterotypic TIR:TIR domain interactions, preventing them from being recruited to the activated receptors. Recent modeling and docking analyses have indicated that the BB loop region of ST2 is important for interactions with both MyD88 and MAL [122].

SIGIRR, also known as TIR-8, consists of a single extracellular Ig domain, a transmembrane region, and an intracellular TIR domain followed by a long cytoplasmic tail (Fig. **2**). Ligands have not been identified for SIGIRR yet and the TIR domain lacks two conserved residues that have been shown to be essential for IL-R1 signaling (C222 and L305 in SIGIRR, corresponding to S447 and Y536 in IL-1RI) [123]. SIGIRR is highly expressed on dendritic and epithelial cells but not primary macrophages [124], and both the domain architecture and expression pattern are evolutionarily conserved among vertebrates [125]. Current evidence suggests that SIGIRR inhibits NF-κB activation mediated by IL-1 family members, and can also dampen NF-κB activation induced by TLR 4, 5, 7 and 9 (reviewed in [126]). Co-immuno-precipitation experiments using deletion mutants have shown that both the Ig and TIR domains are important for inhibiting IL-1 signaling, while only the TIR domain is required for inhibiting TLR-dependent signaling [124, 127]. In both cases the TIR domain appears to inhibit signaling by preventing MyD88-dependent recruitment of downstream signaling components such as IRAKs and TRAF6, and docking studies using homology models of SIGIRR, TLR4 and TLR7 TIR domains suggest that the BB loop region of SIGIRR is involved in blocking the interaction between the receptor TIR domains and the MyD88 TIR domain [128]. SIGIRR deficiency is associated with hypersusceptibility to autoimmune diseases such as systemic lupus and colitis [129, 130], and understanding the molecular details of how SIGIRR inhibits IL-1 and TLR signaling may lead to novel therapeutic opportunities for immune mediated diseases.

Microbial Effectors Mimicking TLR Adaptors

Increasing evidence suggests that both bacterial and viral pathogens can directly inhibit TLR signaling by secreting

molecules that interact with components of the TLR signaling pathways, including the TIR domain-containing adaptor proteins. Bacterial TIR domain proteins from *Brucella melitensis* (TcpB and Btp1), the uropathogeinc *E. coli* strain CFT073 (TcpC) and *Salmonella enterica* (TlpA) have been shown to suppress TLR-mediated signaling in NF-κB reporter assays [131-133]. TcpB and Btp1 are identical except for a single amino acid difference (A138V) in the region predicted to harbour the αA helix. All of these proteins have a C-terminal TIR domain and TcpC also has N-terminal transmembrane region (Fig. **2**). TcpB/Btp1 and TlpA do not contain any annotated domain at the N-terminus, but secondary structure analyses suggest that this part of the protein has a substantial α-helical character, and TcpB requires this region for binding to phospholipids [134]. Both TcpB and TcpC inhibited TLR2- and TLR4-induced NF-κB activation and were shown to bind directly to MyD88 in pulldown assays [131]. Neither protein inhibited TLR3/TRIF-mediated activation of type-1-IFN by the TLR3 ligand poly(I:C), and a direct interaction with TRIF was not observed. However, a recent study has shown that TcpC can inhibit both MyD88 and TRIF-dependent signaling pathways, and transcriptomic analyses identified targets downstream of MyD88, where MyD88- and TRIF-dependent innate immune responses may converge [135].

TcpC reduced pro-inflammatory cytokine secretion, increased bacterial burden and tissue damage in urinary tract infection (UTI) models in mice. A positive correlation was also observed between UTI severity and TcpC-expressing bacteria in clinical samples, providing significant support to the relevance of TcpC as a virulence factor [131]. TlpA is also required for virulence, but this does not appear to be the case for Btp1, because a knockout mutant is not significantly attenuated in the mouse model of brucellosis [133]. However, Btp1 down-modulates maturation of infected dendritic cells, suggesting a role for this protein in the establishment of immune-tolerance and chronic infection. TcpB localizes to the plasma membrane through association with phosphoinositides, and it has thus been hypothesized that TcpB may subvert TLR signaling by acting as a MAL mimetic [134]. However, a recent study by Sengupta *et al*. [136] showed that the presence of TcpB resulted in poly-ubiquitination-dependent degradation of phosphorylated MAL, suggesting that TcpB does not mimic MAL but instead targets the adaptor for proteolytic degradation, thereby suppressing TLR-dependent signaling. TcpB has also been shown to associate with microtubules and can modulate microtubule dynamics by acting as a stabilization factor [137]. A mutant (G158A) in the predicted BB loop region of TcpB has a reduced ability to both stabilize microtubules and to suppress NF-κB activation, suggesting that microtubule dynamics may play a role in TcpB-mediated subversion of TLR signaling.

Vaccinia virus encodes several proteins, A46, A52, B14, N1 and K7, which can interact with different components of the TLR signaling pathways, thereby preventing an immune response. A52 and K7 have been shown to bind to TRAF6 and IRAK2, while B14 targets the IKK kinase complex (reviewed in [138]). K7 can also bind to the RNA helicase DDX3, preventing induction of a type-1-IFN response. Structures have been solved for A52, B14, N and K7 and they all adopt a Bcl-2 like fold [139-141]. A46 was initially

believed to have a TIR domain-like fold but secondary and tertiary structure predictions suggest that it adopts an α-helical Bcl-2 like fold similar to A52, B14, N1 and K7 [142]. This is also supported by recent biophysical studies demonstrating that the C-terminal region of A46 has an α-helical structure [143]. A46 can inhibit NF-κB, MAPK, and IRF3 activation by interacting with the adaptor proteins MyD88, MAL, TRAM and TRIF [94, 144]. Surface plasmon resonance studies indicate that the interaction with MAL has a dissociation constant (K_d) in the micromolar range [143], which is consistent with reported K_ds for TIR:TIR domain interactions [102, 105].

Decoy Peptides and Small Molecule Compounds Targeting TLR Adaptor Proteins

The BB loop region plays a critical role in mediating TIR:TIR domain interactions during activation of a TLR-dependent immune response, and several reports have described the successful use of BB loop decoy peptides and small molecule mimetics to inhibit TLR signaling. Horng and colleagues [24] first reported a BB-loop decoy peptide in 2001. In this study, a 14-amino-acid peptide consisting of the BB loop from MAL was fused to the antennapedia homeodomain from Drosophila (to facilitate delivery into the cell) and was shown to specifically inhibit activation of NF-κB induced by TLR4, but not by TLR9. Seven amino-acid peptides derived from MyD88 and the IL-18R receptor BB loops have been reported to inhibit homodimerization of both full-length MyD88 and the TIR domain alone in GST-pulldown assays and co-immuno-precipitation experiments [145]. A cell-permeable variant of the MyD88 heptapeptide could also prevent MyD88 homodimerisation in live cells, and significantly reduced NF-κB activity induced by IL-1. Cell-permeable BB-loop decoy peptides from the adaptors MAL, MyD88, TRAM and TRIF and the receptors TLR2, TLR4, TLR1 and TLR6 have also been systematically tested in two reports by Toshchakov and colleagues [146, 147]. The adaptor-based peptides could suppress TLR4-induced gene expression, and MAPK and transcription factor activation. The strongest inhibitory effect was observed with the TRAM and MyD88-derived BB-loop peptides, while the MAL peptide was the weakest of the four tested. This observation is consistent with recent structural data that revealed that MAL adopts a different conformation in the BB loop region compared to other TIR domain containing proteins. Peptides based on the BB-loop region of the receptors TLR4 and TLR2 could inhibit TLR signaling induced by agonists of TLR4 and TLR2, but not by TLR3. Furthermore, the TLR4 BB-loop peptide inhibited both MyD88- and TRIF-dependent signaling pathways, which is in agreement with modeling and interaction studies indicating that MAL and TRAM bind to the same or overlapping sites on TLR4. The BB-loop peptide based on the identical regions in TLR1 and TLR6 failed to inhibit both TLR4- and TLR2-mediated signaling. This is consistent with reports showing that both TLR1 and TLR6 act as co-receptors for TLR2 [148, 149], and do not engage directly with the downstream adaptor MyD88 [150].

An 11-amino acid peptide from A46 virus called VIPER (viral inhibitor peptide of TLR4) has been shown to inhibit TLR4-mediated immune responses when fused to a cell

penetrating delivery sequence [151]. Pulldown assays showed that the VIPER peptide could interact with the adaptors MAL and TRAM, but not MyD88, TRIF, or the TIR domain of TLR4, which indicates that VIPER prevents TLR4 signaling by blocking the interaction of MAL and TRAM with TLR4. However, surface plasmon resonance studies did not detect an interaction between the VIPER peptide and purified MAL, suggesting that the interaction is of very low affinity and that the effects of the VIPER peptide on suppression of TLR4 signaling involve different molecular mechanisms compared to full-length A46 [143].

Synthetic small molecule BB-loop mimetics have also been developed. The AS-1 compound [152], which is based on the conserved BB-loop tripeptide sequence [(F/Y)-(V/L/I)-(P/G)] has been shown to be capable of disrupting the interaction between MyD88 and the IL-1RI/IL-1RAcP (interleukin-1 receptor accessory protein) complex, while compound ST2825, based on the BB-loop heptapeptide, prevented homo-dimerisation of MyD88 [152-154]. A non-BB loop-based compound, TAK-242, developed by Takeda Pharmaceuticals, is a cyclohexane derivative that selectively binds to the TLR4 TIR domain by direct binding to C747 in the predicted αC helix and it inhibits signaling by interfering with the interactions between TLR4 and the bridging adaptors MAL and TRAM [155].

THERAPEUTIC TARGETING OF TLR PATHWAYS

The central location of TLR pathways in the processes of infection and immunity places these pathways and therefore the TLR adaptor proteins and their regulators as attractive therapeutic targets. In principle, different conditions would benefit from either activating or inhibiting TLR signalling; activating TLR pathways would be predicted to be beneficial for infectious diseases and vaccines, while targeting inflammatory diseases, allergies, autoimmunity, sepsis and cancer would require inhibition of TLR signaling. The central role of TLR pathways in complex essential processes represents a double-edged sword; it may be difficult to target specific conditions without side-effects, and to achieve a desired therapeutic end-point, the therapeutic targets need to be selected carefully.

The original observations that TLRs responded to microbial products prompted investigations aiming to modulate these receptors to either enhance or reduce responses to pathogens such as viruses, bacteria and protozoa and determine their potential as therapeutic targets. Because TLRs respond to microbial products, it seemed logical that enhancement of signaling might lead to better activation of immune cells, resulting in an enhanced immune response. Thus TLR ligands have been investigated as potential adjuvants, enhancing the activity of vaccines. To this end, a number of natural and synthetic ligands have been studied. Monophosphoryl lipid-A analogs have been developed that activate TLR4, and lipid-A mimetics have been successfully used as adjuvants in a number of vaccines including hepatitis B and some cancers [156-158]. Imiquimod is a quinolin derivative that acts as a TLR7 agonist. This compound, in the form of a cream, has been licensed for use in skin conditions such as Bowen's disease, actinic keratoses, basal cell carcinoma, and genital warts [159-164]. Its mechanism of action

is thought to involve enhancing the production of IL-1, IL-6, TNF and IFNs. TLR9 agonists such as CpG oligodeoxynucleotides (ODNs) are also an exciting possibility as novel adjuvants for both infectious disease vaccines and cancer vaccines [165, 166]. The use of CpG ODNs as TLR agonists is also being investigated as a possible therapy for asthma, with the aim of switching the response to allergens from a Th2 response (causing the chronic symptoms) to a Th1 response (which is more likely to resolve) [167-169]. For more detailed information on TLRs as therapeutic targets, the reader is referred to [170-174].

Comparatively less work has been done to establish the therapeutic potential of inhibitors of TLR signaling pathways, as the disease targets are more difficult to define. If aberrant TLR signaling plays a role in chronic diseases by, for example, sustained activation of NF-κB, then antagonists or inhibitors may be therapeutically useful. The most obvious method for such inhibition would be to antagonise ligand: receptor binding, but blocking the intracellular signaling events would give the same outcome, therefore enzymes such as IKK and IRAK have been targeted. However, the drawbacks to targeting enzymes such as these are that (a) it has been historically difficult to generate highly selective kinase inhibitors; and (b) these enzymes are functional in pathways other than TLR signaling. Thus, other targets within the TLR signaling pathway such as blocking TIR domain interactions may be at least as effective, and possibly more selective, alternative means of inhibiting cellular responses. Indeed, some small molecule inhibitors and decoy peptides show therapeutic promise. As described previously, the AS-1 compound [152] has been shown to both protect the myocardium from ischaemia/reperfusion injury and prevent cardiac hypertrophy in mice [175], while the ST2825 compound prevents left ventricular dilation and hypertrophy after experimental acute myocardial infection in mice [176]. Administration of the AS-1 compound to mice can also attenuate *Staphylococcal* enterotoxin B-induced pro-inflammatory cytokine production and increase the survival rate from toxic shock-induced death, suggesting that BB-loop mimetics may be used as a therapeutic approach to prevent toxic shock [177]. Both the MAL 14-amino-acid peptide used by Horng and colleagues [24] and the MyD88 heptapeptide have also shown activity in different experimental settings (reviewed in [178]), which provides additional support to the usage of BB loop-derived peptides or small molecules as a platform for therapeutic drug development.

The non BB-loop compound TAK-242 inhibited the pro-inflammatory response in a mouse endotoxin shock model, as well as the symptoms associated with endotoxaemia in a guinea-pig model of sepsis, suggesting that it may be a promising drug candidate for the treatment of sepsis [179, 180]. However, this compound has not yet shown efficacy in human disease [155, 179, 181, 182].

The microbial effectors TlpA, TcpB, TcpC and A46, and the mammalian receptors ST2L and SIGIRR8 appear to interact with and block specific receptor or adaptor TIR domains involved in TLR signaling. Therefore, determining the exact interfaces between these naturally occuring inhibitors and the receptor or adaptor TIR domains may provide us with the foundation to engineer new small molecule or pep-

tide-based inhibitors that efficiently block specific TLR signaling pathways.

Structural analysis will be required to confirm that BCAP indeed contains a TIR domain, and further work is needed to elucidate its specific role in acute and chronic inflammation before it could be considered a therapeutic target.

CONCLUSIONS

A more detailed understanding of the structural basis of homotypic and heterotypic TIR domain interactions in TLR signaling, and how ST2, SIGIRR and microbial effectors specifically inhibit these interactions, may provide us with the rationale for designing additional therapeutic agents for specific TLR signaling pathways. This may allow us to target specific conditions and achieve specific desired therapeutic outcomes.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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