Published in final edited form as:
Cytokine. 2008 April ; 42(1): 1–7.

The involvement of the interleukin-1 Receptor-Associated Kinases (IRAKs) in cellular signaling networks controlling inflammation

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Abstract

Innate immunity and inflammation plays a key role in host defense and wound healing. However, Excessive or altered inflammatory processes can contribute to severe and diverse human diseases including cardiovascular disease, diabetes and cancer. The interleukin-1 receptor associated kinases (IRAKs) are critically involved in the regulation of intra-cellular signaling networks controlling inflammation. Collective studies indicate that IRAKs are present in many cell types, and can mediate signals from various cell receptors including Toll-Like-Receptors (TLRs). Consequently, diverse downstream signaling processes can be elicited following the activation of various IRAKs. Given the critical and complex roles IRAK proteins play, it is not surprising that genetic variations in human IRAK genes have been found to be linked with various human inflammatory diseases. This review intends to summarize the recent advances regarding the regulations of various IRAK proteins and their cellular functions in mediating inflammatory signaling processes.

Keywords

Innate immunity; cellular signaling network; IRAK; inflammation; regulation

INTRODUCTION

Following the discovery of Toll-Like-Receptors (TLRs) and related downstream signaling molecules, the field of innate immunity and inflammation has drawn immense interest. Human hosts can specifically respond to distinct non-self or abnormally-processed self molecules via TLRs and illicit complex yet specific responses through the expression of diverse cellular mediators such as cytokines, chemokines, complement factors, co-stimulatory molecules, as well as other numerous inflammatory mediators [1]. TLR and related intracellular signaling molecules are widely expressed in almost all types of cells and tissues. Consequently, innate immunity and inflammation play a key role in not only mediating host defense, but also in facilitating host metabolic and physiological processes. For example, alterations in innate immunity signaling processes have been linked with the pathogenesis of atherosclerosis and stroke [1–3], hypertension [2], diabetes and obesity [2,4,5], Parkinson's [6] and Alzheimer's [7] diseases, as well as cancer [1,8–10].

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Interleukin-1 receptor associated kinases (IRAK-1, 2, M, and 4) are intracellular kinases that can be recruited to the TLR complex and mediate diverse downstream signaling. Molecular and cellular analyses reveal that distinct IRAK proteins are differentially regulated and play unique roles in mediating downstream signaling processes [10–13]. In particular, IRAK-4 appears to be the critical kinase necessary for the classical NF κ B activation pathway [14–16]. A recent study indicates that IRAK-2 is also involved in the classical NF κ B pathway by facilitating TRAF6 ubiquitination [13]. In contrast, IRAK-1 is selectively involved in enhancing transcriptional activities of IRF5/7 as well as p65/RelA by facilitating their phosphorylation [11,17–20]. On the other hand, IRAK-M deactivates TLR-mediated NF κ B activation and may help to prevent excessive expression of cellular inflammatory mediators [21]. Besides regulating the activities of transcription factors, IRAKs may also participate in the regulation of cellular events not directly related with gene transcription. For example, IRAK-4 was shown to phosphorylate NADPH oxidase p47phox and contribute to cellular oxidative response in neutrophils [22]. Recent advances further reveal that IRAK family molecules are not limited to TLR-mediated innate immunity signaling processes. Selected IRAK molecules can also associate with protein partners involved in T cell and B cell receptor-mediated signaling pathways, indicating that IRAK proteins are critical for both innate and adaptive immune signaling [23,24]. Furthermore, IRAK molecules are implicated in the regulation of hepatocytes [25,26], neuronal cells [27], endothelial cells [28], and epithelial cells [29,30]. Collectively, cumulative studies indicate that IRAK molecules and related cellular signaling networks are involved in mediating diverse physiological processes.

Consequently, studies employing transgenic mice as well as human population-based studies have revealed that genetic variations in various IRAK genes are linked with diverse diseases such as infection, atherosclerosis, sepsis, auto-immune diseases, and cancer [8,31–34]. In this review, we intend to summarize the updated understanding of the differential regulation and function of IRAK family proteins within the cellular signaling network and their involvements in the pathogenesis of various human inflammatory diseases.

SIGNALING NETWORKS INVOLVING IRAK-1

IRAK-1 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 [35]. 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 ~76KD. IRAK-1 is expressed ubiquitously in diverse human tissues and cells. By radiation hybrid analysis, Thomas *et al.* mapped the murine IRAK-1 gene to Xq29.52-q29.7 and human IRAK-1 gene to Xq28 [36]. IRAK-1 protein contains an N-terminal death domain, a central serine/threonine kinase domain, and a C-terminal serine/threonine rich region. There are a putative nuclear localization sequence (NLS at aa 503–508) and a nuclear exit sequence (NES at aa 518–526)[27]. Using human THP-1 cells, primary blood mononuclear cells, as well as mice splenocytes, we have demonstrated that there are two signature forms of IRAK-1; the unmodified 80KD form, and the modified 100KD form [37]. IRAK-1 modification consists of phosphorylation, ubiquitination, and sumoylation [27,38,39]. Depending upon the nature of its modification, IRAK-1 may perform distinct functions including activation of IRF5/7[11, 18,20], NF κ B[17,40,41], and Stat1/3 [34,42] (figure 1).

There are multiple pathways and steps leading to fully activated NF κ B [43]. The first step involves the classical pathway causing the activation of IKK α / β complex, which contributes to I κ B α phosphorylation and degradation, and subsequent nuclear translocation of p65/RelA. The second step involves IKK ϵ /TBK-1 dependent p65/RelA phosphorylation, which is independent of the classical pathway and I κ B α degradation. Recent evidence indicates that IRAK-1 contributes to NF κ B activation by facilitating p65/RelA phosphorylation, but not the

classical pathway leading to I κ B α degradation and p65 nuclear translocation [17]. In agreement with this, we have also demonstrated that IRAK-1 deficient cells exhibit compromised p65/RelA phosphorylation, yet normal I κ B α degradation following TLR2-ligand Pam₃CSK₄ challenge [44]. IRAK-1 mediated IKK ϵ activation may also lead to phosphorylation and activation of STAT-related transcription factors [45].

Perhaps one of the major functions of IRAK-1 is to mediate the ligand-stimulated phosphorylation of IRF5/7 [11,20]. Studies from both Akira's and Golenbock's groups convincingly demonstrate that IRAK-1 deficiency leads to diminished activation of IRF5 and IRF7. Consequently, interferon alpha4 gene expression is dramatically decreased in IRAK-1^{-/-} cells following the stimulation with TLR7 and TLR9 ligand. In contrast, NF κ B-mediated gene expressions such as TNF α seem to be un-altered in IRAK-1^{-/-} cells upon challenge with agonists for TLR 7/8 as well as TLR9 [11]. Future efforts are needed to define new and physiologically relevant substrates for IRAK-1.

Besides mediating TLR signaling, IRAK-1 also participates in the regulation of adaptive immune response. For example, T cell co-stimulatory molecule CD26 can trigger the association of IRAK-1 with caveolin on antigen presenting monocytes, which is responsible for the subsequent expression of co-stimulatory molecule CD86 [23].

IRAK-1 is regulated at multiple levels. First, IRAK-1 protein can undergo covalent modifications including phosphorylation, ubiquitination, and sumoylation [34,38,39]. Upstream kinases such as IRAK-4 may contribute to the initial phosphorylation of IRAK-1 at Threonine 381 [46]. Following such event, IRAK-1 can be quickly activated and exhibit self-phosphorylation within its Pro/Ser rich region. This self-phosphorylation may subject the IRAK-1 molecule to subsequent ubiquitination and proteasome-mediated degradation. IRAK-1 degradation may serve as a negative feedback mechanism to prevent excessive inflammatory signaling process. Indeed, IRAK-1 degradation correlates with reduced host response to endotoxin, and has been correlated with endotoxin tolerance observed in septic leukocytes [37,47–51]. Intriguingly, we observed that IRAK-1 also gets sumoylated following either LPS or Pam₃CSK₄ challenge [27,34]. Sumoylated IRAK-1 enters the nucleus and contributes to Stat3 activation and selected gene expression [34]. The dynamic balance of IRAK-1 phosphorylation, sumoylation and ubiquitination may therefore regulate cellular IRAK-1 protein levels and contributes to its diverse yet distinct functions.

IRAK-1 is also regulated through the differential splicing process, which gives rise to three distinct isoforms of IRAK-1 (IRAK-1a, 1b, and 1c). IRAK-1b derives from alternative splicing and deletion of 90bp within exon 12, which yields an in-frame deletion of 30 amino acids (residues 514–543) [52]. IRAK-1c is due to alternative splicing and deletion of exon 11 and part of exon 12 [53]. IRAK-1b exists in minute amount (less than 1% of IRAK-1) in most human cells and tissues with unknown function. On the other hand, the full length IRAK-1 and IRAK-1c are abundantly expressed in human leukocytes and most tissues [27,53]. In contrast to IRAK-1, both IRAK-1b and IRAK-1c are stable and do not undergo covalent modification following various stimulations [27,52]. Overexpression of IRAK-1c blocks IL-1 β induced MAP kinase activation, suggesting that IRAK-1c may serve as a negative regulator of inflammation. Intriguingly, IRAK-1 is absent and IRAK-1c is the predominant form in young human brain tissues [27,53]. The absence of full length IRAK-1 may help keep the human brain in an immune-privileged state. In contrast to young humans, we recently found that both IRAK-1 and IRAK-1c are equally present in brain tissues obtained from aged humans [27]. This may bear significant implication in terms of aging. Increased chronic inflammation is a hallmark of the aging process as evidenced by local infiltration of inflammatory cells such as macrophages, and higher circulatory levels of pro-inflammatory cytokines, complement components and adhesion molecules. Consequently, aging is often accompanied by increasing

incidences of chronic inflammatory diseases such as Alzheimer's or Parkinson's disease. The molecular mechanisms contributing to the chronic inflammatory state during cellular senescence and the aging process is not clearly understood. Our finding that the full-length IRAK-1 and IRAK-1c are equally present in aged human brains may provide at least a partial explanation for the aging process. Future studies determining the mechanism of IRAK-1 mRNA differential splicing and the functions of different IRAK-1 splice forms are warranted.

Besides regulating innate and adaptive immune signaling, IRAK-1 protein is also implicated in regulating other cellular and physiological functions. IRAK-1 is widely expressed in numerous cells and tissues including neuronal cells, hepatocytes, endothelial cells, as well as epithelial cells [26,54–56]. Consequently, IRAK-1 and related cellular signaling networks may play a critical role in regulating diverse physiological processes. Further studies are clearly warranted to further define these complex signaling networks that IRAK-1 is involved in.

Given the significant and diverse roles IRAK-1 play, it is not surprising that variations in IRAK-1 gene will lead to diverse inflammatory diseases. Indeed, deletion of the IRAK-1 gene in mice decreases the risk of Experimental Autoimmune Encephalomyelitis (EAE) [33]. We have found that the IRAK-1 protein in leukocytes from human atherosclerosis patients is constitutively activated/sumoylated and localizes in cell nucleus [34]. Furthermore, our human population-based study indicates that genetic variation in IRAK-1 gene correlates with the severity of atherosclerosis and serum C reactive protein levels [2]. There are two IRAK-1 haplotypes and a rare variant haplotype (~10% of human population) contains three exon single nucleotide polymorphisms (SNPs). Humans harboring the variant IRAK-1 gene tend to have higher serum CRP levels and are at higher risk for diabetes and hypertension [2]. IRAK-1 gene variation is also linked to the risk of sepsis. Arcaroli et al. demonstrated that sepsis patients with the rare variant IRAK-1 haplotype have increased incidence of shock, prolonged requirement for mechanical ventilatory support, and greater 60-day mortality [32].

SIGNALING NETWORKS INVOLVING IRAK-2

IRAK-2 was initially identified by Dixit's group based on the search of the human expressed sequence tag (EST) database for sequences homologous to IRAK-1 [57]. 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. The human IRAK-2 gene is mapped to chromosome 3 at position 3p25.3–3p24.1. Upon overexpression, IRAK-2 can associate with MyD88 as well as TRAF6, and activate NFκB-dependent reporter gene expression. Intriguingly, IRAK-2, instead of IRAK-1 can also interact with another distinct TLR intracellular adaptor molecule Mal/TIRAP [58]. Dominant negative IRAK-2 can block Mal/TIRAP-induced signaling while dominant negative IRAK-1 fails to do so. These studies suggest that IRAK-2 may selectively be recruited by Mal/TIRAP to participate in subsequent NFκB activation. A recent study further confirmed that indeed IRAK-2 instead of IRAK-1 is primarily involved in TRAF-6 ubiquitination and NFκB activation [59] (figure 2). Besides activating NFκB, IRAK-2 also participates in the regulation of cellular apoptosis [60]. Dominant negative IRAK-2 can diminish LPS-induced macrophage apoptosis [60].

O'Neil's group has identified the murine IRAK-2 gene located at chromosome 6 position E3 [61]. In contrast to its human counterpart which only encodes one single transcript, the murine IRAK-2 gene can generate four alternatively spliced isoforms (designated as IRAK-2a, 2b, 2c, and 2d) that have various N-terminal deletions. Upon overexpression, IRAK-2a and IRAK-2b can activate, while IRAK-2c and IRAK-2d inhibit NFκB activation. Alternative splicing of the IRAK-2 gene in mice instead of humans reflects the difference between human and murine TLR signaling processes and innate immunity regulations. The physiological function of

IRAK-2 is poorly defined. Intriguingly, it was reported that several cases of human liver tumors harbor Hepatitis B Virus (HBV) DNA insertion near the IRAK-2 gene, which implies that IRAK-2 and related cellular signaling pathways may regulate human carcinogenesis [25]. Further studies are needed to determine the biochemical regulation of IRAK-2 and its participation in various cellular signaling pathways.

SIGNALING NETWORKS INVOLVING IRAK-M

Using a similar EST search, Wesche *et al.* identified a murine EST sequence which encodes a polypeptide sharing significant homology with IRAK-1 [62]. Human IRAK-M gene is mapped to chromosome 12 at position 12q14.1–12q15, and its murine homolog is mapped to chromosome 10. 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 kDa. Northern blot analysis revealed that this IRAK-M transcript is primarily present in the peripheral blood leukocytes and monocytic cell lines. Initial studies revealed that IRAK-M overexpression can activate NFκB activity [62]. Strikingly, later studies using IRAK-M^{-/-} cells indicate otherwise. IRAK-M^{-/-} macrophages exhibit enhanced NFκB activity and elevated expression of various inflammatory cytokines upon stimulation with several TLR ligands, indicating that IRAK-M may actually attenuate NFκB activation [21]. Phenotypically, IRAK-M^{-/-} mice develop severe osteoporosis, which is associated with the accelerated differentiation of osteoclasts, an increase in the half-life of osteoclasts, and their activation [63]. These studies indicate that IRAK-M may help to attenuate TLR signaling and prevent excessive inflammation. Recently, we have observed that IRAK-M primarily affects the alternative NIK-IKKα/IKKα-RelB NFκB pathway, instead of the classical TAK1-IKKα/IKKβ-RelA pathway [44, unpublished observation](figure 2).

Although IRAK-M may play a pivotal role in preventing excessive activation of NFκB and subsequent inflammatory response, such mechanisms may also be exploited by tumor cells or bacteria to evade active immune surveillance. Sepsis syndrome is initiated by dissemination of bacteria or bacterial products (endotoxin) in blood circulation [64]. The host develops an endotoxin-tolerant state in which blood leukocytes can no longer exhibit inducible NFκB activation and expression of selected inflammatory cytokines, such as TNF-α and IL-6 [65]. Suppressed expression of inflammatory cytokines further puts the host in danger of secondary infection. The suppressed state is caused by deactivation of the innate immunity signaling process, including persistent degradation of IRAK-1 and elevated IRAK-M protein levels in blood leukocytes [37,66–68]. Analogously, deactivation of innate immunity may also tolerate tumor growth and progression. For example, through a yet-to-be-determined mechanism, tumor-associated macrophages fail to express pro-inflammatory cytokines, such as IL12p40 and TNFα, and not only tolerate, but also facilitate tumor growth instead of mounting an efficient tumor cell-killing defense [69–71]. A recent study indicates that deactivated macrophages incubated with tumor cells exhibit increased IRAK-M protein levels [9]. Tumor hosts also have compromised antigen presentation, decreased function of effector T cells, and exacerbated inhibitory function of negative T regulatory cells in both circulating leukocytes as well as within tumor tissues, which is reflective of suppressed adaptive immunity. Given the evidence indicating the role of IRAK-M in deactivating both innate and adaptive immunity signaling, we hypothesize that cancer cells may exploit the inhibitory function of IRAK-M to evade host immune surveillance.

Indeed, our recent study indicates that IRAK-M disruption contribute to enhanced tumor rejection [10]. IRAK-M^{-/-} mice are resistant to tumor growth upon inoculation with transplantable tumor cells. Mechanistically, we observed that immune cells from IRAK-M^{-/-} mice were responsible for the anti-tumor effect, since adoptive transfer of splenocytes

from IRAK-M^{-/-} mice to wild type mice transferred the tumor-resistant phenotype. Upon tumor cell challenge, there were elevated populations of CD4⁺ and CD8⁺ T cells and a decreased population of CD4⁺ CD25⁺ Foxp3⁺ regulatory T cells in IRAK-M^{-/-} splenocytes. Furthermore, we observed that IRAK-M deficiency leads to elevated proliferation and activation of T cells and B cells. Enhanced NFκB activation caused by IRAK-M deficiency may explain the elevated activation of immune cells. Mechanistically, we recently observed that IRAK-M selectively attenuates the alternative NIK-RelB NFκB pathway, instead of the classical TAK-1/p65 NFκB pathway [44, unpublished observation].

SIGNALING NETWORKS INVOLVING IRAK-4

IRAK-4 is the last IRAK family member being identified [14]. Human IRAK-4 gene is mapped to chromosome 12 at position 12p11.22, and its murine homolog is mapped to chromosome 15. Full-length IRAK-4 cDNA encodes a protein with 460 amino acids and a calculated molecular mass of 52 kDa. In contrast to IRAK-1 or IRAK-M deficient mice, IRAK-4^{-/-} mice exhibit severe impairment in NFκB activation and expression of various inflammatory cytokines upon challenges with several TLR ligands [15]. Overexpression of kinase-dead IRAK-4 mutant strongly diminishes IL-1/LPS induced NFκB activation, pointing to the essential role of its kinase activity [14]. MyD88 is critically involved in recruiting IRAK-4 into the TLR4 complex [14]. These studies indicate that IRAK-4 is the primary kinase in the TLR signaling process essential for mediating NFκB activation. Recently, Suzuki *et al.* reported that IRAK-4 is also critically involved in T cell receptor (TCR)-induced T cell proliferation through NF-κB activation [24]. T cell responses *in vivo* are significantly impaired in IRAK-4 deficient mice. Upon TCR stimulation, IRAK-4 is recruited to T cell lipid rafts, where it can associate with Zap70 and activate protein kinase C. This finding indicates that there is an intricate connection between innate and adaptive immune system activation, and IRAK-4 may be directly involved in the cross talk between the two systems.

Intriguingly, a recent finding indicates that IRAK-4 can directly phosphorylate the NADPH oxidase p47phox in neutrophils [22]. This finding opens up a brand new aspect of IRAK-mediated cellular regulation, and suggests that IRAK family proteins may be capable of phosphorylating multiple structurally related, yet functionally distinct substrates. Besides mediating NFκB and related transcription factor activation, IRAK substrates may also be involved in regulating other totally distinct cellular events.

Because of the central role IRAK-4 plays in mediating NFκB activation and innate immunity signaling, humans carrying IRAK-4 gene variation may be prone to microbial infections. Indeed, a study by Picard *et al.* revealed that IRAK-4 mutations are present in three children suffering from persistent pyogenic bacteria infection and poor inflammatory responses [72]. These patients did not respond to IL-1β, IL-18, or any of the TLR1-6 or 9 ligands, as assessed by activation of NF-κB and p38-MAPK, and induction of IL-1β, IL-6, IL-12, TNFα, and IFN-γ. It is intriguing that the spectrum of infections was relatively narrow, with most infections caused by Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pneumoniae*. The patients showed poor sign of inflammatory response and the frequency of infection decreased with age, potentially due to the compensatory action of adaptive immunity. 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* [73]. This patient also exhibits deficient responses in a skin blister model of aseptic inflammation. Cloning and sequencing of the IRAK-4 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, therefore precluding expression of full-length IRAK-4. Recently, a case of invasive,

systemic, extraintestinal Gram-negative *Shigella* infection was reported in a patient with inherited IRAK-4 deficiency [31]. This case indicates that although the pyogenic Gram-positive bacteria *Staphylococcus* and *Pneumococcus* remain the most frequent pathogens associated with IRAK-4 deficiency, Gram-negative bacteria such as *Shigella* may threaten humans with IRAK-4 deficiency and cause severe illness and mortality.

CONCLUDING REMARKS

In summary, collective research efforts regarding gene expression, message splicing, protein structure and function, signal transduction, cellular activation of human and murine immune cells, as well as genetic variation in humans, have led to extensive understanding of the complex roles and regulations of IRAK family proteins. It is apparent that although IRAK proteins share some common structural features, they are clearly not redundant and each has a unique and distinct function. Furthermore, individual members may have multiple roles depending upon their modification and cellular and tissue distribution. Besides mediating various TLR signaling pathways, IRAKs are also involved in other signaling networks in diverse tissues and cells such as adipocytes, hepatocytes, muscle cells, endothelial cells, and epithelial cells. Conceivably, these molecules pose viable targets for designing new therapeutic strategies for various human inflammatory diseases.

Acknowledgements

This work is supported by NIH grants to L.L.

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Figure 1.
IRAK-1 mediated intracellular signaling network

Figure 2.
Involvement of IRAK-2, M, and 4 in the differential regulation of NFκB pathway