The Toll receptor family and microbial recognition

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The survival of multicellular organisms is dependent on their ability to recognize invading microbial pathogens and to induce a variety of defense reactions. Recent evidence suggests that an evolutionarily ancient family of Toll-like receptors plays a crucial role in the detection of microbial infection and the induction of immune and inflammatory responses.

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The immune system of vertebrates has two components: innate immunity and adaptive immunity. The innate immune system is phylogenetically ancient and presumably exists in all multicellular organisms, whereas the adaptive immune system is approximately 500 million years old and is found only in vertebrates. These two systems utilize two very different mechanisms for host defense. The innate immune system relies on a set of germline-encoded receptors that are expressed on a wide variety of cells, particularly effector cells like macrophages and neutrophils, as well as the surface epithelial cells situated at host–environment boundaries. By contrast, the adaptive immune system is critically dependent on a sophisticated somatic gene rearrangement and diversification process that generates millions of antigen receptors with random specificities. These receptors are expressed in a clonal fashion on two specialized cell types of hematopoietic origin, T and B cells.

Although the field of innate immunity goes back as far as the end of the 19th century, when Elie Metchnikoff made his first observations of phagocytosis by starfish larvae cells in 1884, we still know very little about the molecular mechanisms of innate immune recognition and the receptors involved. From the little that we do know, it is clear that innate immune recognition is mediated by structurally and functionally diverse receptors that can directly trigger a variety of host defense mechanisms such as complement activation, phagocytosis and expression of proinflammatory genes[1–4]. Innate immune recognition is directed at conserved microbial structures that are relatively invariant within a given class of microorganisms. These structures are called pathogen-associated molecular patterns (PAMPs), and the receptors of the innate immune system that directly recognize PAMPs are called pattern-recognition receptors (PRRs). PAMPs have several characteristic features important for their immunogenic activities. First, they are produced by microorganisms but not by host cells, which allows the host organism to discriminate between self molecules and microbial-associated non-self. Second, they are usually essential for the survival or pathogenicity of the microorganism, and have therefore been conserved over the course of evolution of the pathogen, even though their recognition by the host immune system creates a negative selective pressure. Finally, PAMPs often, although not always, represent a 'molecular signature' of a pathogen class. Therefore recognition of PAMPs by the innate immune system not only signals the presence of infection, but also provides valuable information regarding the type of infecting pathogen. These three characteristics of PAMPs have a certain predictive value: microbial products that have the first two features are likely to be the targets of innate immune recognition. This is true of many of the best characterized PAMPs, which include lipopolysaccharide (LPS), peptidoglycan and zymosan. It is worth noting that the number of antigens recognized by the adaptive immune system is not finite; novel antigenic determinants are continuously generated during evolution, particularly in the case of viral pathogens. The number of PAMPs, however, is finite and corresponds to the number of specificities that can be recognized collectively by all the PRRs of the host organism.

In the past ten years, a number of PRRs that mediate microbial opsonization, complement activation and phagocytosis have been characterized[5,6]. However, the receptors responsible for the induction of immune and inflammatory genes have been unknown until recently. This situation changed dramatically with the discovery of the immune function of both the *Drosophila* and mammalian Toll receptors.

The Toll pathway in *Drosophila* immunity

Components of the Drosophila Toll pathway

The first member of the Toll family was identified in *Drosophila* during a screen for embryonic polarity genes[7]. Cloning of the Toll gene revealed that it encodes a transmembrane protein with a large extracellular domain consisting of so-called leucine-rich repeats and a cytoplasmic domain referred to as the Toll/IL-1R domain or TIR domain because of its homology to the cytoplasmic domain of the mammalian interleukin 1 receptor (IL-1R)[7,8]. Similar genetic screens

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have also led to the identification of several other signaling components of the Toll pathway, which have been ordered in a linear pathway[9]. *Drosophila* Toll is activated by the endogenous protein ligand Spatzle, which pre-exists in the vitelline fluid as an inactive precursor. The proteolytic processing and activation of Spatzle is induced by a spatially controlled cascade of serine proteases that creates a gradient of active Spatzle with the highest concentration at the ventral side of the egg chamber. The active Spatzle is thought to bind to Toll directly and to induce intracellular signaling events, many details of which are still unknown. The receptor-proximal component of the Toll pathway appears to be the adaptor protein Tube, which recruits and activates the serine/threonine protein kinase Pelle. The activation of Pelle leads, by an unknown mechanism, to the degradation of the inhibitor of κB (IκB) family member Cactus. This, in turn, results in the release of the *Drosophila* nuclear factor κB (NF-κB) proteins Dorsal and Dif and their translocation into the nucleus, where they activate transcription of target genes.

Toll proteins in Drosophila innate immunity

Drosophila Toll was identified as a gene product involved in the control of dorso-ventral polarity in developing embryos. Remarkably, however, the intracellular events of the Toll pathway show a high degree of similarity with the mammalian NF-κB pathway initiated by the IL-1R (Ref. 9), suggesting that the Toll receptor could also be involved in *Drosophila* immunity. This idea was further supported by the finding that a number of antimicrobial peptides rapidly induced in response to infection are encoded by genes containing NF-κB-binding sites in their promoters[10]. Indeed, a series of elegant experiments performed by Jules Hoffmann and associates has demonstrated that the block of the entire Toll pathway, starting from Spatzle and going down to Cactus, is involved in the induction of immune responses in *Drosophila*[11]. Even more surprisingly, the loss-of-function mutations in the Toll gene specifically affected only antifungal responses, whereas antibacterial responses remained relatively intact. The susceptibility to fungal infection in Toll mutant flies was found to be owing to a selective failure to induce the expression of the major antifungal peptide drosomycin[11]. These studies convincingly demonstrated the essential role of the Toll pathway in fly immunity. Moreover, at least in flies, Toll-mediated recognition appeared to discriminate between different types of infection. Antimicrobial peptides, a major component of the host defense of flies, mediate their microbicidal effects by interactions with the cell walls of pathogens. Because the structure of the cell wall is quite different between different classes of microorganisms (e.g. Gram-positive versus Gram-negative bacteria), particular peptides are most efficient against a particular class of pathogen. For example, drosomycin has antifungal activity, whereas attacin kills Gram-negative bacteria most effectively[10]. Infection of *Drosophila* with different classes of pathogens – Gram-positive or Gram-negative bacteria, or fungi – leads to the predominant induction of peptides that have activity against the class of pathogen used for infection, such that fungal infection leads to the induction of drosomycin, for example, but not of attacin[12]. This remarkable observation suggests that the immune system of *Drosophila* can discriminate between different classes of pathogenic microorganisms. Moreover, analysis of fly strains that carry mutations in various components of the Toll pathway suggested that the discrimination between pathogen class is mediated by the Toll and Toll-like receptors[12,13]. In a different system, analysis of fly mutants has revealed that the specificity of the target genes (antimicrobial peptides) induced upon infectious challenge is determined, at least in part, by the selective activation of different members of the NF-κB family, Dif versus Dorsal, both of which are downstream components of the Toll pathway[14]. Taken together, these findings suggest that Toll and other members of the *Drosophila* Toll family not only play a major role in pathogen detection, but also distinguish between different types of infection to induce only the appropriate effector responses.

The molecular mechanism of pathogen recognition by members of the Toll family in *Drosophila* is presently unknown. The Toll receptor itself does not appear to recognize fungal pathogens directly; rather, the active form of Spatzle is generated in response to fungal infection, which leads to the activation of Toll[11,15]. This mechanism is similar to that of Toll regulation during development, except that a different set of serine proteases is triggered upon infection[11]. The identity of these proteases is currently unknown, but the protein(s) that functions upstream of the protease cascade is presumably endowed with the capacity of pattern recognition, perhaps similar to the mechanism by which mannan-binding lectin induces the lectin pathway of complement activation in mammals. Whether other members of the *Drosophila* Toll family are regulated in a similar manner is currently unknown.

Toll-like receptors in mammalian immune recognition

Toll proteins in mammalian innate immunity

The innate immune system in mammals and other vertebrates plays two crucial roles. First, it contains the infection prior to the induction of adaptive immune responses, which can take four to five days. Second, the innate immune system controls the activation of adaptive immunity and determines the type of effector responses that are appropriate for the infecting pathogen[1]. Both functions critically depend on the ability of the innate immune system to detect the presence of infectious microorganisms and to induce a set of endogenous signals, such as inflammatory cytokines and chemokines. These signals, in turn, recruit and activate antigen-specific lymphocytes and induce their differentiation into effector cells. Based on studies performed in the past two years it is becoming increasingly clear that a family of Toll-like

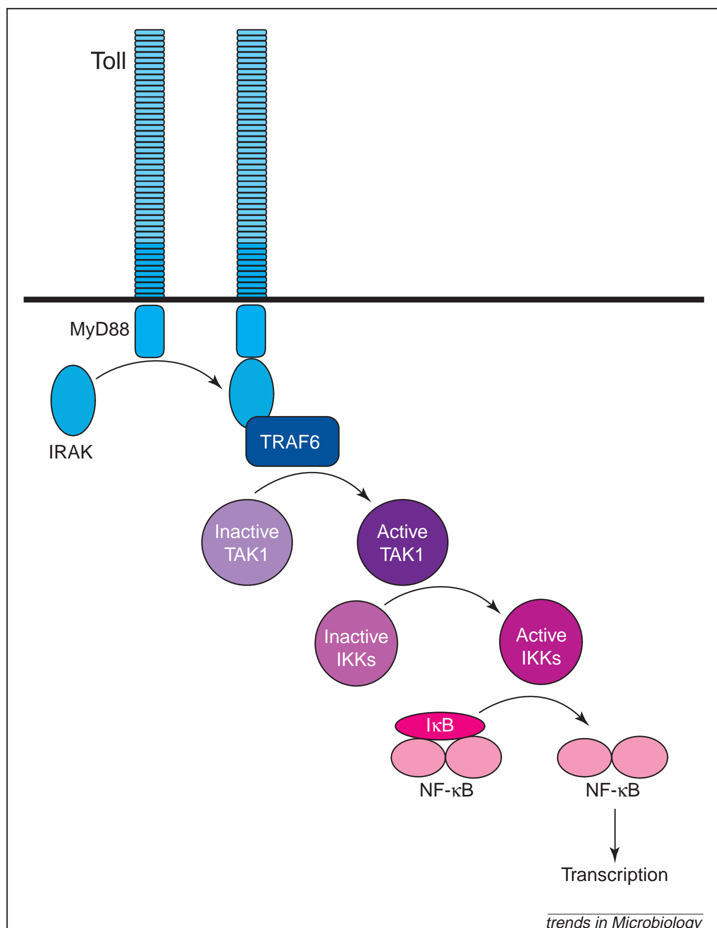

Fig. 1. Toll signal transduction pathway. Ligation of the Toll receptor results in recruitment of the adaptor protein, MyD88. MyD88 consists of an amino-terminal death domain and a carboxy-terminal TIR domain. The TIR domain of MyD88 is recruited through a homophilic interaction with the TIR domain of Toll. The death domain of MyD88 interacts with the death domain of the serine/threonine kinase IRAK. Recruitment of IRAK to the receptor complex results in its autophosphorylation and association with another adaptor protein, TRAF6. TRAF6, in turn, associates with and activates a MAP kinase kinase kinase, TAK-1. Via one or more intermediate steps TAK-1 activation leads to the phosphorylation and activation of the IκB kinases (IKK). IKKs phosphorylate the NF-κB inhibitor, IκB, inducing its degradation and release of NF-κB. Upon release from IκB, NF-κB translocates to the nucleus where it activates transcription of a wide variety of immune and inflammatory genes. Abbreviations: IκB, inhibitor of κB; IL-1R, interleukin 1 receptor; IRAK, IL-1R-associated kinase; MAP, mitogen-activated protein; NF-κB, nuclear factor κB; TIR, Toll/IL-1R.

receptors (TLRs) plays such a crucial role in the induction of adaptive immunity in mammals.

The Toll signaling pathway

The first characterized member of the mammalian Tolls, now referred to as TLR4, has been shown to induce the NF-κB signaling pathway, similar to its *Drosophila* homolog[16]. A constitutively active mutant of *TLR4* was shown to induce the expression of inflammatory cytokines and chemokines. Moreover, the co-stimulatory molecules of the B7 family, which are required for activation of naive T cells by antigen-presenting cells, were also induced by constitutive signaling via TLR4 (Ref. 16). Therefore, mammalian TLRs represent a crucial link between pathogen detection and the induction of adaptive immune responses.

The signaling pathway initiated by human TLR4 is remarkably similar, although presumably not identical, to that of IL-1R (Fig. 1). Both TLR4 and IL-1R recruit an adapter protein, MyD88, upon ligation[17–20]. MyD88 consists of an amino-terminal death domain and a carboxy-terminal TIR domain. MyD88 is associated with a serine/threonine protein kinase IRAK (IL-1R-associated kinase), which contains an amino-terminal death domain[17,19]. A homophilic interaction between MyD88 and IRAK death domains recruits IRAK to the receptor complex and leads to IRAK autophosphorylation. Autophosphorylated IRAK, in turn, forms a complex with another adaptor protein, TRAF6, which results in TRAF6 oligomerization[21]. Although the mechanism of TRAF6 action is not well understood, its oligomerization leads to the activation of TAK-1, a member of the mitogen-activated protein (MAP) 3-kinase family[22]. The events following TAK-1 activation are not fully understood, but the net result is the activation of the IκB kinases IKK1 and IKK2 (Ref. 22). Activated IKKs phosphorylate the IκB protein and induce its degradation through the proteasome pathway, thus releasing NF-κB, which is then free to translocate to the nucleus where it cooperates with other transcription factors to induce the expression of a wide variety of target genes[23]. This pathway parallels, to an extent, that of *Drosophila* Toll, except that a mammalian Tube homolog has not yet been identified, and it appears that mammalian MyD88 is functionally analogous to Tube.

Ligand specificity of mammalian TLRs

The ligands for most mammalian TLRs are currently unknown, although two members of the Toll family, TLR2 and TLR4, have been implicated in recognition of a variety of microbial products. The first indication that human TLRs are involved in PAMP recognition came from *in vitro* studies in which transfection of TLR2 conferred LPS responsiveness to an otherwise unresponsive cell[24,25]. However, subsequent studies revealed that, although in Chinese hamsters *TLR2* is non-functional owing to a frameshift mutation, cells derived from the hamsters are fully responsive to LPS (Ref. 26). The first convincing proof of the involvement of TLRs in microbial recognition came from the analysis of the LPS-unresponsive C3H/HeJ mouse strain, which was shown by positional cloning to harbor a mutation in the gene that encodes mouse TLR4 (Refs 27,28). TLR4-mediated LPS recognition was later found to depend on an additional protein called MD-2, which lacks a transmembrane domain but forms a complex with the ectodomain of TLR4 (Ref. 29). These findings were further confirmed by experimental deletion of *TLR4* in mice, which resulted in a complete loss of LPS responsiveness[30]. Deletion of *TLR2*, alternatively, resulted in the loss of peptidoglycan recognition and responsiveness, with no impairment in LPS responses[31]. *In vitro* studies have also implicated TLR2 in the recognition of a wide variety of other microbial products, including

the components of Gram-positive bacterial cell walls, zymosan and bacterial lipoproteins, as well as mycobacteria and glycolipids derived from mycobacterial cell walls[32–42]. It will be important to confirm these findings using primary cells derived from TLR-knockout mice, as it is highly unlikely that TLR2 mediates recognition of all these structurally unrelated microbial products. So far, using macrophages derived from TLR2-deficient mice, Gram-positive bacteria, peptidoglycan and bacterial lipoproteins have been shown to signal in a TLR2-dependent manner[31,43]. Lipoteichoic acid (LTA), however, was shown to signal through TLR4, similar to LPS (Ref. 31). It should be emphasized that neither *in vitro* transfection studies nor targeted deletion of individual TLRs allows us to assign a specificity of recognition to a single Toll receptor, because it is very likely that at least some TLRs form heterodimers that influence or determine ligand specificity. Nevertheless, based on what has already been established, one can draw some significant conclusions. First, it appears that, unlike *Drosophila* Tolls, which seem to discriminate between different classes of pathogens, members of the mammalian Toll family can be involved in recognition of PAMPs derived from diverse classes of microorganisms. TLR4, for example, mediates recognition of both LPS and LTA, components of Gram-negative and Gram-positive bacterial cell walls, respectively. Second, a given TLR can be involved in recognition of structurally unrelated stimuli. For example, TLR2 is required for recognition of the chemically distinct peptidoglycan and lipoproteins. This probably reflects the possibility that TLR2 can form heterodimers with other TLRs, the combination of which confers a particular specificity.

TLRs and phagocytosis

Another interesting aspect of TLR function was uncovered when the cellular localization of TLR2 was monitored following macrophage stimulation with zymosan, a presumed TLR2 ligand. In these studies, TLR2 was shown to localize first to phagocytic cups and subsequently to phagosomes upon phagocytosis of zymosan[44]. This striking observation suggests that macrophages use TLRs to determine the content of phagosomes. In addition to phagocytosis of microorganisms, macrophages also play a major role in phagocytosis and clearance of apoptotic cells. However, unlike bacteria and zymosan, apoptotic cells do not induce an inflammatory reaction when phagocytosed by macrophages. Depending on the nature of the phagocytosed material, TLRs can be differentially recruited into phagosomes. It will be important to investigate whether other TLRs are similarly recruited to the phagosomes, and whether the recruitment is dependent on the TLR specificities.

Conclusions and perspectives

Although the characterization of mammalian TLR functions is in its very early stages, it is already becoming clear that this receptor family plays a central role in innate immune recognition. The mechanism of TLR-mediated recognition of microbial products is currently unknown. Genetic complementation studies suggest that, at least in the case of TLR4, recognition of LPS might involve direct binding between TLR4 and LPS (Refs 45,46). It is important to determine whether MD-2 associates, in addition to TLR4, with other TLRs, and whether TLRs can form heterodimers that dictate ligand specificities. It is assumed that, in addition to TLR2 and TLR4, other TLRs are involved in microbial recognition, and research in the near future will undoubtedly uncover the specificities of all TLRs for their corresponding microbial products. It would be interesting to correlate TLR specificities with their expression profiles as this information could shed some light on the logic of innate immune recognition.

Once the specificities of all the TLRs for their microbial ligands are established, we will face a new set of questions. First, how does the immune system distinguish between pathogenic and commensal microorganisms? The answer to this question is not at all obvious at the moment, as TLR ligands (PAMPs) appear to be shared by all microorganisms, pathogenic or otherwise. Second, how is the recognition of PAMPs by TLRs translated into the induction of appropriate effector responses? The effector responses of the adaptive immune system are determined by the nature of the infecting microorganisms. For example, intracellular pathogens induce so-called T helper type 1 (Th1) effector responses, whereas extracellular pathogens usually induce Th2 responses. PAMPs recognized by TLRs can be found in either category of pathogens and therefore recognition of PAMPs by TLRs, by itself cannot distinguish between the different types of infection. The third, related question is do different TLRs activate identical signaling pathways and cellular responses? This does not seem to be the case in *Drosophila*, and it is important, therefore, to decipher the logic behind the signaling networks that translates the detection of pathogens into a coordinated set of defense responses. There are obviously many more questions that will need to be addressed in the future. It is safe to predict that functional characterization of the Toll receptor family will bring our understanding of host–pathogen interactions to a new level.

Questions for future research

- What are the specificities of different TLRs?
- What is the mechanism of TLR-mediated recognition? Do TLRs recognize microbial products directly, or is recognition mediated by an endogenous ligand?
- Can TLRs distinguish between intracellular and extracellular pathogens?
- Can TLRs distinguish between pathogenic and commensal microorganisms? What is the mechanism?
- How is TLR-mediated recognition translated into effector responses of the host immune system?
- Did pathogenic microorganisms evolve strategies to avoid recognition by TLRs?

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References

1 Janeway, C.A., Jr (1989) Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harbor Symp. Quant. Biol.* 54, 1–13
2 Hoffmann, J.A. *et al.* (1999) Phylogenetic perspectives in innate immunity. *Science* 284, 1313–1318
3 Fearon, D.T. *et al.* (1996) The instructive role of innate immunity in the acquired immune response. *Science* 272, 50–53
4 Medzhitov, R. and Janeway, C.A., Jr (1997) Innate immunity: impact on the adaptive immune response. *Curr. Opin. Immunol.* 9, 4–9
5 Epstein, J. *et al.* (1996) The collectins in innate immunity. *Curr. Opin. Immunol.* 8, 29–35
6 Stahl, P.D. and Ezekowitz, R.A. (1998) The mannose receptor is a pattern recognition receptor involved in host defense. *Curr. Opin. Immunol.* 10, 50–55
7 Hashimoto, C. *et al.* (1988) The Toll gene of *Drosophila*, required for dorsal–ventral embryonic polarity, appears to encode a transmembrane protein. *Cell* 52, 269–279
8 Gay, N.J. and Keith, F.J. (1991) *Drosophila* Toll and IL-1 receptor. *Nature* 351, 355–356
9 Belvin, M.P. and Anderson, K.V. (1996) A conserved signaling pathway: the *Drosophila* toll-dorsal pathway. *Annu. Rev. Cell Dev. Biol.* 12, 393–416
10 Meister, M. *et al.* (1997) Antimicrobial peptide defense in *Drosophila. Bioessays* 19, 1019–1026
11 Lemaitre, B. *et al.* (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. *Cell* 86, 973–983
12 Lemaitre, B. *et al.* (1997) *Drosophila* host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proc. Natl. Acad. Sci. U. S. A.* 94, 14614–14619
13 Williams, M.J. *et al.* (1997) The 18-wheeler mutation reveals complex antibacterial gene regulation in *Drosophila* host defense. *EMBO J.* 16, 6120–6130
14 Wu, L.P. and Anderson, K.V. (1998) Regulated nuclear import of Rel proteins in the *Drosophila* immune response. *Nature* 392, 93–97
15 Levashina, E.A. *et al.* (1999) Constitutive activation of toll-mediated antifungal defense in serpin-deficient *Drosophila*. *Science* 285, 1917–1919
16 Medzhitov, R. *et al.* (1997) A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388, 394–397
17 Muzio, M. *et al.* (1997) IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science* 278, 1612–1615
18 Muzio, M. *et al.* (1998) The human toll signaling pathway: divergence of nuclear factor kappaB and JNK/SAPK activation upstream of tumor necrosis factor receptor-associated factor 6 (TRAF6). *J. Exp. Med.* 187, 2097–2101
19 Wesche, H. *et al.* (1997) MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* 7, 837–847
20 Medzhitov, R. *et al.* (1998) MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol. Cell* 2, 253–258
21 Cao, Z. *et al.* (1996) TRAF6 is a signal transducer for interleukin-1. *Nature* 383, 443–446
22 Ninomiya-Tsuji, J. *et al.* (1999) The kinase TAK1 can activate the NIK-IκB as well as the MAP kinase cascade in the IL-1 signalling pathway. *Nature* 398, 252–256
23 Karin, M. and Delhase, M. (2000) The IκB kinase (IKK) and NF-κB: key elements of proinflammatory signalling. *Semin. Immunol.* 12, 85–98
24 Yang, R.B. *et al.* (1998) Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. *Nature* 395, 284–288
25 Kirschning, C.J. *et al.* (1998) Human toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide. *J. Exp. Med.* 188, 2091–2097
26 Heine, H. *et al.* (1999) Cutting edge: cells that carry a null allele for toll-like receptor 2 are capable of responding to endotoxin. *J. Immunol.* 162, 6971–6975
27 Poltorak, A. *et al.* (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282, 2085–2088
28 Qureshi, S.T. *et al.* (1999) Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *J. Exp. Med.* 189, 615–625
29 Shimazu, R. *et al.* (1999) MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J. Exp. Med.* 189, 1777–1782
30 Hoshino, K. *et al.* (1999) Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the LPS gene product. *J. Immunol.* 162, 3749–3752
31 Takeuchi, O. *et al.* (1999) Differential roles of TLR2 and TLR4 in recognition of Gram-negative and Gram-positive bacterial cell wall components. *Immunity* 11, 443–451
32 Underhill, D.M. *et al.* (1999) Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc. Natl. Acad. Sci. U. S. A.* 96, 14459–14463
33 Schwandner, R. *et al.* (1999) Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J. Biol. Chem.* 274, 17406–17409
34 Sellati, T.J. *et al.* (1999) Activation of human monocytic cells by *Borrelia burgdorferi* and *Treponema pallidum* is facilitated by CD14 and correlates with surface exposure of spirochetal lipoproteins. *J. Immunol.* 163, 2049–2056
35 Aliprantis, A.O. *et al.* (1999) Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science* 285, 736–739
36 Brightbill, H.D. *et al.* (1999) Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* 285, 732–736
37 Flo, T.H. *et al.* (2000) Human toll-like receptor 2 mediates monocyte activation by *Listeria monocytogenes*, but not by group B streptococci or lipopolysaccharide. *J. Immunol.* 164, 2064–2069
38 Hirschfeld, M. *et al.* (1999) Cutting edge: inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by toll-like receptor 2. *J. Immunol.* 163, 2382–2386
39 Lien, E. *et al.* (1999) Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J. Biol. Chem.* 274, 33419–33425
40 Yoshimura, A. *et al.* (1999) Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. *J. Immunol.* 163, 1–5
41 Means, T.K. *et al.* (1999) Human toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. *J. Immunol.* 163, 3920–3927
42 Means, T.K. *et al.* (1999) The CD14 ligands lipoarabinomannan and lipopolysaccharide differ in their requirement for Toll-like receptors. *J. Immunol.* 163, 6748–6755
43 Takeuchi, O. *et al.* (2000) Cutting edge: preferentially the R-stereoisomer of the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 activates immune cells through a toll-like receptor 2- and MyD88-dependent signaling pathway. *J. Immunol.* 164, 554–557
44 Underhill, D.M. *et al.* (1999) The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* 401, 811–815
45 Lien, E. *et al.* (2000) Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J. Clin. Invest.* 105, 497–504
46 Poltorak, A. *et al.* (2000) Physical contact between lipopolysaccharide and toll-like receptor 4 revealed by genetic complementation. *Proc. Natl. Acad. Sci. U. S. A.* 97, 2163–2167