Signal transduction pathways activated by the IL-1 receptor family: ancient signaling machinery in mammals, insects, and plants

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Abstract: Interleukin-1 (IL-1) is a central regulator of the immune and inflammatory responses. Recently, significant advances have been made in the area of IL-1 receptors and IL-1 signal transduction. A family of proteins has been described that share significant homology in their signaling domains with the Type I IL-1 receptor (IL-1RI). These include the IL-1 receptor accessory protein (IL-1AcP), which does not bind IL-1 but is essential for IL-1 signaling; a *Drosophila* protein Toll; a number of human Toll-like receptors (hTLRs); the putative IL-18/IL-1-γ receptor IL-1Rrp (IL-1 receptor-related protein); and a number of plant proteins. All appear to be involved in host responses to injury and infection. These homologies also extend to novel signaling proteins implicated in IL-1 action. Two IL-1 receptor-associated kinases, IRAK-1 and IRAK-2, which have homologs in *Drosophila* (Pelle) and plants (Pto), have been implicated in the activation of the transcription factor, nuclear factor κB (NF-κB). IRAK-1 has also been implicated in AP1 induction, Jun amino-terminal kinase (JNK) activation, and IL-2 induction. It recruits the adapter protein TRAF6 to the IL-1 receptor complex via an interaction with IL-1AcP. TRAF6 then relays the signal via NF-κB-inducing kinase (NIK) to two I-κB kinases (IKK-1 and -2), leading to NF-κB activation. Progress has also been made on other IL-1-responsive kinases, including JNK and p38 MAP kinase, with the latter having a role in multiple responses to IL-1. The remarkable conservation between diverse species indicates that the IL-1 system represents an ancient signaling machine critical for responses to environmental stresses and attack by pathogens. *J. Leukoc. Biol.* 63: 650–657; 1998.

Key Words: *NF-κB · Toll · IRAK · TRAF6 · N protein · p38 MAP kinase*

INTRODUCTION

Much attention has focused on the cytokine interleukin-1 (IL-1) because of its critical role in the immune and inflammatory responses. A large number of studies have led to the view that, along with tumor necrosis factor (TNF), IL-1 is a determining cytokine in the pathogensis of inflammatory diseases such as rheumatoid arthritis [1]. It is one of the most pleiotropic cytokines, and has effects on nearly every cell in the body. On activation by IL-1, cells display an inflamed phenotype in that a large number of genes become expressed and most of these code for proteins that participate directly in immune reactivity and inflammation. The expression of over 90 genes has been shown to be affected by IL-1, and these include genes for other cytokines, cytokine receptors, acute-phase reactants, growth factors, tissue remodeling enzymes, extracellular matrix components, and adhesion molecules [2]. The receptor system and signal transduction pathway that leads to such changes in gene expression have been the subject of intense scrutiny over the past 10 years. This has been a controversial area, with several second messengers and protein kinases being implicated as key components [2]. The last year, however, has seen a remarkable improvement in our understanding of how IL-1 signals. An intriguing theme that has emerged is that there are a number of IL-1 receptor-like molecules in mammals, insects, and plants which all share the common feature of being involved in responses to infection and that are likely to result in the activation of transcription factors belonging to the NF-κB family. Most of the immune and inflammatory genes induced by IL-1 are NF-κB-regulated, making it a key mediator of IL-1 effects in cells. Similarly, protein kinases that have been shown to be involved in IL-1 signaling in mammalian cells have homologs important for host defense and development in insects, disease resistance in plants, or stress responses in yeast. Work in such diverse areas as *Drosophila* developmental genetics, yeast genetics, and plant diseases has therefore unexpectedly contributed to the area of IL-1 signaling, and the continuing accumulation of information in expressed sequence-tagged (EST) databases is likely to uncover added complexity in the IL-1 receptor family and associated signaling components.

Abbreviations: IL-1, interleukin 1; TNF, tumor necrosis factor; EST, expressed-sequence tagged; IL-1RI, Type I IL-1 receptor; IL-1RAcP, IL-1 receptor accessory protein; IL-1Rrp, IL-1 receptor-related protein; IRAK, IL-1 receptor-activated kinase; NF-κB, nuclear factor-κB; IKK, IκB kinase; NIK, NF-κB-inducing kinase; TRAF6, TNF receptor-associated factor-6; JNK, Jun amino-terminal kinase; TIP kinase, TNF/IL-1-activated protein kinase.

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It is highly likely that the IL-1 signaling system defines an ancient signal transduction machine that unites plants, insects, and mammals in terms of their response to attack, either by environmental stress or infection. This review will focus on recent work in the area of IL-1 receptors and signal transduction, and will particularly address the emerging IL-1 receptor family and the signals that regulate the transcription factor NF-κB and stress-activated protein kinases.

TYPE 1 IL-1 RECEPTOR (IL-1R1) AND TOLL DEFINE THE IL-1 RECEPTOR SUPERFAMILY

There are three ligands in the IL-1 family, IL-1α, IL-1β, and the IL-1 receptor antagonist (IL-1RA). The effects of IL-1 are mediated by IL-1α and IL-1β with IL-1RA acting as a true receptor antagonist. The major receptor that mediates the effects of IL-1 is the Type I IL-1 receptor (IL-1RI). When its gene was cloned in 1988 it was found to have no homologs in the database, and no clue as to how it might signal was apparent from its structure [3]. A second receptor, termed IL-1RII, was cloned in 1991 [4]. The extracellular portion of IL-1RII was highly homologous to that of IL-1RI, but somewhat surprisingly, it was found to have a short cytoplasmic tail and was found not to signal, but instead in soluble form acted as a decoy receptor [5, 6]. Also in 1991 an intriguing observation was made by Gay and Keith [7], who pointed to the fact that the cytoplasmic domain of IL-1RI shared a highly significant homology with the cytoplasmic region of the *Drosophila melanogaster* protein Toll. Toll is a transmembrane protein whose only known role at that time was in the establishment of dorsoventral polarity in the *Drosophila* embryo. Its ligand is a protein called Spatzle. The Toll/IL-1RI homology was particularly compelling because the target for the Toll pathway during development is Dorsal. This transcription factor is a member of the Rel/NF-κB family, which in *Drosophila* is complexed to Cactus, an ankyrin repeat-containing protein homologous to mammalian I-κB, the inhibitory protein that retains NF-κB in the cytosol. This suggested that the IL-1 signaling pathway to NF-κB was conserved in *Drosophila*, with Toll/Cactus/Dorsal representing the respective homologs of IL-1RI/IκB/NF-κB. Genetic techniques had revealed that two other components were involved in Toll signaling, Tube and Pelle. As discussed below, IL-1 receptor-associated kinase (IRAK) appears to be a homolog of Pelle. A system used in mammals to regulate immune and inflammatory genes therefore appeared to have a counterpart in the regulation of dorsoventral polarity regulation in *Drosophila*. It has since become clear, however, that Toll, Tube, Pelle, Cactus, and another Rel/NF-κB family member (possibly Dif) are critical for anti-microbial peptide production in the adult fly [8], thereby implying that this signaling system for defense is conserved between insects and mammals. The ligand in each system is different, with IL-1 being the trigger in mammals and Spatzle being the trigger in *Drosophila*. This striking conservation of pathways in the immune responses of mammals and insects as well as in *Drosophila* development testifies to the efficiency of the IL-1R/NF-κB pathway in driving rapid transcriptional activity.

A number of proteins were subsequently described that have homologies to IL-1RI and/or Toll. All of the members are homologous in their cytoplasmic (signaling) domains. Most members of the family also fit into one of two subgroups based on extracellular homologies. One subgroup contains members that are homologous to IL-1RI, whereas the other subgroup members are more like Toll. An exception to this is a protein termed MyD88, which is cytosolic, has the IL-1RI/Toll signaling domain, and also a death domain. Recent evidence suggests that it may have an adapter role, complexing to IL-1RAcP (see below). The features of each of the members of the family are shown diagrammatically in **Figure 1A**. Those belonging to the IL-1R1 subgroup all have immunoglobulin domains extracellularly. They include the IL-1 receptor accessory protein (IL-1AcP) [9] and the putative interferon-γ-inducing factor (also known as IL-1-γ or IL-18) receptor IL-1 receptor-related protein (IL-1Rrp) [10]. Those belonging to the Toll group have leucine-rich repeats and include another *Drosophila* protein termed 18-wheeler, which has also been shown to have a role in immunity [11], five human Toll-like receptors (hTLRs), and most interestingly, four plant genes involved in disease resistance [12]. These include N protein from tobacco, which is required for resistance to the pathogen tobacco mosaic virus, and L6 from flax, which confers resistance to flax rust. Neither L6 nor N protein are transmembrane receptors but may interact with their ligands in the cytoplasm [12]. The link with disease resistance in plants is further implied by the discovery of Pto, a protein in tomato that confers resistance to *Pseudomonas syringae*. It is intriguing that Pto would appear to be a homolog of Pelle and IRAK [13], implying that components of the IL-1/Toll system of defense are also present in plants.

Human Toll-like receptors (hTLRs) and innate immunity

The uncovering of hTLRs, of which two have been published to date [14, 15] and a further three are in the database, is of some interest. Janeway et al. [15] have indicated that hToll (hTLR4) may be critical for innate immunity and have provided evidence for hTLR4 as being a trigger for macrophage activation. These molecules and their as yet unknown ligands may therefore be important signals that link innate and acquired immunity, further emphasizing the importance of the IL-1 system for both immunity and inflammation. hTLRs are likely to become an area of intense investigation because they may be initiators of the inflammatory cascade.

Conserved motifs in the IL-1 receptor family

Given the extent of the homology in the cytoplasmic region of the family, it has been possible to predict which regions are likely to be critical for signaling. In a study comparing eight members of the family, six distinct regions of conservation were identified [16]. We have performed a similar alignment of 10 members of the family and have also found a highly conserved tryptophan residue, as shown in Figure 1B. The most carboxyl-terminal of the homology motifs (numbered VI in Fig. 1B) was identified previously by deletion analysis and site-directed mutagenesis as being essential for IL-1 signaling. The significance of the homologies is unknown but it is most likely that

they are important for interactions with signaling proteins and/or trafficking.

Apart from these motifs, Hopp has shown that both IL-1RI and IL-1RAcP contain a domain commonly found in GTPases [17]. Whether either receptor functions as a GTPase has not been demonstrated, although there have been reports of increased GTP binding and hydrolysis in IL-1-treated cells [18]. Evidence for a low-molecular-weight G protein in IL-1 signaling is discussed below.

IL-1AcP and IL-1Rrp/IL-18 receptor

For IL-1RI to signal, it must form a complex with another IL-1 receptor family member IL-1AcP [9]. This protein was discovered through the use of an antibody that blocked IL-1 binding to IL-1RI but recognized a different protein, identified as IL-1AcP [9]. It appears that IL-1AcP is essential for signaling, because a cell line, EL4.D6/76, which does not respond to IL-1 was shown to be lacking IL-1AcP [19]. A number of IL-1 responses could be restored in the cells by transfecting IL-1AcP into the cells. IL-1AcP may be required for receptor internalization [20]. Further evidence for a critical role for IL-1AcP has come from transfection studies in 293 cells. Activation of NF-κB is only possible in these cells if they are co-transfected with IL-1AcP [21]. Another important aspect of IL-1AcP is the observation that IRAK interacts with this protein and not IL-1R1 [21, 22]. This implies that the role of IL-1AcP is to recruit IRAK to IL-1RI. This may have relevance for the current debate on the identity of the IL-18 receptor. IL-18 was originally named IL-1-γ because of its similarity to IL-1-β, which was based on structural analysis and fold recognition studies [23]. IL-18 shares many of the effects of IL-1 and it has recently been shown that IL-1Rrp may be its receptor [24]. The affinity of IL-18 for IL-1Rrp is in the nanomolar range, however, and others have suggested that IL-1Rrp may be the accessory protein for the actual receptor, which has been purified but is yet to be identified [25]. Because it has recently been shown that IRAK is required for IL-18 signaling [26], it will be of interest to determine whether IRAK interacts directly with IL-1Rrp. A possible criterion for identifying receptor accessory proteins in the IL-1R family could be interaction with IRAK. The expression of IL-1 or IL-18 receptors may have a critical role in the amplification of Th1 and Th2 responses because it has recently been shown that IL-18 regulates Th1 cells, whereas IL-1 appears more important for Th2 cells [26]. This may be due to differential receptor expression.

THE PATHWAY TO NF-κB: IRAK-1, IRAK-2, AND TRAF6

As mentioned above, NF-κB is a critical transcription factor for the immune and inflammatory responses because a large number of genes are NF-κB regulated. As a result, the mechanism by which agents such as IL-1 activate NF-κB has been the subject of much scrutiny and in the last year several novel proteins have been characterized that participate in the activation process. These are shown in **Figure 2**. The key event

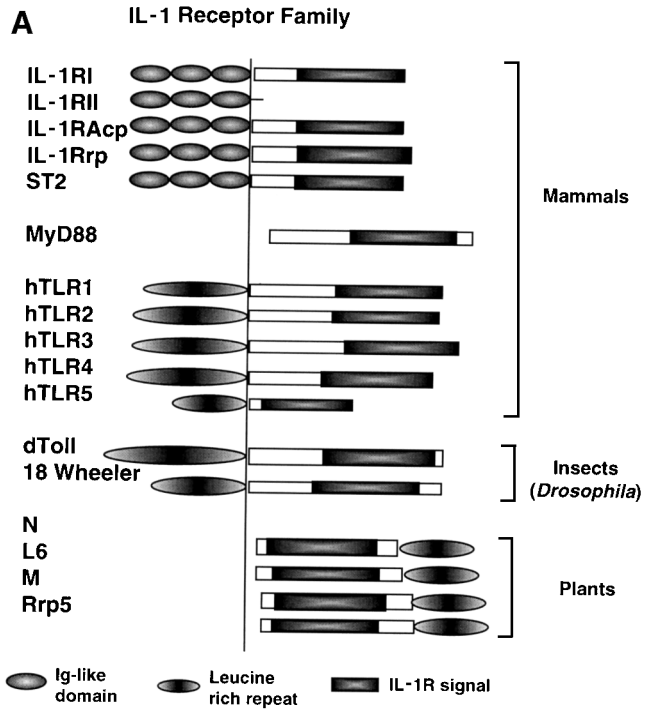

Fig. 1. The IL-1 receptor family. (A) Representative mammalian, *Drosophila*, and plant members of the IL-1 receptor family. Different domain structures are highlighted. (B; see next page) Clustal sequence alignment of the signaling domains of 10 mammalian IL-1 receptor family members. Boxes represent conserved motifs and were originally described by Mitcham et al. [16]. *Highly conserved tryptophan residue.

is the phosphorylation and degradation of the inhibitory protein IκB. This leads to the release of NF-κB, which translocates to nucleus, binds to its consensus sequence on target genes, and up-regulates their expression. Two kinases responsible for IκB phosphorylation have been identified and named IκB kinase (IKK) 1 and 2 (or IKKα and β) [27–30]. Evidence has been presented that both are involved in NF-κB activation by IL-1 [28, 29]. In addition, an upstream regulator of IKKs has been identified and termed NF-κB-inducing kinase (NIK). NIK is also required for NF-κB activation by IL-1 [31]. It is likely that NIK and IKKs are part of a large complex of proteins, with a molecular weight in the region of 900,000, which has been termed the signalsome [30], and is the regulator of NF-κB activation in cells.

Upstream of NIK, attention has focused on two proteins, IRAK and TRAF6. IRAK was originally identified as a protein kinase activity that co-precipitated with IL-1R1 [32]. This was subsequently named IRAK, the gene was cloned, and as mentioned above, was found to be homologous to the *Drosophila* protein Pelle, implying that IRAK would participate in NF-κB activation [33]. Activation of NF-κB was not shown directly in the study reporting the cloning of IRAK. Circumstantial evidence for a role for IRAK in NF-κB activation came from a study describing the cloning of TRAF6 [34]. This protein is a member of the TNF receptor-associated factor (TRAF) family of proteins, but does not interact with TNF receptors. Instead it appears to associate with IL-1RI via IRAK. Furthermore, TRAF6 is critical for NF-κB activation by IL-1 and has been shown to interact with NIK [28]. Its association with IRAK has

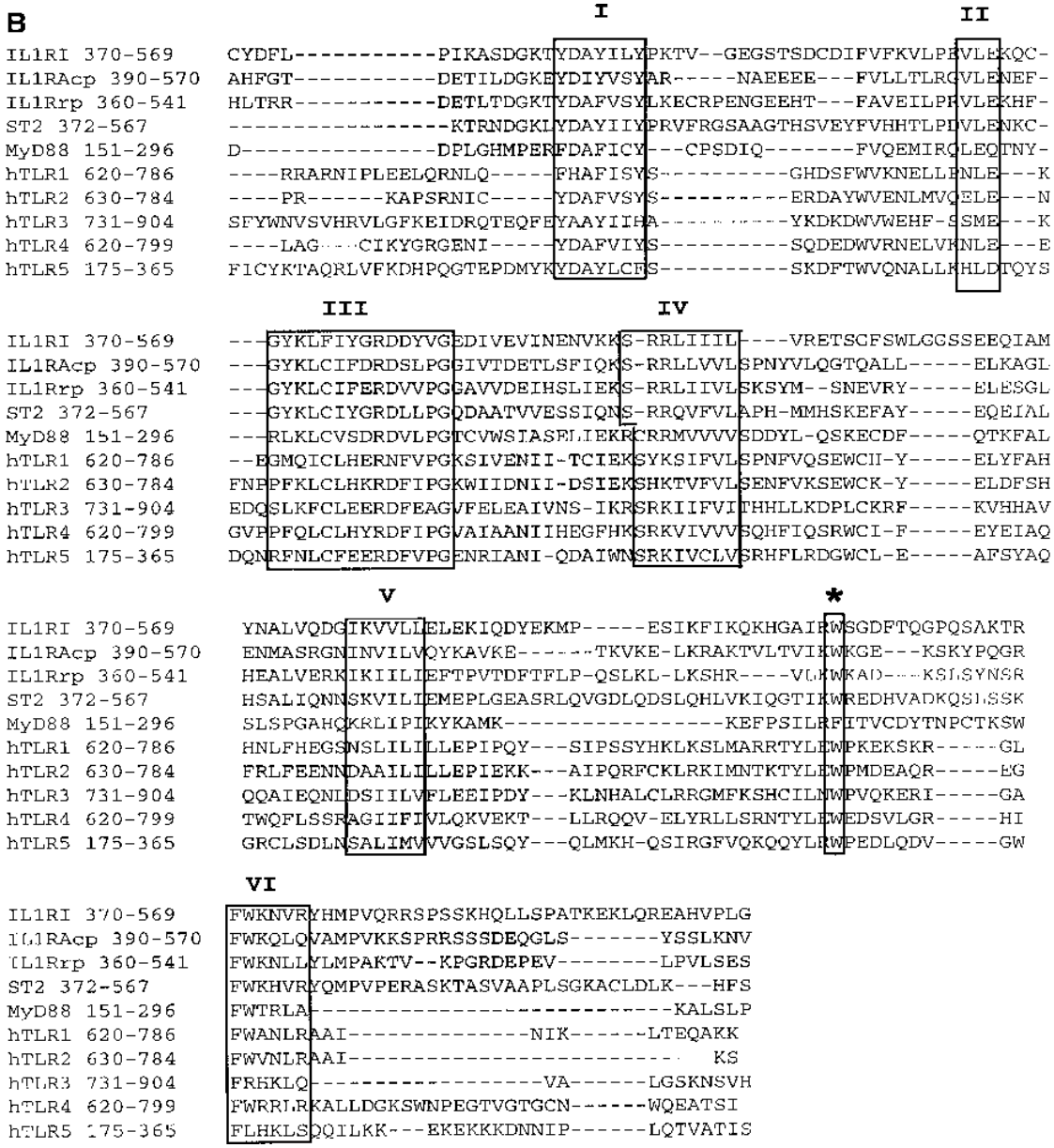

Figure 1 (continued)

been demonstrated by immunoprecipitation, whereby an immunoprecipitating antibody to TRAF6 will co-immunoprecipitate IRAK in IL-1-treated cells [34]. An antibody to IL-1RI will only co-immunoprecipitate IRAK in IL-1-treated cells, however, and not TRAF6 [34], implying that IRAK recruits TRAF6 to the IL-1 receptor complex. We have provided direct evidence for activation of NF-κB by IRAK, in that transfection of EL4.NOB-1 cells with IRAK activates NF-κB [35]. In addition, NF-κB activation in EL4 stably transfected with IRAK under an inducible promoter has been demonstrated [36]. We have also directly shown that TRAF6 lies downstream of IRAK because a dominant negative mutant of TRAF6 inhibits NF-κB activation by IRAK and also IL-1 [35]. This implies that the pathway activated by IL-1, which culminates in NF-κB activation, has the following components in the following order: IL-1RI/IL-1AcP, IRAK, TRAF6, NIK, and IKK1 and 2.

Most recently, however, added complexity has been indicated. First, and as mentioned above, IRAK does not associate with IL-1RI, but instead complexes with IL-1AcP [21, 22]. Whether IRAK is pre-associated with IL-1AcP or whether IL-1 treatment is required to drive the recruitment is uncertain because evidence for both models has been presented [21, 22]. There is also evidence that upon recruitment, IRAK is hyperphosphorylated and degraded [37]. This is likely to be a controlling mechanism and may explain why some IL-1 responses (e.g., NF-κB activation in some cell types) are transient. The work on IRAK phosphorylation also provided a suggestion that there was second type of IRAK in cells [37]. This may be the recently described IRAK-2, the gene for which was found in an EST database [38]. IRAK-2 represents a new member of the IRAK family, which therefore currently comprises IRAK-1 and -2 in mammals, Pelle in *Drosophila*, and Pto in the tomato. IRAK-2 has been shown to associate with IL-1RI and not IL-1AcP, and overexpression of IRAK-2 activates

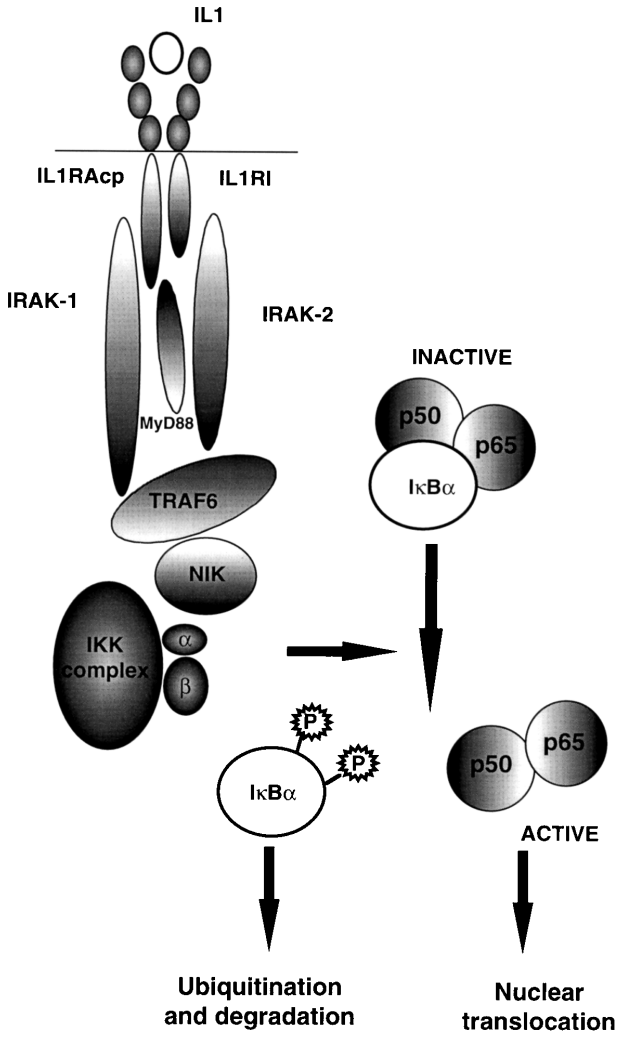

Fig. 2. Activation of NF-κB by IL-1. A multiprotein complex involving IL-1RI/IRAK-2, IL-1RAcP/IRAK-1, and MyD88 is engaged by IL-1 via interaction with IL-1RI in mammalian cells. TRAF6 is recruited to the complex and activates NIK, which activates IKKs, culminating in the phosphorylation of IκB followed by its ubiquitination and degradation. This releases NF-κB, which translocates to the nucleus. Details are given in the text.

NF-κB in a TRAF6-dependent manner [38]. Furthermore, the cytosolic protein MyD88, which is a member of the IL-1 receptor family (as mentioned above) has been shown to associate with IL-1RAcP and via its death domain to IRAK-2 [38]. A model for NF-κB activation comprising all of these observations is shown in Figure 2. A number of outstanding questions remain, however. Is the kinase activity of IRAK-1 required for signaling? What is the role of hyperphosphorylation of IRAK-1? As mentioned previously, is the role of IL-1AcP (or other possible AcPs) IRAK-1 recruitment? What would be the purpose of an association between IRAK-2 and MyD88, given that IRAK-2 can be recruited via IL-1RI? A word of caution may be necessary because much of the evidence for this model is based on overexpression studies; at no point in the article on IRAK-2 is IL-1 actually used to activate cells [38]. Future work on IL-1-treated untransfected cells or indeed extracts prepared from IL-1-treated animals, will be required to confirm this model. At any event, a picture is beginning to emerge whereby addition of IL-1 to cells leads to the formation of a multiprotein complex involving IL-1RI, IL-1AcP, IRAK, and TRAF6, which then engages another multiprotein complex whose known components are NIK and IKKs, ultimately leading to NF-κB activation.

IRAK AND TRAF6 REGULATE AP1 AND IL-2 GENE EXPRESSION

Another outstanding question is whether IRAK or TRAF6 mediate effects other than NF-κB activation. Because they are the most receptor-proximal components so far described, it is possible that they will be involved in other IL-1 signals. We have found that transfection of IRAK or TRAF6 into EL4 cells induces an AP1-linked reported gene, and that the effect of IL-1 or IRAK here is inhibited by a dominant-negative TRAF6 (**Fig. 3A**). In addition, Martin et al. [36] have shown that in EL4 stably transfected with IRAK, a more prolonged activation of Jun amino-terminal kinase (JNK) by IL-1 is observed. In addition, TRAF6 has been shown to activate JNK [39], providing a possible mechanism for our observations on AP1, although whether JNK is involved in AP1 activation by IL-1 has not been investigated. In addition, we have found that IRAK can regulate the IL-2 gene promoter. This effect, and that of IL-1, is also dependent on TRAF6 because dominant-negative TRAF6 inhibited the response to both IRAK and IL-1 (Fig. 3B). Finally, we [35] and others [36] have found that IRAK is involved in IL-2 production. Again, this effect is TRAF6-dependent and is probably at the level of transcription. It therefore seems likely that the effects of IRAK and TRAF6 are not restricted to NF-κB.

ACTIVATION OF PROTEIN KINASES BY IL-1

Apart from NF-κB, the other area that has seen much investigation is the effect of IL-1 on protein phosphorylation [see ref. 40 for reviews]. Four protein kinase pathways are responsive, as shown in **Figure 4**. Much work has been done on MAP kinase cascades. IL-1 activates the three major cascades and recent work has attempted to identify upstream regulators and downstream targets. The ability of IL-1 to activate p42/p44 MAP kinase in some cell types was unexpected because this pathway is normally associated with growth factor action, and IL-1 does not activate Ras, which is an important regulator of this pathway. IL-1 has also been shown to activate MEK, the upstream regulator of p42/p44 MAP kinases. An inhibitor of MEK, PD98059 has been tested on IL-1 action and has been found to block IL-2 production in EL4, suggesting a role for MEK in this process [41].

Regarding p54 MAP kinase/JNK, as mentioned above TRAF6 may be involved in its regulation [39]. There are currently three isoforms of this enzyme. Most notably, the JNK1 isoform is the only MAP kinase activity so far detected in tissues from IL-1-treated rabbits [42]. Its upstream regulator in response to IL-1 is MKK7, the activity of which is increased both in vivo and in vitro [43].

Much attention has focused on p38 MAP kinase. A strong

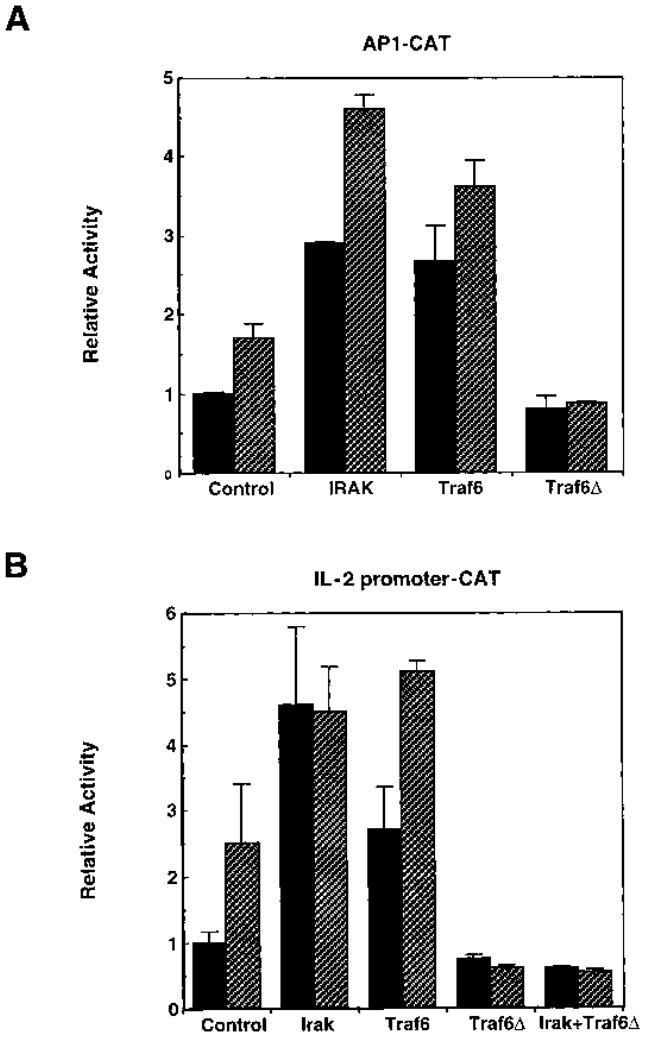

Fig. 3. Effects of IRAK, Traf6, and dominant-negative TRAF6 (TRAF6Δ) on reporter gene expression. EL4.NOB-1 cells were transfected with an (A) AP1-CAT or (B) IL-2 promoter-CAT reporter plasmid and the relevant expression plasmids. After recovery samples, 1×10^6 cells, were left untreated or stimulated with IL-1α (10 ng/mL) for 24 h. Extracts were prepared and 40-μg amounts were incubated in buffer containing 1 mM acetyl CoA and 0.3 mCi [^{14}C]chloramphenicol. Acetylated and non-acetylated complexes were resolved by silica gel thin-layer chromatography in 19:1 (v/v) chloroform/methanol. The separated products were quantified using image analysis. Chloramphenicol acetyl transferase activities relative to control are shown. Both IRAK and TRAF6 drive AP1- and IL-2 promoter-linked reporter gene expression, TRAF6;cD inhibiting the response to both IRAK and IL-1. Solid bars, −IL-1; hatched bars, IL-1.

phosphorylation change that occurs in cells treated with IL-1 is increased phosphorylation of the heat shock protein hsp27. The kinase responsible for this is MAPKAP-kinase-2, which is a target for p38 MAP kinase [44]. Like JNK, p38 MAP kinase is a stress-activated protein kinase and is responsive to a wide variety of stresses, including osmotic shock, heat, UV light, and protein synthesis inhibitors. In baker's yeast, the p38 homolog is HOG-1, which is activated in response to osmotic shock and leads to the up-regulation of genes involved in glycerol biosynthesis, whose expression is required for countering the osmotic imbalance. This is therefore another example of evolutionary conservation, whereby a stress-response system in yeast is found in the IL-1 system in mammals. Human p38 can substitute for HOG-1 when transfected into HOG-1-deficient yeast mutants [45], thereby illustrating the extent of the conservation. Four different p38 MAP kinases have been identified and MKK3 and MKK6 are putative upstream regulators [46]. In terms of what might lie downstream, a specific inhibitor of p38 MAP kinase, SB203580 has been utilized [46]. It has been shown to block multiple IL-1 responses, including induction of IL-2, IL-8, human Immunodeficiency virus long terminal repeat function, prostaglandin production, protease expression, and the glucose transporter GLUT4 [41, 46–48]. This suggests that p38 MAP kinase has a determining role in the expression of several IL-1-responsive genes. The precise target for p38 MAP kinase in such expression is still unknown.

In addition to these three kinase systems, another kinase has been shown to be activated by IL-1. This has been termed TNF/IL-1-induced protein kinase (TIP kinase) because it is the only kinase so far shown to be exclusively regulated by IL-1 and TNF [49]. It is a cytosolic enzyme of about 100 kDa that may be part of larger aggregates of 200–600 kDa. The gene for this protein has yet to be cloned, although the site it phosphorylates has been mapped and indicates that TIP kinase corresponds to a new type of dual-specificity protein kinase, directed toward a novel sequence motif, which may be related to the mixed lineage kinase family [49].

IS A LOW-MOLECULAR-WEIGHT G PROTEIN INVOLVED IN IL-1 SIGNALING?

Low-molecular-weight G proteins are important upstream regulators of MAP kinases. Studies with dominant negative mutants have shown that Rac1 may be involved in the activation of JNK and p38 MAP kinase by IL-1 [50]. In addition, we have provided evidence for the involvement of a low-molecular-weight G protein in IL-1 signaling. This is based on the use of lethal toxin (LT), a virulence factor from *Clostridium sordelii*,

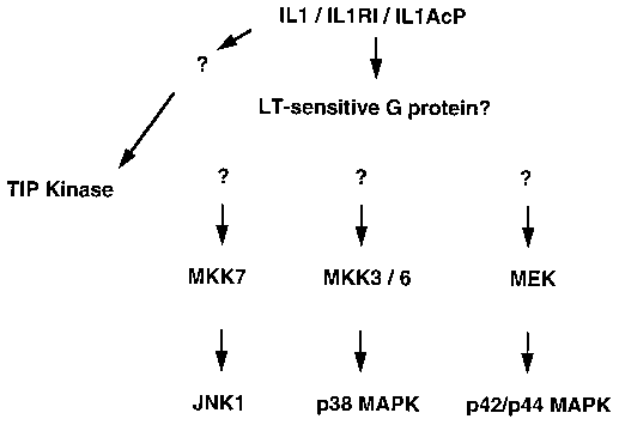

Fig. 4. IL-1 activates multiple protein kinases in mammalian cells. Three MAP kinase cascades are IL-1 responsive and evidence has been presented for the involvement of a lethal toxin-sensitive G protein in the activation of p38 and p42/p44 MAP kinases [52]. In addition, a novel protein kinase, termed TNF/IL-1-activated protein kinase (TIP kinase), has been described [49] that may be related to mixed lineage kinases. It is a 95-kDa protein, but may occur in a complex of 200–600 kDa. So far, only TNF and IL-1 have been shown to activate TIP kinase. Of the kinases shown, only MKK7 and JNK1 have been shown to be activated in vivo [42, 43].

TABLE 1.

	Mammals	Insects	Plants	Yeast
Receptors	IL-1RI, IL-1RAcP, IL-1Rrp MyD88, ST2, hTLR1, hTLR2 hTLR3, hTLR4, hTLR5	Toll, 18-Wheeler	N, L6, M, Rpp5	?
Protein kinases	IRAK 1 and 2 p38 MAP kinase	Pelle	Pto	Hog-1, Sty-1
Rel family transcription factors	p50, p52, p65/RelA, c-Rel	Dorsal, Dif, Relish	?	?
IκB-like inhibitors	IκBα, IκBβ, IκBγ, IκBϵ	Cactus	?	?

The IL-1 signaling system is conserved in different species, with IL-1 receptor family members occurring in mammals, insects (*Drosophila melanogaster*), and plants [tobacco (N), flax (L6 and M) and *Arabidopsis* (Rpp5)]. Each of these proteins participates in defence against pathogens, and in the case of *Drosophila*, Toll is also involved in development. Similar protein kinases also occur in different species, with mammalian IRAK-1 and IRAK-2 (mammals), Pelle (*Drosophila*), and Pto (tomato) all showing significant homology, and in the case of the IRAKs and Pelle, participating in the activation of Rel/NF-κB transcription factors. Homologs of the stress-activated MAP kinase p38 occur in *Saccharomyces cerevisiea* (Hog-1) and *Saccharomyces pombe* (Sty-1). All three are sensitive to osmotic shock, with p38 MAP kinase being an important signal for IL-1. Finally, members of the Rel/NF-κB family of transcription factors and their inhibitors occur in mammals and *Drosophila*. They are a key target of the IL-1 receptor family, regulating genes involved in immunity and being activated via IRAKs and Pelle. Rel family members have yet to be found in plants. See text for further details.

which glucosylates and thereby inactivates Ras, Rac, and Rap [51]. We have found that LT inhibits p38 and p42/p44 MAP kinase activation by IL-1 in EL4 cells [52]. The toxin glucosylates a protein of 20 kDa in the cells. Preliminary evidence suggests that this G protein is not Ras, Rac, or Rap, but may be novel. In addition, we and others have found that Rac1, instead of being directly involved in IL-1 signaling, may synergize with IL-1, both in IL-2 induction and activation of JNK [52, 53].

CONCLUSIONS

The last two years have seen a marked improvement in our understanding of the components important for IL-1 signaling. Additional members of the IL-1 receptor family have been described and their species distribution has indicated that the IL-1 signaling system is evolutionarily conserved. **Table 1** summarizes the homologs of IL-1R1 and signaling components activated by IL-1, in mammals, insects, plants, and yeast. The most striking conclusion from these homologies is that certain components were present in the unicellular common ancestor of both plants and animals, and probably served as environmental stress sensors and/or in the resistance mechanisms to bacterial or other pathogens. In multicellular organisms, these functions were retained and broadened, such that the IL-1 system became central in the response of the organism to infection and injury. Future findings in this area will therefore not only help our understanding of how IL-1 works, but will also have consequences for the mechanisms underlying innate immunity in multicellular organisms and disease resistance in plants. It is likely that further information will be obtained from EST databases that will be of use. A clear goal for the future will be to determine which components are important in vivo. A definitive picture of IL-1 signaling will therefore emerge, which may ultimately lend itself to therapeutic manipulation.

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