PROGRESS

The molecular basis of the host response to lipopolysaccharide

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Abstract | Lipopolysaccharide (LPS), which is produced by Gram-negative bacteria, is a powerful activator of innate immune responses. LPS binds to the proteins Toll-like receptor 4 (TLR4) and MD2 to activate pro-inflammatory signalling pathways. The TLR4–MD2 receptor complex is crucial for the host recognition of Gram-negative bacterial infection, and pathogens have devised many strategies to evade or manipulate TLR4–MD2 activity. The TLR4–MD2 signalling pathway is therefore potentially an important therapeutic target. This Progress article focuses on recent exciting data that have revealed the structural basis of TLR4–MD2 recognition of LPS.

Endotoxins were first recognized in the nineteenth century and are associated primarily with Gram-negative bacteria. They are distinguished from exotoxins, because they do not diffuse into culture media but are released upon lysis of the bacterial cell. We now know that the principal endotoxin is lipopolysaccharide (LPS), a complex glycolipid that is the major component of the Gram-negative outer membrane. LPS is a powerful activator of innate immune responses and is responsible for endotoxic shock, which is the often fatal complication of sepsis.

It is only in the past few years that the molecular mechanisms by which LPS initiates signalling responses in immune system cells have been elucidated. In this Progress article, we focus on recent work that has revealed the structural basis for the recognition of LPS by immune system cells and how this explains the diverse patterns of interaction and response that are observed between Gram-negative pathogens and their hosts.

Variations in lipid A

The general structure of bacterial LPS consists of a hydrophobic lipid A domain (FIG. 1; see also Supplementary information S1 (figure)), an oligosaccharide 'core' and a distal polysaccharide (the O antigen)[1]. The lipid A moiety alone is sufficient to activate the innate immune response; adaptive (antibody) responses are generated to the O antigen polysaccharide later in the course of an infection. Lipid A consists of a diglucosamine diphosphate headgroup that is substituted with a variable number of acyl chains, ranging from four to eight. There is also variability within and between bacterial species in the composition of the headgroup and the length and saturation of the acyl chains. Nevertheless, many bacteria make lipid A species that are similar to *Escherichia coli* lipid A, which contains a diglucosamine diphosphate headgroup and six acyl chains. In general, such hexa-acyl lipid A molecules are powerful immunostimulants.

Changes in the number of acyl chains and in the phosphorylation status of the headgroup can have a profound influence on the biological activity of lipid A. Lipid IVa, for example, which is an intermediary in the biosynthetic pathway for *E. coli* lipid A, has only four acyl chains and is an agonist in mice and horses but an antagonist in humans[2,3] (see BOX 1 for definitions). *Rhodobacter sphaeroides* lipid A has five acyl chains and is an antagonist in humans and mice but an agonist in horses[4]. The synthetic compound eritoran (also known as E5564) has four acyl chains and is an antagonist in all species examined so far[5,6].

The importance of the number of acyl chains for the activity of lipid A-like structures is illustrated by the properties of a library of synthetic monosaccharide lipid A mimetics (the aminoalkyl glucosaminide phosphate compounds) in which both the number and carbon length of the acyl chains are crucial to generate an active compound[7]. The explanation for why underacylated lipid A causes less cell activation than hexa-acylated lipid A is likely to involve an alteration to the interaction between lipid A and the LPS receptor complex, such that receptor activation is reduced[8]. Structural modifications of lipid A, such as changes in the phosphorylation status, also affect its biological activity. Of particular interest is monophosphoryl lipid A from *Salmonella enterica* subsp. *enterica* serovar Minnesota, which is a variant form of lipid A that is only weakly active at the LPS receptor complex compared with fully phosphorylated lipid A[9].

A further complication in understanding lipid A biology is that much of the literature describes experiments using LPS isolated from bacteria that contain a mixture of lipid A structures. For example, LPS isolated from *Porphyromonas gingivalis* is highly heterogeneous, which possibly accounts for reports that *P. gingivalis* LPS can activate two innate immune receptors simultaneously[10].

Detecting lipopolysaccharide

It is 20 years since Janeway proposed the existence of pattern recognition receptors (PRRs)[11]. Janeway argued that, in order to generate an antibody response to a pathogen, it is necessary for a second signal to be produced by antigen-presenting cells in addition to the primary signal of antigen presentation itself, and that this second signal would be provided by the cellular recognition of conserved structures that are associated with pathogenic microorganisms. He singled out LPS as the molecule that fulfills this role, as it was known to be a powerful stimulator of immune system cells, causing the production of pro-inflammatory cytokines and acute-phase proteins.

LPS from infecting bacteria is extracted and solubilized by a serum protein, LPS-binding protein (LBP)[12]. LBP then transfers the LPS to a leukocyte extrinsic membrane protein, CD14 (REF. 13). The main role

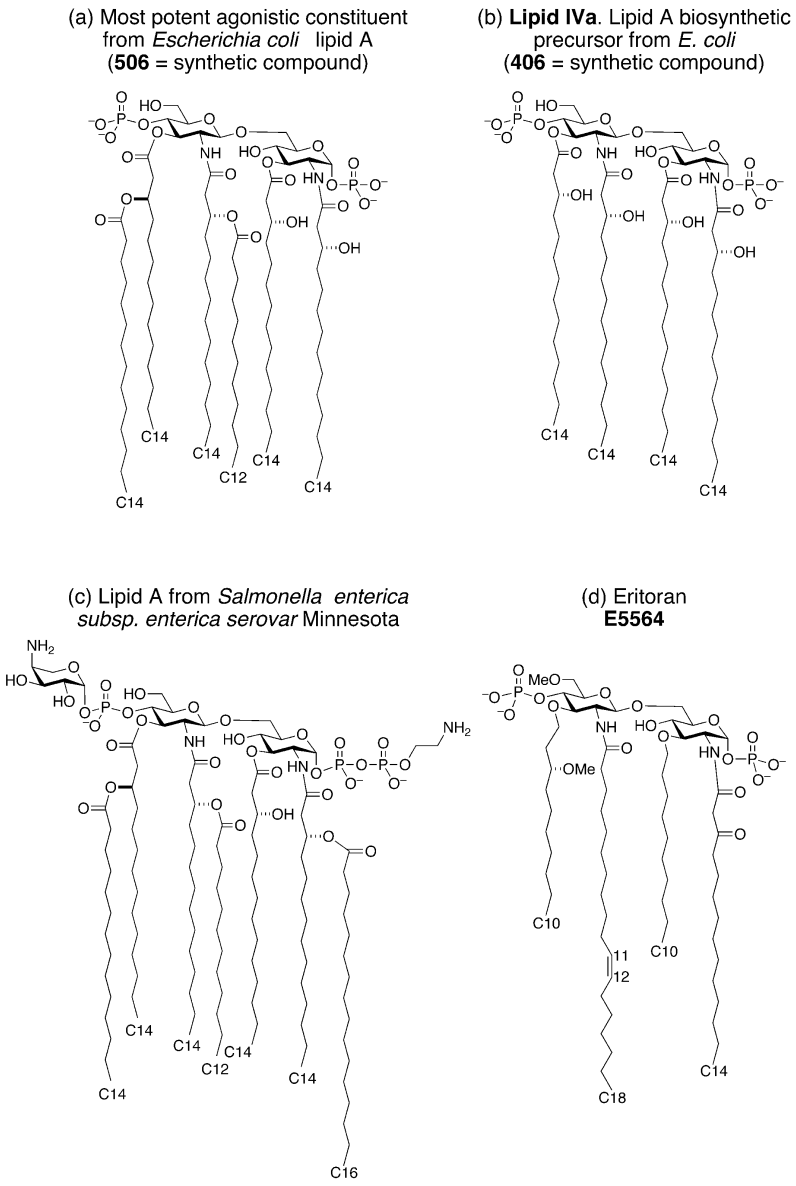

Figure 1 | **Lipid A structures. a** | *Escherichia coli* lipid A. **b** | Lipid IVa, the precursor of *E. coli* lipid A. **c** | Lipid A from *Salmonella enterica* subsp. *enterica* serovar Minnesota. **d** | The synthetic lipid A analogue eritoran (also known as E5564).

for CD14 is to enhance the sensitivity of myeloid cells to LPS, reducing the binding affinity to picomolar concentrations[14]. As a consequence, mice without CD14 are resistant to endotoxic shock[15]. The identity of the PRR that signals in response to LPS, Toll-like receptor 4 (TLR4), was not established until 1999. TLR4 was one of the first TLRs to be identified[16], and mapping studies in the LPS-resistant mouse strains C3H/HeJ and C57BL/10ScNJ identified *Tlr4* as the gene encoding the LPS receptor[17,18]. The role of TLR4 in LPS signalling was confirmed in *Tlr4*$^{-/-}$ mice, which were shown to be hyporesponsive to LPS[19]. Subsequently, mutations in the human *TLR4* were shown to be associated with hyporesponsiveness to inhaled LPS[20]. Further genetic and biochemical studies revealed that expression of TLR4 alone does not confer LPS responsiveness, and that an additional co-receptor protein, MD2 (also known as LY96), is required[21]. Like the *Tlr4* knockouts, mice lacking MD2 do not respond to LPS[22] and are resistant to endotoxic shock. It is now clear that many different cell types express TLR4, CD14 and MD2, including cells of the monocyte–macrophage lineage, lymphoid cells and cells that are not part of the immune system, such as epithelial, endothelial and vascular smooth-muscle cells.

The Toll-like receptor 4–MD2 complex

The identification of the receptors involved in LPS signalling was an important advance in our understanding of innate immunity but revealed little about the molecular basis of pattern recognition or the mechanism and regulation of signal transduction by TLR4. In the past 2 years, dramatic advances in our understanding of these processes have been made as a result of structural analyses.

TLR4 has the characteristic features of a class 1 transmembrane receptor, with an extracellular domain, a single membrane-spanning helix and a globular cytoplasmic domain, the TIR (Toll interleukin-1 receptor) domain. The extracellular domain contains leucine-rich repeat (LRR) motifs and an associated capping structure. LRR motifs are found in ~250 human proteins and fold into curved, solenoidal structures[23]. The LRR framework has a propensity to evolve binding specificity for a wide range of biological molecules, including proteins, lipids and carbohydrates. As with other class 1 transmembrane receptors, signal transduction is expected to require the stimulus-induced dimerization of two receptor molecules[24].

An important step forward in our understanding of LPS recognition by the TLR4–MD2 heterodimer came with the discovery that MD2 belongs to a small family of lipid-binding proteins, the MD2-related lipid recognition family[25]. These proteins fold into a β-sandwich structure that is similar to that formed by the immunoglobulin domains of antibody molecules. Modelling studies suggested that binding to LPS is mediated by the intercalation of the lipid A acyl chains into the hydrophobic core of the MD2 β-sandwich[26,27]. This model was confirmed by structural analyses of MD2 bound to lipid IVa and of a TLR4–MD2 heterodimer in complex with the antagonist eritoran[28,29]. As shown in FIG. 2a,b, the four acyl chains of eritoran and lipid IVa are fully accommodated in the MD2 structure and occupy approximately 90% of the solvent-accessible volume of the LPS-binding pocket. Two of the acyl chains are in the fully extended conformation in the binding pocket, but two are bent in the centre. The diglucosamine backbones are fully exposed to solvent[30]. In both the lipid IVa–MD2 and eritoran–TLR4–MD2 structures the ligand does not induce a conformational change in the receptor, but this is to be expected as these molecules are antagonists. The TLR4 extracellular domain forms a rigid, curved solenoid, as expected, with MD2 bound at two conserved sites in

Box 1 | **The pharmacological activity of ligands at the Toll-like receptor 4–MD2 complex**

Many authors use a range of pharmacological terms to describe the activity of different Toll-like receptor 4 (TLR4)–MD2 ligands, sometimes incorrectly. Incorrect usage could have important consequences, as the pharmacological activity of a compound has precise implications for receptor behaviour. For example, a ligand binds to a receptor but does not necessarily have activity. The following list defines compounds that bind to TLR4–MD2 and describes their activity.

- Agonist (for example, *Escherichia coli* lipid A): a ligand that binds to a receptor and induces a physiological response (efficacy). Agonist binding to TLR4–MD2 induces a change in the conformation of the receptor, which presumably induces the association of the TIR (Toll interleukin-1 receptor) domains to recruit the adaptor proteins and induce activation of the signalling pathways.
- Antagonist (for example, eritoran/E5564): a ligand that binds to the receptor binding site but does not induce changes in receptor conformation or any signalling activation. These drugs inhibit cellular responses by preventing access of an agonist to the receptor binding site, and they do not have any other effect on the receptor.
- Partial agonist (for example, monophosphoryl lipid A): a ligand that binds to a receptor binding site to induce some conformational change without leading to full activation of the receptor, thus resulting in incomplete signalling. In contrast to lipid A, monophosphoryl lipid A lacks the phosphate group at position 1 and is therefore unable to contact positively charged residues on the surface of both MD2 and TLR4 (REF. 34). In the presence of a partial agonist, a full agonist is unable to bind to the receptor; the agonist therefore seems to be antagonized, and the partial agonist acts like an antagonist (sometimes these drugs are incorrectly described as agonist–antagonists).
- Inverse agonist (there are no known examples for TLR4–MD2): a ligand that binds to the receptor binding site, but reverses the constitutive activity of the receptor. Inverse agonists have the reverse effect of agonists and induce negative efficacy.

These definitions were originally coined for ligands acting on G protein-coupled receptors but have been extended to embrace the activity of compounds binding to all receptors[66]. Receptors exist in equilibrium between an active and an inactive state ($R \leftrightarrow R^*$). An agonist will stabilize R* (the active conformation), an inverse agonist will stabilize R (the inactive conformation) and an antagonist will not alter the equilibrium.

It is probable that these definitions will hold true for receptors other than G protein-coupled receptors, such as Toll-like receptors.

the amino-terminal region of TLR4. The entrance to the LPS-binding pocket is on the opposite side of MD2, which is exposed to solvent.

Although this work was an important step forward, it was unclear from the TLR4–MD2 heterodimer structure how the binding of hexa-acyl lipid A induced the dimerization that is required to initiate signal transduction. One important clue came from the finding that mutation of the H155 and F126 residues of MD2 did not affect LPS binding but did abolish the ability of the TLR4–MD2 heterodimer to form the activated heterotetramer, suggesting that these residues form part of the dimerization interface[29]. Further clues came from a study that investigated why lipid IVa is an agonist for horse TLR4 but an antagonist for human TLR4 (REF. 3). This work showed that the species differences are due to amino acid sequence variations in both MD2 and TLR4. A short region in the horse MD2 (residues 57–107) is sufficient to confer responsiveness to lipid IVa when transplanted into a human MD2 framework. Equally, a region in the carboxyl terminus of the horse TLR4, between LRRs 14 and 18, was found to be essential for signalling activity in response to lipid IVa. Strikingly, a horse mutant with a single change at TLR4 residue 285, from arginine to glycine (this being the residue that is found at the equivalent position in the human protein), lost the ability to signal in response to lipid IVa.

On the basis of these results, a structural model for the activated, heterotetrameric TLR4–MD2 complex was generated by protein–protein docking methods. This indicated that there are two regions of contact between the TLR4–MD2 heterodimers (FIG. 2c,d). The first interface involves the MD2 residue F126 and a hydrophobic region of the TLR4 ectodomain at L444. The second interface is on the lateral surfaces of the two ectodomain molecules, centred on LRR 16, the region that was identified as important for signalling in the mutagenesis study. This arrangement of the two TLR4–MD2 heterodimers brings the C-terminal, juxtamembrane sequences of the ectodomains into close proximity and has a similar 'M'-shaped conformation to that of TLR1 and TLR2 complexed by tri-acylated lipid and that of the TLR3 ectodomain bound to double-stranded RNA[31,32]. This suggests that the formation of active TLR complexes follows a common mechanism (FIG. 2d).

This general model of activation has now been confirmed by the elucidation of a high-resolution structure for TLR4–MD2 bound to hexa-acyl lipid A and by mutagenesis studies of the predicted interface residues[33,34] (FIG. 2c). When accommodating lipid A with more than four acyl chains, there is no conformational change in MD2, and this causes the acyl chain at the C2 carbon position to be exposed on the surface of the MD2 structure. Together with the MD2 residue F126, this creates a hydrophobic patch that forms the dimerization interface with TLR4, an interaction that involves L444 and the nearby residues F440 and F463 of TLR4. This forces the glucosamine backbone upwards, repositioning the phosphate groups to contact positively charged residues of both TLR4 subunits. The second dimerization interface is also as predicted by the model, with the lateral surfaces of two ectodomains creating an extensive area of protein–protein interaction that is centred on LRR 16. The TLR4 single-nucleotide polymorphisms that reduce responsiveness to lipid A (D299G and T399I) are located far away from the N-terminal MD2-binding site and the TLR4 dimerization interfaces[20,35] (FIG. 2d). Thus, the mechanism that causes hyporesponsiveness remains unclear, but the mutations could either affect the cooperative binding of lipid A or alter the conformational changes that occur during ligand-induced signal transduction. In this regard, it should be noted that formation of the active TLR4–MD2 complex changes the curvature of the TLR4 solenoid, and mutations that increase rigidity could have a substantial effect on the kinetics of receptor activation.

Other exogenous and endogenous ligands

The interaction between lipid A and the TLR4–MD2 complex is now well understood, but several ligands other than lipid A have been identified as TLR4 agonists. These include endogenous ligands (such as high mobility group protein B1 (HMGB1), heat shock protein 60 (HSP60), HSP70, the type III repeat extra domain A of fibronectin, hyaluronic acid oligosaccharides, heparin sulphate polysaccharide fragments and fibrinogen), other pathogen-derived ligands (such as *Streptococcus pneumoniae* pneumolysin, *Chlamydia pneumoniae* Hsp60, mouse mammary tumour virus envelope proteins and respiratory syncytial virus fusion (F) protein), the house dust mite protein Der p 2

(an allergen associated with asthma) and the plant ligand taxol[36]. It seems that *C. pneumoniae* Hsp60, the respiratory syncytial virus F protein[35] and the type III repeat extra domain of fibronectin[37] all require MD2 for activation of TLR4, but Der p 2 (REF. 38) does not. There is some controversy about how these proteins activate TLR4–MD2; in particular, activation could be due to contaminating LPS. Much of the recent work on these ligands, a good example being a study on Der p 2 (REF. 38), shows both LPS-dependent and LPS-independent activation of TLR4–MD2. The molecular and structural basis for the activation of TLR4–MD2 by these putative ligands remains to be determined, but they probably, directly or indirectly, promote the association of the C termini of the receptor ectodomains.

Downstream consequences of signalling

The biological activity of TLR4 that is induced by ligand binding involves the dimerization or oligomerization of receptor chains[39]. This, in turn, results in conformational changes in the receptor and homodimerization of the two cytoplasmic TIR domains[24]. Fluorescence resonance energy transfer (FRET) microscopy showed that the TIR domains of TLR9 undergo a notable positional change on ligand binding[40]; it is likely that this also occurs with other TLRs on dimerization. The association of the TIR domains would provide a scaffold to recruit specific adaptor proteins to form an active signalling complex.

There are five adaptor proteins that function in TLR signalling, and they all contain TIR domains[41]. Activated TLR4 recruits two adaptor protein pairs, TRAM–TRIF (also known as TICAM2 and TICAM1, respectively) and MAL–MyD88 (REF. 41). MAL and TRAM are thought to engage directly with TLR4 and act as 'bridging adaptors' for the recruitment of MyD88 and TRIF, respectively. MAL is required for rapid activation of the transcription factor nuclear factor-κB (NF-κB) and the production of pro-inflammatory cytokines such as tumour necrosis factor. TRAM stimulates sustained NF-κB activity but also activates interferon regulatory factor 3 (IRF-3). IRF-3 induces the expression of a distinct set of genes to NF-κB, such as the genes encoding interferon-β and CC-chemokine ligand 5 (CCL5; also known as RANTES)[41]. Activation of MAL–MyD88 causes the production of large amounts of pro-inflammatory cytokines, whereas TRAM–TRIF signalling causes adjuvanticity[9].

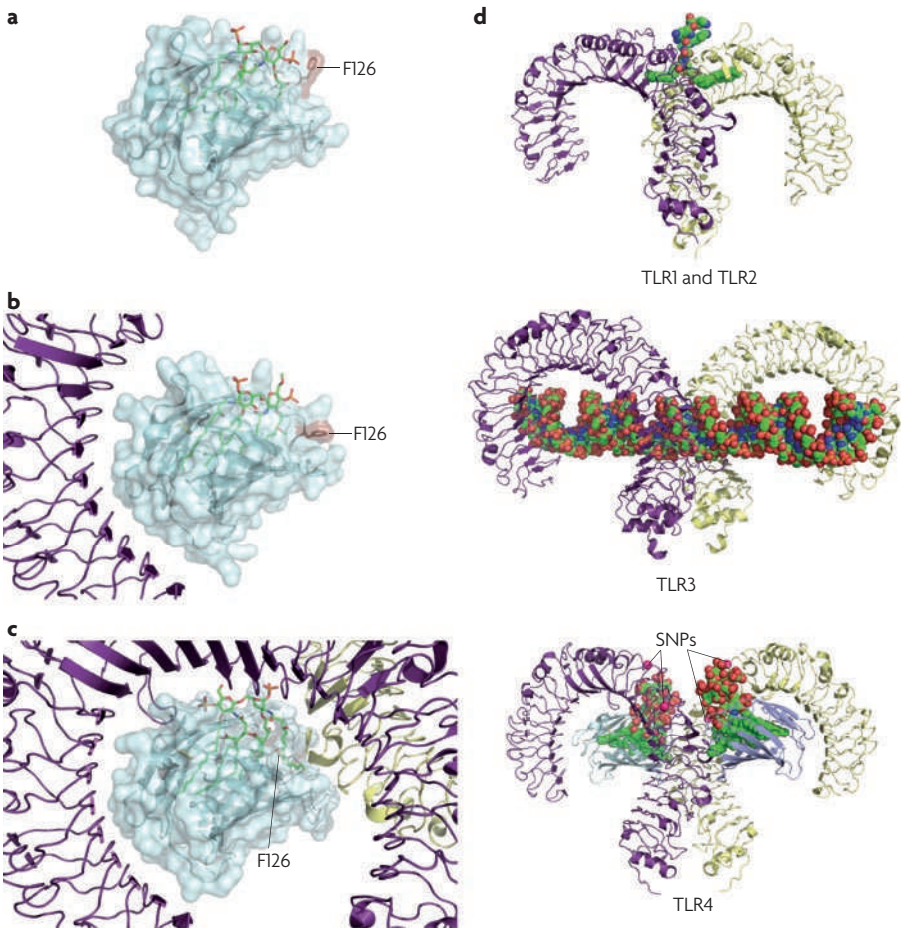

Figure 2 | **MD2 and Toll-like receptor crystal structures. a** | The crystal structure of the complex between MD2 (also known as LY96) and lipid IVa (Protein Data Bank entry 2E59). MD2 is shown in cyan as a semi-transparent molecular surface, and lipid IVa is shown in a stick representation (carbon atoms in green, oxygen atoms in red, phosphate atoms in orange and nitrogen atoms in blue). The two phosphorylated glucosamine headgroups of lipid IVa are solvent exposed. The acyl chains are buried in the hydrophobic cavity of MD2. Residue F126 is crucial for signalling and is represented in its inactive conformation. Residue H155 is surface exposed and located on the other side of MD2 (not shown). **b** | The complex formed between eritoran (also known as E5564), Toll-like receptor 4 (TLR4) and MD2 (Protein Data Bank entry 2Z65). The binding mode of eritoran is similar to that of lipid IVa. TLR4 is shown in purple, MD2 is shown in cyan as a semi-transparent molecular surface and eritoran is shown in a stick representation. **c** | A close-up view of the active TLR4–MD2–lipopolysaccharide (LPS) complex (Protein Data Bank entry 3FXI) reveals a different binding mode for LPS, involving MD2 and two TLR4 molecules. The MD2 proteins are in the same orientation as in parts **a** and **b**. The TLR4 molecules are shown in purple and yellow, MD2 is shown in cyan as a semi-transparent molecular surface and LPS is shown in a stick representation. **d** | Active TLR complexes. The top panel shows TLR1 and TLR2 in complex with a tri-acylated lipopeptide (Protein Data Bank entry 2Z7X). TLR1 is shown in purple, TLR2 is shown in yellow and the ligand is shown in a sphere representation. The middle panel shows TLR3 in complex with double-stranded RNA (Protein Data Bank entry 3CIY). The TLR3 ectodomains are shown in yellow and purple, and the RNA ligand is shown in a sphere representation. The bottom panel shows the TLR4–MD2–LPS complex. The TLR4 ectodomains are shown in yellow and purple, MD2 in blue, and LPS in a sphere representation. The TLR4 single-nucleotide polymorphisms (SNPs) are indicated.

The results of mutagenesis and molecular modelling studies suggest that ligand-induced dimerization of the TLR4 extracellular domains leads to concerted protein conformational changes that, in turn, lead to self-association or rearrangement of the cytoplasmic TIR domains, thereby creating a new molecular surface for the recruitment of signalling adaptor proteins[42] (FIG. 3). This model predicts that MAL and TRAM bind to the same region in the TLR4 dimer interface, thus explaining why cell-permeable peptides that target the TIR BB loop outcompete

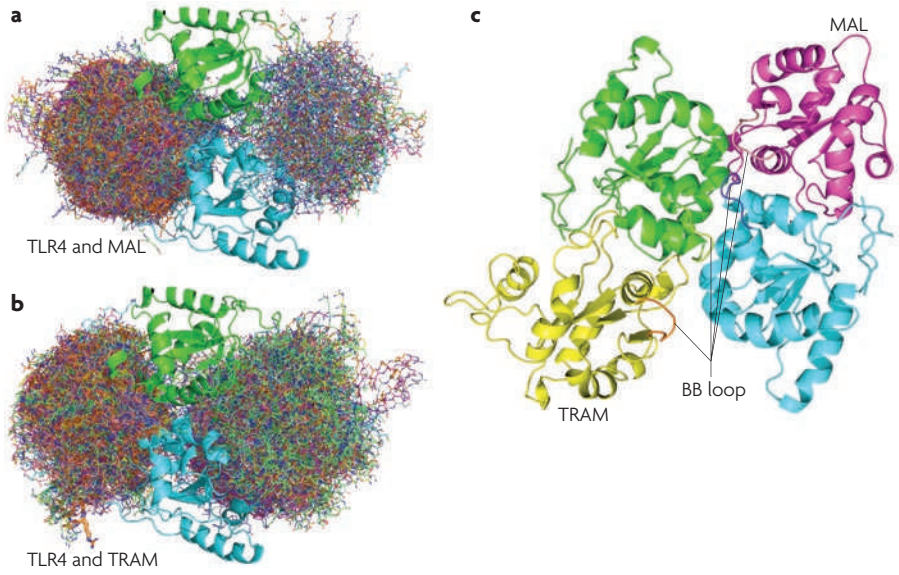

Figure 3 | **Docking model of MAL and TRAM binding at the Toll-like receptor 4 homodimer interface.** The Toll-like receptor 4 (TLR4) protomers are shown as green and cyan ribbons. **a**,**b** | Docked MAL (**a**) and TRAM (also known as TICAM2) (**b**) are shown as stick models of the 50 best docking solutions that were generated by Global Range Molecular Matching Methodology (GRAMM). **c** | A high-resolution complex of the TLR4 dimer with MAL and TRAM. The position of each BB loop is labelled. Figure is reproduced, with permission, from *Nature Immunology* REF. 44 © (2006) Macmillan Publishers Ltd. All rights reserved.

both MAL-directed and TRAM-directed responses simultaneously[43]. However, the model does not resolve the question of whether a single activated receptor dimer can stimulate both the MAL-directed and TRAM-directed pathways simultaneously, or whether adaptor engagement is mutually exclusive (which would require positive cooperativity). Each activated receptor has two symmetry-related adaptor-binding sites so, in principle, either hypothesis is feasible.

MAL and TRAM are both regulated by covalent modification. MAL is phosphorylated by Bruton tyrosine kinase (BTK); phosphorylation is required for MAL to signal and subsequently leads to its degradation[44]. MAL also contains a phosphatidylinositol-4,5-bisphosphate-binding domain that localizes it to the plasma membrane[45]. Similarly, TRAM is myristoylated at its N terminus, which localizes it to the plasma membrane[46], and it undergoes phosphorylation by protein kinase Cε (PKCε), which is required for signalling[47]. On activation, TLR4 traffics to the early endosome, and it is here that TRIF is recruited to activate TBK1, leading to activation of IRF-3 (REFS 45,48). *E. coli* lipid A activates both MAL–MyD88 and TRAM–TRIF signalling. By contrast, monophosphoryl lipid A from *S.* Minnesota strongly activates the TRAM–TRIF signalling pathway but only weakly activates MAL–MyD88, owing to diminished recruitment of MyD88 to TLR4 (REF. 9). The net effect of monophosphoryl lipid A is adjuvant activity, rather than the profound inflammatory response that is seen with hexa-acylated lipid A. It may be that other lipid A structures will also be able to induce differential activation of the two TLR4 signalling pathways.

Manipulation by pathogens

The importance of TLR4–MD2 signalling in the response to Gram-negative pathogens makes this signalling pathway an ideal target for bacterial manipulation. A good example of this is the ability of some pathogens to modify their lipid A structures to alter their detection by the host[49]. *Pseudomonas aeruginosa* modifies the structure of its LPS on invasion of host tissues: environmental isolates of this bacterium produce penta-acylated LPS, which does not activate human TLR4, whereas the hexa-acylated LPS structures that are produced during adaptation to the cystic fibrosis airway are highly pro-inflammatory[49]. Interestingly, the penta-acylated LPS activates mouse TLR4, and the interaction mediating this activation was mapped to amino acids 285–366 of TLR4, which contain the dimerization domain[49]. Another pathogen, *Yersinia pestis*, synthesizes lipid A with poor TLR4 activation capacity when grown at 37 °C but produces highly active lipid A when grown at 26 °C. The LPS that is produced at the higher temperature can antagonize the LPS that is produced at the lower temperature, suggesting that *Y. pestis* can produce a mixture of stimulatory and non-stimulatory LPS species[50]. These data imply that the production of highly active LPS allows the host to detect the pathogen quickly and to activate immune signalling, but that the pathogen can evade these antibacterial defences by lipid A modification[50]. It is unclear whether pathogens have evolved the ability to change their lipid A structure in order to evade the host, or whether this simply reflects a change in the bacterium to accommodate different environmental niches[51]. Pathogens also produce other compounds that modify TLR signalling; for example, *P. aeruginosa* produces *N*-(3-oxododecanoyl)-L-homoserine lactone, which downregulates TLR4–MD2-induced signalling[52]. These strategies may be useful for immune evasion of TLR4–MD2 by Gram-negative pathogens.

Several microbial proteins have been reported to bind to TLR4 (with or without MD2). As discussed above, several protein ligands from bacteria (for example, pneumolysin and *C. pneumoniae* Hsp60) and viruses (mouse mammary tumour virus envelope proteins and respiratory syncytial virus F protein) activate TLR4 signalling. It is unclear as yet how the interaction of these proteins with TLR4 influences virulence. Recently, Der p 2 has been shown to facilitate signalling by interacting with TLR4 and reconstituting LPS-driven TLR4 signalling in the absence of MD2. A mouse model challenge with Der p 2 led to experimental allergic asthma in wild-type and MD2-deficient, but not TLR4-deficient, animals[38,53]. How Der p 2 interacts with TLR4 and TLR4–MD2 remains to be clarified, but the production of proteins that sensitize TLR4–MD2 signalling may also be a useful strategy to aid pathogen survival. Other proteins, such as the nematode protein ES-62, form a complex with TLR4, sequestering PKCα. This causes proteasome-independent degradation of PKCα, and this reduces mast cell activation[54].

Several viral and bacterial proteins interfere with TLR signalling. Recently, bacterial proteins containing bacterial TIR domains have been found and characterized in *Salmonella enterica* subsp. *enterica* serovar Enteritidis, *Paracoccus denitrificans*, *E. coli* and *Brucella melitensis*. These proteins possibly interfere with MyD88 recruitment to TLR4, may interact with mammalian TIR domains and have been shown to reduce NF-κB activation in cellular assays[55–58]. The TlpA protein from *S.* Enteritidis and

TCP pilus biosynthesis protein C (TcpC) from *E. coli* both seem to be important in virulence and in causing host pathology[55,57]. Several other bacterial proteins subvert host signalling pathways, and many of these will also affect TLR4 signalling, suggesting that this pathway is a rich target for microbial manipulation.

Therapeutic interventions

The TLR4–MD2 signalling pathway is an important therapeutic target, not only in infectious diseases but also in other diseases with an inflammatory aetiology, such as cancer, atherosclerosis, asthma and autoimmune conditions. The elucidation of the activated TLR4–MD2 complex structure should accelerate the process of the rational identification of new agonists and antagonists. Many antagonists of TLR4–MD2 have been identified already, some of which are based on the lipid A structures and other small-molecule inhibitors[5,59–61]. The principal aim of using TLR4 antagonism as a therapy has, so far, been to treat septic shock. One concern with this approach is that mice that cannot signal in response to LPS show markedly increased susceptibility to Gram-negative infections[62]. One inhibitor, eritoran, is in clinical trials for the treatment of sepsis[63]; these trials were initially not very promising, owing to poor patient selection, but recent data suggest a trend towards reduced mortality[64]. It will be interesting to see how successful selective TLR4 antagonists, or TLR4-specific antibodies[65], will be in treating a complex, multifactorial disease like septic shock. Agonists of TLR4, in particular those that activate TRAM or TRIF signalling, are also being developed as adjuvants. Several compounds, including the aminoalkyl glucosaminide phosphate compounds and monophosphoryl lipid A, are in development or in use as vaccine adjuvants[7,9]. In addition, targeting the lipid A biosynthetic pathway in bacteria has established a new class of antibiotic[1]. Finally, compounds that target the lipid A interaction with TLR4 in the host could lead to the development of a new class of anti-inflammatory therapies for use in infectious and non-infectious diseases.

Conclusions and perspectives

The structural data on ligand recognition by TLR4–MD2 have begun to explain the underlying mechanisms whereby diverse patterns of LPS and Gram-negative pathogens may be detected by their hosts. Host recognition of LPS through TLR4–MD2 is crucial for the control of bacterial infection, but disturbances in the activation of this signalling pathway can lead to sepsis and pathogen evasion. The recent elucidation of the molecular mechanisms by which the TLR4–MD2 complex recognizes LPS should facilitate the pharmacological analysis of this receptor complex and the design of new therapeutic compounds to target this signalling pathway. It is now clear that TLR4–MD2 can be activated by ligands other than LPS, and this may underlie the aetiology of allergic conditions such as asthma. Understanding the structural basis of how these proteins interact with TLR4 or TLR4–MD2 could lead to the generation of new compounds to treat important allergic and inflammatory diseases. We are, however, still left with important unanswered questions, such as how is the selective activation of either MAL–MyD88 or TRAM–TRIF signalling achieved, how do ligands other than LPS activate TLR4–MD2, and what are the physiological reasons for species selectivity in response to lipid A structures? The recent advances that have been made in structure–function analyses should allow many of these questions to be resolved in the near future.

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Competing interests statement
The authors declare no competing financial interests.

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