Mini Review

Novel Role and Regulation of the Interleukin-1 Receptor Associated Kinase (IRAK) Family Proteins

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The interleukin-1 receptor associated kinases (IRAKs) sit at the bottle neck for the Toll-like-receptor (TLR) mediated signal transduction process controlling host innate immune response. However, the exact role and regulation of IRAKs are still in the early stage and not fully understood. This review intends to summarize the recent advancement in this important topic and points out areas that need further intensive investigation. *Cellular & Molecular Immunology*. 2005;2(1):36-39.

Key Words: IRAK, innate immunity, signaling, TLR

Introduction

With the revelation of TLR and related downstream signaling molecules, the field of innate immunity has recently drawn immense interest. Human hosts can specifically react to distinct non-self molecules *via* innate immunity signaling and illicit highly specific responses (1). TLR-mediated signaling has been shown to control diverse cellular processes including expression of both pro- as well as anti-inflammatory mediators, regulation of cellular apoptosis, as well as cell differentiation. The specificity of TLR signaling is achieved through differential recruitment of adaptor molecules such as MyD88, Mal/TIRAP, TRIF and TRAM. These molecules may subsequently recruit and mediate the activation of IRAK family kinases. To date, four IRAK genes have been identified in the human genome, namely IRAK1, IRAK2, IRAK-M and IRAK4. Initially, it was thought that all IRAKs might play a somewhat redundant role in activating transcription factor NF-κB. As the field evolves, it has been gradually realized that each distinct IRAK may have highly specific downstream target(s), and therefore, contribute to the specific activation of cellular response upon distinct TLR ligand challenge.

Regulation and function of IRAK1

IRAK1 was first identified by Cao et al. through biochemical purification of the IL-1 dependent kinase activity that co-immunoprecipitates with the IL-1 type 1 receptor (2). Micropeptide sequencing and subsequent cDNA library screening yielded a full length cDNA clone encoding a protein with 712 amino acids and a predicted molecular size of ~76 kD (2). IRAK1 message is expressed ubiquitously in diverse human tissues. By radiation hybrid analysis, Thomas et al. mapped the murine IRAK1 gene to Xq29.52-q29.7 (3). Since TLRs share the TIR domain with IL-1 receptor, it was hypothesized that IRAK1 might also participate in TLR mediated signaling. Subsequent works including ours have confirmed that indeed various TLR ligands can activate endogenous IRAK1 kinase activation (4-7). Biochemically, we and others have shown that IRAK1 undergoes covalent modification likely due to phosphorylation and ubiquitination upon IL-1 or LPS challenge (5, 8, 9). Its modification may eventually lead to its subsequent degradation (9). Using human THP-1 cells, primary blood mononuclear cells, as well as mice splenocytes, we have confirmed numerous previous studies that there are indeed two signature forms of IRAK1, one being the unmodified 85 kD form, and the other being modified (phosphorylated and/or ubiquitinated) 100 kD form. In consistent with previous studies, upon LPS challenge, we have observed that the level of the modified IRAK1 form increases, while the unmodified form decreases (10). With regard to its subcellular distribution, there is only one published report showing that IL-1β stimulation may lead to IRAK1 nuclear localization (11). Yet this report has been largely ignored by the field. We have further analyzed the distribution of IRAK1 in the fractionated cellular and nuclear extracts. Strikingly, the majority of the 85 kD form exists in the cytoplasm fraction. In sharp contrast, the modified IRAK1 is mainly present in the nucleus. We have also noticed from the previous study reporting IRAK1 nuclear localization that the apparent molecular weight of

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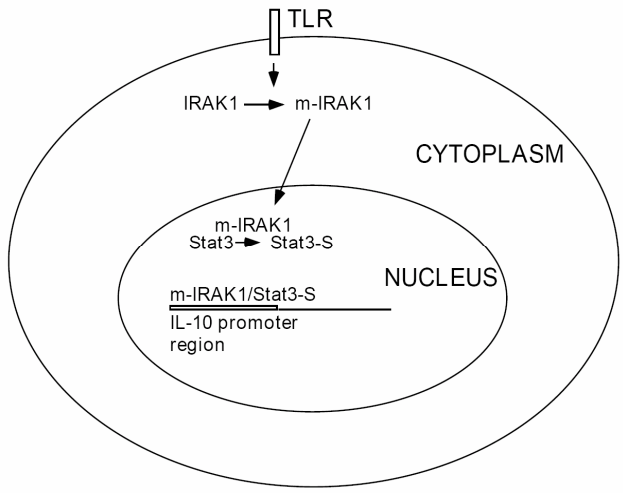

Figure 1. Schematic illustration of the novel function of IRAK1.

nuclear IRAK1 is ~100 kD, consistent with our present finding. However, the authors of that report did not elaborate in their publication the different migration patterns of nuclear and cytoplasmic IRAK1 (11). The fact that the modified IRAK1 primarily appears inside the nucleus suggests that LPS-induced IRAK1 modification such as phosphorylation and/or ubiquitination may be critical for its trafficking into the nucleus and its subsequent function.

Functionally, IRAK1 can form a complex with MyD88, as well as TRAF6 (4). Since the most apparent downstream target of LPS signaling is the activation of NF-κB, IRAK1 is the apparent candidate to fulfill such role. Therefore, IRAK1 has historically been linked with IL-1/LPS mediated NF-κB activation. Yet the majority of published evidence supporting the role of IRAK1 in mediating IL-1/LPS-induced NF-κB activation has been derived from studies employing cell lines with IRAK1 overexpression (12-14). Upon overexpression, both the wild type and the kinase-dead IRAK1 (which has a point mutation in the ATP-binding pocket [K239S] or in the catalytic site [D340N]) can strongly induce NF-κB reporter activation (12, 14). The fact that despite being an active kinase, its kinase activity is not required for its function, raises the concern that IRAK1 may perform other novel unidentified function besides activating NF-κB.

Intriguingly, our study has presented evidence indicating that IRAK1 plays a novel and critical role in Stat3 mediated IL-10 gene expression. IL-10 induction is severely compromised in IRAK1 deficient cells upon LPS challenge (10). IRAK1 forms a complex with Stat3 as well as the IL-10 promoter element in the nucleus upon LPS challenge (Figure 1). It has been documented that Stat3 is responsible for increased IL-10 gene expression upon LPS challenge (15). LPS can induce Stat3 phosphorylation at both serine 727 and tyrosine 705 residues (15), and Stat3 phosphorylation at both Y705 and S727 are critical for its maximum transcriptional activity (16). Our study concurs with these studies. Over-expression of Stat3 with serine 727 to alanine mutation or tyrosine 705 to phenylalanine mutation fails to mediate LPS induced IL-10 gene reporter activity. Janus kinase 3 (JAK3) has been shown to be the main kinase responsible for Stat3 tyrosine phosphorylation (17). To date, the mechanism for LPS induced Stat3 serine 727 phosphorylation is not clear. In consistent with previous reports, we have documented that LPS can induce Stat3 S727 and Y705 phosphorylation in wild type murine splenocytes (10). Strikingly, we have observed that although LPS-induced Y705 phosphorylation is normal, LPS-induced Stat3 S727 phosphorylation is greatly compromised in IRAK1 deficient splenocytes. Furthermore, we have observed that nuclear Stat3 serine phosphorylation is completely absent in IRAK1 deficient splenocytes. In contrast, there is an increase in nuclear Stat3 serine phosphorylation in wild type splenocytes upon LPS challenge. The increased Stat3 serine phosphorylation inside the nucleus upon LPS challenge correlates well with our observation that LPS facilitates IRAK1 and Stat3 interaction inside the nucleus. We have documented such interaction using mice splenocytes, human THP-1 cells, as well as human peripheral blood mononuclear cells. Furthermore, our *in vitro* analyses indicate that IRAK1 can directly use Stat3 as its substrate and induce Stat3 serine 727 phosphorylation. This is one of the first evidence revealing the biological substrate of IRAK1. Taken together, our data indicate that IRAK1 is essential for Stat3 serine phosphorylation inside the nucleus.

Besides activating Stat3 which is critical for IL-10 gene expression, our study also reveals the intriguing phenomenon that IRAK1 may directly serve as a transcriptional regulator for IL-10 gene transcription. Using the Chip assay, our study showed that endogenous nuclear IRAK1 could specifically bind with IL-10 promoter element *in vivo* upon LPS challenge (10). Further detailed studies are warranted to decipher the mechanism for IRAK1 binding with the IL-10 promoter element and subsequent regulation of IL-10 gene expression. IL-10 expression is completely absent in healthy human blood cells. However, it has long been noticed that IL-10 levels are elevated in the blood sera of atherosclerosis patients. Elevated IL-10 levels may be a self-protective mechanism preventing excessive inflammation and limiting the progression of atherosclerosis. The mechanism for the increased IL-10 gene expression is not clear. Our data indicate that IRAK1 protein in the atherosclerosis patient blood samples is constitutively modified and localized in the nucleus. Elevated IRAK1 modification and nuclear localization may lead to elevated IL-10 gene expression (10).

Regulation and function of IRAK2

IRAK2 was initially identified by Dixit's group based on the search of the human expressed sequence tag (EST) database for sequences homologous to IRAK1 (18). Subsequent screening of a human umbilical vein endothelial cell cDNA library resulted in the isolation of a full-length cDNA clone which encodes a 590-amino acid protein with a predicted size of 65 kD. Northern blot analysis revealed a single IRAK-2

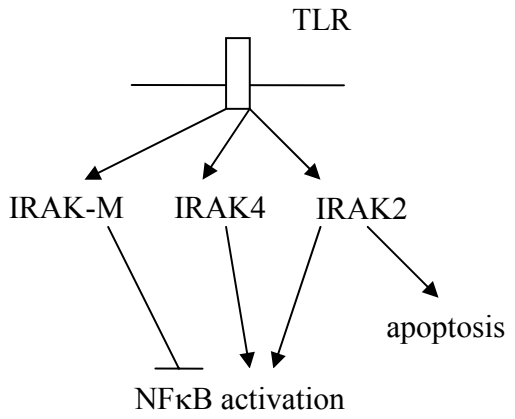

Figure 2. Putative functions of IRAK2, IRAK-M and IRAK4.

transcript expressed in a variety of tissues whose size is consistent with that of the cDNA. Upon overexpression, IRAK2 can associate with MyD88 as well as TRAF6, and activate NF-κB-dependent reporter gene expression. Intriguingly, IRAK2, instead of IRAK1 can also interact with another distinct TLR intracellular adaptor molecule Mal/TIRAP (Fitzgerald et al., 2000). Dominant negative IRAK2 can block Mal/TIRAP-induced signaling while dominant negative IRAK1 fails to do so. These studies suggest that IRAK2 may selectively be recruited by Mal/TIRAP and participate in NF-κB activation. Besides activating NF-κB, IRAK2 also participates in the regulation of cellular apoptosis (19) (Figure 2). Dominant negative IRAK2 can diminish LPS-induced macrophage apoptosis (19).

Recently, O'Neil's group has identified the murine *Irak2* gene (20). In contrast to its human counterpart which only encodes one single transcript, the murine *Irak2* gene can generate four alternatively spliced isoforms (designated as *Irak2a, 2b, 2c* and *2d*) that have various N-terminal deletions. Upon overexpression, *Irak2a* and *Irak2b* could activate, while *Irak2c* and *Irak2d* inhibit NF-κB activation. Alternative splicing of the *Irak2* gene in mice instead of human reflects the distinct difference in human and murine TLR signaling process and innate immunity regulation.

Regulation and function of IRAK-M

Using the similar EST search, Wesche et al. first identified a murine EST sequence which encodes a polypeptide sharing significant homology with IRAK1 (13). Screening of human peripheral blood leukocyte library with this EST sequence resulted in the isolation of a full length cDNA clone that encodes a protein with 596 amino acids and a calculated molecular mass of 68 kD. Northern blot analysis revealed that IRAK-M transcript is primarily present in the peripheral blood leukocytes and monocytic cell lines. Initial studies revealed that IRAK-M overexpression can activate NF-κB activity (13). Strikingly, later studies using IRAKM$^{-/-}$ cells indicate otherwise. IRAKM$^{-/-}$ macrophages exhibit elevated expression of various inflammatory cytokines upon stimulation with several TLR ligands (21). IRAKM$^{-/-}$ mice show increased inflammatory responses to *Salmonella typhimurium* infection (21). Furthermore, endotoxin tolerance, a phenolmenon often observed in leukocytes with prolonged endotoxin treatment or human blood cells from sepsis patients, is significantly reduced in IRAKM$^{-/-}$ cells. IRAK-M levels are elevated in human monocytes isolated from septic patients, as well as healthy human monocytes and murine macrophages treated with LPS. This and other studies indicate that IRAK-M plays a negative role in TLR signaling and NF-κB activation (Figure 2).

Regulation and function of IRAK4

Lastly, yet another EST search has yielded another human cDNA sequence that encodes a distinct polypeptide sharing significant homology with IRAK (22). Full-length cDNA obtained *via* PCR encodes a protein with 460 amino acids and a calculated molecular mass of 52 kD. In contrast to IRAK1 or IRAK-M knockout mice, IRAK-4 knockout mice exhibit severe impairment in NF-κB activation and expression of various inflammatory cytokines upon challenges with several TLR ligands (23). Overexpression of kinase-dead IRAK4 mutant strongly diminishes IL-1/LPS induced NF-κB activation, pointing to the essential role of its kinase activity. MyD88 is critically involved in recruiting IRAK4 into the TLR4 complex. These studies indicate that IRAK4 is the primary kinase in the TLR signaling process essential for mediating NF-κB activation (Figure 2).

Because of the central role IRAK4 plays in mediating NF-κB activation, deficiency in IRAK4 may conceivably cause severe human diseases. A study by Picard et al. revealed that IRAK4 mutations were present in three children suffering from persistent pyogenic bacteria infection and poor inflammatory responses (24). These patients did not respond to IL-1β, IL-18, or any of the TLR1-6 or 9 ligands tested, as assessed by activation of NF-κB and p38-MAPK, and induction of IL-1β, IL-6, IL-12, TNF-α and IFN-γ. In a separate study, a patient was identified who suffered from recurrent bacterial infections and failed to respond to Gram-negative LPS *in vivo*, and whose leukocytes were profoundly hyporesponsive to LPS and IL-1 *in vitro* (25). This patient also exhibited deficient responses in a skin blister model of aseptic inflammation. Cloning and sequencing of IRAK4 gene revealed that this patient expresses a "compound heterozygous" genotype, with a point mutation (C877T in cDNA) and a two-nucleotide, AC deletion (620-621del in cDNA) encoded by distinct alleles of the IRAK-4 gene. Both mutations encode proteins with an intact death domain, but a truncated kinase domain, thereby precluding expression of full-length IRAK-4.

In summary, it seems evident that each individual IRAK plays a distinct role in the innate immunity signaling process and that their interplay determines specific cellular response.

Seemingly redundant, studies to date have shown that in fact distinct IRAKs may have specific roles in the signaling pathways leading to cellular apoptosis (IRAK2), NF-κB activation and inflammatory cytokine expression (IRAK4 and 2), Stat3 activation and IL-10 expression (IRAK1), and inhibition of NF-κB activation (IRAK-M). Further investigation of the exact function and regulation of each IRAK may help significantly for understanding the process of innate immune response and related human diseases.

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