Review

Summary and comparison of the signaling mechanisms of the Toll/interleukin-1 receptor family

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Abstract

The Toll/interleukin-1 (IL-1) receptor (TIR) family comprises two groups of transmembrane proteins, which share functional and structural properties. The members of the IL-1 receptor (IL-1R) subfamily are characterized by three extracellular immunoglobulin (Ig)-like domains. They form heterodimeric signaling receptor complexes consisting of receptor and accessory proteins. The members of the Toll-like receptor (TLR) subfamily recognize alarm signals that can be derived either from pathogens or the host itself. TLRs possess leucine-rich repeats in their extracellular part. TLRs can form dimeric receptor complexes consisting of two different TLRs or homodimers in the case of TLR4. The TLR4 receptor complex requires supportive molecules for optimal response to its ligand lipopolysaccharide (LPS). A hallmark of the TIR family is the cytoplasmic TIR domain that is indispensable for signal transduction. The TIR domain serves as a scaffold for a series of protein–protein interactions which result in the activation of a unique signaling module consisting of MyD88, interleukin-1 receptor associated kinase (IRAK) family members and Tollip, which is used exclusively by TIR family members. Subsequently, several central signaling pathways are activated in parallel, the activation of NFκB being the most prominent event of the inflammatory response. Recent developments indicate that in addition to the common signaling module MyD88/IRAK/Tollip, other molecules can modulate signaling by TLRs, especially of TLR4, resulting in differential biological answers to distinct pathogenic structures. Subtle differences in TLR signaling pathways are now becoming apparent, which reveal how the innate immune system decides at a very early stage the direction in which the adaptive immune response will develop. The creation of pathogen-specific mediator environments by dendritic cells defines whether a cellular or humoral response will be activated in response to the pathogen.

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1. Introduction

In mammals, challenge by bacteria and viral pathogens results in a rapid activation of the immune system to mount a fast and adequate response. The activation of specialized receptors for pathogen-associated structures on sentinel cells is translated into the production and release of endogenous alarm mediators. The master cytokines interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα), but also IL-12 and IL-18, are rapidly released by these cells of first line defense. They orchestrate the acute response required to support the local phagocytes in their effort to control the pathogens before they can spread systemically. Interestingly, the receptors recognizing many pathogen-associated structures, the human Toll-like receptors (TLRs),
and the receptors for the endogenous pro-inflammatory cytokines IL-1 and IL-18, share a cytoplasmic motif, the Toll/IL-1 receptor (TIR) domain, which is required for initiating intracellular signaling. The TIR family not surprisingly seems to utilize very similar signaling mechanisms to activate downstream effector mechanisms. While some components of the downstream signaling machinery like the adapter TNF receptor associated factor 6 (TRAF6) are shared by other receptors of proinflammatory cytokines, one signaling module is exclusively employed by the TIR family. This consists of MyD88, interleukin-1 receptor associated kinase (IRAK) family members and Tollip.

Thus, these receptors are not only grouped into one family because of the sharing of TIR domains and utilization of the MyD88/IRAK module but also functionally, as they include pivotal alarm receptors of the innate immune system. It needs to be stressed, however, that both IL-1 and IL-18 are not just mediators of the innate immune response. Both cytokines are centrally involved in the development, proliferation and differentiation of T helper cells and B cells. Thus, they represent key elements at the interface of innate and adaptive immune responses.

The aim of this review is to critically discuss the present concepts of signal initiation in the TIR family and highlight apparent differences emphasizing the initial receptor-proximal events rather than downstream effects.

2. The family of TIRs

Members of the family of TIRs have been described in vertebrates, invertebrates and plants. Some family members are important at certain developmental stages in ontogeny, the best-characterized molecule being Drosophila Toll, which is involved in the embryonic development and dorsal–ventral polarity in insects.

The main functional reason to group these molecules is that, as far as is known, all members of the TIR family are involved in protection against viral, bacterial or protozoal infections in adult organisms of animals or plants alike. They are crucial mediators of host defense (reviewed in Ref. [1]) and can be dubbed alarm receptors of the host defense system.

In addition to their biological function, there is also a clear structural reason to group these diverse molecules into one family: they share a common intracellular motif which is essential for the signaling of these receptors or adapters. This motif is called the TIR motif or the TIR domain (reviewed in Ref. [2]). With the exception of the type II IL-1 receptor (IL-1R), which does not signal, all transmembrane or cytoplasmic members of the TIR family contain this TIR domain. Signaling of this family of receptors is similar but not identical in vertebrates and invertebrates (reviewed in Ref. [3]).

The TIR family may be roughly grouped into three groups defined by their structural features (Fig. 1).

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**Fig. 1.** The human TIR family. The TIR family consists of three subfamilies, two transmembrane receptor subfamilies and the MyD88 adapter family. (Soluble receptors are not depicted for the sake of clarity.)
2.1. The IL-1R subfamily

The IL-1R subfamily comprises type I transmembrane molecules and soluble forms, which are characterized by extracellular immunoglobulin (Ig)-like domains. The prototypes of this family are the IL-1R type I [4] and its respective coreceptor molecule, the IL-1RAcP [5]. A few years ago, the subfamily member formerly known as IL-1Rlp1 [6] was identified as the α chain of the IL-18 receptor [7]. Subsequently, the IL-18 receptor β chain (IL-18RαcP) was cloned and turned out to be a structural and functional homologue to IL-1RAcP [8]. In both cases, the receptor chains (type I IL-1R, or IL-18RαcP) contain the ligand binding site, whereas the respective coreceptors IL-1RAcP or IL-18Rβ are incapable of binding the cytokine alone.

A series of orphan receptors share the features of the receptors for IL-1 and IL-18 (compared in Ref. [9]). They also contain three extracellular Ig-like domains, they are type I transmembrane molecules and possess a TIR domain in their cytoplasmic part. One family member, single Ig-domain containing IL-1R related (SIGIRR), possesses a single extracellular Ig-like domain [10], whose function is unclear. It is very likely that they are receptors or coreceptors for novel members of the IL-1 family [11–13]. For the two new forms, IL-16 and IL-1e, it was very recently shown that they function as agonist (IL-1e) and as antagonist (IL-16) of IL-1Rlp2, possibly constituting a novel ligand/receptor pair of the IL-1R subfamily [14].

The IL-1R subfamily also contain a cytoplasmic TIR domain [15]. Its function seems to be of a regulatory nature. Two mechanisms have been described for type II IL-1R which modulate the responsiveness of cells to IL-1: One is ligand sequestration [16], and the other one is competition for the coreceptor [17]. The type II IL-1R thus functions as a true decoy receptor (reviewed in Ref. [18]). The transmembrane molecules of the IL-1R subfamily are depicted on the left side of Fig. 1. Soluble IL-1Rs or the IL-18 binding protein have regulatory functions by sequestering IL-1 or IL-18; they do not initiate signal transduction.

2.2. The TLR subfamily

The TLR subfamily comprises a growing number of molecules which share leucine-rich repeat domains (reviewed in Refs. [1,19,20]). These molecules are also type I transmembrane molecules in animals, but seem to be cytoplasmic molecules in plants [21]. The members of the Toll-like subfamily also contain a cytoplasmic TIR domain which is essential for signal generation (an exception may be RP-105). The prototype of this subfamily is the Drosophila Toll protein. In the ontogeny of the fruit fly, this receptor is activated by its protein-ligand Spätzle, which initiates a signaling cascade resulting in the activation of the transcription factor Dorsal and induction of dorsal–ventral patterning [22]. In the adult fly, Toll is involved in anti-fungal protection [23], but the ligands activating Toll-mediated host defense mechanisms are not known. 18 wheeler was reported to be the insect counterpart responsible for anti-bacterial reactions [24]. This has been questioned recently as discussed in Ref. [25]. In insects, presently a total of nine TLRs were described [26] which play a role in host defense and development. Comparison of human expressed sequence tags with the Drosophila Toll sequence identified a human Toll homologue, which upon overexpression activated proinflammatory signaling pathways [27]. In this seminal publication, it was suggested for the first time that this molecule, later dubbed human TLR4, was a receptor for pathogens [27].

The human TLRs have now been identified as receptors for pathogen-associated structures (reviewed in Ref. [28]). The prototypes of this subfamily are TLR2 and TLR4. The discovery that TLRs are receptors for pathogen-associated membrane patterns and that these receptors belong to the TIR family, opened a new and permanently growing field and revived research in innate immunity (commented in Ref. [29]). TLRs recognize a variety of molecules derived from pathogens such as bacterial cell wall components and heat shock proteins (TLRs 1,2,4,6) [28–38], flagellins (TLR5) [39,40], viral dsRNA and polyI:C (TLR3) [41], and small anti-viral compounds (TLR7) [42], and CpG-DNA (TLR9) [43], but also eukaryotic proteins which appear after destruction of cells or tissues such as heat shock proteins [44] or fibrinogen [45], signs of wounding, often being a consequence of infection. In that sense, maybe TLRs should be more appropriately addressed as danger sensors, supporting the notion that the immune system differs between non-dangerous and dangerous structures rather than between self and non-self (reviewed in Ref. [46]).

Discussion of the exploding number of important publications identifying novel ligands for TLR family members would go beyond the scope and aim of this review. The evolving overall picture suggests two basic principles. First, TLRs recognize more than one ligand, which often do not share any obvious structural similarity and which can be derived from pathogens or the host. Second, TLRs form receptor complexes that may consist of at least two different TLRs, or in the case of the lipopolysaccharide (LPS) receptor, of a TLR4 homodimer requiring MD-2 [47] and CD14 (reviewed in Ref. [48]) to bind LPS. Presently, 10 coding family members have been definitely described in humans (reviewed in Refs. [49,50]). Anecdotal rumors claim even more TLRs. It is tempting to speculate that different combinations of individual TLRs will define different specificities for pathogen-associated structures.

The TLR subfamily members are differentially expressed in tissues or immune competent cells with a certain degree of specificity (Refs. [51–53], summarized in Ref. [19]). An increasing number of recent reports describe regulation of TLRs in several cell types and tissues, mainly on mRNA level, by microbial signals and cytokines demonstrating that...
regulating of TLRs adds a further level of complexity to innate immunity [54–57].

2.3. The mammalian molecule MyD88

The mammalian molecule MyD88 clearly does not fit the classification of either the IL-1R subfamily or the TLR subfamily and defines a third subfamily. Very recently, a MyD88-like protein was identified independently by two groups, one calling it MyD88-like adapter protein or MyD88-adapter-like (Mal) [58], the other TIRAP for TIR domain-containing adapter protein [59]. Neither MyD88 nor Mal/TIRAP are receptors. They are ubiquitously expressed cytoplasmic molecules characterized by a TIR domain. MyD88 contains an N-terminal death domain and is an indispensable adapter in the signaling cascade of all TIR family members [60–64]. Mal/TIRAP is 75 amino acids shorter in the N-terminus and thus lacks a death domain. It was reported to interact with TLR4 [58,59].

3. TIR complexes

3.1. The IL-1R complex

The type I IL-1R binds IL-1α, IL-1β and the receptor antagonist IL-1Ra (reviewed in Ref. [65]). The crystal structure of the extracellular part of type I IL-1R bound to IL-1 was determined [66,67]. The ligated type I IL-1R is recognized by IL-1RAcP [5]. A model for the interaction of the extracellular parts of type I IL-1R and IL-1RAcP with IL-1β was proposed [68]. The result of this interaction is the formation of a heterodimeric transmembrane receptor complex (Fig. 2A). Both molecules are required for initiation of signal transduction, lack of the coreceptor results in non-responsiveness to IL-1 [69–72]. Soluble type I or soluble type II IL-1R is involved in regulation of IL-1 responsiveness by sequestering IL-1. Ligand transfer for IL-1 has not been reported.

IL-18 binds to the IL-18 receptor α chain (IL-1Rrp1) [7], and the ligated receptor is recognized by the IL-18 β chain (AcPL) [8], forming a heterodimeric transmembrane receptor (Fig. 2B). Again both receptor chains are needed for signal transduction.

Although the molecules of the IL-1R complex and the IL-18 receptor complex are closely related, they cannot substitute for each other. The receptors are specific for their respective ligands and the coreceptors recognize only the respective ligated receptors. Of interest is the considerable
difference in affinities of the receptor complexes for their respective ligands: The affinity of the IL-1R complex for IL-1α or IL-1β is in the range of 0.2 nM [15], whereas the affinity of the IL-18 receptor complex for IL-18 is around 20 nM [7,8,73]. A soluble IL-18 binding protein exists which can efficiently neutralize IL-18 [74,75].

The depicted models of functional heterodimers are minimalistic ones. It is unclear whether the heterodimeric receptor complexes of the TIR family form oligomers or larger complexes, as has been described for other families of cytokine receptors. A study employing fluorescence resonance energy transfer (FRET) suggested that type I IL-1R clusters upon stimulation of cells with IL-1 [76]. Further characterization of different receptor complexes with biophysical means will help to clarify this issue.

3.3. The heterodimeric TLR complexes

The principle of generating heterodimers for signal transduction through the membrane is also utilized by family members of the TLRs. Recent publications suggest that TLR1 and TLR6 serve as coreceptors or modulators for TLR2 [77,78]. This fits nicely as no ligand for TLR1 alone has been described to date; thus, it could play the coreceptor role of IL-1RAcP or IL-18Rβ chain in the IL-1R subfamily. In addition, collaboration of TLR2 and TLR6 in the recognition of soluble tuberculosis factor and *Borrelia burgdorferi* outer surface protein A lipoprotein was described [79], which would suggest that more heterogeneous TLR complexes are conceivable. Comparative reconstitution and complementation experiments are required to clarify this important issue. The ability to recognize so many diverse pathogen-associated structures by a limited number of TLRs could be potentially achieved by different combinations of TLRs. The prototype of a heterodimeric TLR complex is depicted in Fig. 2C, although no cross-linking studies have been published yet to define ligand binding sites in either of the two participating TLRs (for TLR4, see below). Besides the participation of CD14 in TLR2 signaling upon LPS stimulation [32,80,81], no additional extracellular or transmembrane molecules were reported to be required for TLR signal transduction. However, it cannot be excluded that ubiquitously expressed proteins participate in these receptor complexes, which have not yet been identified. It is also unclear whether different subsets of TIR domains are required for signal initiation, for example, contributed by different receptor-like TLRs and accessory-protein-like TLRs as in the IL-1 and IL-18 receptor complexes.

3.4. The homodimeric TLR4 complex

TLR4 is somewhat different to the other family members. Formation of homodimers or homo-oligomers seems to be sufficient to initiate intracellular signaling [27], and TLR4 requires the help of extracellular proteins to function as receptor for LPS. Clearly, TLR4 is required, but not sufficient for activation of the receptor complex by LPS. The co-expression of MD-2 is required for optimal TLR4 surface

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**Fig. 3.** The homodimeric TLR4 complexes (the LPS receptor complex). (A) The TLR4/MD-2 complex. TLR4 is co-expressed on the cell surface with MD-2 (which may be soluble, not shown). Bacterial lipopolysaccharide is bound to LBP and then transferred to CD14, which can be found either bound to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor or in a soluble form (not shown). All three molecules, TLR4, MD-2, and CD14, participate in LPS binding. It is assumed that homodimerization must take place to allow signal transduction. One type of TIR domain seems to suffice in the TLR4 homodimer. (B) The alternative B lymphocyte TLR4 complex. In this LPS complex on B cells, which can be expressed in parallel to the TLR4/MD-2 complex, the Toll-like protein RP-105 together with MD-1 play the role of MD-2. It is unclear whether CD14 participates in this type of LPS receptor in the same fashion as shown for TLR4/MD-2. This alternative TLR4 complex activates the src-kinase Lyn and PKCβ isoforms in addition to the conventional TLR4 signaling module.
expression [82] and function [47,83]. MD-2 is associated with TLR4 on the extracellular side (sequence alignment identifies a potential membrane spanning region; however, MD-2 was also reported to be released in a soluble form remaining associated with TLR4). Its function is unclear, although direct binding of LPS was demonstrated [84]. LPS must be "presented" to TLR4/MD-2 by CD14, either in a membrane-bound or soluble form. CD14 is well established as a very potent and high-affinity LPS binding protein (LBP). Optimal loading of CD14 with LPS is achieved if the LBP is present (reviewed in Ref. [48]). Thus, the LPS receptor complex is a multisubunit complex on the extracellular face of the plasma membrane (Fig. 3A). Cross-linking studies demonstrated that LPS interacted with all three components of the TLR4/MD-2/CD14 complex [82]. It was reported that the deletion of the first hundred N-terminal amino acids in TLR4 containing five leucine-rich repeats abolished LPS binding, but did not affect MD-2 binding to TLR4 [82]. Signal transduction through the membrane is achieved by homodimerization. In this case, one type of TIR domain (that of TLR4) seems sufficient to initiate the signaling machinery dependent on MyD88 [27] and Mal/TIRAP [58,59].

In B lymphocytes, a TLR molecule termed RP-105 was identified which interacts with MD-1 in a fashion similar to TLR4 with MD-2 [85]. Studies revealed that RP-105/MD-1 may substitute for MD-2 in the TLR-4 complex and thus form a different LPS receptor complex [86]. Signaling of this TLR4/RP-105/MD-1 complex may be partially different to TLR4/MD-2, for example, the activation of the src-family member Lyn was described under these conditions [87]. It is presently unclear how the attenuation of TLR4 signaling is achieved in the complex with RP-105/MD-1 and whether CD14 is directly involved in such an LPS receptor complex. RP-105 was also identified in dendritic cells.

3.5. Other TLR receptor complexes

Presently, no information is available on the molecular composition of the TLR9 receptor complex, which recognizes bacterial CpG-DNA [43,88]. It was reported that this receptor does not interact with the ligand on the plasma membrane, but in endosomal compartments. This requires that the ligands are transported to TLR9 and possibly that they are modified before optimal binding and signaling can occur. Whether TLR9 does this all by itself, or if other molecules participate in the process, is presently unclear. No information is available on the composition of TLR3, TLR5, TLR7 or TLR10 receptor complexes.

4. Signaling of the TIR family: the MyD88/IRAK/Tollip module

The TIR domain is required for signal transduction by all TIR family members. In the heterodimeric receptor complexes, both individual TIR domains of receptor and coreceptor need to be present. Deletion of critical amino acids in the TIR domain or the whole TIR domain in either type I IL-1R [89] or IL-1RaCP [90] results in loss of IL-1 responsiveness. This has been shown for TLRs as well [80,91].

We will discuss the molecular events leading to signal generation at the active IL-1R complex as prototype for the whole TIR family. Although a series of questions remains open, we suggest a working model that accommodates the published features of MyD88, IRAK, and Tollip.

4.1. The first step: recruiting IRAK-1 to the receptor complex by protein–protein interactions

After ligand binding and formation of the heterodimeric receptor complex, presumably the close spatial association of the two TIR domains allows homotypic protein–protein interactions of the TIR domains. As a consequence, the conformation of the TIR domains is altered, or the combination of the cytoplasmic domains creates novel scaffolds. This is allowing an interaction of the adapter proteins MyD88 and Tollip with the TIR domains at conserved surface patches, as suggested from the crystal structure of TIR domains [91].

In this model, MyD88 associates with the TIR domains of receptor and coreceptor [92,93] possibly as a dimer [94] and thus introduces death domains into the active receptor complex. IRAK-1, an IL-1R associated serine/threonine-specific protein kinase activity identified in 1994 [95] and cloned in 1995 [96], rapidly translocates to the active receptor complex and interacts with its N-terminal death domain with MyD88 [92–94]. Tollip, which is found associated with IRAK-1 in unstimulated cells, is most likely interacting with the N-terminal, death domain containing portion of IRAK-1 (Ref. [97, Kollewe and Martin, unpublished data). In the resting state, Tollip may serve as an IRAK-1 silencer, possibly by inhibiting IRAK-1 dimerization. Upon IL-1 stimulation, the Tollip/IRAK-1 complex is translocating to the active IL-1R complex. This brings the death domains of IRAK-1 and MyD88 into close contact. The two death domains interact most likely in a fashion similar to the Drosophila homologues Tube and Pelle [98]. Presumably, this results in the dislocation of Tollip, thus releasing IRAK-1, which is anchored at the active receptor complex via the death domain protein–protein interaction with MyD88. We assume that at this stage, dimerization of IRAK-1 molecules occurs, as dimers can be found in over-expression systems that are stabilized via phosphorylation-insensitive homotypic death domain interactions (Kollewe and Martin, unpublished data).

4.2. The second step: IRAK-1 autophosphorylation and departure from the receptor complex

Very rapidly, IRAK-1 auto- and/or cross-phosphorylates itself at the active receptor complex, resulting in phosphor-
ylation in the kinase domain. In vivo, the very recently identified IRAK-4 is most likely the kinase that initially phosphorylates IRAK-1 in the activation loop (see Ref. [8], Note Added After the Reviewing Process). Subsequently, IRAK-1 catalyzes multiple (auto)phosphorylations in its ProST region, a domain between death and kinase domain which is rich in proline, serine and threonine residues. The introduction of several negative charges might be the cause for the release of hyperphosphorylated IRAK-1 from the receptor complex into the cytoplasm. At this stage, IRAK-1 likely interacts with TAB2 (see below).

4.3. The third step: formation of the IRAK/TRAF6 signalosome

In the cytoplasm, IRAK-1 interacts with the downstream adapter TRAF6. The details of the molecular interaction of TRAF6 with IRAK-1 are unresolved. However, it was shown that a truncated form containing the TRAF-N and TRAF-C domains responsible for homo- and hetero-oligomerization of TRAF family members (and for interaction with other receptor complexes, e.g., CD40) is sufficient to interact with IRAK-1, although it cannot transduce the signal further, resulting in a dominant negative effect on IL-1 stimulated NF-κB activation [99]. In parallel, IRAK-1 also interacts transiently with membrane-associated TAB2, an adapter protein for the MAPKKK TGFβ-activated kinase 1 (TAK1) [100–102] in the mammalian mitogen-activated protein kinase (MAP-K) signaling cascades (reviewed in Ref. [103]). It was suggested that IRAK-1 has the function to introduce TAB2 to a complex consisting of TRAF6 with TAK1 and its activator protein TAB1 [104]. In this multiprotein complex, TRAF6 presumably allows the formation of novel type of polyubiquitin chains and has polyubiquitinated itself in collaboration with the ubiquitin-conjugating enzyme complex (E2) consisting of Ubc13 and Uev1A. TRAF6 functions as an E3 ubiquitin ligase in this process [105].

Recently, it was proposed that IRAK-1 and TAB2 remain at the membrane and that TRAF6 translocates to the membrane to become activated [106]. Previously, it had been shown that hyperphosphorylated IRAK-1 and TRAF6 can be detected in the cytosol [99].

4.4. The fourth step: downstream signaling events

By an unresolved mechanism, polyubiquitinated TRAF6 directly facilitates full activation of TAK1, which activates itself by autophosphorylation in its activation loop [107] and then in turn phosphorylates IkB-kinase β (IKKβ) specifically at S177 and S181 and MKK6 at S207 and T211, thus activating the NF-κB pathway as well as p38 and, surprisingly, c-Jun N-terminal kinase (JNK) [108], which is normally not phosphorylated by MKK6 (reviewed in Ref. [103]). Deletion of TRAF6 completely abrogates signaling of the TIR family [109,110], demonstrating that TRAF6 is an essential key element in signaling of TIR family members. As it was shown that oligomerization of TRAF6 is sufficient to activate subsequent signaling pathways [111], the task of IRAK-1 may be twofold: First, to serve as a scaffold for TRAF6 oligomerization, which may be sufficient for TRAF6 auto-polyubiquitinylation. And second, to translocate the pivotal TAB2 to TAK1, which is then fully activated by the modified TRAF6 serving as coactivator (Fig. 4). The central role for TAK1 was also demonstrated for LPS signaling [112].

An additional pathway via the adapter molecule ECSIT (evolutionary conserved signaling intermediate in Toll pathways) was described to directly interact and activate MEKK1 resulting also in NFκB and AP-1 activation. Expression of dominant negative versions of ECSIT inhibited constitutive signaling of a CD4/TLR4 chimeric receptor suggesting involvement of ECSIT also in the TLR subfamily [113].

NFκB-inducing kinase (NIK) was originally proposed as a kinase linking TRAFs with IKK activation [114], but was later shown to be completely dispensable for IL-1 and TNF signaling [115]. The role of NIK is still not completely clear, but it appears to be involved in lymphotixin β signaling.

Finally, direct contact of the adapter molecule p62, which interacts with the atypical protein kinase Cζ (PKCζ), was identified in this multiprotein complex (see below).

4.5. The last step: the fate of IRAK-1 and turning the signalosome off

The fate of hyperphosphorylated IRAK-1 is proteolytic degradation (Refs. [116,117], reviewed in Ref. [118]). After hyperphosphorylation, IRAK-1 is rapidly proteolytically cleaved, and it was reported that this is dependent on its phosphorylation state [116,117]. It can be speculated that upon degradation of IRAK-1, the multiprotein signalosome centering around TRAF6 dissociates, thus limiting the duration of the IL-1 response. Indeed, several downstream events like the activation of NFκB or JNK and p38 kinases are transient after IL-1 stimulation.

The individual steps involved in termination of the signaling complex still await detailed clarification.

In summary, it seems that IRAK-1 plays a crucial role in facilitating the multiprotein signaling complex or signalosome. IRAK-1 is linking the active receptor complex to the central adapter and coactivator protein TRAF6. Thus, IRAK-1 is the switch turning the signalosome on (by allowing TRAF6 oligomerization and by introducing TAB2).

IRAK-1 may also be the switch turning the signalosome off by its autoinduced removal via degradation. Interestingly, the enzymatic activity of IRAK-1 as a protein kinase does not seem to be required for this function, although it might have a regulatory role. Consistent with this model, no substrate for IRAK other than itself and possibly Tollip [119] has been identified (reviewed in Ref. [118]).
Presently, there is no indication whatsoever that the mechanisms discussed here for the IL-1R complex are different for the IL-18 receptor complex. As far as investigated, no qualitative differences in the major signal pathways activated by IL-18 were revealed to those reported for IL-1 [73].

5. Are there differences to these initial signaling events in TLR signaling?

Pathogen-associated structures are very diverse and they cause a multitude of biological responses in animals or cell cultures. In the past decades, effects for LPS were reported which cannot be achieved with IL-1, suggesting that differences in the signaling mechanisms of TLRs and the IL-1R exist. These may be due to differential receptor expressions on different cell types in cell mixtures. In clearly defined cell lines, no systematic study has been published in which a consequent comparison of IL-1 and LPS stimulated signaling events or induced genes was investigated. Such a global comparison will ultimately allow to answer the question whether the two subfamilies actually use identical pathways and in fact induce identical gene expression profiles in the same cellular context.

5.1. The MyD88 knock out phenotype

However, some differences were already found by comparing IL-1 and LPS effects in MyD88 knock out mice which suggest that there are MyD88-dependent pathways and MyD88-independent pathways. Knocking out MyD88 results in the loss of IL-1 and IL-18 signal transduction as far as tested [63]. Signaling of TLRs is also severely affected in animals or cell lines lacking MyD88 [64] or in cells in which dominant-negative versions of MyD88
were expressed [120]. In MyD88 knock out mice or in dendritic cells derived thereof, LPS or CpG-DNA stimulated pro-inflammatory cytokine production and the induction of costimulatory molecules such as CD40 were totally lost. Nevertheless, LPS or CpG-DNA caused caspase-dependent IL-18 release from pro-IL-18 and some features of dendritic cell maturation (Refs. [121,122], reviewed in Ref. [123]). These data suggest that at least some TLRs can signal via yet unknown pathways in the absence of MyD88 as shown for TLR4 activating PKR [59]. It was shown that the MyD88-like adapter protein Mal/TIRAP can supplement MyD88 in TLR4 signaling thus explaining some of the effects observed in the MyD88 knock out situation [58,59].

5.2. The IRAK-1 knock out phenotype

IRAK-1 knock out animals or cells were investigated with respect to IL-1 [124,125], IL-18 [126] and LPS responses [125,127]. Although signaling upon stimulation with these ligands was reduced, the mice or cells derived from these were not completely defective in IL-1 response. It was suggested that this residual signaling was due to IRAK-2 and/or IRAK-M, two other molecules of the small IRAK family, which can substitute for IRAK-1 in overexpression experiments (Refs. [92,128,129], reviewed in Ref. [118]). The biological function of these kinase-inactive family members is presently unclear. It was proposed that kinase-inactive forms may prolong IL-1 signaling because their half life may be altered similar to that of an IRAK-1 splice variant lacking enzymatic activity [117].

5.3. Further possible differences

There are reports demonstrating that activation of TLR2 induces apoptosis [130,131], an effect normally not achievable by stimulation of IL-1, although some cell lines like A375 exist in which IL-1 is antiproliferative [132].

Finally, differences may exist in the composition of the intracellular signaling complex, or signalosome. The identification of Mal/TIRAP as an additional adapter protein to MyD88 in TLR4 mediated signaling and the fact that it interacts preferably with IRAK-2 may explain some of the differences observed in MyD88 knock outs [58,59]. Finally, Tollip was reported to be associated with IL-1R or IL-18R complexes [97], and TLR2 or TLR4, where it was proposed to play an inhibitory role [79,119]; however, its role in other TLR complexes needs to be further investigated. Unfortunately, to date, no further information to this molecule is available and a knock out mouse has not been reported yet.

The first systematic comparisons of differential activities mediated by TLR2 and TLR4 in human dendritic cells revealed differences in cytokine and chemokine transcription, although both receptors led to a comparable activation of MAP-K family members and NFκB [133].

6. Signaling events not directly linked to the MyD88/IRAK/Tollip module

Three central downstream signaling pathways are dependent on the MyD88/IRAK/Tollip module and on TRAF6. As discussed above, these are the activation of the IKK complex resulting in the phosphorylation and degradation of IκB and thus the translocation of NFκB to the nucleus, the activation of the JNK pathway resulting in the phosphorylation of AP-1 complexes, and the activation of the p38 pathway, which is also involved in regulating mRNA stability [134].

However, IL-1 and LPS stimulate several other signaling pathways, and the coupling of these to the receptor complexes are not fully understood. It is conceivable that IRAK functions in two different ways in the IL-1R complex. One is the adapter function which is responsible for interaction with TRAF6 (as discussed above). The second function is a speculative one and is independent of IRAK’s signaling properties. By its presence, IRAK-1 may stabilize the active receptor complex due to the strong protein–protein interactions with MyD88 and possibly Tollip. In such a situation, signaling pathways not requiring IRAK-1’s adapter function to TRAF6 could still be dependent on IRAK-1’s presence. Thus, they would appear as dependent on IRAK-1 in the IRAK-1- or MyD88-knock out model systems.

6.1. Involvement of phosphatidylinositol-3 kinase (PI-3-K) in TIR family signaling

Another pathway reported to be involved in IL-1 signaling is the activation of PI-3-K and subsequently of protein kinase B (PKB or Akt kinase) [135–139]. It is unclear whether PI-3-K activation is dependent on IRAK or not, as unfortunately, neither PI-3-K activation directly nor PKB activation was investigated in the MyD88−/−, IRAK −1−/− or TRAF6−/− situation. On one hand, it was suggested that PI-3-K was interacting with IRAK [135]; others suggested that direct interaction with the IL-1R was independent of IRAK [138].

PI-3-K consists of a p110 catalytic subunit and a p85 regulatory subunit. After ligand stimulation, the SH2 domain of p85 interacts with a tyrosine-phosphorylated motif in receptors. For the type I IL-1R, tyrosine 476 was identified as potential phosphorylation site and mutation of this tyrosine resulted in lack of PI-3-K activation [138]. Recently, transient tyrosine phosphorylation of TLR2 at residues 616 and 761 was demonstrated to be critical for interaction of p85 with TLR2 [140].

An additional molecule, the low molecular weight G protein Rac1 of the Rho family was identified as participating in this pathway [140], which was also reported to be involved in IL-1-stimulated p65 NFκB transactivation [141]. Rac1 activation was independent of MyD88 but relying on IRAK-1 and TRAF6 [142]. Rac1 was found to
be dependent on the transient phosphorylation of the same tyrosine in TLR2 required for PI-3-K activation, and it was speculated that Rac1 is involved in the regulation of PI-3-K [140]. Rac1 was also reported to be involved in the rapid formation of focal adhesion points necessary for the activation of MAP-Ks [143]. This pathway may contribute to the IKK-independent control of NFκB activity by direct phosphorylation events (reviewed in Ref. [144]). In that respect, it is interesting to note that nuclear translocation of PI-3-K was also reported upon IL-1 stimulation [139].

6.2. Involvement of other GTP-binding proteins in IL-1 signaling

In the past years, several reports demonstrated that small GTP binding proteins were involved in IL-1 signal transduction. Ras has been shown to differentially control IL-1-induced transcription factor activation [145], and IL-1-induced activation of Raf was discussed to be involved in transformation of cells [146]. Cdc42 was described to be involved in activation of the JNK pathway [147,148]. In most cases, the use of inhibitors or the overexpression of mutant forms indicated participation of small G proteins [149,150]. However, some of these claims have been disputed [151]. A direct association to the IL-1R was reported for Rho, which is possibly involved in cytoskeletal reorganization [152]. The association of Rac with PI-3-K in the receptor complex was discussed above. In summary, the molecular connection of these proteins to the IL-1R complex remains to be defined.

6.3. Involvement of sphingomyelinases in IL-1 signaling

IL-1 may induce ceramide production by stimulating neutral and acid sphingomyelinase activities in certain cell types (reviewed in Ref. [153]). The activation of acid sphingomyelinase by IL-1 was dependent on the presence of IL-1RAcP, while that of neutral sphingomyelinase was not [154]. The consequence of sphingomyelinase activation for downstream signaling events is controversial [155,156], and remains unresolved. In addition, the mechanism by which the two different enzymes couple to the IL-1R complex is unclear. For the p55 TNF receptor, an adapter protein (FAN) was described coupling receptor and neutral sphingomyelinase; however, this protein is not used by the IL-1R [157].

6.4. Involvement of protein kinase C forms in IL-1 signaling

Ceramide (liberated by sphingomyelinase) is able to directly activate the atypical protein kinase Cζ [158,159]. In addition, PKCζ can be activated in a TRAF6-dependent manner via the p62 adapter molecule that interacts with the TRAF domain of TRAF6 [160]. The activation of PKCζ results in the activation of NFκB [161]. PKCζ may also be a target for Ras-mediated effects as these proteins interact [162]. The atypical λ/κ PKC was reported to be involved in phospholipase A2-catalyzed release of arachidonic acid and subsequent activation of NFκB [163,164].

A plethora of articles demonstrate the involvement of PKC isoforms in IL-1 signaling frequently by employing inhibitors. This IL-1-dependent stimulation of PKC activity may be due to the release of diacylglycerol after stimulation of phospholipase activity (Ref. [165], reviewed in Ref. [153]).

To our knowledge, a direct association of PKC isoforms to any member of the TIR family has not yet been reported.

6.5. Involvement of phospholipases in IL-1 signaling

IL-1 stimulation of different forms of phospholipases was reported during the last 10 years [164,166–169]. The rapid release of arachidonic acid and diacylglycerol affects downstream effectors such as PKC isoforms. For example, it was shown that IL-1 can activate PKCζ by activating phosphatidylcholine-specific phospholipase C, resulting in NFκB-dependent ICAM-1 expression [170], and that IL-1 mediated cyclooxygenase type II and prostaglandin E2 biosynthesis is PKCζ-mediated [171].

It is unclear how the formation of the signaling IL-1R complex is translated into phospholipase activation.

6.6. Involvement of casein kinases in IL-1 signaling

IL-1 stimulates casein kinase II, which was reported to phosphorylate directly the p65 subunit of NFκB [172]. A second kinase capable of phosphorylating casein in vitro was specifically activated by TNF and IL-1 [173]. The coupling of these kinases and the relative positioning in the IL-1 and TLR signaling cascade remains unclarified to date.

7. Concluding remarks

The interest in the TIR family has increased dramatically in the years since the discovery that TLRs are sensors of the immune system for pathogen-associated patterns. In the meantime, we have begun to appreciate that TLRs, in general, are recognizing danger signals sensing that something is wrong and needs attention.

An incredible amount of information is rapidly accumulating on TLR ligands, leaving the investigator with the challenge to find an explanation for a basic question in this field: How is specificity for the different types of pathogens achieved?

It seems that groups of pathogen-associated structures address defined TLRs or combinations of TLRs. Gram-positive bacteria require a different handling by the immune system than Gram-negative bacteria, viruses or fungi.

How is this pathogen-specific recognition maintained at the level of the cellular response, if the signal transduction
pathways which result in gene induction are identical? The crucial question is, are they really identical?

First indications point to subtle differences in the composition of the initial signalosome between different TIR family members, which result in an overlapping but distinct set of signaling pathways finally leading to different responses of a given cell type. The most prominent example is the dendritic cell, where different pathogens lead to skewing of subsequent adaptive immune responses. It makes sense to maintain specificity, at least in part, during signal transduction to induce individual gene expression profiles which take into account that different pathogens require specific reactions. It is anticipated that the availability of high-throughput screening systems such as cDNA arrays will clarify some of the present uncertainties in the near future. The search for additional signaling pathways acting in parallel to the ones we discussed here, most prominently to the MyD88/IRAK/TRAF6-dependent pathway, may identify pathogen-specific signaling components. This may give us tools to inhibit undesired effects of pathogen structures, for example, in sepsis or, alternatively, stimulate TLR-specific pathways for a better (and more specific) response, for example, in vaccination protocols.

8. Note added after the reviewing process. Identification of IRAK-4

Since completing this manuscript, one of us (H.W.) has published the identification of IRAK-4, a novel member of the IRAK family [174]. IRAK-4 shows significant sequence homologies to IRAK-1, IRAK-2 and IRAK-M, and like IRAK-2 and IRAK-M, it lacks the C-terminal domain present in IRAK-1. But like IRAK-1, IRAK-4 has a strong autophosphorylative capacity, that is, kinase activity, which in contrast to IRAK-1 is indispensable for IL-1 signaling. The present model is that IRAK-4 is recruited to the active receptor complex via MyD88 and death domain interactions, and then initiates signaling by phosphorylating IRAK-1. This requirement for IRAK-4 can be overcome in in vitro overexpression systems by autophosphorylation of IRAK-1, resulting in constitutive activation of downstream signaling pathways. Interestingly, IRAK-4 cannot substitute for IRAK-1. Knocking out IRAK-4 completely abolishes signaling for the TIR family, as reported very recently, pointing to the key role for IRAK-4 [175].

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References

[14] R. Debets, J.C. Timans, B. Homey, S. Zurawski, T.R. Sana, S. Lo, J. Wignner, G. Edwards, T. Clifford, S. Menon, F.F. Bazan, R.A. Kastelein, Two novel IL-1 family members, IL-1delta and IL-1epsilon, as reported very recently, pointing to the key role for IL-1Rrp [175].


E. Thomassen, T.A. Bird, B.R. Renshaw, M.K. Kennedy, J.E. Sims, Binding of interleukin-18 to the interleukin-1 receptor homologous receptor IL-1Rrp1 leads to activation of signaling pathways similar to those used by interleukin-1, J. Interferon Cytokine Res. 18 (1998) 1077–1088.


J.L. Slack, K. Schooler, T.P. Bonnert, J.L. Mitcham, E.E. Qwarn-


