The interleukin-1 receptor/Toll-like receptor superfamily: signal generators for pro-inflammatory interleukins and microbial products

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Abstract: The interleukin-1 (IL-1) receptor/Toll-like receptor (TLR) superfamily is a recently defined and expanding group of receptors that participate in host responses to injury and infection. The superfamily is defined by the Toll/IL-1 receptor (TIR) domain, which occurs in the cytosolic region of family members, and is further subdivided into two groups based on homology to either the Type I IL-1 receptor or *Drosophila* Toll receptor extracellular domain. The former group includes the receptor for the important Th1 cytokine IL-18, and T1/ST2, which may have a role in Th2 cell function. The latter group includes six mammalian TLRs, including TLR2 and TLR4, that largely mediate the host response to gram-positive and gram-negative bacteria, respectively. Whether bacterial products are actual ligands for TLRs, or whether they generate ligands via as yet unidentified pattern recognition receptors, has yet to be determined. Signaling pathways activated via the TIR domain trigger the activation of downstream kinases, and transcription factors such as NF-κB, and involve the adaptor protein MyD88, which itself contains a TIR domain. *J. Leukoc. Biol.* 67: 508–514; 2000.

Key Words: *TIR domain · MyD88 · TLR4 · innate immunity · signal transduction*

INTRODUCTION

Interleukin-1 (IL-1) is a pro-inflammatory cytokine that has a crucial role in the inflammatory response and in autoimmune diseases such as rheumatoid arthritis. It induces a pro-inflammatory phenotype in cells through engagement with the cell surface Type I IL-1 receptor (IL-1RI), which initiates a signaling cascade culminating in altered gene expression [1]. In 1991, Gay and Keith drew attention to the fact that the IL-1RI had significant homology in its cytosolic region to the *Drosophila melanogaster* protein Toll [2]. Toll was originally shown to have a role in developmental signals but is now known to also be involved in host defense in the adult fly. The description of additional homologs led to the definition of the IL-1R/Toll-like receptor (TLR) superfamily, based on the homologous cytosolic domain, which is now termed the Toll/IL-1R (TIR) domain. The family is divided into two subgroups, depending on whether the extracellular region of a member displays homology to the immunoglobulin-like IL-1RI or the leucine-rich repeat motif of the Toll receptor, although some TIR domain-containing proteins exist that do not obviously fit into either subgroup, such as MyD88.

Previously, we described how the family represents a paradigm for the response to infection and environmental stress in diverse organisms [3]. The conservation across different species of both the TIR domain and downstream signaling mechanisms was discussed, with particular emphasis on the kinases involved in signal transduction. This review will attempt to summarize recent developments in the IL-1R/TLR family.

NEW MEMBERS OF THE FAMILY

Figure 1 illustrates the current published IL-1R/TLR family members. A consensus sequence for the TIR domain is also shown, based on an alignment of 31 family members. Although the TIR domain is a hallmark of the family, relatively little is known as to its specific structure and role in signaling. However, it is highly likely to mediate homotypic interactions with other TIR domains [4, 5]. Although there are other regions of significant homology, the three regions highlighted, called Box 1, 2, and 3, seem to be particularly important. Box 1 is a signature sequence of the family, whereas Box 2 and 3 contain amino acids shown to be important in signaling, based on mutational analysis of mainly IL-1R1 [6], but also now of TLR2 and TLR4 (see below). Sub-consensus sequences for the IL-1R, TLR, and plant branches of the family have also been calculated [7]. Structure predictions for the TIR domain suggest that it might be similar to the bacterial chemotaxis regulator CheY, in which case a role as a conformational trigger for signaling, rather than a passive scaffold upon which receptor complexes are assembled, would be implied [7].

The family broadly splits into those molecules that are IL-1RI-like, in that they have three extracellular immunoglobulin domains, and those that are Toll-like, in that they have extracellular leucine-rich repeats.

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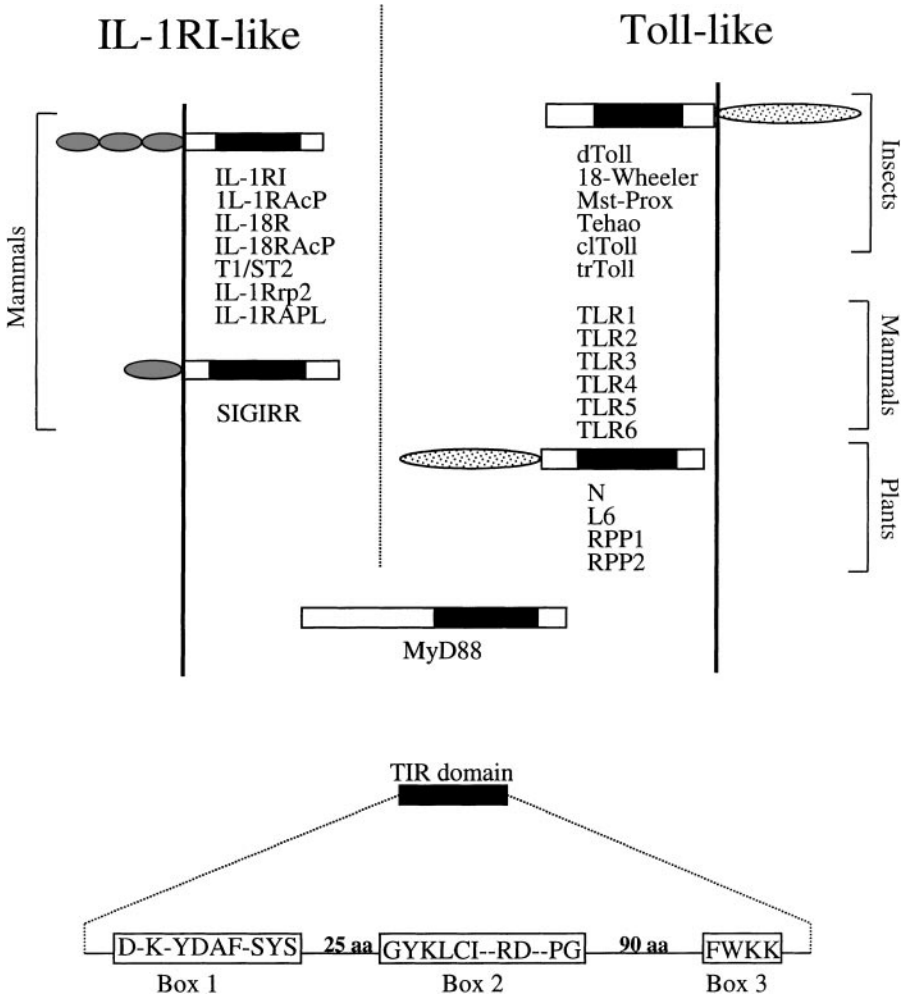

Fig. 1. The IL-1R/TLR superfamily. The family is defined by the presence of the TIR domain (solid bars), which is involved in signaling, and is subdivided into two groups based on homology to either IL-1R-like extracellular immunoglobulin loops (shaded elipses) or Toll-like leucine-rich repeats (stippled elipses). The family includes members from mammals, insects, and plants. A consensus sequence for three conserved regions of the TIR domain, based on the alignment of 31 family members, is shown. See text for details.

IL-1RI-like members

In this subgroup, IL-1RI and the IL-1 receptor accessory protein (IL-1RAcP) have been previously described [reviewed in ref. 3]. The IL-1 receptor-related protein (IL-1Rrp) has been renamed IL-18R, given its now recognized role in IL-18 signaling. Originally identified using degenerate oligonucleotides based on conserved regions in the TIR domain, IL-1Rrp was shown to signal given the fact that a chimeric receptor incorporating the extracellular domain of IL-1R1 fused to the intracellular domain of IL-1Rrp could bind IL-1 and lead to NF-κB activation [8]. However, until recently it was an orphan receptor. Purification and identification of a receptor binding IL-18 with high affinity from a Hodgkin's disease-derived cell line, L428, revealed that it was identical to IL-1Rrp [9]. It is interesting that IL-18 is structurally similar to IL-1 [10], suggesting that both cytokines may bind to their respective receptors using similar structural features. Similar to IL-1, IL-18 also requires an accessory protein to associate with the receptor-ligand complex for signaling, and IL-18RAcP (also known as AcPL) was identified as a novel member of the family. This was shown to be essential for IL-18-mediated induction of NF-κB activity [11]. IL-18 is a key regulator of Th1 cells, in that both IL-18-deficient and IL-18R-deficient mice exhibit impaired interferon-γ (IFN-γ) production and natural killer cell and cytotoxic T cell function [12, 13].

T1/ST2 is an orphan receptor cloned 10 years ago [14, 15]. Its intracellular domain has also been shown to be capable of transducing a signal in that a chimeric receptor incorporating the extracellular domain of IL-1RI fused to the intracellular TIR-containing T1/ST2 domain transduced an IL-1-dependent signal to NF-κB [16]. Interest in T1/ST2 is likely to increase given that it has recently been implicated in directing Th2 cell function [17]. Expression of T1/ST2 was shown to be restricted to Th2 cells, and anti-T1/ST2 monoclonal antibodies attenuated Th2 responses *in vivo* [17]. The ligand and receptor accessory protein for T1/ST2 have yet to be identified.

IL-1Rrp2, cloned in 1996 [18], remains an orphan receptor, although using the same chimeric receptor approach, the intracellular TIR-containing IL-1Rrp2 domain transduced an IL-1-dependent signal to NF-κB [19]. Caution must be urged in using the chimeric receptor approach to assess the potential of intracellular domains in orphan receptors to transduce a signal because signaling effects could be via the recruitment of IL-1RAcP by IL-1 bound to the extracellular region of IL-1R1 [20], rather than due to the presence of the orphan receptor TIR domain per se.

IL-1RAPL (for IL-1 receptor accessory protein-like) is the putative gene product of a novel gene that is highly expressed in the hippocampus, dentate gyrus, and entorhinal cortex, has a role in X-linked mental retardation and may have a specialized role in physiological processes underlying memory and learning ability [21]. It has an obvious TIR domain, and also three

Ig-like extracellular domains, and is most similar to IL-1RAcP. Hence IL-1RAPL may have a role in IL-1 signaling cascades in the central nervous system.

Another novel member of the family is single immunoglobulin IL-1R-related molecule (SIGIRR), which was identified from computer searches of an expressed-sequence tagged (EST) database with the use of the TIR domain [19]. As its name suggests, SIGIRR is so far a unique member of the family in that it only contains one extracellular immunoglobulin domain. Its mRNA is widely expressed. Although its intracellular domain extends that of the typical IL-1R family member by more than 70 amino acids, similar to the length of *Drosophila* Toll, its TIR domain is more closely related to IL-1RI than to Toll. In contrast to the other receptor chimeras mentioned above, IL-1RI-out/SIGIRR-in or IL-1RAcP-out/SIGIRR-in chimeras were unable to signal in response to IL-1. Furthermore, overexpression of SIGIRR failed to inhibit signaling by full-length IL-1RI. Thus the function of SIGIRR remains to be determined. It is interesting that the gene for SIGIRR mapped to 11p15-5, a cluster identified with two syndromes: Beckwith-Wiedemann syndrome, described by a number of childhood abnormalities including high incidence of tumors, and a variant of the Freeman-Sheldon syndrome, distal arthrogryposis type II [19]. The precedence for the role of IL-1R family members in bone metabolism and development hints at a possible role for SIGIRR in distal arthrogryposis type II. Similar to SIGIRR, other novel members of the family have been identified based on TIR-containing ESTs. This includes ESTs from *Caenorhabditis elegans* and strikingly the bacterium *Streptomyces coelicolor*, although these have not yet been characterized [22].

Toll-like members

As mentioned above, the *Drosophila* protein Toll defines the TLR branch of the IL-1R/TLR family. Toll is important in the establishment of dorsoventral polarity in *Drosophila* embryos, whereas in the adult fly it signals anti-fungal defense [23]. In contrast, another Toll-like receptor in *Drosophila*, 18-Wheeler, has been implicated in responses to bacteria [24]. The theme of different TLRs responding to distinct pathogens is now also apparent in the mammalian TLRs (see below). The role of two other *Drosophila* TLRs, MstProx and Tehao, remains to be determined [16, 22]. Toll homologs in other insects have been identified in the primitive dipteran *Clogmia albipunctata* (clToll) and in the beetle *Tribolium casteneum* (trToll). These proteins also have a role in dorsoventral patterning [25]. The plant disease-resistant proteins, N protein, L6, RPP1, and RPP5 are part of a plant subgroup of the family, which will likely expand presently as more plant genome information is now becoming available.

One of the most intensely studied subgroups of the family in the past year has been the mammalian TLRs. Currently, six have been published and described in detail, each displaying distinct mRNA expression patterns [7, 26, 27]. TLR1, also identified as Rsc786 [16] and TIL [28] is expressed ubiquitously and at higher levels than the other TLRs. TLR2 (also described as TIL4) is expressed in lymphoid tissue and monocytes, and particularly strongly in peripheral blood lymphocytes [29, 30]. TLR3 expression is evident in brain, heart, muscle, and lung, with alternative-length transcripts detectable in the pancreas and placenta. TLR4 expression predominates in spleen and lymphocytes, and is also strong in the heart. TLR5 (also described as TIL3) was detected in ovary, peripheral blood monocytes, leukocytes, and prostate. TLR6, the newest member, and most closely related in sequence to TLR1, is expressed predominantly in spleen, thymus, ovary, and lung.

As well as those TLRs above that have been published, a further four TLRs have been cloned by Schering Corp., who have a patent listing *DNAX Toll-like receptors 2-10* (DTLRs 2–10), available from http://patent.womplex.ibm.com as application number WO9850547.

In terms of function, TLR2 and TLR4 are the best characterized of this subgroup, and are now strongly implicated as important mediators in innate immunity (see below). Chimeras of the extracellular domain of Fas fused to the transmembrane and cytosolic domains of TLR2, TLR5, or TLR6 have been shown to activate NF-κB [26, 27], while overexpressing an epitope tagged TLR4, which may act like a subtle positive mutant, also leads to a signal to NF-κB [31]. An IL-1RI-out/TLR1-in chimera was unable, however, to signal NF-κB activation [16].

Recently it has been shown that increased expression and signaling by TLR4 occurs in the injured myocardium [32]. Because no infection is evident in this model, this raises the intriguing possibility that TLR4 may function during inflammation, possibly in response to an endogenous ligand.

DISTINCT ROLES FOR TLR2 AND TLR4 IN THE RESPONSE TO PATHOGENS

Given that *Drosophila* and plant TLRs have both been shown to be involved in the host response to microbial pathogens, it was always likely that the human TLRs would fulfill a similar role. In 1997 Medzhitov et al. showed that a constitutively active mutant of hTLR4 (then referred to as hToll) could induce the expression of the NF-κB-controlled inflammatory cytokines IL-1, IL-6, and IL-8, as well as the expression of B7.1, a co-stimulatory molecule required for the activation of naive T cells [33], thus implicating hTLR4 in innate immunity. Since then, interest has focused on the role of TLRs in responding to different microbial pathogens or their products, most notably lipopolysaccharide (LPS). LPS had been known to induce signals very similar to IL-1, and also to bind to CD14 on macrophages and somehow trigger an innate immune response [34]. CD14 itself is a glycophosphatidylinositol-anchored membrane protein lacking a cytosolic domain, and therefore unlikely to mediate a signal directly. Two groups in 1998 demonstrated that overexpression of TLR2 in mammalian cell culture rendered cells responsive to LPS in a CD14-dependent manner [29, 30]. LPS was also shown to bind to TLR2, although the affinity appeared very low [30]. However, powerful genetic evidence then implicated TLR4, rather than TLR2 in LPS signaling. Two strains of mice, C3H/HeJ and C57BL/10ScCr, were known to be hyporesponsive to LPS and highly susceptible to gram-negative infection. The responsible allele in both cases was shown to map to the gene encoding TLR4 [35, 36]. The former strain displayed a missense mutation in the Tlr4 gene,

predicted to replace a highly conserved proline in Box 2 of the TIR domain with a histidine. The latter strain displayed a null mutation of *Tlr4*. Thus disruption of TLR4 was sufficient to suppress LPS signaling, suggesting that it might have a more important role than TLR2 in mediating the LPS signaling pathway because TLR2 was normal in these mice. TLR4 has also recently been shown to require the presence of another proximal novel molecule, MD-2, in order to confer LPS responsiveness to cells [37].

Further work involving crosses between mouse strains normal and refractory to LPS activation demonstrated that the proline to histidine point mutation exerts a dominant negative effect on LPS signaling [35, 38], suggesting that the mutated protein is expressed and able to interfere with signaling, and therefore demonstrating the importance of the conserved proline to normal signal transduction. It will be interesting to determine whether the mutated TLR4 serves as a decoy receptor (binding ligand yet failing to signal) analogous to the Type II IL-1 receptor [1], or exerts an inhibitory effect by disrupting formation of a receptor signaling complex.

Both C3H/HeJ and C57BL/10ScCr mice are developmentally and immunologically normal apart from their hyporesponsivity to LPS and inability to counter gram-negative infection, which suggests that like *Drosophila* TLRs, mammalian TLRs will discriminate to a degree between different types of pathogens, and hence not be functionally redundant. This has become more obvious in the case of TLR2 and TLR4. Further work demonstrated that cells that carry a null allele for TLR2 were capable of responding to LPS [39], whereas TLR2 but not TLR4 was involved in responding to gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae* [40, 41]. Evidence has also been presented that TLR2-mediated host defense mechanisms are triggered particularly by bacterial lipoproteins, peptidoglycan and lipotechoic acid [42–44]. TLR2 also mediates activation by *Mycobacterium avium* [40] and *Borrelia burgdorferi* [42], whereas *Mycobacterium tuberculosis* utilizes both TLR2 and TLR4 [41].

Further insights into the mechanism of innate immunity triggering by TLRs came most recently from Underhill et al., who showed that TLR2 was recruited specifically to macrophage phagosomes containing yeast, and that mutations in TLR2 abrogated the inflammatory response to yeast and gram-positive bacteria, but not to gram-negative bacteria [45]. TLR4 was not involved in these responses but was still clearly required for responses to gram-negative bacteria. This study therefore further clarifies the role of TLR2 and TLR4 in host defense, suggesting that TLR2 is more important for signaling the response to yeast and gram-positive bacteria, whereas TLR4 has a primary role in responding to gram-negative bacteria, via recognition of LPS.

However, from results discussed above, and other observations such as that by Brightbill et al. [43] that an antibody to TLR2 inhibited LPS-induced IL-12 secretion from human monocytes, it is apparent that TLR2 can also respond to LPS under certain conditions. It will be interesting to determine whether activation of different human TLRs will give rise to distinct or overlapping patterns of antimicrobial gene expression. Bearing in mind that the history of receptor ligand interaction is that there are generally 3–10 receptors discovered for every ligand, there may be a similar degree of overlap with the TLRs, so that although TLR4 is clearly more important for responses to LPS, TLR2 may be one of a number of low-affinity LPS receptors. However, the true ligands of TLR2 and TLR4 are also still a matter of controversy, and comparison of the *Drosophila* Toll signaling pathway with its mammalian homolog would suggest that these ligands remain to be discovered (see below).

WHAT ARE THE TRUE LIGANDS FOR MAMMALIAN TLRS?

In the developing *Drosophila* embryo, the putative ligand for Toll is Spaetzle. In dorsoventral patterning, the generation of Spaetzle is known to involve cleavage of a pro-form by a protease cascade involving three germline-dependent serine proteases, Gastrulation defective, Snake, and Easter [46]. The existence of an alternative protease cascade that would generate Spaetzle in the response to infection had always been postulated, since deletion of these three proteases does not prevent a Toll-mediated immune response [23]. Levashina et al. recently identified what may be a negative feedback mechanism for this postulated protease cascade [47]. They showed that active Spaetzle was proteolytically generated shortly after immune challenge, and that the active ligand was constitutively present in mutant flies lacking a blood serine protease inhibitor, Spn43Ac. In wild-type cells, immune challenge led to up-regulation of the *Spn43Ac* gene, suggesting that it might function as a negative feedback mechanism to shut down the upstream proteolytic cascade. These results, together with the precedence for a proteolytic cascade leading to Spaetzle formation in development, suggest that non-self recognition by the innate immune system is an event that occurs upstream of Toll, and that the pattern recognition receptor in this pathway remains to be identified.

These results in *Drosophila* are bound to have implications for the role of mammalian TLRs in innate immunity. Furthermore, in the horseshoe crab, a similar proteolytic coagulation cascade, which can be inhibited by serpins similar to Spn43Ac, can be activated by binding of LPS to an upstream multidomain recognition protein [48]. Thus mammalian TLRs may be receptors for Spaetzle-like ligands generated by protease cascades triggered by as yet unidentified pattern recognition receptors, as shown in **Figure 2**, which also illustrates other known parallels in signaling components between Toll and TLRs in host defense. Proteases in the complement and coagulation pathways might be involved, although it might be more likely that a cell membrane-bound cascade is activated because the only soluble factors needed for LPS responsiveness *in vitro* are LPS binding protein and CD14 [49, 50].

SIGNAL TRANSDUCTION PATHWAYS

Information on the intracellular signaling pathways downstream of IL-1RI and TLRs has also increased in the last year. Most

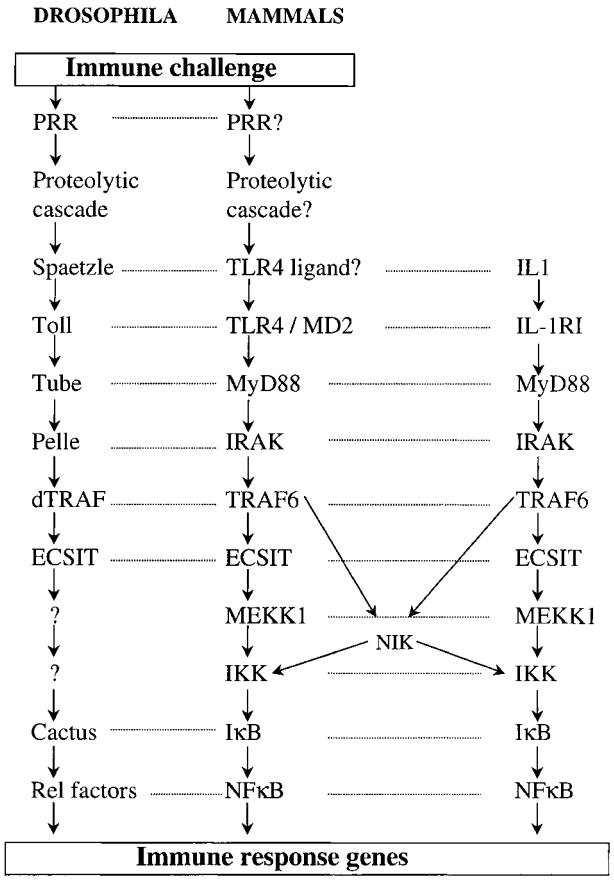

Fig. 2. Flow chart of proposed signaling pathways of *Drosophila* and mammalian host defense involving TLRs. These are represented by *Drosophila* Toll and mammalian TLR4. For comparison, the IL-1RI axis is also shown. Proteins connected by dashed lines display functional, and often structural, similarity. PRR, pattern recognition receptor. See text for details.

notably, although the mammalian TLRs probably resemble *Drosophila* Toll in the upstream arm of the pathway as described above, downstream TLR2 and TLR4 have been shown to be more like the IL-1RI system, in that both pathways require MyD88, IL-1 receptor-associated kinase (IRAK), tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), and NF-κB-inducing kinase (NIK) in order to activate NF-κB [31, 51, 52]. One difference between IL-1RI and TLR4 signaling seems to be that TLR4 can recruit MyD88 directly [31], whereas in order for the IL-1RI receptor complex to bind MyD88, the IL-1RAcP must also be present [5].

MyD88 is the functional homolog of *Drosophila* Tube in that it is an adaptor protein. Like Tube, MyD88 has a death domain involved in recruitment of the downstream serine/threonine kinase (Pelle for Tube and IRAK for MyD88), whereas in contrast to Tube it has a TIR domain, most likely involved in interactions with IL-1R1/TLR family receptor complexes. In fact, MyD88 has recently been confirmed as having a central role in the immune signaling cascades of the IL-1R1/TLR family in that in MyD88 knockout mice, the responses to IL-1, IL-18, and LPS are all abrogated [53, 54]. It is interesting that, in contrast to IL-1 and IL-18, LPS could still activate NF-κB and MAP kinases in these mice, albeit in a delayed manner, suggesting that other TLR/MyD88-independent LPS signaling pathways also exist, a conclusion substantiated by the fact that the C3H/HeJ mice lacking functional TLR4 can also respond in a muted manner to LPS [discussed in ref. 54].

Downstream of MyD88, the important role of IRAK and TRAF6 in these pathways has also been confirmed in knockout studies. Cells from IRAK-deficient mice were shown to be defective in their response to IL-1 and IL-18, whereas TRAF6-defective cells were impaired in responding to IL-1 and LPS [55, 56]. A *Drosophila* TRAF-like protein that is probably a functional analog of TRAF6 has recently been cloned [57]. A novel signaling molecule that interacts with TRAF6, leading to MEKK1 activation in mammals, and to transcription of host defense genes in *Drosophila*, has been characterized and named ECSIT (evolutionarily conserved signaling intermediate in Toll pathways) [58].

Our understanding of the events proximal to NF-κB activation has greatly increased in recent years, with the key kinases that directly and specifically phosphorylate IκB, leading to its degradation and subsequent activation of NF-κB, now identified and well characterized [reviewed in ref. 59]. These kinases, IκB kinase (IKK) α and β, exist as part of a larger IKK signalsome that contains other scaffold and regulatory proteins. IKKα and β form a core IKK complex together with IKKγ (or NEMO, NF-κB-essential modulator), which seems to be the minimal requirement for NF-κB activation. Many upstream kinases have now been shown to be capable of activating the IKK complex *in vitro*, including NIK, MEKK1, TAK1, protein kinase Cζ, and MEKK2 and 3 [60–67], which might explain how diverse stimulants can all activate NF-κB. In terms of IL-1R/TLR pathways, MEKK1 and particularly NIK are probably particularly important [31, 58]. Genetic analysis of IKK function using IKKα and β knockout mice reveals that IKKα is more important in development, whereas IKKβ, but not IKKα, is essential for the response to pro-inflammatory stimuli [reviewed in ref. 68]. It will be interesting to determine which of the IKKs are activated by TLR4. Enteroinvasive bacteria have been shown to activate both IKKα and β in cell culture models, which might be mediated by TLRs [69]. Another group showed that LPS and TNF had differential effects on monocytic IKK complexes, in terms of the kinetics and potency of IKKα and β activation, which translated into differential patterns of IκB degradation and reappearance [70]. A novel LPS-inducible IKK, IKK-i, has been identified that is capable of phosphorylating the relevant serine residues of IκB, is mainly expressed in immune cells, and is responsive to IL-1 and IL-6 in addition to LPS [71].

In summary, although the different IL-1R/TLR family members use similar signaling pathways, there may be subtleties in proteins recruited during signaling. Different receptors may utilize TIR binding proteins distinct from MyD88 that may recognize subtly different TIR domains, kinases that might activate distinct IKK signalsomes, novel IKKs, and particular combinations of NF-κB/Rel factors that would activate distinct subsets of genes. There is recent evidence for the latter case in the *Drosophila* Toll pathway, where distinct dimers of Dorsal, Dif, and Relish activate unique anti-microbial genes [72, 73].

CONCLUSIONS

The past year has seen major advances in our understanding of the overall functions of IL-1R1/TLR family members and how they signal. A number of novel members have been characterized, reflecting the importance of the TIR domain in diverse organisms for responses to injury and infection. There is likely to be a flurry of new members as more genomic DNA sequence for different organisms becomes available. Outstanding issues that remain to be resolved include the predicted role of protease cascades in hTLR signaling, the identification of further TLR activators, and the level of redundancy and specificity in signaling to gene induction by different family members, given that they now seem to employ very similar signaling cascades. Addressing these issues should bring us one step closer to rational therapeutic intervention of IL-1R1 and TLR signaling pathways.

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