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Microreview

Toll receptors and pathogen resistance

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Summary

Toll receptors in insects, mammals and plants are key players that sense the invasion of pathogens. Toll-like receptors (TLRs) in mammals have been established to detect specific components of bacterial and fungal pathogens. Furthermore, recent evidence indicates that TLRs are involved in the recognition of viral invasion. Signalling pathways via TLRs originate from the conserved Toll/IL-1 receptor (TIR) domain. The TIR domain-containing MyD88 acts as a common adaptor that induces inflammatory cytokines; however, there exists a MyD88-independent pathway that induces type I IFNs in TLR4 and TLR3 signalling. Another TIR domain-containing adaptor, TIRAP/Mal has recently been shown to mediate the MyD88-dependent activation in the TLR4 and TLR2 signalling pathway. Thus, individual TLRs may have their own signalling systems that characterize their specific activities.

Introduction

Host defence against pathogens in mammals depends on two types of immunity, innate and adaptive (acquired) immunity (Fearon and Locksley, 1996). Adaptive immunity is a system found only in vertebrates, in which antigen receptors expressed on the surface of B and T lymphocytes recognize foreign antigens. In order to cope with a variety of antigens, B and T cells rearrange genes for immunoglobulin and the T cell receptor, to produce various types of antigen receptors. Lymphocytes bearing receptors that have suitable affinity to a specific antigen show clonal expansion when stimulated with the antigen. Thus, adaptive immunity is a highly sophisticated system to combat microorganisms; however, it requires several steps before the efficient activation, as the recognition of antigens by lymphocytes is preceded by the processing of antigens by antigen-presenting cells and it takes a few days for lymphocytes to clonally expand. Therefore, the host organism has a system to combat the invading pathogens before adaptive immunity is activated. Indeed, invertebrates exhibit efficient host defence by virtue of innate immunity even though they have no adaptive immune system.

Innate immunity, which is phylogenetically conserved and present in almost all multicellular organisms, has been shown to detect invading pathogens and exhibit a first line of host defence (Hoffmann *et al*., 1999). Several lines of evidence demonstrate that Toll receptors play an essential role in the detection of invading pathogens in fruit flies and mammals, and even in plants. In this review, we will describe recent advances in our understanding of the functions of Toll receptors and their signalling pathways.

Toll-like receptors in mammals

A mammalian Toll receptor (now termed TLR4) was shown to induce the expression of genes involved in inflammatory responses (Medzhitov *et al*., 1997). Furthermore, mutation in the *Tlr4* gene was identified in mouse strains that are hyporesponsive to lipopolysaccharide (Poltorak *et al*., 1998). Since then, Toll receptors in mammals have become an object of intensive studies in the immunology field. First, several proteins that have structural similarity to TLR4 were identified and named Toll-like receptors (TLRs) (Rock *et al*., 1998). The TLR family now consists of 10 members (TLR1-TLR10). The cytoplasmic portion of Toll-like receptors shows high similarity to that of the IL-1 receptor family, and is now called the Toll/IL-1 receptor (TIR) domain. In spite of this similarity, the extracellular portions of both receptors are structurally unrelated. The IL-1 receptors possess an Ig-like domain, whereas Toll-like receptors bear leucine-rich repeats (LRRs) in the extracellular domain. Toll-like receptors have been revealed to play essential roles in the recognition of patho-

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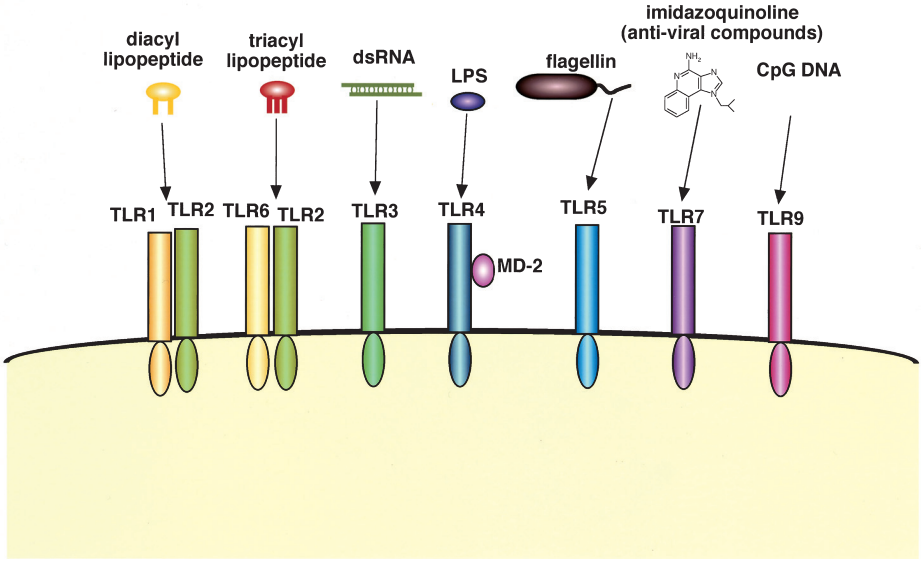

Fig. 1. Host defence in *Drosophila.* In *Drosophila*, the Toll and Imd pathways confer host defence against pathogens. The Toll pathway regulates production of antimicrobial peptides against fungi and Gram-positive bacteria. PGRP-SA is essential for activation of the Toll pathway in response to Gram-positive bacteria. Persephone is involved in the activation of the Toll pathway in response to fungi. PGRP-LC recognizes the invasion of Gram-negative bacteria and is required for activation of the Imd pathway, which is essential for anti-Gram-negative bacterial responses.

gens. Each TLR recognizes specific components of pathogens (Fig. 1).

TLR1, TLR2 and TLR6

TLR2 is involved in the recognition of components from a variety of pathogens. These include lipoproteins from a variety of pathogens, lipoteichoic acid from Gram-positive bacteria, lipoarabinomannan from mycobacteria, glycosylphophatidylinositol anchors from *Trypanosoma Cruzi,* a phenol-soluble modulin from *Staphylococcus epidermis*, zymosan from fungi and glycolipids from *Treponema maltophilum* (Akira *et al*., 2001; Medzhitov 2001). An essential role for TLR2 in the recognition of peptidoglycan and lipoproteins has been shown in TLR2-deficient mice (Takeuchi *et al*., 1999; 2000a). In addition, TLR2 presumably recognizes atypical LPS from *Leptospira interrogans* or *Porphyromonas gingivalis*, both of which differ from the typical LPS of Gram-negative bacteria in several biochemical and physical properties (Hirschfeld *et al*., 2001; Werts *et al*., 2001).

The mechanism by which TLR2 recognizes a variety of microbial components is partly explained by the cooperation of TLR2 with TLR1 and TLR6. The introduction of a dominant negative form of TLR6 into macrophage cell lines inhibited TNF-α production in response to peptidoglycan, but not to bacterial lipopeptides, both of which are recognized by TLR2 (Ozinsky *et al*., 2000). Analyses of TLR6- and TLR1-deficient mice have revealed their important roles in the discrimination of subtle structural differences between lipopeptides. Macrophages from TLR6-deficient mice did not show any production of inflammatory cytokines in response to mycoplasma-derived diacyl lipopeptides. However, these cells showed normal production of inflammatory cytokines in response to triacyl lipopeptides derived from Gram-negative bacteria (Takeuchi *et al*., 2001). In contrast, macrophages from TLR1-deficient mice showed a normal response to mycoplasma-derived diacyl lipopeptides, but an impaired response to triacyl lipopeptides and lipoproteins from mycobacteria. The response to triacyl lipopeptides with an *N*-palmytoyl-*S*-dilauryl cysteine residue was mostly impaired in TLR1-deficient mice (Takeuchi *et al*., 2002). Thus, TLR1 and TLR6 functionally associate with TLR2 and participate in the discrimination of diacyl or triacyl lipopetides. The involvement of TLR1 in the recognition of the outer surface lipoprotein of *Borrelia burgdorferi* was also shown (Alexopoulou *et al*., 2002).

TLR3

The involvement of TLR3 in the recognition of double-stranded RNA (dsRNA) has been demonstrated (Alexopoulou *et al*., 2001). Double-stranded RNA is produced by most viruses during their replication. Double-stranded RNA is known to induce the synthesis of type I interferons (IFN-α/β), which exert antiviral and immunostimulatory activities. Synthetic dsRNA, polyinosinic-polycytidylic acid (poly(I:C)), has a similar immunostimulatory activity to dsRNA. TLR3-deficient mice

showed an impaired response to dsRNA and poly(I:C). Thus, TLR3 is implicated in the recognition of dsRNA.

TLR4

TLR4 is an essential receptor that transduces the signals of lipopolysaccharide (LPS), as demonstrated by a positional cloning of the gene responsible for hyporesponsive to LPS in the C3H/HeJ mouse strain (Poltorak *et al*., 1998; Qureshi *et al*., 1999). The discovery that TLR4 is essential for LPS signalling was confirmed in TLR4 knockout mice (Hoshino *et al*., 1999).

The recognition of LPS by TLR4 requires several additional molecules. Lipopolysaccharide binds to LPS-binding protein (LBP) in serum and the LPS-LBP complex is associated with CD14. CD14 is a glycosylphosphatidylinositol (GPI)-anchored molecule preferentially expressed in monocytes/macrophages and neutrophils. Mice lacking CD14 and LBP show impaired responses to LPS, indicating the important roles of CD14 and LBP in the recognition of LPS (Haziot *et al*., 1996; Jack *et al*., 1997; Wurfel *et al*., 1997). Another molecule is MD-2, a secreted protein that associates with the extracellular portion of TLR4. MD-2 has been shown to enhance the responsiveness to LPS (Shimazu *et al*., 1999). A Chinese hamster ovary (CHO) cell line that is hyporesponsive to LPS has been shown to have a missense mutation in the MD-2 gene (Schromm *et al*., 2001). MD-2-deficient mice showed a severe defect in the responsiveness to LPS (Nagai *et al*., 2002a). Thus, MD-2 is a critical component of the LPS receptor complex. An additional component is involved in the recognition of LPS in B cells. Miyake and colleagues identified RP105 which bears leucine-rich repeats that are structurally related to TLRs in the extracellular portion. RP105 is preferentially expressed in B cells. B cells from RP105-deficient mice showed a severely reduced response to LPS (Ogata *et al*., 2000). A functional association between TLR4 and RP105 was also shown. Thus, RP105 is involved in the recognition of LPS together with TLR4 in B cells. Similar to TLR4, RP105 associates with MD-1 which shows high similarity with MD-2. MD-1-deficient mice showed a defective B cell response to LPS with reduced expression of RP105, indicating that MD-1 is a critical component for the expression of RP105 (Nagai *et al*., 2002b). Thus, several components are implicated in the recognition of LPS, indicating that the functional LPS receptor forms a large complex.

TLR4 is implicated in the recognition of several ligands in addition to LPS. Taxol, a diterpene purified from the bark of the Western yew (*Taxus brevifolia*) that is now used as an anti-tumour agent in the clinic, has been shown to exhibit LPS-like activities in mice, although the structure of Taxol is quite different to that of LPS. TLR4 mediates the LPS-mimetic activity of Taxol (Kawasaki *et al*., 2000; Byrd-Leifer *et al*., 2001). Heat shock protein 60 (HSP60), which is induced by several stressful conditions such as heat shock, ultraviolet radiation, viral or bacterial infection, is an evolutionary conserved molecular chaperone. HSP60 activates the immune cells by acting as 'a danger signal' (Gallucci and Matzinger, 2001). The immuno-stimulatory activity of HSP60 has been shown to be elicited through the recognition by TLR4 (Ohashi *et al*., 2000; Vabulas *et al*., 2001). In addition to HSP60, TLR4 seemingly recognizes HSP70, as mice defective for TLR4 showed impaired production of inflammatory cytokines in response to HSP70 as well as HSP60 (Asea *et al*., 2002; Dybdahl *et al*., 2002; Vabulas *et al*., 2002). Extracellular matrix (ECM) components are produced in response to tissue injury and modulate inflammation-induced tissue remodelling. Components of the ECM such as the extra domain A (EDA) of fibronectins, oligosaccharides of hyaluronic acid, and heparan sulphate have been shown to activate the immune cells via TLR4 (Okamura *et al*., 2001; Johnson *et al*., 2002; Termeer *et al*., 2002). Injury, infection and autoimmunity are often associated with extravascular deposition of fibrinogen and its derivative fibrin. The capacity of fibrinogen to induce the production of chemokines from macrophages has been shown to be elicited through the recognition by TLR4 (Smiley *et al*., 2001). Thus, TLR4 is seemingly implicated in the recognition of several endogenous ligands involved in the inflammatory response.

TLR5

CHO cells, when expressing human TLR5, responded to the culture supernatants of *Listeria monocytogenes*. Purification of the culture supernatants with TLR5-activating ability led to the isolation of flagellin (Hayashi *et al*., 2001). Flagellin is a monomeric constituent of bacterial flagella, a polymeric rod-like appendage extending from the outer membrane of bacteria. Flagellated bacteria, but not non-flagellated bacteria, activated TLR5, indicating that flagellin is a specific ligand for TLR5. Exposure of the basolateral, but not apical, surface of intestinal epithelia to flagellin induces an inflammatory response. Furthermore, TLR5 is expressed exclusively on the basolateral side of the intestinal epithelial cells (Gewirtz *et al*., 2001). This may correlate with the fact that pathogenic Gram-negative bacteria, but not commensal bacteria, translocate flagellin across epithelia, and induce inflammatory responses.

TLR7

TLR7 has recently been demonstrated to be involved in the immune response to synthetic compounds that are approved for treatment of diseases associated with viral

infection (Hemmi *et al*., 2002). Imidazoquinolines were first identified as compounds that have an antiviral activity in guinea pigs infected with herpes simplex virus and are now used in the clinical treatment of genital warts caused by an infection of human papillomavirus. They induce the production of inflammatory cytokines, especially IFN-α. It is TLR7 that is responsible for the imidazoquinoline-induced immune responses. TLR7-deficient mice did not show any response to the imidazoquinolines (Hemmi *et al*., 2002). This study emphasizes that synthetic compounds that activate the TLR family can be utilized for the treatment of infectious diseases.

TLR9

An essential role for TLR9 in the recognition of bacterial CpG DNA, a potent activator of immune cells, has been shown. TLR9-deficient mice did not show any response to CpG DNA (Hemmi *et al*., 2000). Bacterial DNA contains unmethylated CpG motifs, which confer the immunostimulatory activity. In vertebrates, the frequency of CpG motifs is severely suppressed and the cytosine residues of the CpG motifs are highly methylated, which leads to abrogation of the immunostimulatory activity. Synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs also activate immune cells and elicit Th1-like immune responses. Therefore, CpG DNA is now expected to be useful as an adjuvant for a variety of vaccines against infectious diseases, cancer and allergy.

CpG DNA has been shown to be recognized in the endosome after non-specific uptake into the cells (Wagner, 1999). Flow cytometric analysis using monoclonal antibodies against TLR1, TLR2 or TLR4 clearly demonstrated that these TLRs are expressed on the cell surface. In contrast, TLR9 has recently been shown to be localized in the endosomal/vacuolar/vesicular compartment, but not at the cell surface (Ahmad-Nejad *et al*., 2002). This indicates that CpG DNA is not recognized by the same mechanism as in the case of other TLR ligands.

Toll-like receptors in viral recognition

Several lines of evidence have indicated that TLRs are involved in the recognition of virus as well as bacteria. First, Two types of vaccinia viruses have been shown to share amino acid similarity with the cytoplasmic region of TLRs, which causes the interference of TLR-mediated intracellular signalling (Bowie *et al*., 2000). TLR4 and CD14 have been shown to recognize the fusion protein of respiratory syncytical virus (RSV) (Kurt-Jones *et al*., 2000). TLR4-mutated C3H/HeJ and C57BL/10ScCr mice showed a reduced inflammatory response to RSV infection, and accordingly did not clear RSV as effectively as normal mice (Haynes *et al*., 2001). Activation of B cells by murine retroviruses such as mouse mammary tumour virus (MMTV) depends on TLR4. Indeed, the envelope proteins of MMTV and Moloney murine leukaemia virus have been shown to associate with TLR4 (Rassa *et al*., 2002).

TLR3 is involved in the recognition of dsRNA, a representative of viral immunostimulating components, as described above. Thus, TLR3 and TLR4 are presumably involved in the viral recognition. Intriguingly, both TLR3 and TLR4 utilize a unique signalling pathway that is independent of a common adaptor MyD88 (see below for details). TLR3 and TLR4-dependent activation of a unique pathway in response to virus recognition may confer the viral resistance. Furthermore, chemical compounds, which TLR7 recognizes, are used in the treatment of viral infection. Therefore, TLR7 may also be involved in the viral recognition. Elucidation of a natural ligand for TLR7 will shed new light on the role of TLRs in viral recognition.

Toll-like receptors in infectious models

Involvement of TLRs in the resistance to several infectious models has been delineated. TLR4 mutant mice have been shown to be highly sensitive to infection with the Gram-negative bacteria, *Salmonella typhimurium*, as a result of the defective LPS recognition (O'Brien *et al*., 1980). In contrast, TLR2-deficient mice have been shown to be sensitive to infection with the Gram-positive bacteria, *Staphylococcus aureus*, which correlates with the defective recognition of peptidoglycan that is abundantly present in cell walls of Gram-positive bacteria (Takeuchi *et al*., 2000b). In addition, TLR2-deficient mice have been shown to be susceptible to meningitis induced by the Gram-positive bacteria, *Streptococcus pneumoniae* (Echchannaoui *et al*., 2002). Thus, TLR2 and TLR4 are required for the resistance to infection with Gram-positive and Gram-negative bacteria respectively. TLR2- and TLR4-deficient mice are further used for experiments of some infectious models. TLR2-deficient mice showed no inflammatory response to lipoproteins from the spirochetes, *Borrelia burgdorferi,* and accordingly harboured more spirochetes in tissues after infection (Wooten *et al*., 2002). Recent studies indicated that TLR2 and TLR4 are involved in controlling *Mycobacterium tuberculosis* infection (Abel *et al*., 2002; Reiling *et al*., 2002). It now becomes apparent that TLRs are involved in regulating activation of adaptive immunity, which evokes an antigen-specific immune responses against pathogens (Schnare *et al*., 2001). More precise investigation on the involvement of TLRs in several infections will be performed using several TLR-deficient mice in the future.

Toll receptors in *Drosophila*

Drosophila, although they have no adaptive immunity, show an effective host defence against invasion by pathogens through the synthesis of antimicrobial peptides (Hoffman *et al*., 1999). The production of antifungal peptides has been shown to be induced through activation of the signalling pathways via Toll, which was originally identified as a receptor responsible for dorso-ventral patterning in the developing embryo (Lemaitre *et al*., 1996) (Fig. 2). The Toll pathway has also been shown to be involved in the anti-Gram-positive bacterial response (Rutschmann *et al*., 2002). A secreted factor pro-Spätzle is activated through cleavage by a so far unidentified serine protease in response to invasion by fungi and Gram-positive bacteria. The stimulation of Toll by Spätzle activates the cytoplasmic serine/threonine kinase Pelle via the adaptor protein DmMyD88 and Tube. Activation of Pelle promotes degradation of the ankyrin-repeat protein Cactus, which associates with the Rel-type transcription factor Dorsal and DIF in the cytoplasm. Once Cactus is degraded in response to the Toll-mediated signal, Dorsal and DIF translocate into the nucleus, where they induce the transcription of specific target genes (Hoffmann and Reichhart, 2002). Molecules that act upstream of Toll have been revealed. In Gram-positive bacterial infections, a member of the family of petidoglycan recognition proteins, PGRP-SA, has been shown to be essential for activation of Toll (Michel *et al*., 2001). PGRP-SA contains a domain that is required for recognition of peptidoglycan, abundantly present in cell walls of Gram-positive bacteria. Therefore, PGRP-SA may directly recognize an invasion of Gram-positive bacteria. In fungal infections, a serine protease that is encoded by the *Persephone* gene, has been shown to be required for activation of Toll (Ligoxygakis *et al*., 2002). As the *Persephone* gene product has no obvious pathogen recognition motif, an unknown molecule that is responsible for the recognition of fungi acts upstream of this gene product.

Host defence against Gram-negative bacteria has been shown to be mediated by a distinct pathway (Fig. 2). *Imd* mutant flies were highly susceptible to infection by Gram-negative bacteria, but not to fungi (Lemaitre *et al*., 1995). The *imd* gene encodes an adaptor protein containing a death domain that shows similarity to the mammalian receptor interacting protein (RIP) (Georgel *et al*., 2001). In the Imd-mediated pathway, DmIKKβ, DmIKKγ, dTAK1 and Relish have been shown to play important roles in

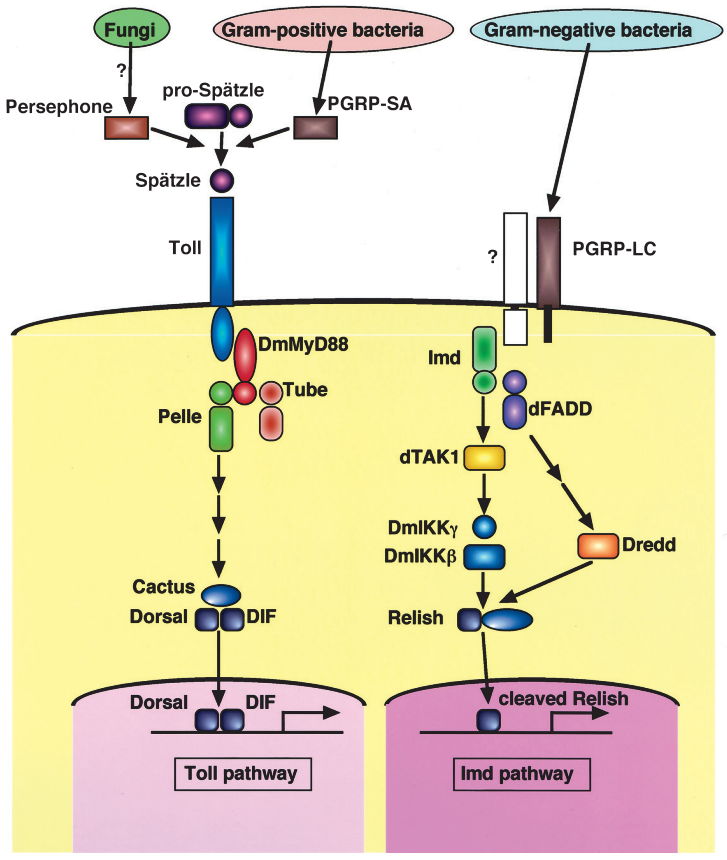

Fig. 2. Toll-like receptors and their ligands. Among the 10 known mammalian TLR family members, TLR1, 2, 3, 4, 5, 6, 7 and 9 have been implicated in the recognition of microbial components. TLR2 is responsible for the recognition of microbial lipopeptides. TLR1 and TLR6 associate with TLR2, and discriminate subtle differences between triacyl and diacyl lipopeptides respectively. TLR4 recognizes LPS. TLR9 is a receptor for CpG DNA, whereas TLR3 is implicated in the recognition of viral dsRNA. TLR5 is a receptor for flagellin. Thus, the TLR family discriminates between specific patterns of bacterial components.

response to Gram-negative bacteria (reviewed in Hoffmann and Reichhart, 2002). A member of the PGRP family, PGRP-LC, has recently been shown to be involved in the activation of the Imd pathway (Choe *et al*., 2002; Gottar *et al*., 2002; Ramet *et al*., 2002). Unlike PGRP-SA, PGRP-LC has a transmembrane portion. Therefore, PGRP-LC may act as a receptor that recognizes Gram-negative bacteria and activates the Imd pathway. Relish, comprising an N-terminal Rel homology domain and a C-terminal inhibitory ankyrin repeat domain, is activated by a cleavage. Dredd, a homologue of mammalian caspase-8, has been shown to be involved in this process (Hoffmann and Reichhart, 2002). Dredd has also been shown to associate with dFADD, a homologue of mammalian FADD (Hu *et al*., 2000). In mammals, the FADD/caspase8-dependent pathway induces apoptosis in the type I TNF receptor (TNF-RI)-mediated signalling. Overexpression of Imd, a homologue of mammalian RIP that associates with TNF-RI, induces apoptosis in *Drosophila*, and *imd* mutant flies are resistant to UV-induced apoptosis (Georgel *et al*., 2001). Thus, analogous to the mammalian TNF-RI-mediated signal pathway, the Imd pathway is presumably involved in the induction of apoptosis as well as the response against Gram-negative bacteria.

Toll-related proteins in plants

There are more than 100 genes that encode proteins containing the TIR domain in plants (Jebanathirajah *et al*., 2002). Most TIR domain-containing proteins observed in plants possess LRRs and a nucleotide binding site (NBS), but not a transmembrane domain. Among the TIR-NBS-LRR family of proteins, the tobacco *N* gene product was first shown to confer resistance to the tobacco mosaic virus in Tomato (Whitham *et al*., 1994). Subsequently, several TIR-NBS-LRR proteins have been shown to be responsible for the pathogen resistance. These include products encoded by the flax *L6* and *M* genes (resistance to *Melampsora lini*), the *Arabidopsis RPP5* and *RPP1* genes (resistance to *Peronospora parasitica*), and the *RPS4* gene (resistance to bacteria) (Baker *et al*., 1997; Jebanathirajah *et al*., 2002). In addition to the TIR-NBS-LRR proteins, the *FLS2* gene encoding a transmembrane receptor-like kinase with LRRs has been shown to be essential for the recognition of flagellin in *Arabidopsis* (Gomez-Gomez and Boller, 2000). Thus, several Toll-related proteins are implicated in the resistance to pathogens in plants.

Signalling pathways of Toll-like receptors

Signalling pathways via TLR in mammals have similar features as those via Toll in *Drosophila*. Host defences to pathogens in mammals, and to fungi and Gram-positive bacteria in *Drosophila* mainly rely on pathways that originate from the TIR domain of TLRs and Toll respectively. In mammals, TLR interacts with MyD88, which has the TIR domain in its C-terminal portion, via interaction between the respective TIR domains. Upon stimulation, MyD88 recruits a death domain-containing serine/threonine kinase, the IL-1 receptor associated kinase (IRAK). IRAK is activated by phosphorylation and then associates with TRAF6, leading to the activation of two distinct signalling pathways, JNK and NF-κB. In *Drosophila*, DmMyD88, Pelle and Dorsal correspond to mammalian MyD88, IRAK and NF-κB respectively. Recent studies revealed that there exists a MyD88-independent pathway in the TLR4 and TLR3 signalling in addition to a MyD88-dependent pathway that is common to all the TLR family (Fig. 3).

MyD88-dependent signalling pathway

In the TLR-mediated signalling pathways, MyD88, IRAKs, and TRAF6 play critical roles, as demonstrated by analysis in gene targeted mice. MyD88-deficient mice showed no production of inflammatory cytokines in response to LPS, peptidoglycan, lipoproteins, CpG DNA, dsRNA, flagellin or the imidazoquinolines (Akira *et al*., 2001). These results indicate that MyD88 is an essential adaptor in the TLR-mediated signalling that leads to production of inflammatory cytokines. There are four IRAK family members: IRAK-1, IRAK-2, IRAK-M and IRAK-4 (Li *et al*., 2002). IRAK-1-deficient mice showed a partially impaired response to LPS (Swantek *et al*., 2000). In contrast, IRAK-4-deficient mice showed almost no inflammatory responses to LPS, peptidoglycan, dsRNA and CpG DNA (Suzuki *et al*., 2002). Among the IRAKs, IRAK-4 is most structurally related to *Drosophila* Pelle. These findings indicate that IRAK-4 is an essential component in the TLR-mediated signalling pathways. Unlike IRAK-1 and IRAK-4 that are ubiquitously expressed, IRAK-M is exclusively expressed in monocyte/macrophages and has no kinase activity (Wesche *et al*., 1999). IRAK-M has also a unique physiological property acting as a negative regulator in the TLR-mediated signalling, as IRAK-M-deficient mice showed increased production of inflammatory cytokine in response to TLR ligands (Kobayashi *et al*., 2002a). TRAF6-deficient mice exhibited an impaired response to LPS, indicating that TRAF6 is a critical component of the TLR4-mediated signalling pathway (Lomaga *et al*., 1999; Naito *et al*., 1999).

MyD88-independent signalling pathway

As described above, MyD88 is essential for the production of inflammatory cytokines in response to a variety of microbial components. Indeed, activation of NF-κB or JNK

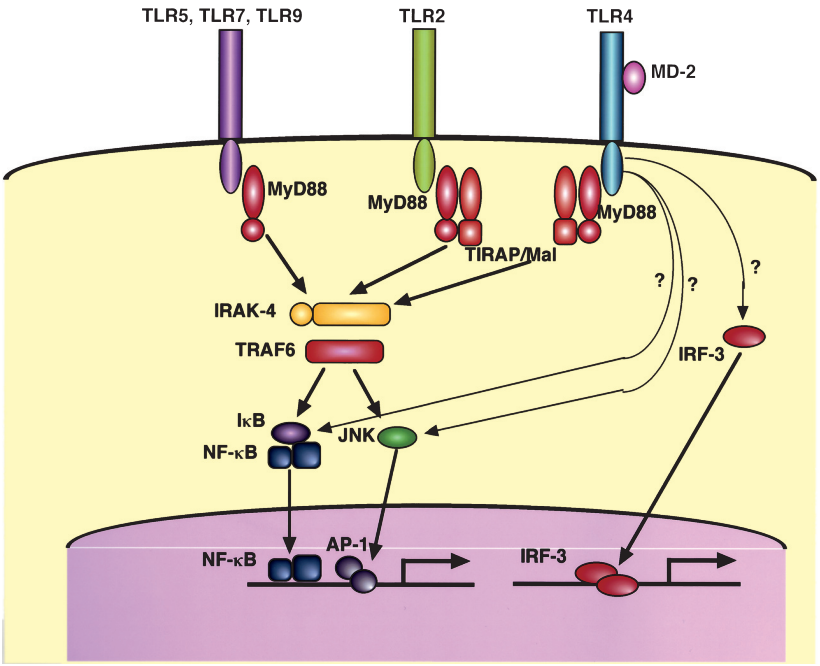

Fig. 3. TLR signalling pathways. MyD88 is an essential adaptor for the induction of inflammatory cytokines via all TLRs. In the case of TLR4 signalling, LPS-induced activation of IRF-3, JNK and NF-κB has been reported, indicating the presence of the MyD88-independent pathway. TIRAP/Mal acts as a second adaptor together with MyD88 in TLR2- and TLR4-mediated signalling.

was not induced in MyD88-deficient macrophages in response to peptidoglycan, lipoprotein, CpG DNA, or the imidazoquinolines. However, LPS stimulation resulted in activation of NF-κB and JNK with delayed kinetics in spite of no production of cytokines in MyD88-deficient macrophages (Kawai *et al.*, 1999). This indicates that although the LPS-induced production of inflammatory cytokines completely depends on the signalling pathway via MyD88, there exists a MyD88-independent component in the TLR4 signalling pathway. MyD88-independent activation of the LPS-TLR4 signalling pathway is evident in several aspects. Dendritic cells from MyD88-deficient, but not from TLR4-deficient, mice showed functional maturation in response to LPS (Kaisho *et al.*, 2001). Lipopolysaccharide stimulation induced caspase-1-dependent cleavage of the IL-18 precursor into its mature form in Kupffer cells from MyD88-deficient mice (Seki *et al.*, 2001). Lipopolysaccharide stimulation of MyD88-deficient macrophages led to the induction of several IFN-inducible genes, such as those encoding IP-10 and GARG16 (Kawai *et al.*, 2001). In addition to LPS, dsRNA induced activation of NF-κB in MyD88-deficient mice (Alexopoulou *et al.*, 2001). Thus, TLR4 as well as TLR3 utilizes the MyD88-independent pathway.

IRF-3, a member of the IRF family of transcription factors, was originally characterized as a factor that is phosphorylated in response to viral infection and dsRNA, and induces the IFN-α/β and IFN-inducible genes (Weaver *et al.*, 1998; Yoneyama *et al.*, 1998). Analysis of IRF-3-deficient mice has revealed an essential role for IRF-3 in viral infection-induced expression of IFN-α/β (Sato *et al.*, 2000). IRF-3 has been shown to be specifically activated in the TLR3 and TLR4 signalling pathways (Kawai *et al.*, 2001; Doyle *et al.*, 2002). Furthermore, LPS stimulation induced activation of IRF-3 in a MyD88-independent manner (Kawai *et al.*, 2001). LPS-induced activation of IRF-3 has been shown to confer induction of IFN-β and subsequent activation of Stat1, which leads to the expression of IFN-inducible genes in macrophages and dendritic cells (Doyle *et al.*, 2002; Hoshino *et al.*, 2002; Toshchakov *et al.*, 2002). Thus, IRF-3 presumably plays an important role in the MyD88-independent pathway of TLR3 and TLR4 signalling.

A second adaptor molecule named TIR domain-containing adaptor protein (TIRAP)/MyD88-adaptor-like (Mal) was identified (Fitzgerald *et al.*, 2001; Horng *et al.*, 2001). Similar to MyD88, TIRAP/Mal possesses a C-terminal TIR domain, and specifically associates with TLR4 through interaction between their respective TIR domains. Initial *in vitro* studies indicated that TIRAP/Mal is a possible adaptor involved in the LPS-induced, MyD88-independent signalling. However, generation of TIRAP/Mal-deficient mice has recently revealed a quite unexpected role of TIRAP/Mal in the TLR signalling (Horng *et al.*, 2002; Yamamoto *et al.*, 2002). TIRAP/Mal-deficient mice showed impaired production of inflammatory cytokines in response to LPS and lipopeptides, although they showed normal response to CpG DNA, dsRNA and the imidazoquinolines. Lipopolysaccharide stimulation of TIRAP/Mal-deficient macrophages led to delayed activation of NF-κB and normal induction of IFN-inducible genes, which was also observed in MyD88-deficient mac-

rophages. Even in mice lacking both TIRAP/Mal and MyD88, IFN-inducible genes were normally induced in response to LPS, indicating that LPS-induced activation of the MyD88-independent signalling was not affected in these mice. Thus, it has been shown that TIRAP/Mal is essential for the MyD88-dependent signalling pathway via TLR2 and TLR4, but not for the MyD88-independent signalling. This study highlighted that the TIR domain-containing molecule acts in the TLR signalling pathway, and some may provide the specificity of the TLR signalling. An unidentified TIR domain-containing molecule may be responsible for the MyD88-independent signalling.

In addition to the TLR3 and TLR4 signalling, TLR7-mediated signalling has been shown to induce the expression of IFN-α/β (Ito *et al*., 2002; Hemmi *et al*., 2002). But unlike TLR3 and TLR4, the TLR7-dependent induction of IFN-α/β is completely dependent on MyD88 (Hemmi *et al*., 2002). CpG DNA that activates TLR9 has been shown to induce IFN-α/β in plasmacytoid dendritic cells (Krug *et al*., 2001). TLR7 and TLR9 are structurally related and presumably exhibit similar biological responses. Stimulation of TLR2 did not induce expression of IFN-α/β (Kawai *et al*., 2001; Hoshino *et al*., 2002). Therefore, there seems to exist a signalling cascade that confers the MyD88-dependent induction of IFN-α/β in some of the TLR signallings. At present, it remains quite obscure how the MyD88-dependent signalling leads to IFN-α/β induction.

An additional molecule that mediates TLR-induced signalling was proposed. Receptor interacting protein-2 (RIP2), possessing a C-terminal CARD domain, was originally identified as a serine/threonine kinase that associates with the TRAFs and with TNF receptor family members such as the type I TNF receptor and CD40 to induce NF-κB activation and apoptosis. RIP2-deficient mice exhibited partially impaired responses to LPS, peptidoglycan and dsRNA (Chin *et al*., 2002; Kobayashi *et al*., 2002b). Furthermore, RIP2 was shown to associate with TLR2, indicating that RIP2 is involved in TLR signalling pathways.

Future prospects

The identification of the Toll receptor has made us aware that innate immunity is a skillful system that senses the invasion of pathogens. However, it remains unclear how TLRs recognize invading pathogens under physiological conditions, as live pathogens do not expose their components that activate the immune cells. Phagocytosis plays a major role in innate immunity (Aderem and Underhill, 1999; Underhill and Ozinsky 2002). Pathogens are digested and degraded in the lysosomal compartment after phagocytosis. The relation between TLRs and phagocytic receptors of pathogens such as Fc receptors, complement receptors and lectins would be one of the most interesting points to be elucidated in the future. Recently obtained evidence indicates that TLRs presumably sense viral invasion as well as bacterial and fungal infection. In the signalling pathway via TLRs, a MyD88-independent pathway that leads to the induction of type I IFNs may play an important role in the antiviral response. The identification of molecules that activate the MyD88-independent signalling would also be of interest. This will clarify the role of TLRs in antiviral responses.

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