

SIGIRR/TIR8: A Negative Regulator of Toll-IL-1R Signaling

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Abstract: Toll-like receptors (TLRs) belong to the Toll-IL-1 receptor superfamily, which is defined by a common intracellular Toll-IL-1 receptor (TIR)-domain. These receptors employ related yet distinct signaling components and downstream pathways, leading to activation of the transcription factors NF- κ B, ATF and IRF3. Recent studies have also begun to unravel how these pathways are negatively regulated. SIGIRR (also known as TIR8), a member of Toll-IL-1R superfamily that does not activate the transcription factors NF- κ B, ATF and IRF3, instead negatively modulates responses. Inflammation is enhanced in SIGIRR-null mice as measured by enhanced chemokine induction after IL-1 injection and a reduced threshold for lethal endotoxin challenge. SIGIRR-deficient mice are more susceptible to DSS-induced inflammatory bowel disease. Cells from SIGIRR-null mice show enhanced activation in response to either IL-1 or certain Toll ligands. Therefore, SIGIRR functions as a biologically important modulator of Toll-IL-1R signaling.

INTRODUCTION

Innate immunity is a first-line of defense against pathogenic microorganisms through the recognition of pathogen-associated molecular patterns by the Toll-like receptors. Consistent with their roles in detecting the invasion of microorganisms, TLRs are expressed in innate immune cells, including macrophages, dendritic cells, B cell, phagocytes and mast cells [1]. In addition, TLRs are also expressed in several other cell types that play important roles in first-line host defense [1]. The mucosal surfaces of the respiratory and intestinal tract are covered by a single layer of epithelial cells, forming a protective barrier against pathogens. In the intestine, the apical surfaces of epithelial cells are continually exposed to bacteria (resident microflora), but this does not result in exaggerated inflammation. These epithelial cells elicit inflammatory responses only against pathogenic bacteria that invade to the basolateral compartment from the apical side. Upon damage of the intestine and colon epithelium (chemical such as DSS treatment, physical such as a cut or a perforating ulcer), these commensals become pathogens, leading to inflammatory responses [20]. The expression of TLRs is regulated spatially and temporally in the mucosal surfaces of the respiratory and intestinal tract, which contributes to capability of the epithelial cells to discriminate between pathogen and resident microflora [41].

Stimulation of the innate immune system by TLRs activates the adaptive immune system through the production of proinflammatory cytokines, including IL-1, IL-6, TNF or IL-12, and the induction of key cell-surface molecules, which drive T cell activation, including MHC, CD40, CD80, and CD86. The cytokines produced, such as IL-1, induce pronounced positive feedback in the immune

system, which, if not well controlled, can cause devastating illnesses such as chronic arthritis and inflammatory bowel diseases. Therefore, it is critical to investigate the detailed molecular mechanisms by which the Toll-IL-1Rs mediate signaling and how the pathways are regulated, with the long-term objective to develop more effective anti-inflammatory small molecule drugs.

Recent studies indicate that SIGIRR functions as a biologically important negative regulator of Toll-IL-1R signaling [55]. SIGIRR-deficient mice had a reduced threshold for lethal endotoxin challenge are more susceptible to DSS-induced inflammatory bowel disease [13,55]. Therefore, the action of SIGIRR probably provides a novel mechanism by which the normal intestinal epithelium regulates innate immune response and inflammation. In addition to SIGIRR, several other negative regulators have been shown to inhibit the Toll-IL-1R signaling, including ST2 [4], IRAKM [31], MyD88s [21], SOCS1 [29] and Triad3A [9]. In this review, we will discuss how SIGIRR and the other negative regulators exert their inhibitory roles on the signaling events mediated by IL-1 and Toll ligands, thereby modulating the innate immune response and inflammatory response.

TOLL-IL-1R SUPERFAMILY

The Toll-IL-1 receptor superfamily, a large family of proteins defined by the presence of an intracellular Toll-IL-1 receptor (TIR) domain, plays crucial roles in the immune response. This superfamily can be divided into two main subgroups based on their extracellular domains, the Immunoglobulin domain (Ig) containing receptors [37] and the Leucine Rich Repeat motif (LRR) containing receptors [35].

1. The Immunoglobulin (Ig) Domain Subgroup

The Ig domain subgroup includes IL-1R1, IL-1RII, IL-1R8, SIGIRR and ST2. IL-1 has been demonstrated to be

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one of the key orchestrators of the immune response, eliciting a wide range of biological responses, including fever, lymphocyte activation, and leukocyte infusion to the site of injury and infection [11]. While IL-1 activates its signaling cascade through IL-1R1, IL-1RII serves as a negative regulator of IL-1 signaling by binding IL-1 and preventing downstream signaling. IL-18 plays important roles in promoting Th1 cell differentiation and NK cell activation. The two orphan receptors, SIGIRR and ST2, have now been shown to function as negative regulators of the signaling pathways mediated by the Toll-IL-1 receptors, which will be further discussed below [13,55].

2. The Leucine Rich Repeat Motif (LRR) Subgroup

The LRR subgroup consists of at least eleven Toll-like receptors (TLRs) [8,17,35,44,50,63]. These receptors have received intense attention because different TLRs were found to be activated by specific pathogen products. While TLR4 has been genetically identified as a signaling molecule essential for the responses to LPS, a component of gram negative bacteria [42], TLR2 responds to mycobacteria, yeast, and gram-positive bacteria [48,49,53,54]. TLR6 associates with TLR2 and recognizes lipoproteins from microplasma. While TLR5 mediates the induction of the immune response by bacterial flagellins [15], TLR9 has been shown to recognize bacterial DNA [17], and TLR3 recognizes dsRNA [2]. While a synthetic compound (imidazoquinoline compound R848) with antiviral activity has been described as a ligand for TLR7 and TLR8 [25,51], recent studies showed that ssRNA is the natural ligand for TLR7/8 [10,16]. The natural ligands for TLR10 and TLR11 are still not known [63].

TOLL-IL-1R SIGNALING

1. IL-1R-mediated Signaling

The Toll-IL-1R superfamily delivers biological activities mainly by activating the transcription of various genes in different target cells. Tremendous effort has been devoted to understanding the signaling pathways mediated by this receptor superfamily. Because their cytoplasmic domains are similar, these receptors may employ related yet distinct signaling components and downstream pathways. Because IL-1R was the first discovered receptor in this superfamily, the IL-1-mediated signaling pathway serves as a “prototype” for other family members. Genetic and biochemical studies revealed that IL-1R mediates a very complex pathway, involving a cascade of kinases organized by multiple adapter molecules into signaling complexes, leading to activation of the transcription factors NF- κ B, ATF and AP-1 [3,39,40]. Based on published studies [18,19,23,24], a model of the IL-1 pathway is postulated (Fig. 1). Upon IL-1 stimulation, adapter molecule MyD88 [56] is first recruited to the IL-1 receptor, followed by the recruitment of two serine-threonine kinases, IRAK4 [32,47] and IRAK [6,33], and the adapter TRAF6 [7], resulting in the formation of the receptor complex (Complex I). During the formation of Complex I, IRAK4 is activated, leading to the hyperphosphorylation of IRAK, which creates an interface for its interaction with adapter Pellino 1 [23]. The formation of Pellino 1-IRAK4-IRAK-TRAF6 causes conformational changes in the receptor complex (Complex I), releasing these signaling molecules from the receptor. The released components then interact with the membrane bound pre-associated TAK1-TAB1-TAB2-TAB3 [24], resulting in the formation of Complex II (IRAK-TRAF6-TAK1-TAB1-TAB2-TAB3). TAK1 and

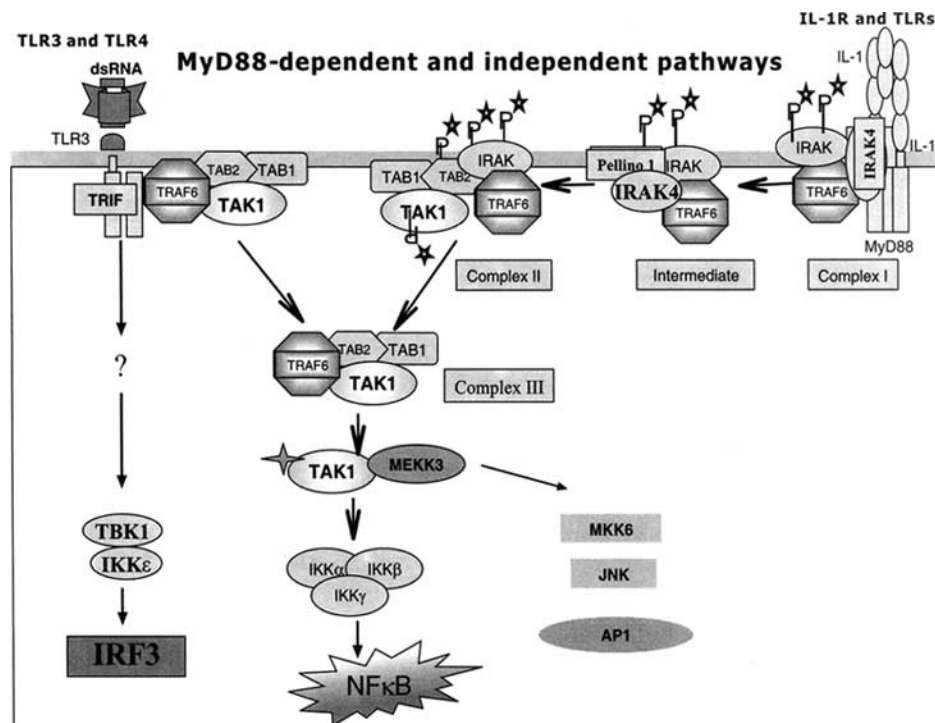


Fig. (1). Model of the Toll-IL-1R-mediated pathways.

TAB2 are phosphorylated in the membrane-bound Complex II, triggering the dissociation and translocation of TRAF6-TAK1-TAB1-TAB2-TAB3 (Complex III) from the membrane to the cytosol. The translocated Complex III interacts with additional factors in the cytosol, leading to TAK1 activation. The activation of TAK1 eventually leads to the activation of I B kinase (IKK), in turn leading to the phosphorylation and degradation of I B proteins, and liberation of NF B to activate transcription in the nucleus [36,43,57,62]. Activated TAK1 has also been implicated in the IL-1-induced activation of MKK6 and JNK [38], leading to the activation of other transcription factors, including ATF and AP1, thereby also activating gene transcription. In addition to TAK1, MEKK1 and MEKK3 have also been implicated in the activation of IKK and MAPK, leading to the activation of NF B and JNK [58,60,61].

2. TLR-mediated MyD88-independent Signaling Pathway

Several Toll-IL-1 receptors also utilize variations of the above common signaling pathway. For example, TLR3 and TLR4 use MyD88-independent pathways to activate the transcription factors NF B and IRF3 [28,49]. IRF3 is part of the IRF family of transcription factors, which play important roles in host defenses against pathogens, immunomodulation, and growth control. IRF3 is required for expression of interferon and the chemokine RANTES in response to viral infection. In unstimulated cells, IRF3 is present in the cytoplasm. Viral infection and Toll ligands (dsRNA and LPS) lead to the phosphorylation of IRF3 and its subsequent dimerization and interaction with the co-activators CREB-binding protein (CBP) and p300. The IRF3 complex then translocates to the nucleus, where it activates promoters containing IRF-binding sites. Recently, the I B kinase (IKK)-related kinases IKK and TANK-binding kinase 1 have been implicated in the phosphorylation and activation of IRF3 [27,46]. The TIR domain-containing adapter inducing IFN- (TRIF) was recently identified as an adapter for TLR3 and TLR4 [26,34]. TRIF-deficient mice are defective in both TLR3- and TLR4-mediated expression of IFN- and activation of IRF3 [34]. Whereas TRIF-deficient mice showed complete loss of TLR3-induced NF B activation, TLR4-mediated NF B activation was only abolished completely in mice deficient in both MyD88 and TRIF [34]. In addition to TRIF, TRAM has also been implicated in TLR4-, but not TLR3-mediated MyD88-independent pathway [12,59].

It has also been shown that double-stranded RNA (dsRNA)-triggered, TLR3-mediated signaling is independent of MyD88, IRAK4 and IRAK [22]. Instead, TRAF6, TAK1 and TAB2 are recruited to TLR3 upon Poly I:C stimulation. TRAF6-TAK1-TAB2 are then translocated to the cytosol, where TAK1 is phosphorylated and activated, leading to the activation of IKK and NF B [22]. It has been recently reported that adapter molecule TRIF recruits TRAF6-TAK1-TAB2-TAB3 to TLR3 through its TRAF6-binding site, which is required for NF B but not IRF3 activation [22,45]. Therefore, dsRNA-induced TLR3/TRIF-mediated NF B and IRF3 activation diverge at TRIF. Based on these published studies of others [22,26,27,34,46], a model for TLR3-mediated signaling has been proposed (Fig. 1). Upon PolyI:C

stimulation, TRIF is recruited to TLR3 through TIR-TIR domain interactions. TRIF then recruits TRAF6 to TLR3 through its TRAF6 binding site, followed by recruitment of TAK1 and TAB2 via their interaction with TRAF6. TRAF6-TAK1-TAB2 then dissociates from the receptor and translocates to cytosol, where TAK1 is activated. Activated TAK1 leads to the activation of IKK and NF B. Independent of TRAF6-TAK1-TAB2, through interaction with unknown intermediate signaling components, TRIF leads to the activation of TBK1/IKK, resulting in IRF3 activation.

NEGATIVE REGULATION OF TOLL-IL-1R-MEDIATED SIGNALING

While the positive regulation of NF B, AP1 and IRF3 through the Toll-IL-1R superfamily has been studied extensively, recent studies have also begun to unravel how these pathways are negatively regulated. Two orphan receptors of the Toll-IL-1R superfamily, SIGIRR [55] (also known as TIR8 [13]) and ST2 [4], have now been identified as negative regulators for the signaling pathways mediated by the members of this receptor superfamily. The other molecules that have been shown to negatively regulate the Toll-IL-1R signaling include IRAKM, MyD88, SOCS1 and Triad3A.

1. SIGIRR

SIGIRR (the single immunoglobulin IL-1 receptor related molecule), also known as TIR8 (Toll-IL-1R 8), was initially identified through database searching for Toll domain containing proteins [51]. SIGIRR represents a unique subgroup of the Toll-IL-1R superfamily, since its extracellular domain consists of a single Ig domain. Although most members of this superfamily have been shown to be capable of activating NF B, AP1 or IRF3 constitutively or after structural modification, this activation has not been observed with SIGIRR. Further, SIGIRR has been shown not to bind IL-1 or to enhance IL-1 signaling, but to inhibit IL-1-mediated signaling. SIGIRR is expressed highly in primary kidney and intestinal epithelial cells and moderately in splenocytes and immature dendritic cells, but is not expressed in primary macrophages, fibroblasts, and endothelial cells [55]. LPS stimulation leads to down regulation of SIGIRR expression in different mouse tissues, suggesting an important regulatory role of SIGIRR in the inflammatory process [55]. In order to understand the biological functions of SIGIRR, SIGIRR (TIR8)-deficient mice were constructed [13,55]. Consistent with SIGIRR's expression pattern, SIGIRR-deficient kidney and intestinal epithelial cells and splenocytes, but not macrophages, exhibit enhanced responsiveness to IL-1 and Toll ligands [55]. Furthermore, SIGIRR (TIR8)-deficient dendritic cells (DC) showed increased cytokine production in response to TLR ligands, including LPS and CpG DNA [13]. Moreover, SIGIRR-deficient mice show an enhanced inflammatory response to IL-1, specifically in the lung and colon and a reduced threshold for lethal endotoxin challenge [55]. Importantly, it has been shown that the SIGIRR-deficient mice are more susceptible to inflammatory bowel disease induced by dextran sulfate sodium (DSS), as compared to wild-type mice [13]. Taken together, these observations indicate that SIGIRR (TIR8) represents a negative pathway

of regulation of the Toll-IL-1R system, expressed in epithelial cells and DC, crucial for tuning inflammation in gastrointestinal tract. The detailed signaling mechanism for SIGIRR is still unclear. The fact that SIGIRR forms a complex with the IL-1 receptor, IRAK and TRAF6 upon IL-1 stimulation suggests that SIGIRR functions through its interaction with the Toll-IL-1 receptor complex [55].

2. ST2

Both SIGIRR and ST2 are TIR containing orphan receptors and do not activate NF- κ B when they are overexpressed. Recently both of them have been shown to be involved in the negative regulation of TLR signaling [4,13,55]. ST2 has been shown to play an important role in Th2 responses [52]. ST2-deficient mice showed increased production of inflammatory cytokines in response to LPS; moreover, they also showed defective induction of LPS tolerance [4]. Overexpression of ST2 was found to inhibit NF- κ B activation, because ST2 associated with, and probably sequestered, MyD88 and TIRAP. Thus, the results from ST2-deficient mice provide a molecular explanation for the function of ST2 in Th2 responses. Inhibition of Toll-IL-1R signaling by ST2 on cells such as macrophages and mast cells will result in a Th2 response because the ability of these receptors to induce a Th1 response would be impaired.

3. Other Negative Regulators

In addition to these inhibitory orphan receptor molecules, several intracellular inhibitory molecules have also been reported for regulating Toll-IL-1R signaling. An IRAK family member, IRAKM (Fig. 2), was implicated in the negative regulation of TLR signaling [30,31]. IRAKM expression is induced upon TLR stimulation and negatively regulates TLR signaling. IRAKM-deficient cells exhibit

increased cytokine production upon Toll-IL-1 stimulation and bacterial challenge, and IRAKM-deficient mice show increased inflammatory responses to bacterial infection. At the molecular level, IRAKM was shown to prevent the dissociation of IRAK and IRAK4 from MyD88 and the formation of IRAK-TRAF6 complexes. The detailed molecular mechanism for how IRAKM negatively regulates Toll-IL-1R-mediated signaling is still unclear. The IRAK family consists of two active kinases, IRAK and IRAK4, and two inactive kinases, IRAK2 and IRAK3. Recent studies showed that two specific splicing variants of murine IRAK2, IRAK2c and IRAK2d also have inhibitory effects on signaling pathways mediated members of the Toll-IL-1R superfamily [14]. In addition, the splicing variant of MyD88, MyD88s, has been demonstrated to act as a negative regulator of Toll-IL-1R signaling by blocking the recruitment of IRAK4 [5,21]. Furthermore, it is reported recently that a Ring finger protein, Triad3A, acts as an E3 ubiquitin-protein ligase and enhances ubiquitination and proteolytic degradation of some TLRs, thereby regulating the intensity and duration of TLR signaling [9]. Moreover, SOCS1, a classical cytokine negative regulator, has also been implicated in TLR signaling. SOCS1 is rapidly induced by LPS and negatively regulate LPS signaling [29]. Taken together, these findings suggest that multiple regulatory mechanisms are probably involved in the downregulation of TLR signaling. It is conceivable that the inhibitory orphan receptors T1/ST2 and SIGIRR may activate an inhibitory signaling pathway(s) by employing some of these intracellular inhibitory molecules.

DISCUSSION

As discussed above, SIGIRR plays an important negative regulatory role in TLR-mediated signaling. The high expression levels of SIGIRR in epithelial cells suggests that

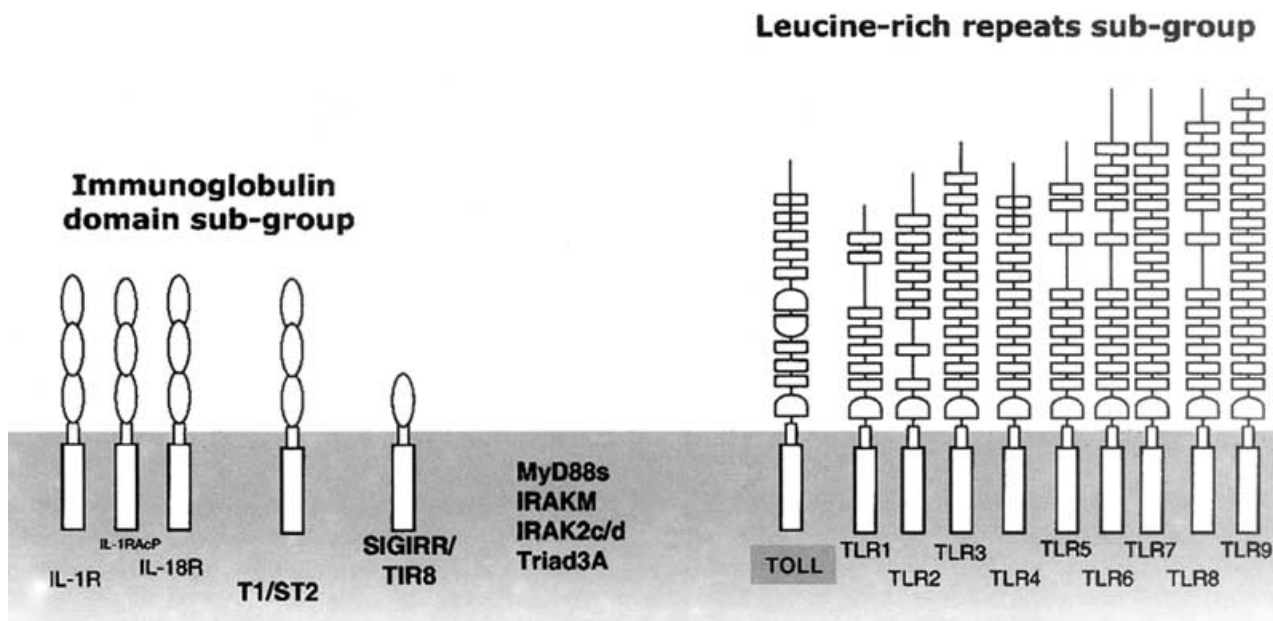


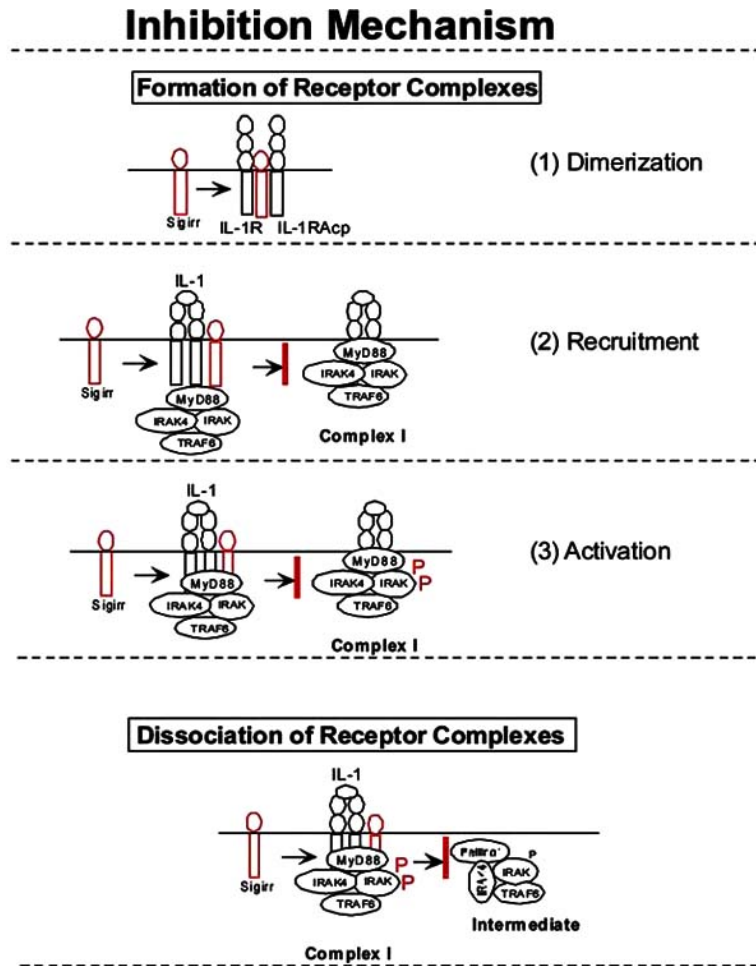
Fig. (2). Negative regulators for the TLR-IL-1R superfamily.

SIGIRR may primarily serve to dampen the immune response in cells, such as colon and lung epithelial cells, that are continually exposed to microorganisms. Indeed, the SIGIRR(TIR8)-deficient mice are more susceptible to develop DSS-induced colitis, indicating that SIGIRR plays an irredundant negative regulatory role in intestinal inflammation by modulating Toll-IL-1R-mediated signaling. SIGIRR expression is down regulated in certain inflammatory conditions, suggesting that the inhibitory role of SIGIRR can be reduced through its down regulation to facilitate more potent immune responses.

One of the future directions is to elucidate its molecular mechanism of SIGIRR's action. Since endogenous SIGIRR

interacts with IL-1R, TLR4, IRAK and TRAF6 upon IL-1 and LPS stimulation, SIGIRR probably exerts its inhibitory effect through its interaction with the receptor complexes. SIGIRR may directly interfere with the formation of the receptor complex (including receptor dimerization, appropriate recruitment and activation of the receptor proximal signaling components) or attenuates the dissociation of the activated signaling components from the receptor, inhibiting the activation of downstream signaling events (Fig. 3A). In addition to (or instead of) imposing direct steric inhibition on the receptor complexes, SIGIRR may actively recruit intracellular inhibitory molecules to the receptors, thereby exerting its inhibitory effects (Fig. 3B).

3A)



3B)

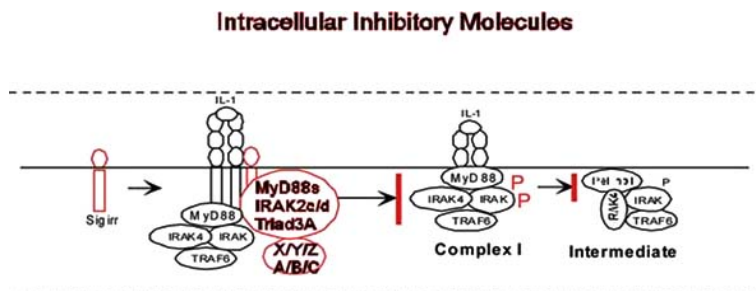


Fig. (3). Modes of inhibition for SIGIRR.

SIGIRR may interact with the known intracellular inhibitory molecules of Toll-IL-1R signaling, including IRAKM, IRAK2c, IRAK2d, MyD88s and Triad3A. SIGIRR may also recruit other novel intracellular inhibitory molecules. As IRAKM has been described to function primarily in macrophages and SIGIRR is not detected in this cell type, it is not likely that these proteins cooperate *in vivo*. However, it is still possible that both of these proteins may be induced under certain conditions and cooperate as negative regulators. Elucidation of the detailed molecular mechanism of SIGIRR's action in the future is critical for understanding the regulatory role of SIGIRR in inflammatory and innate immune responses and essential for evaluating the therapeutic potential of SIGIRR.

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