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REVIEW

SPECIAL ISSUE: CELL BIOLOGY OF THE IMMUNE SYSTEM

Signalling, sorting and scaffolding adaptors for Toll-like receptors

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ABSTRACT

Toll-like receptors (TLRs) are danger-sensing receptors that typically propagate self-limiting inflammatory responses, but can unleash uncontrolled inflammation in non-homeostatic or disease settings. Activation of TLRs by pathogen- and/or host-derived stimuli triggers a range of signalling and transcriptional pathways to programme inflammatory and anti-microbial responses, including the production of a suite of inflammatory cytokines and other mediators. Multiple sorting and signalling adaptors are recruited to receptor complexes on the plasma membrane or endosomes where they act as scaffolds for downstream signalling kinases and effectors at these sites. So far, seven proximal TLR adaptors have been identified: MyD88, MAL, TRIF (also known as TICAM1), TRAM (TICAM2), SARM (SARM1), BCAP (PIK3AP1) and SCIMP. Most adaptors tether directly to TLRs through homotypic Toll/interleukin-1 receptor domain (TIR)–TIR interactions, whereas SCIMP binds to TLRs through an atypical TIR–non-TIR interaction. In this Review, we highlight the key roles for these adaptors in TLR signalling, scaffolding and receptor sorting and discuss how the adaptors thereby direct the differential outcomes of TLR-mediated responses. We further summarise TLR adaptor regulation and function, and make note of human diseases that might be associated with mutations in these adaptors.

KEY WORDS: Toll-like receptor, TLR, Adaptors, Receptor signalling, Innate immune, Cytokine, Inflammation

Introduction

Our innate immune system acts as the first line of defence against invading pathogens and is able to distinguish between 'self' and 'non-self' through a variety of germline-encoded transmembrane pattern recognition receptors (PRRs). PRRs detect evolutionarily conserved molecules that are distinct to specific microbes, termed pathogen-associated molecular patterns (PAMPs), as well as endogenous molecules that become exposed in the context of infection, tissue damage and inflammation known as damage-associated molecular patterns (DAMPs) (Lotze et al., 2007). Of these PRRs, the Toll-like receptor (TLR) family is one of the best studied. Named after their similarities with the toll gene that was first described in *Drosophila melanogaster*, this family of immune receptors is highly conserved across multiple organisms (Anderson et al., 1992). There are currently ten known functional human TLRs (TLRs 1–10) and twelve functional mouse TLRs (TLRs 1–13, where mouse TLR10 is a non-functional pseudogene) (Kawai and Akira, 2010), each recognising different ligands and utilising four canonical adaptors and three regulatory adaptors to induce transcription of anti-pathogenic genes (see Table 1).

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TLRs have their most prominent roles in cells of the innate immune system, such as macrophages, dendritic cells and natural killer cells, but they are also expressed on mucosal epithelial cells, endothelial cells, fibroblasts and adaptive immune cells (B and T cells) (Zarember and Godowski, 2002). TLRs distribute over cell surfaces and within cells and this offers a comprehensive immune defence (see Table 1). TLRs 1, 2, 4, 5 and 6 are displayed on cell surfaces where they are poised to recognise PAMPs representing surface molecules on different pathogens. The lipopolysaccharide (LPS) of Gram-negative bacteria is a prime example and it is recognised in either soluble form or on bacteria themselves, by TLR4 in combination with co-receptors MD-2 (also known as LY96) and CD14 (Gay et al., 2014). TLRs 3, 4 and 7–13 are found on membranes within cells (Fig. 1), including the endoplasmic reticulum (ER) or on endosomes and lysosomes, where they are tasked with identifying intracellular viruses and bacteria through recognition of bacterial components and nucleic acid signatures such as CpG oligodeoxynucleotides (a TLR9 agonist) (Latz et al., 2004) (see Table 1 and Fig. 1).

TLRs are type I transmembrane proteins defined by an extracellular leucine-rich repeat (LRR) domain for ligand recognition, a transmembrane domain (TMD) and a cytoplasmic Toll/interleukin 1 receptor (TIR) homology domain (Gay et al., 2014). TIR domains comprise a conserved five-β-stranded sheet region surrounded by five adjacent α-helices on either side (Núñez Miguel et al., 2007). They are common to both TLRs and to interleukin-1 (IL-1) receptors, which together form the 'interleukin-1 receptor/Toll-like receptor superfamily' (Dunne and O'Neill, 2003). Upon ligand binding, TLRs dimerise to induce a conformational change in the TIR domains, which allows homotypic interactions with other partners, including C-terminal TIR domain-containing TLR signalling adaptor proteins (Figs 1 and 2) (Toshchakov et al., 2011; Yamamoto et al., 2004). Since TLRs themselves are non-enzymatic, the recruitment of signalling adaptors (see Table 1 and 2) provides an essential link to downstream kinases for signal transduction, and thus the adaptors become the crucial players for guiding the outcomes of TLR activation and signalling (O'Neill and Bowie, 2007). Here, we introduce a variety of signalling pathways generated by TLRs, or crosstalk receptors and the adaptors that are involved in signalling and/or the cellular localisation of TLR signalling. We highlight structural and functional properties for seven TLR adaptors and conclude by discussing human diseases where TLR adaptors are emerging as potential disease genes.

TLR signalling pathways and their adaptors

A fundamental role of activated TLRs is to elicit an acute array of transcriptional and translational outputs that combine to generate robust anti-microbial and pro-inflammatory responses at sites of possible infection or danger (Medzhitov, 2001). Thus, TLR signalling can trigger the release of anti-microbial peptides, diverse arrays of cytokines and chemokines and reactive oxygen species. Activated TLRs also induce cellular responses, such as phagocytosis

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Table 1. Toll-like receptors, associated PAMPs and adaptors

TLR	PAMP	Adaptors	Cellular location
TLR1/2	Triacylated lipoproteins	MyD88, MAL, BCAP, SCIMP	Cell surface
TLR2	Bacterial lipoproteins, sporozoite	MyD88, MAL, BCAP, SCIMP	Cell surface
TLR2/6	Diacylated lipoproteins	MyD88, MAL, BCAP, SCIMP	Cell surface
TLR3	dsRNA	TRIF, SARM, SCIMP	Endosome
TLR4	LPS	MyD88, MAL, TRIF, TRAM, SARM, BCAP, SCIMP	Cell surface, endosome
TLR5	Flagellin	MyD88, TRIF	Cell surface
TLR7	ssRNA, imidazoquinolines, guanosine analogs	MyD88	Endosome
TLR8	ssRNA, imidazoquinolines	MyD88	Endosome
TLR9	CpG dsDNA	MyD88, MAL, SCIMP	Endosome
TLR10	Viral glycoproteins, dsRNA	MyD88	Endosome
TLR11	Flagellin, profilin	MyD88	Endosome
TLR12	Profilin	MyD88	Endosome
TLR13	Bacterial 23S rRNA	MyD88	Endosome

Toll-like receptors in human (TLRs 1–10) and mouse (TLRs 1–13, where mouse TLR10 is non-functional). TLR2 can form heterodimers with TLR1 and TLR6 (denoted TLR1/2 and TLR2/6, respectively). For each TLR, the respective pathogen-associated ligands (PAMPs), TLR adaptors for signal transduction, and cellular localisation are listed.

and macropinocytosis, for increased uptake from the surrounding milieu and pathogen ingestion (Condon et al., 2018; Marques et al., 2017; West et al., 2004), whereas TLR-mediated cell survival, death and proliferation responses all contribute to an expansive adaptive immune response (Medzhitov et al., 1997). Importantly, temporally orchestrated outputs from TLRs stage immune responses that transition from release of pro-inflammatory attack mediators to the anti-inflammatory mediators needed to initiate resolution and tissue repair (Mogensen, 2009). As such, the TLRs, their adaptors and resulting programmes of cytokines and chemokines are central to balancing inflammation for homeostasis and in chronic disease settings, where excessive inflammation is most often associated with dysregulation of pro- and anti-inflammatory cytokines (Joosten et al., 2016).

TLR adaptors act proximally to TLRs by binding directly to the TIR domains of the receptor in order to trigger signalling cascades (O'Neill and Bowie, 2007). The canonical adaptors are myeloid differentiation primary response 88 (MyD88), TIR-domain-containing adaptor-inducing interferon-β (TRIF, also known as TICAM1), TRIF-related adaptor molecule (TRAM, also known as TICAM2) and MyD88 adaptor-like (MAL or TIRAP). Recruitment of one or more of these canonical adaptors is essential for maximal

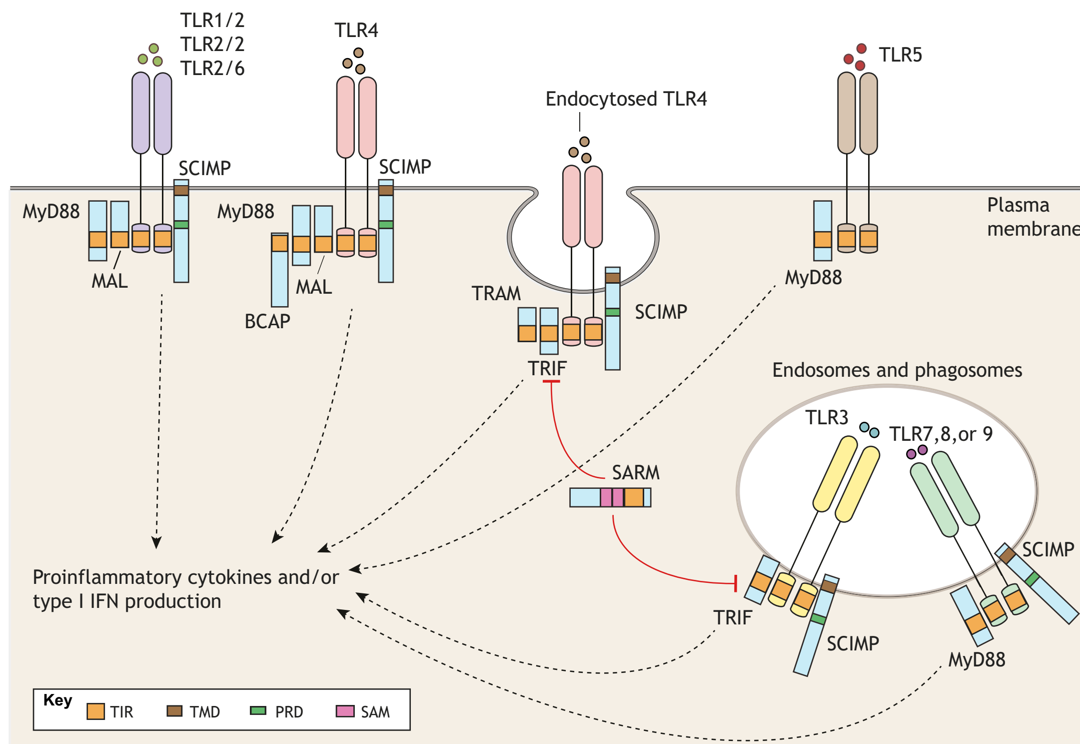

Fig. 1. TLR localisation and their signalling, sorting or scaffolding adaptors. TLRs are positioned on the cell surface or on endosomal compartments for signalling. Upon ligand binding, TLR signalling is initiated by dimerisation of receptors, which allows the recruitment of specific sets of adaptors for different TLRs (MyD88, Mal, TRIF, TRAM, BCAP and SCIMP). SARM negatively regulates TRIF-dependent TLR signalling (red arrows). Engagement of the signalling adaptor stimulates downstream signalling pathways to drive the induction of pro-inflammatory cytokines, and, in the case of the endosomal TLRs, the production of type I interferons (IFNs). Highlighted protein domains are the Toll/interleukin-1 receptor (TIR), transmembrane domain (TMD), proline-rich domain (PRD), and sterile α-motif (SAM) (see key). TLR1/2, heterodimer of TLR1 and TLR2; TLR2/6, heterodimer or TLR2 and TLR6.

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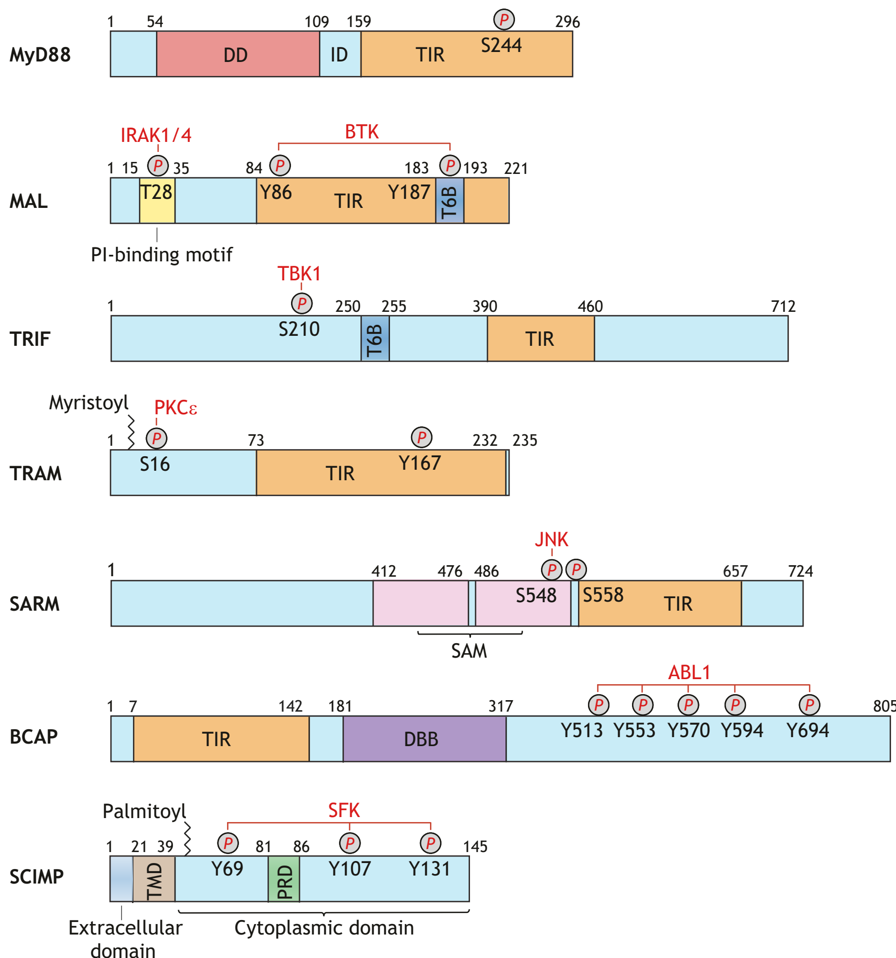

Fig. 2. Schematic diagram of protein domains and motifs in the human TLR adaptors. All TLR adaptors contain a TIR domain for interactions with TLRs and other TIR-containing adaptors, except for SCIMP, which associates with TLRs through a unique TIR–non-TIR interaction. MyD88 is the only adaptor to contain a death domain (DD), which associates with IRAKs to allow myddosome formation. SARM contains two tandem sterile α-motif (SAM) domains, which allow its self-association. Multiple TLR adaptors contain lipid-localising motifs for membrane association: MAL has a phosphatidylinositol (PI)-binding motif, TRAM is myristoylated at its N terminus, and SCIMP contains a transmembrane domain (TMD) followed by two palmitoylation sites. SCIMP also contains a proline-rich domain (PRD) in its cytoplasmic tail, which scaffolds the SFK Lyn for signalling. In addition to these domains, MyD88 contains an intermediary domain (ID), MAL has a TRAF6-binding motif (T6B), and BCAP has a Dof/BANK/BCAP (DBB) domain, which together mediate additional protein–protein interactions. All TLR adaptors are further regulated through phosphorylation and their known upstream kinases are shown in red. IRAK1/2, interleukin 1 receptor-associated kinase 1/2; BTK, Bruton's tyrosine kinase; TBK1, TANK-binding kinase 1; PKCε, protein kinase Cε; JNK, c-Jun N-terminal kinase; ABL1, ABL proto-oncogene 1, non-receptor tyrosine kinase; SFK, Src family kinase.

signalling from any of the TLRs; they broadly serve as signalling or sorting adaptors for the TLR family and their roles and cellular sites of signalling are well entrenched in the literature (O'Neill and Bowie, 2007). Another group of TLR adaptors are those that are recruited, in addition to the canonical adaptors, to serve specific roles, such as providing a scaffold for downstream kinases or acting as a negative regulator of a specific canonical adaptor in order to further modulate signalling. Classified here as 'regulatory adaptors', these include sterile α- and armadillo motif (SARM, also known as SARM1), B-cell adaptor for phosphoinositide 3-kinase (PI3K) (BCAP, also known as PIK3AP1) and SLP65/76 and Csk-interacting membrane protein (SCIMP) (see Table 2 and Fig. 2).

TLR signalling can also be modulated by other classes of signalling adaptors and co-receptor complexes that act distally to TLRs and without tethering directly to the receptors themselves. For example, TLRs engage in crosstalk with complement receptors and G-protein-coupled receptors (GPCRs) to produce antagonistic or synergistic effects in response to receptor stimulation (Hajishengallis and Lambris, 2016; Husted et al., 2017). Furthermore, LPS-induced activation of C5a anaphylatoxin chemotactic receptor 1 (C5aR1) inhibits TLR4-driven expression of IL-12 family proinflammatory cytokines in macrophages through PI3K and extracellular signal-regulated kinase 1 and 2 (ERK1/2, also known as MAPK3 and MAPK1, respectively) signalling (Hawlisch et al., 2005), whereas co-activation of TLR4 and the GPCR TGR5 (also known as GPBAR1) enhances nuclear factor (NF)-κB-mediated proinflammatory cytokine release (Mobraten et al., 2015). We recently showed that the endocytic receptor low density lipoprotein related protein 1 (LRP1) is activated in a crosstalk fashion by ligand-bound TLRs to recruit the small GTPase Rab8a and its effector PI3Kγ, which modulate Akt–mTOR signalling to downregulate pro-inflammatory outputs in macrophages (Luo et al., 2018). In addition, the membrane-associated scaffold and signalling adaptor proteins, adaptor protein phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1) and APPL2, are recruited by distinct Rab GTPases to differentially regulate TLR responses from either cell surface or endocytic membranes to exert opposing effects on Akt–mitogen-activated protein kinase (MAPK) signalling and modulate cytokine outputs (Chau et al., 2015; Yeo et al., 2016). Thus, signalling downstream of ligand-bound dimerised TLRs is driven by both canonical and regulatory proximal adaptors as direct receptor binding partners, and is then further fine-tuned through the additional influences of co-opted receptors and distal adaptors.

TLR trafficking and signalling on cell membranes

TLRs traffic to and from the cell surface through exocytic, endocytic and recycling pathways, and they can encounter pathogens or PAMPs at the plasma membrane, in phagosomes, macropinosomes and other endocytic, degradative or recycling compartments (Gay et al., 2014). TLR family members signal from the cell surface or endosomes and/or phagosomes and adaptors are recruited to these sites. MyD88 and MAL are typically recruited to ligand-activated TLRs on the cell surface (see Table 1), whereas TRIF and TRAM are recruited to endosomal membranes for signalling by intracellular TLRs. In innate immune cells with high rates of plasma membrane turnover, cell surface TLRs are inevitably internalised by multiple

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Table 2. TLR adaptors

Adaptor	Molecular mass (kDa)	Expression	Function	KO phenotype	Reference
MyD88	33	Macrophages, DCs, B cells T cells, NK cells, mast cells, granulocytes, osteoclasts	Early NF-κB activation	Delayed TLR4 signalling; enhanced TLR3 signalling	Kawai et al., 1999; Siednienko et al., 2011
MAL (TIRAP)	24	Macrophages, DCs, B cells T cells, NK cells, mast cells, granulocytes, osteoclasts	Sorting adaptor for MyD88	Reduced TLR2, TLR4 signalling	Yamamoto et al., 2002a
TRIF	76	Macrophages, DCs, B cells T cells, NK cells, mast cells, granulocytes, osteoclasts	Delayed NF-κB activation	Reduced TLR3, TLR4 signalling	Yamamoto et al., 2003a
TRAM	27	Macrophages, DCs, B cells T cells, NK cells	Sorting adaptor for TRIF	Reduced TLR4 signalling	Nilsen et al., 2015; Yamamoto et al., 2003b
SARM	79	Highly expressed in neurons, Macrophages, myeloid progenitor cells	Inhibits MyD88, TRIF	Reduced apoptosis	Mukherjee et al., 2015
BCAP	90	Macrophages, B cells, osteoclasts	Links TLRs to PI3K	Reduced B cell development	Yamazaki et al., 2002
SCIMP	17	Macrophages, DCs, B cells	Signalling scaffold for Src family kinases and effectors	Reduced dectin-1 signalling	Kralova et al., 2016; Luo et al., 2017a

There are seven TLR adaptors, including four canonical adaptors (MyD88, MAL, TRIF and TRAM) and three regulatory adaptors (SARM, BCAP and SCIMP). The stated molecular mass refers to the human protein. The prominent cellular expressions of each adaptor, its function in TLR signalling, and knockout (KO) mouse phenotypes with associated references are provided.

pathways, including in clathrin-coated vesicles and macropinosomes (Barton and Kagan, 2009; Husebye et al., 2006; Kagan et al., 2008; Wall et al., 2017; Zanoni et al., 2011).

TLR4 generates signalling from both plasma membrane and endosomal adaptors during its internalisation. In line with this, inhibition of the GTPase dynamin was found to block the depletion of surface TLR4 and disrupt subsequent endosomal signalling from TRAM for interferon β (IFNβ) production (Kagan et al., 2008). Regulators such as Syk kinase and phospholipase C-γ-2 (PLCγ2) were found to mediate the macropinocytosis of TLR4 complexes (Zanoni et al., 2011). Several Rab GTPases also mediate vesicle trafficking and signalling of TLR4. Rab10 localises on Golgi membranes and enhances TLR4 trafficking to the cell surface, resulting in increased signalling in LPS-activated cells (Wang et al., 2010). TLR4 is redirected out of the macrophage endocytic recycling route under the control of Rab11a to populate the surface of phagosomes during Gram-negative bacterial ingestion for TLR4–TRAM-mediated IRF3 signalling (Husebye et al., 2010). TRAM is also trafficked from recycling endosomes through Rab11a by forming a complex with the Rab11 effector FIP2 for delivery to phagosomes and, indeed, TRAM is necessary for phagocytosis and dispatch of Gram-negative bacteria (Skjesol et al., 2019). On early phagosomes, TRAM acts in dual roles to direct IRF3 signalling, as well as for nucleating polymerisation of the actin cytoskeleton (Skjesol et al., 2019). Rab8a is not involved in TLR4 endocytosis but it is required for TLR-mediated Akt–mTOR signalling from early macropinosomes (Luo et al., 2014; Wall et al., 2017). Finally, Rab7b directs late endosome and/or lysosome trafficking of TLR4 for receptor degradation, as one of the mechanisms for downregulating signalling (Wang et al., 2007). This is facilitated by the transmembrane protein TMED7 which localises with TLR4 in late endosomes where it encounters TRAM adaptor with GOLD domain (TAG) and mediates TAG-dependent disruption of the TRAM-TRIF complex and TLR4 degradation (Doyle et al., 2012).

For intracellular TLRs, their trafficking to endosomal compartments for signalling can take direct or circuitous routes. The multi-membrane spanning chaperone Unc93B1 and other accessory proteins are needed for transport of TLRs including TLR3, TLR5, TLR7 and TLR9 between cell compartments (Huh et al., 2014; Kim et al., 2008; Lee et al., 2013; Pohar et al., 2013). For example, Unc93B1 shepherds newly synthesized TLR9 out of the ER and the receptor is then transported to the cell surface from where it is internalised by adaptor protein-2 (AP-2) into endosomes and lysosomes; in contrast, TLR7 is transported with Unc93B1 from the Golgi directly to endosomes through the AP-4 sorting adaptor (Kim et al., 2008; Lee et al., 2013). Hence, like other receptors, TLRs are constantly trafficked through cellular pathways and recruitment of the signalling adaptors has to be spatially, sequentially and temporally orchestrated on different membrane domains.

Canonical TIR-containing TLR adaptors

MyD88 and MAL

MyD88 was the first of the TIR-containing adaptor proteins found to be involved in signal transduction from all known TLRs, with the exception of TLR3, which signals independently of MyD88 (Yamamoto et al., 2003a). MyD88 is a cytoplasmic adaptor and mice lacking functional MyD88 are noted for their unresponsiveness to bacterial endotoxin (Kawai et al., 1999). MyD88 consists of three functional domains: a death domain, an intermediate domain and a C-terminal TIR domain (Fig. 2). MyD88 interacts with TLRs through its TIR domain (a TIR–TIR interaction) and in so doing it recruits downstream interleukin-1 receptor-associated kinase 4 (IRAK4) through a homotypic death domain protein–protein interaction (Loiarro et al., 2009). Subsequent recruitment of IRAK1 and IRAK2 by IRAK4 results in formation of a ‘myddosome’ complex, which activates PI3K–Akt and MAPK, leading to nuclear translocation of the transcription factor NF-κB and transcription of NF-κB- and AP-1-dependent pro-inflammatory cytokines such as tumour necrosis factor (TNF) and IL-6 (Gay et al., 2011; Lin et al., 2010; Motshwene et al., 2009). The myddosome is a precise, stoichiometric assembly of MyD88 with its downstream IRAK kinases that is nucleated in response to an activated receptor complex for signal transduction. A crystal structure of the complex reveals it consists of six MyD88 death domains and four each from

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IRAK2 and IRAK4, and together they assemble into a left-handed helix (Lin et al., 2010). TLR stimulation induces recruitment of the membrane-localised bridging adaptor MAL through a homotypic TIR domain interaction between MAL and MyD88 (Fig. 2), which acts to concentrate and aggregate IRAKs in the myddosome to control the intensity and duration of TLR signalling (Bonham et al., 2014; Ve et al., 2017). In the case of TLR4, signal intensity relies on the speed of myddosome assembly as well as myddosome number and size as revealed by single-molecule imaging (Latty et al., 2018). Thus, TLR-induced myddosome formation is tightly regulated for the establishment of an activation threshold for dictation of the inflammatory response.

MAL contains a lipid-binding domain at its N-terminus, which interacts with phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2] enriched in microdomains on the cytoplasmic face of the plasma membrane, thereby recruiting MyD88 to the TLR at the membrane for signal transduction (Kagan and Medzhitov, 2006). Disruption of membrane PI(4,5)P_2 dampens signalling from multiple MyD88-dependent TLRs, indicating the requirement for MAL to ensure the correct, lipid-driven spatial distribution of the receptors (Choi et al., 2013; Kagan and Medzhitov, 2006). Moreover, during TLR4 endocytosis, the plasma membrane PI(4,5)P_2 is depleted, causing membrane dissociation of MAL, which then provides the opportunity for subsequent recruitment of the endosomal adaptors TRIF and TRAM (Kagan et al., 2008). Therefore, MyD88 and MAL are key TLR adaptors for recruiting downstream kinases and targeting TLR4 to the appropriate lipid membrane for TLR signalling.

TRIF and TRAM

Following the identification of the MyD88–MAL pathway of TLR activation, a further TIR-containing signalling adaptor, TRIF, was identified as a mediator of MyD88-independent TLR signalling (Yamamoto et al., 2002b). TRIF-mediated signalling is triggered at the early endosome by TLR3 or endocytosed TLR4 to induce a delayed NF-κB response (Fig. 1) (Kagan et al., 2008). This delayed response occurs through TNF receptor-associated factor 6 (TRAF6), TRAF3 and receptor-interacting serine/threonine-protein kinase 1 (RIP1, also known as RIPK1) acting on the MAPK TGF-β activated kinase 1 (TAK1, also known as MAP3K7), which leads to the activation of interferon regulatory factor 3 (IRF3) (O'Neill and Bowie, 2007). Activated IRF3 subsequently translocates to the nucleus and induces transcription of type 1 interferons (IFNs) together with activation of TNF, inducing a late-phase secondary activation of NF-κB (Covert et al., 2005). Viruses such as vaccinia (VACV) and hepatitis C (HCV) have developed strategies for suppressing TRIF-mediated inflammatory signalling through TRIF antagonism, cleavage or degradation to evade host antiviral responses (Li et al., 2005; Liang et al., 2018; Stack et al., 2005). Further to its activation of IRF3 and NF-κB, TRIF has been shown to control TLR-induced apoptosis through RIP1 and caspase-8, providing a mechanism by which macrophages and dendritic cells (DCs) undergo programmed cell death after intracellular bacterial infection (De Trez et al., 2005; Ruckdeschel et al., 2004).

In a similar manner to MyD88–MAL, TRAM acts as a bridging adaptor at the TLR4 TIR domain for the recruitment of TRIF to TLR4 on the endosomal membrane (Figs 1 and 2) (Fitzgerald et al., 2003). TRAM itself is localised to the membrane of endosomes through a protein–lipid interaction mediated by myristolyation of its N-terminus, which is critical for its role in signalling (Rowe et al., 2006). Furthermore, TLR-induced phosphorylation of TRAM at serine-16 by protein kinase Cε (PKCε) is required for TRAM signalling, and PKCε is essential for macrophage activation in response to bacterial infection (Castrillo et al., 2001; McGettrick et al., 2006). These distinct signalling pathways mediated by TRIF and TRAM at the endosome therefore provide additional regulation of the inflammatory response as well as a mechanism for governing pathogen-induced programmed cell death.

Regulatory TLR adaptors

SARM

The fifth TIR-containing adaptor to be discovered and the most highly conserved member of the TIR protein family is SARM, with SARM orthologs found in *C. elegans*, *Drosophila* and mammals (O'Neill et al., 2003). SARM is predominantly expressed in neurons of the central nervous system (CNS) and is a key regulator of neuronal survival through its nicotinamide adenine dinucleotide (NAD)ase activity, accelerating NAD+ depletion leading to axonal degeneration (Carty and Bowie, 2019; Kim et al., 2007). SARM contains two tandem sterile alpha motif (SAM) domains, which allow SARM self-association (Fig. 2), and recent evidence shows that NAD+ cleavage activity is regulated through SAM-mediated self-association of the SARM TIR domain (Horsefield et al., 2019). Unlike the pro-inflammatory effects of MyD88, MAL, TRIF and TRAM, SARM acts as a negative regulator of TLR signalling, inhibiting both TRIF- and MyD88-dependent pathways and transcription factor activation (Carty et al., 2006; Peng et al., 2010). This likely occurs through a homotypic interaction between the BB loop of the SARM TIR domain with the TRIF and MyD88 TIR domains (Carlsson et al., 2016). Although it acts primarily as a negative TLR regulator, SARM can also activate TLR signalling under specific conditions as evidenced by SARM-dependent pro-inflammatory cytokine expression in the CNS following viral infection (Hou et al., 2013; Szretter et al., 2009; Wang et al., 2018). SARM also participates in other PRR pathways, for instance by negatively regulating Nacht, LRR and PYD domains-containing protein 3 (NLRP3) during inflammasome activation to constrain the release of the pro-inflammatory cytokine IL-1β (Carty et al., 2019). Thus, this regulatory adaptor imposes control through its interaction with canonical adaptors.

BCAP – a linker between TLR and PI3K signalling

BCAP is the sixth identified TIR-containing TLR adaptor, and links TLR signalling to the activation of one subfamily of class I PI3Ks (Fig. 3A). PI3Ks belong to a large family of lipid signalling kinases that phosphorylate phosphoinositides, such as PI(4,5)P_2, at the D3 position of the inositol ring, creating D3′ phosphorylated inositol lipids such as phosphatidylinositol-(3,4,5)-trisphosphate [PI(3,4,5)P_3] (De Craene et al., 2017). PI3Ks are evolutionarily conserved from yeast to mammals and are divided into classes I, II and III, according to their molecular structure, cellular regulation and *in vivo* substrate specificities (Bilanges et al., 2019). Importantly, D3-phosphoinositides are key cellular signalling messengers and play a substantial role in the immune system, including the promotion of cell survival, proliferation, macrophage M2 polarisation and protein synthesis through activation of Akt–mTOR (Hawkins and Stephens, 2015).

BCAP was first identified in B cells where it acts as a scaffold for the class I PI3K regulatory subunit p85 (encoded by *PIK3R1* and *PIK3R2*) to recruit and activate PI3K catalytic subunits p110α, p110β or p110δ (PIK3CA, PIK3CB and PIK3CD, respectively) through its phosphorylated tyrosine site within four YxxM motifs during B-cell receptor (BCR) ligation (Okada et al., 2000). In addition, BCAP is highly expressed in other hematopoietic cells, including macrophages, DCs and natural killer (NK) cells. In

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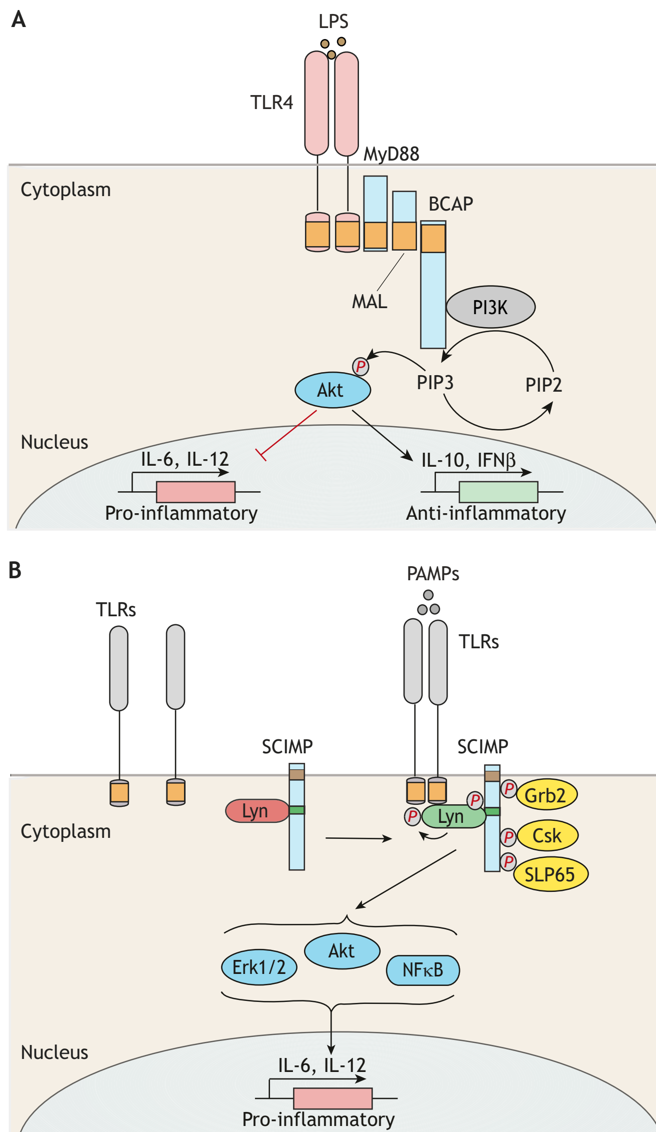

Fig. 3. Regulation of TLR signalling by BCAP and SCIMP. (A) BCAP links TLR signalling to PI3K activation. BCAP is engaged to MyD88 through its TIR domain by ligand-activated TLR4. BCAP tethers class I PI3K to TLRs for Akt activation. PI3K–Akt activation has regulatory effects on the outcome of TLR signalling, including limiting pro-inflammatory cytokine secretion and promoting anti-inflammatory cytokine production. (B) SCIMP-mediated TLR signalling pathway. SCIMP associates with multiple TLRs and the Src-family kinase Lyn in response to PAMPs. As a result, SCIMP acts as a scaffold to recruit the effectors Grb2, Csk, and SLP65 and to enable phosphorylation of specific TLR tyrosine residues to propagate the activation of downstream kinases (ERK1/2, Akt and NF-κB) for the selective production of inflammatory cytokines.

macrophages, BCAP is constitutively phosphorylated and associated with the p85 subunit of PI3K for class I PI3K recruitment, which downregulates pro-inflammatory TLR responses (Ni et al., 2012). PI3K-mediated transitions from $PI(4,5)P_2$ to $PI(3,4,5)P_3$ at the membrane result in a depletion of membrane-anchored MAL and reduction of MyD88-dependent TLR signalling (Halabi et al., 2017). BCAP tethers TLRs to class IA PI3Ks through a typical TIR domain at its N terminus (Halabi et al., 2017). The TIR domain of BCAP can also interact with other TLR adaptors including MyD88 and MAL (Troutman et al., 2012). BCAP-deficient macrophages from knockout mice (*Pik3ap1*$^{-/-}$) produce increased levels of proinflammatory cytokines TNF, IL-12p40 and IL-6 in response to TLR4, TLR7, and TLR9 agonists (Troutman et al., 2012). Consistently, BCAP-deficient mice have increased recruitment of TNF-producing DCs and monocytes to the spleen early after infection with *Salmonella typhimurium* and an increase in the severity of dextran sodium sulphate (DSS)-mediated colitis (Troutman et al., 2012). Thus, BCAP acts as a key negative regulator of TLR-driven inflammation through the PI3K–Akt pathway (Fig. 3A), although the exact downstream targets and mechanisms are not yet described.

SCIMP – a non-TIR, transmembrane TLR adaptor

SCIMP is a member of the palmitoylated transmembrane adaptor protein (pTRAP) family and is the seventh TLR signalling adaptor described here. SCIMP comprises a short extracellular domain, a long intracellular domain containing multiple tyrosine phosphorylation sites, a proline-rich motif, and two palmitoylation sites (Fig. 2). Other pTRAPs include linker for activation of T-cells family member 1 (LAT1, also known as SLC7A5), phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG1), non-T-cell activation linker (NTAL, also known as LAT2 and LAB), and Lck-interacting transmembrane adaptor (LIME1), some of which have well-known roles in T and B cell receptor signalling (Curson et al., 2018). During T and B cell receptor signalling, these pTRAP family members work collaboratively and somewhat redundantly to form nanoclusters for the organisation of protein interactions to trigger and modulate signalling (Curson et al., 2018). pTRAP redundancy is also evident in signalling mediated by the tetrameric IgE receptor complex FcεRI. In response to activation of immune receptor FcεRI by multivalent antigens bound on IgE, multiple pTRAPs have a redundant role in FcεRI-mediated signalling. For example, knockout of LAT and NTAL reduced but did not abolish FcεRI-mediated production of IL-3, IL-6 and TNF in mast cells (Zhu et al., 2004). SCIMP was first described in B cells and dendritic cells (Draber et al., 2011; Kralova et al., 2016) and has also been shown by us to be the first transmembrane and non-TIR-containing TLR adaptor in macrophages (Luo et al., 2017a). Indeed, SCIMP is predominantly expressed in macrophages where it acts as a novel, 'universal' signalling adaptor for multiple members of the TLR family including both surface and endosomal TLRs (Luo et al., 2017a; Luo et al., 2019).

SCIMP was initially discovered in B cells through sequence analysis of its C-terminal Src kinase (Csk) SH2 binding domain, and binding sites for the adaptor proteins growth factor receptor-bound protein 2 (Grb2) and Src homology 2 domain-containing leukocyte protein of 65 kDa (SLP65) and SLP76, as well as a palmitoylation motif and an interaction with major histocompatibility complex class II (MHCII) (Draber et al., 2011). Interestingly, SCIMP-deficient mice have no defects in leukocyte development or MHCII signalling in B cells (Kralova et al., 2016). In dendritic cells, SCIMP has also been shown to associate with the PRR dectin-1, which is involved in fungal β-glucan recognition (Kralova et al., 2016). SCIMP was shown to be required for propagation of dectin-1 ERK signalling through recruitment of the Src family kinase (SFK) Syk (Kralova et al., 2016), although the exact model for this interaction remains unclear. Dectin-1 is also known to interact with TLR2 and 4 during the anti-β-glucan response (Yadav and Schorey, 2006), and SCIMP could potentially be implicated in this interaction.

For TLR signalling, SCIMP acts as a membrane-bound scaffold to mediate intracellular signal transduction (Luo et al., 2017a)

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(Fig. 3B). SCIMP is localised on intracellular membranes and on the macrophage plasma membrane, with high concentrations in dynamic cell-surface projections such as filopodia and membrane ruffles (Luo et al., 2017a). SCIMP binds directly to ligand-bound dimerised TLR4 through a unique TIR–non-TIR interaction, which likely precedes and does not require the binding of MyD88 (Luo et al., 2017a). Mechanistically, the SCIMP proline-rich domain (PRD) scaffolds the SFK Lyn to phosphorylate both SCIMP itself and LPS-activated TLR4, driving signalling and transcription in a highly selective manner. Amongst a panel of cytokines tested, SCIMP regulates the secretion of the pro-inflammatory cytokines IL-6 and IL-12p40, but not TNF and others (Luo et al., 2017a). A more extensive survey of cytokines is needed to determine whether IL-6 and IL-12 are truly the only cytokines driven by SCIMP. In addition, SCIMP is also recruited for signalling downstream of other TLRs, including both plasma membrane and intracellular TLRs (Luo et al., 2019). Like other pTRAPs such as LAT1, NTAL and PAG, SCIMP has the potential to orchestrate multiple protein interactions and/or trafficking of its binding partners at receptor signalling sites.

In TLR-activated mouse macrophages, SCIMP itself is rapidly phosphorylated by Lyn at three tyrosine residues (Fig. 3B), Y58, Y96 and Y120 (Y69, Y107 and Y131 in the human protein), thereby allowing TLR-activated SCIMP to act as a scaffold to recruit the effectors Grb2, Csk and SLP65 at each site, respectively (Luo et al., 2019, 2017b) (Fig. 3B). Grb2 and SLP65, which contain multiple protein-binding motifs, have been implicated in B cell signalling (Wienands et al., 1998), whereas Csk is a known negative regulator of Lyn (Okada et al., 1991). Therefore, TLR4 and Csk are temporally scaffolded by phosphorylated SCIMP as a potential on–off mechanism for TLR phosphorylation. Therefore, this positions SCIMP as a proximal universal TLR adaptor that brings its effectors Lyn, Grb2, Csk and SLP65 to TLR signalling. Lyn is required for SCIMP and TLR phosphorylation and recruitment of other effectors, whereas the precise roles of other SCIMP remain to be characterised. Together, SCIMP and its effectors produce a capacity for selective pro-inflammatory cytokine responses.

TLR adaptors and human diseases

TLR pathways and their canonical adaptors are associated with a number of human diseases including infection, sepsis, inflammatory, allergic and autoimmune diseases, as well as cancer (Marshak-Rothstein, 2006). Dysregulation of TLR pathways is often central to these pathologies through altered production of type I interferons, IL-6, IL-1β and TNF (Chen et al., 2016; Eftychi et al., 2019; Zwicky et al., 2019). As examples, autosomal recessive MyD88 deficiency results in an increased predisposition to recurrent pyogenic bacterial infection through impaired TLR and IL-1R responses, whereas leaving the immune response to other microbes unaffected (Picard et al., 2011; von Bernuth et al., 2008). TRIF dysregulation and deficiency has been linked to weakened responses to multiple viral and some bacterial infections through impaired TLR3–TLR4 responses (Ullah et al., 2016). Disease-associated single-nucleotide polymorphisms (SNPs) have been identified, which potentially link several of the TIR-containing TLR adaptors, MyD88, MAL, TRIF, TRAM and BCAP, to various diseases (Andiappan et al., 2011; Chu et al., 2019; George et al., 2010; Liu et al., 2015; Mekonnen et al., 2018; Zhang et al., 2011; Zhou et al., 2017). Like these TIR adaptors, mutation(s) in the non-TIR-containing TLR adaptor SCIMP and its effectors are emerging as being relevant in human inflammatory and autoimmune diseases. Genome-wide association studies (GWASs) have uncovered potential associations between SCIMP, a key positive regulator of TLR pathways, and a number of immune-linked human diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and Alzheimer's disease (AD) (see Box 1) (Dozmorov et al., 2014; Heneka et al., 2015; Jansen et al., 2019; Lambert et al., 2013; Liu et al., 2017; Moreno-Grau et al., 2019). SCIMP was identified as one of the top five genes with the largest number of epigenomic elements enriched in the promoter regions of SLE- and RA-associated gene sets, indicating that transcriptional regulation of SCIMP might be altered in autoimmune diseases (Dozmorov et al., 2014). The association of multiple TLR adaptors with a growing number of inflammation- and immune-linked diseases warrants further studies to determine whether and how these disease associations act through TLR-driven inflammation.

Conclusions and perspectives

This Review summarises the regulation and function of the seven known TLR adaptors that are involved in TLR-mediated signalling responses. MyD88, MAL, TRIF and TRAM adaptors, which are utilised by different TLRs, initiate both pro-inflammatory and anti-inflammatory responses. SARM plays a negative role in MyD88- and TRIF-mediated signalling, and BCAP is exclusively involved in TLR-induced PI3K–Akt signalling. The palmitoylated, transmembrane- and lipid-raft-localised SCIMP is likely upstream of all the other TLR adaptors, where it scaffolds the SFK Lyn and other effectors and is responsible for TLR tyrosine phosphorylation and activation. Given the well-characterised roles of other pTRAPs in T and B cell receptor signalling, SCIMP and other pTRAPs warrant further investigation in PRR pathways in innate immune cells. Overall, multiple adaptor families are required to shape signalling and inflammatory responses emanating from TLRs (see Tables 1 and 2). The TLR pathways demonstrate the importance of spatiotemporal regulation for adaptor recruitment with receptor–adaptor complexes at the plasma membrane and on endosomes and/or macropinosomes (Fig. 1) generating different signalling pathways and transcriptional outcomes to control inflammation. The association of TLR adaptors with infectious and autoimmune

Box 1. GWAS associations of SCIMP with Alzheimer's disease

Increasing evidence links the pathogenesis of AD to innate immune dysfunction and neuroinflammation (Heneka et al., 2015), and SNPs in several upstream promoter regions and non-coding sites proximal to SCIMP have been associated with AD in recent bioinformatics studies. A meta-analysis of multiple GWAS studies identified SCIMP as a genome-wide significant suggestive signal for AD risk in European populations (rs7225151; OR=1.10; P-value=3.7×10^{-7}) (Lambert et al., 2013). GWAS by proxy (GWAX) analysis of common diseases in 116,196 individuals from the UK Biobank identified SCIMP as a novel risk locus for AD (rs77493189; OR=1.11; P-value=9.6×10^{-10}) (Liu et al., 2017). Recently, a large-scale GWAS of clinically diagnosed AD cases as well as individuals with one or both parents diagnosed with AD (AD-by-proxy), consisting of 71,880 cases and 383,378 controls, revealed multiple significantly associated, immune-linked AD risk genes including SCIMP (rs113260531; P-value=1×10^{-9}) (Jansen et al., 2019). Furthermore, another recent GWAS confirmed an association between SCIMP and AD in a sample of 4120 AD cases and 3289 control individuals (rs7225151; OR=1.11; P-value=9.45×10^{-9}) (Moreno-Grau et al., 2019). It is noteworthy that the odds ratios and other measures of SCIMP association with AD predict a low disease risk. Nevertheless, SCIMP, other TLR adaptors and the receptor pathways are increasingly emerging in inflammation that underlies AD and other chronic diseases.

diseases highlights the importance of their biology and molecular mechanisms, which may ultimately provide avenues for novel therapeutic targets in immune-linked disease.

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