

Review article

The Toll receptor family

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Toll is a *Drosophila* gene that is essential for ontogenesis and antimicrobial resistance in the insect (1, 2). Genetic analysis has revealed that this gene controls dorsal-ventral polarization in the fruit fly, as well as immunity to fungal infections. The recognition of sequence homology between the intracellular domain of Toll and that of the mammalian IL-1 receptor (IL-1R) merged classical *Drosophila* genetics with more conventional cytokine/innate immunity research (3). A Toll homologue was then identified in man and mice; it induces cytokine production and expression of co-stimulatory molecules (4). Subsequently, several Toll-like receptors (TLR) have been identified and cloned (TLR1 to 9). Here we will briefly review TLR structure, ligand recognition, signal transduction, and expression pattern, and speculate about their functional role.

The *Drosophila* Toll receptor

Insects respond to infection with antimicrobial peptides rapidly produced by the fat body and hemocytes; *Drosophila* is the insect in which the humoral response to infection is best understood (5). In particular, upon microbial invasion, a signaling cascade by “immune receptors” activates the antimicrobial peptides gene. Two distinct membrane-bound receptors have been identified in the fruit fly that play a crucial role in the natural immune response; namely, Toll and 18-Wheeler. After infection, the ligand of Toll, Spätzle, binds to the cognate receptor and recruits the adapter protein Tube and the Ser/Thr kinase Pelle; this subsequently results in phosphorylation and subsequent

degradation of Cactus and release of active Dorsal (2), an event that finally drives transcription of antimicrobial peptides genes.

Recent genetic analysis of the role of Toll in antifungal resistance suggests that activation of a protease upstream of Toll is essential for the production of antimicrobial peptides; these results suggest that Toll may not function as a pattern recognition receptor itself; rather, it may be a downstream component in the immunity cascade after microbial invasion (6).

The *Drosophila* gene product 18-Wheeler is a membrane-bound receptor that also plays a role in the innate immune response of *Drosophila* through a similar signaling pathway (7). Significantly, Toll and 18-Wheeler belong to the same gene family and share sequence similarity. Both Toll and 18-Wheeler are type I transmembrane receptors with an extracellular domain containing several leucine-rich repeats (LRR), and a cytoplasmic portion intriguingly similar to the cytoplasmic domain of the human interleukin-1 receptor (IL-1R) (3).

The Toll receptor family in mammals

IL-1 is a primary proinflammatory cytokine that generates systemic and local responses to infection, injury, and immunologic challenges (8). A connection between the IL-1R (in mammals) and Toll (in *Drosophila*) has been proposed on the basis of the sequence similarity shared by their intracellular portions (3, 9). Moreover, Toll induces Dorsal activation,

which is a homologue of the human NF- κ B transcription factor that is strongly induced upon IL-1 stimulation of the cells (8).

Given this, it was reasonable to assume the existence of a yet to be identified orthologue of Toll in man. As expected, Janeway's group has recently identified and cloned the first human homologue of *Drosophila* Toll (4). After this, by systematic BLAST searches of partial cDNAs databases, several other Toll homologues have been identified. Up to now, nine human and mouse cDNAs encoding Toll-like receptors (TLR) have been cloned (4, 10–12) (see Table 1 to retrieve TLR sequences from the NCBI database: <http://www.ncbi.nlm.nih.gov/>).

Toll receptor family structural features

Toll-like receptors (TLR) are type I transmembrane proteins (i.e., the C-terminal portion is intracellular, and the N-terminal portion is extracellular with a single transmembrane domain); they have been grouped into the same gene family by their sequence similarity. Six human members have been partially characterized, namely, TLR1 to TLR6 (4, 10–12), but there is evidence for the existence of many more in private and public databases (Table 1). All of them show peculiar structural features. Several leucine-rich repeats (LRR) are present in the extracellular domain of the molecule; TLR cross the cytoplasmic membrane once, and their intracellular portion is extremely similar to the cytoplasmic domain of both the *Drosophila* Toll and the IL-1 receptor (IL-1R) (3).

Although a specific ligand for the *Drosophila* Toll receptor has been proposed on the basis of genetic analysis (namely, Spätzle), no Spätzle orthologue has been identified in vertebrates yet; in this respect, human TLR are still orphan receptors. On the other hand, several microbial molecules or pathogen-associated molecular patterns (PAMP, see paragraph below), such as lipoproteins and lipopolysaccharides, and whole live or killed organisms have been found to bind specifically and/or activate TLR as “opportunistic ligands” (see Table 2 for a schematic representation) (Refs. 13–29). It is important to note that direct evidence of binding to TLR4 has been provided only for lipopolysaccharide (LPS) (30). Other microbial components have yet to be formally identified as TLR ligands; nevertheless, they all specifically activate either TLR2 or TLR4 by triggering signaling, gene activation, and immune response to infection. These observations point to the crucial role of TLR in mediating innate immunity.

It is important to note that an extracellular protein, MD-2, binds to TLR4, and confers LPS responsiveness on the cells (27). In addition, it has been recently

reported that the intracellular protein HSP60 can bind and stimulate TLR4 when added to the cell culture (28). However, a canonical cytokine-like TLR4 ligand is still missing.

The Toll receptor family signaling pathway

As previously mentioned, TLR are type I orphan receptors with an extracellular portion containing LRR, and a cytoplasmic domain significantly similar to the intracellular portion of the IL-1R (3, 10); these observations suggest that IL-1R and TLR may use an analogous molecular framework for signaling.

IL-1 triggers the activation of various transcription factors, including nuclear factor kappa B (NF- κ B), which is essential to drive transcription of several secondary inflammatory cytokine genes (8). The molecular events occurring from the IL-1R signaling complex to the induction of NF- κ B activity flow through a biochemical cascade of protein–protein interactions. After binding to IL-1, the IL-1R and the IL-1R accessory protein (IL-1RAcP) couple, and their corresponding cytoplasmic portions aggregate to form an active IL-1R signaling complex that includes, at least, the adapter molecule MyD88 and two putative Ser/Thr kinases, namely, IRAK and IRAK2 (31–33). It is interesting to note that the intracellular domain of the IL-1R and IL-1RAcP interact with the adapter MyD88 via a so-called homophilic interaction wherein similar domains bind to each other.

MyD88 has a modular structure; the C-terminal domain that binds the IL-1R can be also called the TIR module (for Toll-IL-1R module); on the other hand, the N-terminal portion is the “death domain” module (dd module) that was first found in apoptotic proteins but has been recently identified as an interaction domain. The dd module of MyD88 recruits both IRAK and IRAK-2 to the IL-1 receptor signaling complex (again by a homophilic interaction). Further downstream, IRAK and IRAK2 interact with the adapter molecule TRAF6 that bridges them to the protein kinases TAK-1 and NIK (32, 34–36). These, and other protein kinases yet to be identified, will finally activate the I- κ B kinase complex (including the two I- κ B kinases IKK α and IKK β and the scaffold protein NEMO/IKK γ) that directly phosphorylates I- κ B α (32–42) (Fig. 1 presents a schematic representation).

Since TLR4 shares sequence similarity with distinct members of the IL-1R family, we have investigated, on the basis of our previous efforts on IL-1, the signaling pathway activated by TLR4. We found that TLR4 utilizes most of the IL-1 signaling mediators. In particular, the cytoplasmic tail of TLR4 recruits the adapter protein MyD88 and the Ser/Thr kinase IRAK (but not IRAK-2) to the signaling complex. IRAK subsequently interacts with TRAF6, which bridges to

the NF- κ B inducing kinase NIK. Finally, NIK activates the IKK complex, leading to NF- κ B activation (43) (Fig. 1).

The Toll receptor family and innate immunity

The complex of defense responses generated upon encounter with microorganisms or foreign antigens is collectively referred to as immunity or the immune response (44, 45). A number of primary immune defense mechanisms are present before, and independently of, exposure to infectious agents or foreign macromolecules; are not enhanced by subsequent exposures; and are activated by distinct molecular structures that are invariant and essential constituents of many different microorganisms (and are therefore called pathogen-associated molecular patterns [PAMP]) (45). These primary defense mechanisms are generally referred to as innate immunity and fulfill two different tasks: they provide a first line of defense against invasion by microorganisms, and (as anticipated by theoretical biologists and later experimentally confirmed) (46) they generate the instructive signals that are required for the induction of a slower acting, more persistent, and specific form of immune response, the adaptive immunity. Innate immune recognition is mediated by either soluble or cell-membrane-associated recognition molecules. One of them, CD14, is present both in the serum and as a surface-bound protein on myelomonocytic cells: by binding LPS and transferring it to the membrane of macrophages, it sets in motion the cellular response to LPS (47).

In addition to this, the Toll receptor family has been recently recognized as a central component of the innate immune system (48, 49). The first TLR family member to be identified, namely, TLR4, was later shown to induce the expression of proinflammatory cytokines and costimulatory molecules, which in turn are required

Table 1. Human Toll-like receptors cDNAs at NCBI database. (<http://www.ncbi.nlm.nih.gov/>)

Name	Gene bank accession number
TLR1	U88540; NM-003263
TLR2 = TIL4	U88878; NM-003264; AF051152
TLR3	U88879; NM-003265
TLR4 = hToll	U88880; NM-003266; U93091
TLR5 = TIL3	U88881; AF051151
TLR6	NM-006068; AB020807
TLR7	AF240467; AF245702; NM-016562
TLR8	NM-016610; AF245703; AF246971
TLR9	AF245704; NM-017442
TLR9 isoform A (alternative splicing)	AF259262
TLR9 isoform B (alternative splicing)	AF259263

for naive T-cell activation (4). Subsequently, several TLR have been identified, mainly by sequence homology (10–12). Shortly after the identification of so many TLR family members, independent research led to the identification of TLR4 as the critical transducer of LPS-elicited signals (25); in the following months, several groups identified TLR4 or TLR2 as receptors for various microbial components such as lipoteichoic acid, mannans of the yeast wall, peptidoglycan, bacterial lipoproteins, and components of the mycobacterial cell wall (Table 1) (13–29, 50–55). Given all these observations, it is apparent that TLR play a key role as fine regulators of the molecular switch from innate to adaptive immune.

The Toll receptor family expression pattern

Despite the assumption that TLR mediate innate immune response, their expression pattern in immunocompetent cells has been ill defined. TLR belong to a numerous family still expanding; their number may reflect specialized functions, redundancy, and/or differential expression and roles in different cell types. We

Table 2. Toll-like receptors and their corresponding activators

TLR2 activators	TLR4 activators
Soluble peptidoglycan (sPGN) (from <i>Staphylococcus aureus</i>) (13, 14)	Lipopolysaccharide (LPS) (from <i>S. minnesota</i> , <i>E. coli</i>) (25, 26)
Heat-killed <i>S. aureus</i> , <i>Streptococcus pneumoniae</i> (14)	Synthetic lipid A (<i>E. coli</i> type) (26)
Lipopeptides (from <i>Mycoplasma fermentans</i>) (15, 16)	Lipoteichoic acid (LTA) (from <i>S. aureus</i> and <i>St. sanguis</i>) (26)
Viable <i>Borrelia burgdorferi</i> (16)	MD2 (27)
Whole <i>Mycobacterium avium</i> (16)	HSP60 (28)
Whole <i>S. aureus</i> (16)	Taxol (29)
Lipoteichoic acid (LTA) (from <i>Bacillus subtilis</i> , <i>St. pyogens</i> , <i>St. sanguis</i>) (13)	
Lipopeptides (from <i>Bo. burgdorferi</i>) (16, 17)	
Lipopeptides (from <i>Treponema pallidum</i>) (16)	
Lipoarabinomannans (LAM) (from avirulent mycobacterium) (18)	
Heat-killed <i>Listeria monocytogenes</i> (19)	
Synthetic bacterial lipopeptide (sBLP) (20)	
Murein lipoprotein (MLP) (from <i>Escherichia coli</i>) (20)	
19-kDa lipoprotein antigen (from <i>Mycobacterium tuberculosis</i> H ₃₇ Rv) (21)	
Whole <i>M. tuberculosis</i> (22)	
Viable <i>M. tuberculosis</i> (23)	
Yeast particle zymosan (24)	

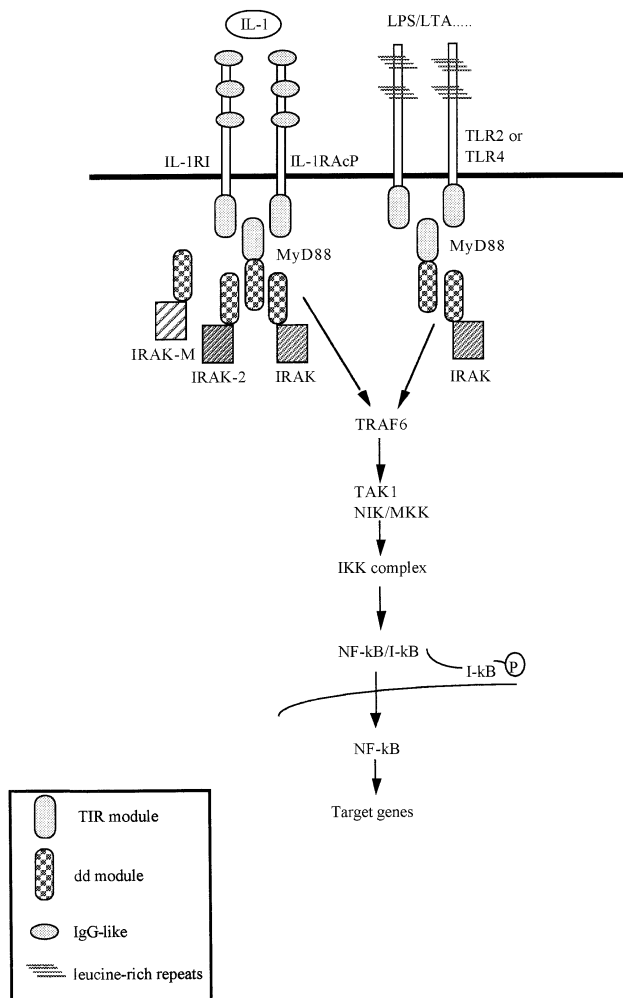


Figure 1. Overview of IL-1 and TLR signaling pathways: signaling cascade that flows from signaling complex to activation of NF- κ B. TIR indicates Toll/interleukin-1 receptor domain; dd indicates death domain.

recently designed a study to characterize systematically the pattern of expression of the first five TLR in human leukocytes. The results obtained demonstrate differential expression and regulation of TLR and suggest a novel classification of these molecules.

We separated fresh human monocytes, T cells, NK cells and polymorphonuclear granulocytes (PMN), B cells, Th1 or Th2 lymphocytes, and dendritic cells (DC). Total RNA was extracted from the cells and analyzed by Northern blot to detect specific TLR transcripts. TLR1 mRNA was ubiquitously expressed. In contrast, TLR2 to 5 showed a restricted pattern of expression; in particular, TLR2, TLR4, and TLR5 were present in monocytes, PMN, and DC. TLR3 was exclusively expressed by DC, but absent in all the other leukocytes analyzed (56).

Exposure to bacterial products, such as LPS, or to primary cytokines, such as IFN- γ , increased TLR4 expression. In contrast, TLR2 was unaffected by LPS

but increased by distinct Gram-positive bacterial components. All in all, these observations suggest that TLR4 and TLR2 expression can be differentially regulated at sites of infection or inflammation, either directly by bacterial components, or indirectly by primary cytokines (56).

After culture in the presence of GM-CSF, IL-4 or IL-13 for days, precursor monocytes differentiate into DC. Upon additional exposure to inflammatory signals (such as TNF- α , IL-1 β , or LPS), they undergo functional maturation. DC are unique in that they have the whole repertoire of five characterized TLR receptors and are the only cell type to express appreciable levels of TLR3 mRNA. This full repertoire may reflect the unique role of DC in sensing pathogens and causing transition from innate to specific immunity (56).

Collectively, these observations suggest that it may be useful to classify TLR by their pattern of expression in ubiquitous (TLR1), restricted (TLR2, TLR4, and TLR5), and specific (TLR3) TLR molecules.

Concluding remarks

TLR are a family of transmembrane receptors some of which have been clearly demonstrated to play a key role in innate immunity. TLR2 and TLR4 signaling pathways have been extensively studied. The recently identified TLR signaling mediators may be important pharmacologic targets, and the future identification of all the components of the LPS signaling cascade will be an invaluable platform for designing therapeutic interventions.

In spite of the fact that *Drosophila* Toll has a putative cognate ligand (Spätzle), no Spätzle orthologue has been identified and no cytokines have been shown to bind and/or activate any vertebrate TLR; in this respect, TLR are still orphan receptors. On the other hand, several microbial components act as opportunistic ligands.

Finally, why are there so many TLR, with similar structure and, perhaps in part, similar ligand recognition (e.g., TLR2 and TLR4 for LTA)? We may speculate that redundancy may render at least certain TLR functions in mammals robust; i.e., relatively insensitive to quantitative or qualitative alterations of individuals members of the TLR family. Alternately, strict regulation of their expression in certain cell types, and within certain microenvironments, may confer some "specificity" on the innate immune system.

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