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MINIREVIEW

Toll Receptors: a Central Element in Innate Immune Responses

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Innate immunity is an evolutionarily ancient system that provides multicellular organisms with immediately available defense mechanisms against a wide variety of pathogens without requiring prior exposure. Hallmarks of innate immune responses include the ability to (i) recognize structures that are present in large groups of microorganisms and are distinct from self, (ii) activate effector mechanisms that will destroy within hours most microorganisms encountered in life, and (iii) activate and orientate an adaptive immune response that, through clonal expansion of lymphocytes, will be aimed specifically at persistent microorganisms (25, 45, 65).

Recognition of microbial determinants by elements of the innate immune system sets in motion early humoral and cellular mechanisms for defense against pathogens. The rapid inflammatory reaction that follows infection is principally mediated by monocytes, neutrophils, and endothelial cells and can be reproduced in vitro in the absence of components of the adaptive immune response. Bacterial products directly elicit the upregulation of adhesion molecules on vascular endothelial cells, contributing to the recruitment of leukocytes to the focus of infection. β_2 integrins are also upregulated on neutrophils in response to bacterial products, and they participate in both trafficking of leukocytes into infected tissue and helping to dispose of the offending organisms. In addition, the activation of leukocytes that occurs upon ligation of adhesion-promoting receptors stimulates the production of reactive oxygen intermediates that contribute to the clearance of bacteria from tissues. Bacterial products also induce the synthesis and release of proinflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), that amplify the response to infection. Innate immune recognition induces secretion of effector cytokines, such as IL-12, that control CD4-T-cell differentiation, upregulation of costimulatory molecules on antigen-presenting cells that are necessary for T-cell activation, and signals that are necessary for B-cell proliferation. Thus, the innate response to microbial challenge controls and instructs the adaptive immune response.

The Toll family of receptors, a family that is conserved throughout evolution from flies to humans, plays a central role in the initiation of cellular innate immune responses. The Toll family of receptors provides us with the long sought transmembrane molecules linking the extracellular compartment, where contact with and recognition of microbial pathogens occurs, and the intracellular compartment, where signaling cascades leading to cellular responses are initiated. In this review, we will particularly focus on the molecular mechanisms that allow Toll receptors to transduce a signal.

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THE TOLL FAMILY OF RECEPTORS

Toll and its mammalian homologs are type I transmembrane proteins, with an ectodomain consisting of leucine-rich repeats (LRRs) and one or two cysteine-rich regions. The intracellular domain of Toll-related receptors contains a Toll/IL-1 receptor (TIR) domain, based on homology of the region with a similar intracellular domain of the IL-1 receptor (IL-1R). As described below, the TIR domain in mammalian Toll-related receptors provides the initial internal scaffold for the interaction of the members of a well-defined signaling cascade.

The first member of the family to be identified was named Toll, in which mutations disrupted the establishment of dorsoventral polarity in *Drosophila melanogaster* embryos (10, 33). Toll also participates in innate immune responses of adult *Drosophila*, as flies with mutations in Toll have a dramatically reduced ability to activate expression of an antifungal peptide and to survive fungal infection (55). A mutation in 18-Wheeler, another *Drosophila* Toll-related receptor, rendered *Drosophila* larvae more susceptible to bacterial infection (119), confirming the role for this type of receptor in host defense. Searches in the *Drosophila* genome have revealed the existence of seven additional Toll-related genes (*toll-3* to *toll-9*), but the role of these additional receptors in innate immune responses remains to be defined. To date, only Toll-5 has been characterized as a participant in the antifungal immunity of *Drosophila*. Expression in insect cells of a chimeric construct in which the truncated ectodomain from Toll was fused to the transmembrane and intracellular domains of Toll-5 activates the promoter for drosomycin, an antifungal peptide (109). Similar experiments expressing chimeric 18-Wheeler in S2 cells failed to detect activity of the attacin promoter, suggesting that 18-Wheeler is not sufficient to promote production of antibacterial peptides.

Following the discovery of a role for Toll in *Drosophila* host defense, Medzhitov et al. reported the cloning of human Toll and showed that a constitutively activated construct of human Toll transfected into human cell lines induced the activation of NF-κB and the expression of genes controlled by NF-κB. The constitutively active human Toll also induced the expression of the costimulatory molecule B7.1 that is required for activation of naïve T cells (66). The search for other members of the family led to the identification over the past 3 years of nine

more human Toll-related proteins (16, 22, 96, 108). The 10 human Toll-related proteins characterized so far are referred to as Toll-like receptors (TLRs) 1 to 10, with human Toll renamed TLR4. In the mouse, so far only TLRs 1 to 9 have been identified. The Toll family of proteins appears to be conserved throughout evolution, since proteins homologous to Toll have now been described not only in *Drosophila* but also in a variety of organisms, including plants (21, 118). In addition, three other proteins, RP105, Nod1, and Nod2, which will be discussed below, share structural and functional homology with members of the Toll family.

SPECIFICITY OF TLRS TOWARD MICROBES

The first observations for a direct role of TLRs in mediating innate immune responses to microbes came from two studies showing that expression of human TLR2 endowed an otherwise unresponsive human cell line with the ability to activate NF-κB in response to lipopolysaccharide (LPS; endotoxin) (50, 122). TLR2 has since been shown to confer responsiveness to a wide variety of gram-positive bacterial cell wall components as well as to lipoproteins that are found in gram-positive and gram-negative bacteria and *Mycoplasma* spp. (12, 37, 59, 63, 100, 106, 111, 123) (see Table 1). TLR2 also enables responses to yeast particles (87). In mice, TLR2 is necessary for in vivo responses to cell walls from several gram-positive bacteria and to preparations of peptidoglycan (PGN) (105). TLR2-deficient mice are also highly susceptible to infection by *Staphylococcus aureus* (104), illustrating the importance of this receptor in mounting a defense against infection. Surprisingly, given the initial link between TLR2 and responses to LPS, TLR2-deficient mice and cell lines from Chinese hamsters that are deficient for TLR2 respond normally to LPS (35, 105). However, LPS preparations from *Escherichia coli* and *Salmonella* lose their ability to induce TLR2-dependent responses after removal of contaminant proteins by multiple phenol extractions (38), suggesting that the lipopeptides rather than the LPS are responsible for cellular activation via TLR2. It is possible, however, that some types of LPS can activate TLR2. In the studies mentioned above, all of the forms of LPS tested were from *Enterobacteriaceae*. The structure of lipid A, a moiety that possesses most of the biological activities of LPS and anchors LPS to the external membrane of gram-negative bacteria, is well known and conserved across species (94, 95). Recently, LPS was isolated from *Leptospira interrogans*, a spirochete that produces an atypical LPS differing from gram-negative LPS in several biochemical, physical, and biological properties (115). *L. interrogans* LPS induced cytokine secretion in a macrophage-like cell line, and this was inhibited by anti-TLR2 antibodies. Moreover, TLR2-deficient mice did not respond to leptospiral LPS (115). As is the case with many studies using purified LPS, the possibility remains that a non-LPS contaminant copurified with *L. interrogans* LPS and that the contaminant activated the cells through TLR2. However, if confirmed, these studies suggest that LPS from other organisms, with as yet unknown structural properties, could trigger an innate immune response through TLR2.

A strong indication that TLR4 is critical for in vivo responses to LPS came from mapping the receptor to the narrowly defined *lps* locus on chromosome 4 of mice. This locus was long associated with hyporesponsiveness to LPS and susceptibility to gram-negative infection in C3H/HeJ and C57BL/10ScCr mice, and a mutation in TLR4 was identified in both of these strains (91, 93). The importance of TLR4 for responses to LPS and gram-negative bacteria was later confirmed by the observation that targeted disruption of the TLR4 gene renders mice unresponsive to LPS and that this defect can be reversed in macrophages derived from TLR4-deficient mice by reintroducing a wild-type copy of the gene (40). Hyporesponsiveness to LPS in a small group of humans is also linked to a missense mutation in TLR4 (9). Given the importance of TLR4 for responses to LPS in vivo, it was surprising that transfection of HEK293 cells with human TLR4 does not confer the ability to respond to LPS but does constitutively activate NF-κB (50). This apparent contradiction was resolved by the discovery that coexpression with MD-2, a soluble protein tethered to the cell surface through its physical association with human TLR4, allowed activation of NF-κB by TLR4 in response to LPS to levels well above those observed in the absence of bacterial agonists (101). Recently, a Chinese hamster ovary cell line defective in responses to LPS was identified as having a point mutation in MD-2, further supporting the importance of this protein for TLR4-mediated responses to endotoxin (99). Human TLR4 thus associates with MD-2 to form a complex that is responsive to LPS, and the same is true for the murine TLR4 and MD-2 (3, 101).

DNA from a variety of organisms, including bacteria and yeast, contains motifs that are rare among mammals and are recognized as foreign, eliciting responses associated with host defense (1). TLR9 was recently demonstrated to be necessary for both in vitro and in vivo responses to foreign DNA (36). Akira and coworkers challenged macrophages, dendritic cells, and B cells from TLR9-deficient mice with CpG DNA and found that they failed to respond. Further, the TLR9-deficient animals themselves were resistant to toxic shock elicited by CpG DNA (36). While these results clearly indicate that TLR9 is required for responses to CpG oligonucleotides, further studies will be required to determine to what extent TLR9 contributes to host defense against infection or the varied immunomodulatory activities attributed to CpG DNA, including its ability to act as an adjuvant in eliciting an adaptive immune response skewed toward antibody production and Th1-type cytokines (15, 90).

While a great deal of work has been concentrated on the important roles of TLR4 and TLR2 in responses to pathogenic challenge, the functions of other TLRs are still under investigation. When TLR5 is transfected into CHO cells, it confers the ability to activate NF-κB in response to bacterial flagellin (34), thus implicating TLR5 in initiating or enhancing responses to flagellated bacteria. An increasing number of observations suggest that TLR1 and -6 may either interact with TLR2 or at least influence responses mediated primarily by TLR2. Expression of TLR1 enhances TLR2-dependent NF-κB activation in response to soluble factors from *Neisseria meningitidis* (120). In addition, the TLR2-dependent NF-κB activation in response to phenol soluble modulin (PSM) is inhibited by the coexpression of TLR1 (32). Expression of a dominant-negative construct of TLR6 inhibits TLR2-dependent NF-κB activation in response to PGN and PSM but not to Pam_3CSK_4 lipopeptide (32, 87). Macrophages from TLR6-deficient mice

respond to PGN just as well as wild-type cells but are deficient in their response to macrophage-activating lipopeptide 2 (MALP-2) (107). Even if some of these observations are apparently contradictory, taken together they suggest that TLR6 may contribute positively to the ability of TLR2 to recognize or respond to certain bacterial components.

The intriguing results for TLR1 and -6 and TLR2 contribute to the growing notion that different TLRs could combine to recognize foreign agonists. Such combinations could alter not only the specificity of the determinants recognized but also the nature and intensity of the signals generated. Nine TLRs forming either homo- or heterodimers give rise to 2^9, or more than 500, possible combinations. Studies with RP105, a protein with structural homology to the extracellular domain of TLRs (see below), suggest that TLRs could potentially associate into complexes larger than dimers, thus opening up the possibility for a very large combinatorial repertoire. This might, in turn, allow specific responses to a large number of structurally distinct bacterial agonists. To date, the main observations supporting the idea for an association between TLRs are the functional studies in which TLR1 and -6 are expressed with TLR2 and an additional report of coimmunoprecipitation of TLR2 and TLR6 that suggests a physical association between TLR2 and TLR6 in the absence of bacterial components (87). All of these observations have been made using transfected cell lines that overexpress the receptors examined, and they will need to be confirmed in primary cells.

There is also evidence that TLRs can be activated by nonbacterial products, such as taxol (48, 62), heat shock protein 60 (84), or the extracellular domain A of fibronectin (85), as well as by oxidative stress (29). A summary of the specificity of TLRs for both bacterial products and nonbacterial products is provided in Table 1.

TOLL PROTEINS AND RECOGNITION

One model for the initiation of signaling by TLRs in response to microbial products is based on studies in *Drosophila*. Recognition of fungal determinants occurs upstream of *Drosophila* Toll and induces a proteolytic cascade, resulting in the maturation of an endogenous peptidic ligand for Toll. Genetic and biochemical studies suggest that a ligand for *Drosophila* Toll is a proteolytic fragment of the protein Spätzle comprising the C-terminal 106 amino acid residues (18, 76). Mutants that do not express Spätzle cannot activate the Toll pathway and are defective in their development (76) and immune response (55). During *Drosophila* development, Spätzle is cleaved by the protease Easter, which is itself activated by the protease Snake, but neither Easter nor Snake are required for activation of the Toll pathway during the immune response (55). Serine proteases seem to be critical, however, since a loss-of-function mutation in the gene encoding the blood serine protease inhibitor Spn43Ac leads to the constitutive cleavage of Spätzle and activation of the Toll-mediated antifungal defense (57).

Parallels can be drawn between the protease cascade in *Drosophila* and the clotting cascade induced in response to LPS in a primitive arthropod, *Limulus*, the horseshoe crab. Both of the *Drosophila* serine proteases, Easter and Snake, display structural similarities to proteases from the *Limulus* clotting cascade (102), and several protease inhibitors that fall into the same class as Spn43Ac can specifically inhibit the proteases of the clotting cascade (57). Finally, while Spätzle does not display significant primary structural homology with *Limulus* coagulogen, or incidentally with any mammalian proteins identified or sequenced to date, sequence analyses do predict that the core disulfide bond arrangement of the cleaved Spätzle is highly similar to that of coagulogen and vertebrate nerve growth factor (18, 74). While the parallels between events upstream of Toll and the *Limulus* clotting cascade are intriguing, there is to date little support for this model with regard to activation of TLRs in mammalian cells.

A second model has therefore been proposed in which recognition of microbial products would occur upon their binding directly to TLRs or a protein associated with TLRs. Two studies support the idea that TLR4 itself is sufficient to confer the ability to recognize LPS. Both took advantage of the species-specific pharmacology of one or two lipid A analogues, *Rhodobacter spheroides* lipid A (RSLA) and lipid IVa. Both compounds display LPS mimetic activity in the hamster but act as antagonists of LPS in human cells, while in the mouse lipid IVa is an agonist and RSLA is an antagonist (19, 30). Cells that heterologously express TLR4 from different species reacted to lipid A analogues with the pharmacological specificity corresponding to the species from which the TLR4 transgene originated (58, 92), suggesting that TLR4 itself confers recognition of the structures and implying that the receptor must make physical contact to do so.

Another approach that has been pursued is to demonstrate physical proximity of molecules putatively involved in recognition of bacterial agonists. Many attempts to identify a receptor for LPS using an iodinated, cross-linkable derivative of LPS have been made, and this approach has yielded equivocal results in the past. A recent study with such a probe has, however, demonstrated that LPS interacts with TLR4 and MD-2 (17).

The role of MD-2 in recognition of bacterial LPS is less clear. Transfection of MD-2 in 293 cells expressing ectopic TLR2 confers the ability to activate NF-κB in response to LPS preparations that have been reextracted with phenol and hence contain a lower amount of contaminating lipopeptides (23). This would support a model whereby MD-2 determines reactivity toward LPS. However, in this study, expression of MD-2 also induced a dramatic increase in expression of TLR2 and enhanced responses to gram-positive bacteria and PGN that do not depend on MD-2. Ectopic expression of MD-2 could thus render the cells more sensitive to agonists without altering the specificity of the reaction.

TLRS AND INTRACELLULAR SIGNALING

Bacterial products, including LPS, induce intracellular signaling that results in activation of transcription factors, such as NF-κB and AP-1, and modulation of cytokine production. In the first demonstration that the intracellular portion of human TLRs could activate signaling pathways involved in innate immune responses, the extracellular domain of CD4 was used to replace that of TLR4, presumably causing constitutive dimerization of the TLR4 TIR domains (66). Expression of the chimeric receptors in Jurkat cells induced NF-κB activation and cytokine secretion (66). Since this initial experiment, a

TABLE 1. Agonists reported to activate TLRs

Agonist	TLRs	Necessity in vivo[b]	Activity in vitro	References
Bacterial products				
LPS				
E. coli, Salmonella spp. (all gram-negative bacteria tested so far except one)	TLR4/MD2	+	Activates	91, 93, 99, 101, 105
	TLR4 plus RP105/MD1 (B cells)	+	Activates	81
L. interrogans	TLR2		Activates	115
Lipoproteins, lipopeptides	TLR2		Activates	
Borrelia burgdorferi				37
Treponema pallidum				59
Mycobacterium fermentans				80, 106
Mycobacterium tuberculosis				12
Lipopeptides				
Synthetic Pam_3CSK_4	TLR2		Activates	6
MALP2	TLR2 plus TLR6		Activates	107
PGN	TLR2	+	Activates	68, 100, 105, 123
	TLR6		Facilitates	87
AraLAM[a]	TLR2		Activates	64
PSM				32
	TLR2		Activates	
	TLR6		Facilitates	
	TLR1		Inhibits	
Lipoteichoic acids	TLR2 (human)		Activates	86, 100
	TLR4 (mouse)	+		105
CpG DNA	TLR9	+	Activates	36
Flagellin	TLR5	+	Activates	34
Bacteria and extracts				
E. coli, Salmonellae	TLR4	+	Activates	40
S. aureus, Clostridium diphteriae, Neisserria coeliaca	TLR2	+	Activates	59, 105
N. meningitidis	TLR2		Activates	120
	TLR1		Facilitates	
Micrococcus luteus	TLR2		Activates	114
Listeria monocytogenes	TLR2	+	Activates	28
Mycobacteriae				
M. avium	TLR2		Activates	59, 63
M. tuberculosis	TLR2 and TLR4		Activates	12, 64, 111
M. bovis	TLR2		Activates	64
Treponema				98
T. maltophilum	TLR2		Activates	
T. brennaborense	TLR2 and TLR4		Activates	
Nonbacterial products				
Yeast	TLR2		Activates	110
Respiratory syncytial virus	TLR4	+	Activates	53
Hsp60	TLR4		Activates	84
Extracellular domain A	TLR4		Activates	85
Oxidative stress	TLR2		Activates	29
Taxol	TLR4/MD2		Activates	48

[a] AraLAM, arabinolipoarabinomannan.
[b] Plus signs indicate that the TLR is necessary in vivo.

variety of studies have demonstrated that TLR2, -5, -6, and -9 are also capable of activating NF-κB when they are expressed in transfected cells (34, 36, 50, 66, 108, 122). In addition, TLR2, -4, -6, and -9 have been shown to activate AP-1 (29, 36, 77, 108).

Characterization of a major signaling pathway linking TLRs to NF-κB has advanced rapidly, based on the homology of the TLR intracellular domains with that of IL-1R. Upon binding IL-1, the IL-1R associates with IL-1R accessory protein (IL-1RacP) (31, 41, 117), and their corresponding cytoplasmic portions cooperate (13, 116) to form an active signaling complex that includes the adapter protein MyD88 and IL-1R activated kinase (IRAK). MyD88, a previously described myeloid differentiation marker, has a modular structure with a carboxy-terminal TIR domain that interacts with the TIR domains of IL-1R and IL-1RAcP via homophilic interaction and an amino-terminal death domain module that recruits IRAK to the signaling complex by its own death domain (13, 14, 116). IRAK, a member of the family of serine/threonine innate immunity kinases (52), autophosphorylates and binds to TRAF6, an adapter molecule necessary for NF-κB activation by IL-1 and LPS (60). This in turn leads to the activation of the IκB kinase (IKK) complex and the phosphorylation of IκB. Degradation of phosphorylated IκB by the proteasome then allows

NF-κB to translocate to the nucleus. The IKKs are thought to be activated by a MAP kinase kinase kinase. Candidates for this kinase include NF-κB inducing kinase (NIK), which can bind directly to TRAF6 (61), and MEKK-1, which would be bridged to TRAF6 through evolutionarily conserved signaling intermediate in Toll (ECSIT) pathways (51).

Similarly, the initial step in TLR-mediated signaling is thought to involve the association of the intracellular domains of at least two TLR molecules (121), although, as discussed earlier, the nature of the ligands that induce this association is not completely clear. The majority of the work on the signaling cascade downstream of TLRs has been done with dominant-negative constructs of the components of the IL-1R signaling system. Using this method, it has been suggested that there is very nearly identity between the two pathways leading to NF-κB activation, with MyD88, IRAK, TRAF6, NIK, ECSIT, and components of the IKK complex required for the translocation of NF-κB to the nucleus induced by TLRs (50, 67, 77, 114). MyD88 is also required for activation of AP-1 by constitutively active CD4-TLR4 (67, 77). However, downstream of MyD88, the AP-1 activation pathway requires further elucidation. In addition, recent work indicates the existence of signaling pathways from TLRs that do not depend on MyD88 (4), suggesting that a multiplicity of pathways originates from TLR, with some convergent on NF-κB and others generating different endpoint effectors.

Other proteins are likely to be involved in the signaling pathway for activation of NF-κB. For example, a melanoma cell line deficient in the actin-binding protein filamin fails to activate NF-κB in response to TNF or when it is transfected with constitutively active TLR4 or TRAF6 (56). The defect appears to be caused by the lack of filamin, as reintroduction of filamin restores NF-κB activation by TNF and constitutively active TLR4 or TRAF6. Physical interaction of filamin with *Drosophila* Toll and Tube (24), a protein that carries out functions partially analogous to the adapter protein MyD88, and with human TRAF2 (56), MKK-4 (SEK-1), an upstream regulator of AP-1, and p38 (62) has been observed either in yeast two-hybrid assays or through coimmunoprecipitation. In light of these results, Leonardi and colleagues (56) have proposed that filamin provides a scaffold upon which a TRAF-dependent signaling complex can assemble for the activation of NF-κB.

While a great deal of effort has been expended on elucidating the signaling mechanisms shared with IL-1R that lead to transcriptional regulation in response to bacterial products, less has been done on the role of TLRs in mediating the activation of p38. Activation of p38 is a hallmark response to lipopolysaccharide and other bacterial products, and the kinase can regulate cytokine production both transcriptionally and at a posttranscriptional level (54). In addition, p38 MAP kinase activity is required for cellular processes that do not require de novo protein synthesis but nonetheless contribute to responses to pathogens, such as the upregulation of integrin-mediated adhesion by neutrophils and enhancement of their ability to produce reactive oxygen intermediates (20, 79). We have recently demonstrated that TLR2 functions in activating p38 and its downstream effectors in response to TLR2-specific effectors (112). It remains to be demonstrated whether other TLRs can mediate p38 activation in response to other stimuli. Monocytes from a MyD88-deficient mouse are still able to activate p38 in response to LPS (47), although they do so more slowly than wild-type cells, raising the possibility that TLRs can engage an as yet uncharacterized pathway to activate p38. Small GTP-binding proteins of the Ras superfamily have been shown to activate p38, and toxins that act on these proteins inhibit p38 activation by IL-1 (69, 88). A recent study demonstrated that TLR2 can regulate the transcriptional activity of NF-κB in the nucleus through a Rac1/PI3K/Akt-dependent pathway (8), suggesting the possibility that TLR2 may also utilize small GTP-binding proteins to activate p38.

A link between TLR2 and apoptosis has been suggested by observing that a bacterial lipopeptide induced apoptosis of 293 cells when the cells were transfected with TLR2 (6). Apoptosis could be inhibited by MyD88, Fas-associated death domain protein, and caspase-8 dominant-negative constructs (7), suggesting that TLR2 may directly activate caspase pathways. The relevance of such a response in controlling the innate immune response remains to be assessed.

It is likely that as more intracellular signaling pathways involving TLRs are identified, differences will arise between the various receptors. The sequence conservation among TIR intracellular domains for TLRs is generally in the range of 20 to 30%, and the length of these domains is also quite variable. Despite the low sequence homology, the change of one single conserved residue of the TIR in TLR2, -4, and -6 (Pro to His) makes these proteins either inactive or active as dominant-negative mutants (32, 91, 93, 110). In TLR2, the Pro681-to-His change does not have a global effect on the structure of the protein but is sufficient to render TLR2 unable to activate NF-κB and to prevent the TIR domain from interacting with the adapter MyD88 (121). However, the low sequence homology among TLRs could well account for differences in efficiency of signaling between the receptors for a given pathway or for differences in the pathways initiated by different receptors. Analysis via X-ray crystallography of two closely related TIRs, from TLR1 and TLR2, having 50% amino acid identity, shows a similar structural backbone but significant differences in the loop structures between the main structural elements (121). The residues appearing on certain loops are quite different for these two TIRs, and these may contribute to important functional differences.

MOLECULES STRUCTURALLY AND FUNCTIONALLY RELATED TO TLRS

Three proteins, RP105, Nod1, and Nod2, are structurally related to TLRs and have been suggested to play a role in responses to LPS. Like TLRs, RP105 is a type I transmembrane protein with an ectodomain consisting of LRRs and one cysteine-rich region (73, 97). In addition, like TLR4, RP105 associates through its ectodomain with a soluble protein homologous to MD-2, MD-1 (70, 71). However, the short intracellular domain of RP105 does not contain a TIR domain and shares no homology with those of TLRs. RP105 is expressed primarily on mature peripheral B cells (72), in contrast to TLR4, which is not abundantly expressed on this cell type (2). The observation that mice deficient in either RP105 or TLR4 fail to mount a full B-cell proliferative and humoral response to LPS (81, 91, 93) has led to the hypothesis that RP105 cooperates with TLR4 on B cells to enable responses to LPS.

Supporting this hypothesis, Ogata and colleagues have shown that cells expressing RP105 and MD-1 activate NF-κB in response to LPS through TLR4 in the absence of MD-2 (81). While no direct interaction between RP105 and TLR4 has been demonstrated, the results suggest the possibility that TLRs can associate with heterodimeric complexes containing proteins other than true TLRs.

Nod1 and Nod2 are both cytosolic proteins that could act as intracellular functional equivalents of TLRs. Nod1 and -2 were recently shown to endow 293 cells with the ability to activate NF-κB in response to LPS independently of TLR4, MyD88, and TRAF6 (44). Transfection with Nod2 also enabled the cells to respond to PGN, while transfection with Nod1 did not (44), suggesting that the proteins may confer a specificity to the response. Nod1 and -2 are composed of one and two N-terminal caspase recruitment domains (CARD), respectively, a central nucleotide binding domain, and multiple LRRs at the C terminus (11, 43, 83). The LRR domains have a regulatory function on the activity of the CARD (44, 83) and are necessary for the activation of NF-κB in response to LPS (44). The CARD is the effector domain that, upon dimerization, associates with the CARD of the protein kinase RIP-like interacting CLARP kinase (RICK) that in turn activates NF-κB (44, 83). Nod family members may thus function as proteins that activate innate immune responses to a wide array of products from pathogens. It is worth noting that BLAST searches of public databases indicate that the human genome contains at least 20 Nod-like genes (44). The mechanisms by which exogenously added bacterial agonists such as LPS would reach the cytosol, be recognized, and induce activities via Nod proteins are not well understood. Such a system could, however, be useful for sensing intracellular pathogens. A recent study demonstrated that LPS either microinjected or presented inside the cell by the invasive pathogen *Shigella flexneri* can stimulate NF-κB activation (89), supporting the idea that an intracellular system for recognizing LPS may exist. In addition, mutations in human Nod2 have been linked with susceptibility to Crohn's disease (42, 82), an inflammatory bowel disease thought to be due to an abnormal inflammatory response to enteric microflora (26).

CONCLUSION AND PERSPECTIVES

In the 4 years that have followed the discovery of the first human TLR, characterization of the biological activities of TLRs has shown them to be important components of the innate immune system in mammals. They provide a family of transmembrane proteins that transduce an intracellular signal following recognition of microbial determinants in extracellular compartments. TLRs are activated by chemically diverse microbial products such as the following: TLR4 by LPS, TLR9 by bacterial DNA, TLR2 by a variety of gram-positive cell wall products, and TLR5 by flagellin. In addition, TLR1 and -6 may combine with TLR2 to mediate responses to some microbial products (32, 87, 107). The specific expression of TLR3 in dendritic cells and its downregulation in cells stimulated by bacterial products suggest a role for this receptor in maturation of these cells. TLR3 has also recently been shown to activate NF-κB in response to double-stranded RNA (5). The mechanisms of interaction of bacterial agonists with TLRs are not yet clearly understood, but TLR4 appears to play a direct role in recognition of LPS (58, 92), and a recent review mentioning data not yet published suggests a direct role for TLR9 in recognition of oligodeoxynucleotides (113). With the exception of *S. aureus* (104), little is known on the role of TLRs in resistance to infectious agents. We can nevertheless predict that as TLR-deficient mice and blocking antibodies become more widely available their role will be tested in animal models of infectious challenge.

Our understanding of the intracellular signaling pathways activated by TLRs has greatly advanced. Different TLRs have been shown to activate similar pathways. For example, TLR2, -4, -5, -6, and -9 are capable of activating NF-κB. The observation that maturation of dendritic cells induced by CpG DNA and TLR9 requires MyD88, while that induced by LPS and TLR4 does not (46), suggests that two TLRs may utilize different pathways to elicit a similar response. Very recently, a TIR domain-containing adapter protein was characterized that may play a role in LPS-induced maturation of dendritic cells (27, 39). A dominant-negative form of this protein inhibited NF-κB activation and dendritic cell maturation activated by TLR4 or LPS but did not inhibit responses of IL1R1 or to the TLR9 agonist CpG DNA (27, 39). It is thus likely that differences in the signaling cascades initiated by the intracellular domains of different TLRs will be found, given their primary structural differences. The cellular expression pattern of TLRs may influence not only the ability of a given cell type to respond to a particular agonist but also the kind of response promoted by the agonist. While a preliminary view of cellular expression has emerged (78), it is incomplete, and more information is likely to be forthcoming.

The elucidation of the role and specificity of TLRs has also been advanced by a better understanding of the chemical structure of bacterial agonists. Most bacterial agonists are also vital structural components for the bacteria themselves. For example, the lipid A moiety of LPS is not only able to elicit biological responses in mammalian cells but also serves as a structure essential for the integrity of the outer membrane of gram-negative bacteria. The structure of lipid A and other agonists is consequently conserved, and they thus represent good targets for innate immune responses. Some variations in the structure of lipid A are, however, possible, and they result in altered biological activity. Canonical lipid A from *Enterobacteriaceae* which contains six fatty acids is recognized by TLR4 as an agonist, but lipid A from *R. spheroides*, with four fatty acids, is an antagonist for TLR4. Further, lipid A from *L. interrogans* may be an agonist for TLR2. Similarly, lipoteichoic acids from various species, strains, or preparations have been shown to have differences in immunostimulatory activities, and these appear to correlate with their lipophilicity and pathogenicity (49, 75, 103). It is thus possible that some pathogenic bacteria have developed ways to interact with TLRs and escape the innate immune response. Since all TLRs may not be equivalent for stimulating and orienting the adaptive immune response, tampering with TLRs may allow bacteria to also act on the adaptive immune response. Better characterization of the chemical structure of bacterial agonists will help define the structural requirements for activation or inhibition of TLRs.

REFERENCES

1. **Aderem, A., and D. A. Hume.** 2000. How do you see CG? Cell **103:**993–996.
2. **Akashi, S., H. Ogata, F. Kirikae, T. Kirikae, K. Kawasaki, M. Nishijima, R.**

Shimazu, Y. Nagai, K. Fukudome, M. Kimoto, and K. Miyake. 2000. Regulatory roles for CD14 and phosphatidylinositol in the signaling via toll-like receptor 4-MD-2. Biochem. Biophys. Res. Commun. **268:**172–177.
3. **Akashi, S., R. Shimazu, H. Ogata, Y. Nagai, K. Takeda, M. Kimoto, and K. Miyake.** 2000. Cell surface expression and lipopolysaccharide signaling via the toll-like receptor 4-MD-2 complex on mouse peritoneal macrophages. J. Immunol.**164:**3471–3475.
4. **Akira, S., K. Takeda, and T. Kaisho.** 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat. Immunol. **2:**675–680.
5. **Alexopoulou, L., A. Czopik Holt, R. Medzhitov, and R. A. Flavell.** 2001. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature **413:**732–738.
6. **Aliprantis, A. O., R. B. Yang, M. R. Mark, S. Suggett, B. Devaux, J. D. Radolf, G. R. Klimpel, P. Godowski, and A. Zychlinsky.** 1999. Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. Science **285:**736–739.
7. **Aliprantis, A. O., R. B. Yang, D. S. Weiss, P. Godowski, and A. Zychlinsky.** 2000. The apoptotic signaling pathway activated by toll-like receptor-2. EMBO J. **19:**3325–3336.
8. **Arbibe, L., J. P. Mira, N. Teusch, L. Kline, M. Guha, N. Mackman, P. J. Godowski, R. J. Ulevitch, and U. G. Knaus.** 2000. Toll-like receptor 2-mediated NF-kappa B activation requires a Rac1-dependent pathway. Nat. Immunol. **1:**533–540.
9. **Arbour, N. C., E. Lorenz, B. C. Schutte, J. Zabner, J. N. Kline, M. Jones, K. Frees, J. L. Watt, and D. A. Schwartz.** 2000. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat. Genet. **25:** 187–191.
10. **Belvin, M. P., and K. V. Anderson.** 1996. A conserved signaling pathway: the Drosophila toll-dorsal pathway. Annu. Rev. Cell. Dev. Biol. **12:**393–416.
11. **Bertin, J., W. J. Nir, C. M. Fischer, O. V. Tayber, P. R. Errada, J. R. Grant, J. J. Keilty, M. L. Gosselin, K. E. Robison, G. H. Wong, M. A. Glucksmann, and P. S. DiStefano.** 1999. Human CARD4 protein is a novel CED-4/Apaf-1 cell death family member that activates NF-kappaB. J. Biol. Chem. **274:**12955–12958.
12. **Brightbill, H. D., D. H. Libraty, S. R. Krutzik, R. B. Yang, J. T. Belisle, J. R. Bleharski, M. Maitland, M. V. Norgard, S. E. Plevy, S. T. Smale, P. J. Brennan, B. R. Bloom, P. J. Godowski, and R. L. Modlin.** 1999. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. Science **285:**732–736.
13. **Burns, K., F. Martinon, C. Esslinger, H. Pahl, P. Schneider, J. L. Bodmer, F. Di Marco, L. French, and J. Tschopp.** 1998. MyD88, an adapter protein involved in interleukin-1 signaling. J. Biol. Chem. **273:**12203–12209.
14. **Cao, Z., W. J. Henzel, and X. Gao.** 1996. IRAK: a kinase associated with the interleukin-1 receptor. Science **271:**1128–1131.
15. **Chu, R. S., O. S. Targoni, A. M. Krieg, P. V. Lehmann, and C. V. Harding.** 1997. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity. J. Exp. Med. **186:**1623–1631.
16. **Chuang, T., and R. J. Ulevitch.** 2001. Identification of hTLR10: a novel human Toll-like receptor preferentially expressed in immune cells. Biochim. Biophys. Acta**1518:**157–161.
17. **da Silva Correia, J., K. Soldau, U. Christen, P. S. Tobias, and R. J. Ulevitch.** 2001. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex: transfer from CD14 to TLR4 and MD-2. J. Biol. Chem. **276:**21129–21135.
18. **DeLotto, Y., and R. DeLotto.** 1998. Proteolytic processing of the Drosophila Spatzle protein by easter generates a dimeric NGF-like molecule with ventralising activity. Mech. Dev. **72:**141–148.
19. **Delude, R. L., R. Savedra, Jr., H. Zhao, R. Thieringer, S. Yamamoto, M. J. Fenton, and D. T. Golenbock.** 1995. CD14 enhances cellular responses to endotoxin without imparting ligand-specific recognition. Proc. Natl. Acad. Sci. USA **92:**9288–9292.
20. **Detmers, P. A., D. Zhou, E. Polizzi, R. Thieringer, W. A. Hanlon, S. Vaidya, and V. Bansal.** 1998. Role of stress-activated mitogen-activated protein kinase (p38) in beta 2-integrin-dependent neutrophil adhesion and the adhesion-dependent oxidative burst. J. Immunol. **161:**1921–1929.
21. **Dixon, M. S., C. Golstein, C. M. Thomas, E. A. van Der Biezen, and J. D. Jones.** 2000. Genetic complexity of pathogen perception by plants: the example of Rcr3, a tomato gene required specifically by Cf-2. Proc. Natl. Acad. Sci. USA **97:**8807–8814.
22. **Du, X., A. Poltorak, Y. Wei, and B. Beutler.** 2000. Three novel mammalian toll-like receptors: gene structure, expression, and evolution. Eur. Cytokine Netw. **11:**362–371.
23. **Dziarski, R., Q. Wang, K. Miyake, C. J. Kirschning, and D. Gupta.** 2001. MD-2 enables Toll-like receptor 2 (TLR2)-mediated responses to lipopolysaccharide and enhances TLR2-mediated responses to Gram-positive and Gram-negative bacteria and their cell wall components. J. Immunol. **166:** 1938–1944.
24. **Edwards, D. N., P. Towb, and S. A. Wasserman.** 1997. An activity-dependent network of interactions links the Rel protein Dorsal with its cytoplasmic regulators. Development **124:**3855–3864.
25. **Fearon, D. T., and R. M. Locksley.** 1996. The instructive role of innate immunity in the acquired immune response. Science **272:**50–53.
26. **Fiocchi, C.** 1998. Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology **115:**182–205.
27. **Fitzgerald, K. A., E. M. Palsson-McDermott, A. G. Bowie, C. A. Jefferies, A. S. Mansell, G. Brady, E. Brint, A. Dunne, P. Gray, M. T. Harte, D. McMurray, D. E. Smith, J. E. Sims, T. A. Bird, and L. A. O'Neill.** 2001. Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. Nature **413:**78–83.
28. **Flo, T. H., O. Halaas, E. Lien, L. Ryan, G. Teti, D. T. Golenbock, A. Sundan, and T. Espevik.** 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.
29. **Frantz, S., R. A. Kelly, and T. Bourcier.** 2001. Role of TLR-2 in the activation of nuclear factor-kappa B by oxidative stress in cardiac myocytes. J. Biol. Chem. **276:**5197–5203.
30. **Golenbock, D. T., R. Y. Hampton, N. Qureshi, K. Takayama, and C. R. Raetz.** 1991. Lipid A-like molecules that antagonize the effects of endotoxins on human monocytes. J. Biol. Chem. **266:**19490–19498.
31. **Greenfeder, S. A., P. Nunes, L. Kwee, M. Labow, R. A. Chizzonite, and G. Ju.** 1995. Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. J. Biol. Chem. **270:**13757–13765.
32. **Hajjar, A. M., D. S. O'Mahony, A. Ozinsky, D. M. Underhill, A. Aderem, S. J. Klebanoff, and C. B. Wilson.** 2001. Functional interactions between toll-like receptor (TLR) 2 and TLR1 or TLR6 in response to phenol-soluble modulin. J. Immunol. **166:**15–19.
33. **Hashimoto, C., K. L. Hudson, and K. V. Anderson.** 1988. The Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. Cell **52:**269–279.
34. **Hayashi, F., K. D. Smith, A. Ozinsky, T. R. Hawn, E. C. Yi, D. R. Goodlett, J. K. Eng, S. Akira, D. M. Underhill, and A. Aderem.** 2001. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature **410:**1099–1103.
35. **Heine, H., C. J. Kirschning, E. Lien, B. G. Monks, M. Rothe, and D. T. Golenbock.** 1999. Cells that carry a null allele for toll-like receptor 2 are capable of responding to endotoxin. J. Immunol. **162:**6971–6975.
36. **Hemmi, H., O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda, and S. Akira.** 2000. A Toll-like receptor recognizes bacterial DNA. Nature **408:**740–745.
37. **Hirschfeld, M., C. J. Kirschning, R. Schwandner, H. Wesche, J. H. Weis, R. M. Wooten, and J. J. Weis.** 1999. Inflammatory signaling by Borrelia burgdorferi lipoproteins is mediated by toll-like receptor 2. J. Immunol. **163:**2382–2386.
38. **Hirschfeld, M., Y. Ma, J. H. Weis, S. N. Vogel, and J. J. Weis.** 2000. Repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. J. Immunol. **165:**618–622.
39. **Horng, T., G. M. Barton, and R. Medzhitov.** 2001. TIRAP: an adapter molecule in the Toll signaling pathway. Nat. Immunol. **2:**835–841.
40. **Hoshino, K., O. Takeuchi, T. Kawai, H. Sanjo, T. Ogawa, Y. Takeda, K. Takeda, and S. Akira.** 1999. Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J. Immunol.**162:**3749–3752.
41. **Huang, J., X. Gao, S. Li, and Z. Cao.** 1997. Recruitment of IRAK to the interleukin 1 receptor complex requires interleukin 1 receptor accessory protein. Proc. Natl. Acad. Sci. USA **94:**12829–12832.
42. **Hugot, J.-P., M. Chamaillard, H. Zouali, S. Lesage, J.-P. Cezard, J. Belaiche, S. Almer, C. Tysk, C. A. O'Morain, M. Gassull, V. Binder, Y. Finkel, A. Cortot, R. Modigliani, P. Laurent-Puig, C. Gower-Rousseau, J. Macry, J.-F. Colombel, M. Sahbatou, and G. Thomas.** 2001. Association of NOD2 leucine-rich repeat variants with suceptibility to Crohn's disease. Nature **411:**599–603.
43. **Inohara, N., T. Koseki, L. del Peso, Y. Hu, C. Yee, S. Chen, R. Carrio, J. Merino, D. Liu, J. Ni, and G. Nunez.** 1999. Nod1, an Apaf-1-like activator of caspase-9 and nuclear factor-kappaB. J. Biol. Chem. **274:**14560–14567.
44. **Inohara, N., Y. Ogura, F. F. Chen, A. Muto, and G. Nunez.** 2001. Human nod1 confers responsiveness to bacterial lipopolysaccharides. J. Biol. Chem. **276:**2551–2554.
45. **Janeway, C. A., Jr.** 1992. The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol. Today **13:**11–16.
46. **Kaisho, T., O. Takeuchi, T. Kawai, K. Hoshino, and S. Akira.** 2001. Endotoxin-induced maturation of myd88-deficient dendritic cells. J. Immunol. **166:**5688–5694.
47. **Kawai, T., O. Adachi, T. Ogawa, K. Takeda, and S. Akira.** 1999. Unresponsiveness of MyD88-deficient mice to endotoxin. Immunity **11:**115–122.
48. **Kawasaki, K., S. Akashi, R. Shimazu, T. Yoshida, K. Miyake, and M. Nishijima.** 2000. Mouse toll-like receptor 4.MD-2 complex mediates lipopolysaccharide-mimetic signal transduction by Taxol. J. Biol. Chem. **275:** 2251–2254.
49. **Kengatharan, K. M., S. De Kimpe, C. Robson, S. J. Foster, and C. Thiemermann.** 1998. Mechanism of gram-positive shock: identification of peptidoglycan and lipoteichoic acid moieties essential in the induction of nitric oxide synthase, shock, and multiple organ failure. J. Exp. Med. **188:**305–315.
50. **Kirschning, C. J., H. Wesche, T. Merrill Ayres, and M. Rothe.** 1998. Human

toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide. J. Exp. Med.**188:**2091–2097.
51. **Kopp, E., R. Medzhitov, J. Carothers, C. Xiao, I. Douglas, C. A. Janeway, and S. Ghosh.** 1999. ECSIT is an evolutionarily conserved intermediate in the Toll/IL-1 signal transduction pathway. Genes Dev. **13:**2059–2071.
52. **Kopp, E. B., and R. Medzhitov.** 1999. The Toll-receptor family and control of innate immunity. Curr. Opin. Immunol. **11:**13–18.
53. **Kurt-Jones, E. A., L. Popova, L. Kwinn, L. M. Haynes, L. P. Jones, R. A. Tripp, E. E. Walsh, M. W. Freeman, D. T. Golenbock, L. J. Anderson, and R. W. Finberg.** 2000. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. Nat. Immunol. **1:**398–401.
54. **Lee, J. C., J. T. Laydon, P. C. McDonnell, T. F. Gallagher, S. Kumar, D. Green, D. McNulty, M. J. Blumenthal, J. R. Heys, S. W. Landvatter, et al.** 1994. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature **372:**739–746.
55. **Lemaitre, B., E. Nicolas, L. Michaut, J. M. Reichhart, and J. A. Hoffmann.** 1996. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell **86:**973–983.
56. **Leonardi, A., H. Ellinger-Ziegelbauer, G. Franzoso, K. Brown, and U. Siebenlist.** 2000. Physical and functional interaction of filamin (actin-binding protein-280) and tumor necrosis factor receptor-associated factor 2. J. Biol. Chem. **275:**271–278.
57. **Levashina, E. A., E. Langley, C. Green, D. Gubb, M. Ashburner, J. A. Hoffmann, and J. M. Reichhart.** 1999. Constitutive activation of toll-mediated antifungal defense in serpin-deficient Drosophila. Science **285:**1917–1919.
58. **Lien, E., T. K. Means, H. Heine, A. Yoshimura, S. Kusumoto, K. Fukase, M. J. Fenton, M. Oikawa, N. Qureshi, B. Monks, R. W. Finberg, R. R. Ingalls, and D. T. Golenbock.** 2000. Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. J. Clin. Investig. **105:** 497–504.
59. **Lien, E., T. J. Sellati, A. Yoshimura, T. H. Flo, G. Rawadi, R. W. Finberg, J. D. Carroll, T. Espevik, R. R. Ingalls, J. D. Radolf, and D. T. Golenbock.** 1999. Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. J. Biol. Chem. **274:**33419–33425.
60. **Lomaga, M. A., W. C. Yeh, I. Sarosi, G. S. Duncan, C. Furlonger, A. Ho, S. Morony, C. Capparelli, G. Van, S. Kaufman, A. van der Heiden, A. Itie, A. Wakeham, W. Khoo, T. Sasaki, Z. Cao, J. M. Penninger, C. J. Paige, D. L. Lacey, C. R. Dunstan, W. J. Boyle, D. V. Goeddel, and T. W. Mak.** 1999. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes Dev. **13:**1015–1024.
61. **Malinin, N. L., M. P. Boldin, A. V. Kovalenko, and D. Wallach.** 1997. MAP3K-related kinase involved in NF-kappaB induction by TNF, CD95 and IL-1. Nature**385:**540–544.
62. **Marti, A., Z. Luo, C. Cunningham, Y. Ohta, J. Hartwig, T. P. Stossel, J. M. Kyriakis, and J. Avruch.** 1997. Actin-binding protein-280 binds the stress-activated protein kinase (SAPK) activator SEK-1 and is required for tumor necrosis factor-alpha activation of SAPK in melanoma cells. J. Biol. Chem. **272:**2620–2628.
63. **Means, T. K., E. Lien, A. Yoshimura, S. Wang, D. T. Golenbock, and M. J. Fenton.** 1999. The CD14 ligands lipoarabinomannan and lipopolysaccharide differ in their requirement for Toll-like receptors. J. Immunol. **163:** 6748–6755.
64. **Means, T. K., S. Wang, E. Lien, A. Yoshimura, D. T. Golenbock, and M. J. Fenton.** 1999. Human toll-like receptors mediate cellular activation by Mycobacterium tuberculosis. J. Immunol. **163:**3920–3927.
65. **Medzhitov, R., and C. A. Janeway, Jr.** 1997. Innate immunity: the virtues of a nonclonal system of recognition. Cell **91:**295–298.
66. **Medzhitov, R., P. Preston-Hurlburt, and C. A. Janeway, Jr.** 1997. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature **388:**394–397.
67. **Medzhitov, R., P. Preston-Hurlburt, E. Kopp, A. Stadlen, C. Chen, S. Ghosh, and C. A. Janeway, Jr.** 1998. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol. Cell **2:**253–258.
68. **Michelsen, K. S., A. Aicher, M. Mohaupt, T. Hartung, S. Dimmeler, C. J. Kirschning, and R. R. Schumann.** 2001. The role of Toll-like receptors (TLRs) in bacteria-induced maturation of murine dendritic cells—PGN and LTA are inducers of DC maturation and require TLR2. J. Biol. Chem. **276:**25680–25686.
69. **Minden, A., A. Lin, F. X. Claret, A. Abo, and M. Karin.** 1995. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell **81:**1147–1157.
70. **Miura, Y., R. Shimazu, K. Miyake, S. Akashi, H. Ogata, Y. Yamashita, Y. Narisawa, and M. Kimoto.** 1998. RP105 is associated with MD-1 and transmits an activation signal in human B cells. Blood **92:**2815–2822.
71. **Miyake, K., R. Shimazu, J. Kondo, T. Niki, S. Akashi, H. Ogata, Y. Yamashita, Y. Miura, and M. Kimoto.** 1998. Mouse MD-1, a molecule that is physically associated with RP105 and positively regulates its expression. J. Immunol. **161:**1348–1353.
72. **Miyake, K., Y. Yamashita, Y. Hitoshi, K. Takatsu, and M. Kimoto.** 1994. Murine B cell proliferation and protection from apoptosis with an antibody against a 105-kD molecule: unresponsiveness of X-linked immunodeficient B cells. J. Exp. Med.**180:**1217–1224.
73. **Miyake, K., Y. Yamashita, M. Ogata, T. Sudo, and M. Kimoto.** 1995. RP105, a novel B cell surface molecule implicated in B cell activation, is a member of the leucine-rich repeat protein family. J. Immunol. **154:**3333–3340.
74. **Mizuguchi, K., J. S. Parker, T. L. Blundell, and N. J. Gay.** 1998. Getting knotted: a model for the structure and activation of Spatzle. Trends Biochem. Sci. **23:**239–242.
75. **Morath, S., A. Geyer, and T. Hartung.** 2001. Structure-function relationship of cytokine induction by lipoteichoic acid from Staphylococcus aureus. J. Exp. Med.**193:**393–397.
76. **Morisato, D., and K. V. Anderson.** 1994. The spatzle gene encodes a component of the extracellular signaling pathway establishing the dorsal-ventral pattern of the Drosophila embryo. Cell **76:**677–688.
77. **Muzio, M., G. Natoli, S. Saccani, M. Levrero, and A. Mantovani.** 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.
78. **Muzio, M., N. Polentarutti, D. Bosisio, M. K. Prahladan, and A. Mantovani.** 2000. Toll-like receptors: a growing family of immune receptors that are differentially expressed and regulated by different leukocytes. J. Leukoc. Biol. **67:**450–456.
79. **Nick, J. A., N. J. Avdi, P. Gerwins, G. L. Johnson, and G. S. Worthen.** 1996. Activation of a p38 mitogen-activated protein kinase in human neutrophils by lipopolysaccharide. J. Immunol. **156:**4867–4875.
80. **Nishiguchi, M., M. Matsumoto, T. Takao, M. Hoshino, Y. Shimonishi, S. Tsuji, N. A. Begum, O. Takeuchi, S. Akira, K. Toyoshima, and T. Seya.** 2001. Mycoplasma fermentans lipoprotein M161Ag-induced cell activation is mediated by Toll-like receptor 2: role of N-terminal hydrophobic portion in its multiple functions. J. Immunol. **166:**2610–2616.
81. **Ogata, H., I. Su, K. Miyake, Y. Nagai, S. Akashi, I. Mecklenbrauker, K. Rajewsky, M. Kimoto, and A. Tarakhovsky.** 2000. The toll-like receptor protein RP105 regulates lipopolysaccharide signaling in B cells. J. Exp. Med. **192:**23–29.
82. **Ogura, Y., D. K. Bonen, N. Inohara, D. L. Nicolae, F. F. Chen, R. Ramos, H. Britton, T. Moran, R. Karaliuskas, R. H. Duerr, J.-P. Achkar, S. R. Brant, T. M. Bayless, B. S. Kirschner, S. B. Hanauer, G. Nunez, and J. H. Cho.** 2001. A frameshift mutation in *NOD2* associated with suceptibility to Crohn's disease. Nature**411:**603–606.
83. **Ogura, Y., N. Inohara, A. Benito, F. F. Chen, S. Yamaoka, and G. Nunez.** 2001. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappa B. J. Biol. Chem. **276:**4812–4818.
84. **Ohashi, K., V. Burkart, S. Flohe, and H. Kolb.** 2000. Heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. J. Immunol. **164:**558–561.
85. **Okamura, Y., M. Watari, E. S. Jerud, D. W. Young, S. T. Ishizaka, J. Rose, J. C. Chow, and J. F. Strauss III.** 2001. The EDA domain of fibronectin activates toll-like receptor 4. J. Biol. Chem. **276:**10229–10233.
86. **Opitz, B., N. W. Schroder, I. Spreitzer, K. S. Michelsen, C. J. Kirschning, W. Hallatschek, U. Zahringer, T. Hartung, U. B. Gobel, and R. R. Schumann.** 2001. Toll-like receptor (TLR)-2 mediates Treponema glycolipid and lipoteichoic acid (LTA)-induced NF-kappa B translocation. J. Biol. Chem. **276:**22041–22047.
87. **Ozinsky, A., D. M. Underhill, J. D. Fontenot, A. M. Hajjar, K. D. Smith, C. B. Wilson, L. Schroeder, and A. Aderem.** 2000. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. Proc. Natl. Acad. Sci. USA **97:**13766–13771.
88. **Palsson, E. M., M. Popoff, M. Thelestam, and L. A. O'Neill.** 2000. Divergent roles for Ras and Rap in the activation of p38 mitogen-activated protein kinase by interleukin-1. J. Biol. Chem. **275:**7818–7825.
89. **Philpott, D. J., S. Yamaoka, A. Israel, and P. J. Sansonetti.** 2000. Invasive Shigella flexneri activates NF-kappa B through a lipopolysaccharide-dependent innate intracellular response and leads to IL-8 expression in epithelial cells. J. Immunol. **165:**903–914.
90. **Pisetsky, D. S.** 1996. Immune activation by bacterial DNA: a new genetic code. Immunity **5:**303–310.
91. **Poltorak, A., X. He, I. Smirnova, M. Y. Liu, C. V. Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton, and B. Beutler.** 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science **282:**2085–2088.
92. **Poltorak, A., P. Ricciardi-Castagnoli, S. Citterio, and B. Beutler.** 2000. Physical contact between lipopolysaccharide and Toll-like receptor 4 revealed by genetic complementation. Proc. Natl. Acad. Sci. USA **97:**2163–2167.
93. **Qureshi, S. T., L. Lariviere, G. Leveque, S. Clermont, K. J. Moore, P. Gros, and D. Malo.** 1999. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). J. Exp. Med. **189:**615–625.
94. **Raetz, C. R.** 1990. Biochemistry of endotoxins. Annu. Rev. Biochem. **59:** 129–170.
95. **Rietschel, E. T., T. Kirikae, F. U. Schade, U. Mamat, G. Schmidt, H.**

Loppnow, A. J. Ulmer, U. Zahringer, U. Seydel, F. Di Padova, et al. 1994. Bacterial endotoxin: molecular relationships of structure to activity and function. FASEB J.**8:**217–225.
96. **Rock, F. L., G. Hardiman, J. C. Timans, R. A. Kastelein, and J. F. Bazan.** 1998. A family of human receptors structurally related to Drosophila Toll. Proc. Natl. Acad. Sci. USA **95:**588–593.
97. **Roshak, A. K., K. M. Anderson, S. D. Holmes, Z. Jonak, B. Bolognese, J. Terrett, and L. A. Marshall.** 1999. Anti-human RP105 sera induce lymphocyte proliferation. J. Leukoc. Biol. **65:**43–49.
98. **Schroder, N. W., B. Opitz, N. Lamping, K. S. Michelsen, U. Zahringer, U. B. Gobel, and R. R. Schumann.** 2000. Involvement of lipopolysaccharide binding protein, CD14, and Toll-like receptors in the initiation of innate immune responses by Treponema glycolipids. J. Immunol. **165:**2683–2693.
99. **Schromm, A. B., E. Lien, P. Henneke, J. C. Chow, A. Yoshimura, H. Heine, E. Latz, B. G. Monks, D. A. Schwartz, K. Miyake, and D. T. Golenbock.** 2001. Molecular genetic analysis of an endotoxin nonresponder mutant cell line. A point mutation in a conserved region of md-2 abolishes endotoxin-induced signaling. J. Exp. Med. **194:**79–88.
100. **Schwandner, R., R. Dziarski, H. Wesche, M. Rothe, and C. J. Kirschning.** 1999. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. J. Biol. Chem. **274:**17406–17409.
101. **Shimazu, R., S. Akashi, H. Ogata, Y. Nagai, K. Fukudome, K. Miyake, and M. Kimoto.** 1999. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J. Exp. Med. **189:**1777–1782.
102. **Smith, C. L., and R. DeLotto.** 1992. A common domain within the proenzyme regions of the Drosophila snake and easter proteins and Tachypleus proclotting enzyme defines a new subfamily of serine proteases. Protein Sci. **1:**1225–1226.
103. **Takada, H., Y. Kawabata, R. Arakaki, S. Kusumoto, K. Fukase, Y. Suda, T. Yoshimura, S. Kokeguchi, K. Kato, T. Komuro, N. Tanaka, M. Saito, T. Yoshida, M. Sato, and S. Kotani.** 1995. Molecular and structural requirements of a lipoteichoic acid from *Enterococcus hirae* ATCC 9790 for cytokine-inducing, antitumor, and antigenic activities. Infect. Immun. **63:**57–65.
104. **Takeuchi, O., K. Hoshino, and S. Akira.** 2000. TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. J. Immunol.**165:**5392–5396.
105. **Takeuchi, O., K. Hoshino, T. Kawai, H. Sanjo, H. Takada, T. Ogawa, K. Takeda, and S. Akira.** 1999. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity **11:**443–451.
106. **Takeuchi, O., A. Kaufmann, K. Grote, T. Kawai, K. Hoshino, M. Morr, P. F. Muhlradt, and S. Akira.** 2000. 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.
107. **Takeuchi, O., T. Kawai, P. F. Muhlradt, M. Morr, J. D. Radolf, A. Zychlinsky, K. Takeda, and S. Akira.** 2001. Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int. Immunol. **13:**933–940.
108. **Takeuchi, O., T. Kawai, H. Sanjo, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, K. Takeda, and S. Akira.** 1999. TLR6: a novel member of an expanding toll-like receptor family. Gene **231:**59–65.
109. **Tauszig, S., E. Jouanguy, J. A. Hoffmann, and J. L. Imler.** 2000. Toll-related receptors and the control of antimicrobial peptide expression in Drosophila. Proc. Natl. Acad. Sci. USA **97:**10520–10525.
110. **Underhill, D. M., A. Ozinsky, A. M. Hajjar, A. Stevens, C. B. Wilson, M. Bassetti, and A. Aderem.** 1999. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature **401:**811–815.
111. **Underhill, D. M., A. Ozinsky, K. D. Smith, and A. Aderem.** 1999. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Proc. Natl. Acad. Sci. USA **96:**14459–14463.
112. **Vasselon, T., W. A. Hanlon, S. D. Wright, and P. A. Detmers.** Toll-like receptor 2 (TLR2) mediates activation of stress-activated MAP kinase p38. J. Leukoc. Biol., in press.
113. **Wagner, H.** 2001. Toll meets bacterial CpG-DNA. Immunity **14:**499–502.
114. **Wang, Q., R. Dziarski, C. J. Kirschning, M. Muzio, and D. Gupta.** 2001. Micrococci and peptidoglycan activate the TLR2-myd88-IRAK-TRAF-NIK-IKK-NF-kappaB signal transduction pathway that induces transcription of interleukin-8. Infect. Immun. **69:**2270–2276.
115. **Werts, C., R. I. Tapping, J. C. Mathison, T. H. Chuang, V. Kravchenko, I. Saint Girons, D. A. Haake, P. J. Godowski, F. Hayashi, A. Ozinsky, D. M. Underhill, C. J. Kirschning, H. Wagner, A. Aderem, P. S. Tobias, and R. J. Ulevitch.** 2001. Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. Nat. Immunol. **2:**346–352.
116. **Wesche, H., W. J. Henzel, W. Shillinglaw, S. Li, and Z. Cao.** 1997. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. Immunity **7:**837–847.
117. **Wesche, H., C. Korherr, M. Kracht, W. Falk, K. Resch, and M. U. Martin.** 1997. The interleukin-1 receptor accessory protein (IL-1RAcP) is essential for IL-1-induced activation of interleukin-1 receptor-associated kinase (IRAK) and stress-activated protein kinases (SAP kinases). J. Biol. Chem. **272:**7727–7731.
118. **Whitham, S., S. P. Dinesh-Kumar, D. Choi, R. Hehl, C. Corr, and B. Baker.** 1994. The product of the tobacco mosaic virus resistance gene N: similarity to toll and the interleukin-1 receptor. Cell **78:**1101–1115.
119. **Williams, M. J., A. Rodriguez, D. A. Kimbrell, and E. D. Eldon.** 1997. The 18 wheeler mutation reveals complex antibacterial gene regulation in Drosophila host defense. EMBO J. **16:**6120–6130.
120. **Wyllie, D. H., E. Kiss-Toth, A. Visintin, S. C. Smith, S. Boussouf, D. M. Segal, G. W. Duff, and S. K. Dower.** 2000. Evidence for an accessory protein function for Toll-like receptor 1 in anti-bacterial responses. J. Immunol. **165:**7125–7132.
121. **Xu, Y., X. Tao, B. Shen, T. Horng, R. Medzhitov, J. L. Manley, and L. Tong.** 2000. Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. Nature **408:**111–115.
122. **Yang, R. B., M. R. Mark, A. Gray, A. Huang, M. H. Xie, M. Zhang, A. Goddard, W. I. Wood, A. L. Gurney, and P. J. Godowski.** 1998. Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. Nature **395:**284–288.
123. **Yoshimura, A., E. Lien, R. R. Ingalls, E. Tuomanen, R. Dziarski, and D. Golenbock.** 1999. Recognition of gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. J. Immunol. **163:**1–5.

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