

IRAKs: Key Regulatory Kinases of Innate Immunity

Nobutaka Suzuki, Shinobu Suzuki and Takashi Saito*

Laboratory for Cell Signaling, RIKEN Research Center for Allergy and Immunology, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan

Abstract: Toll-like receptors (TLRs), interleukin 1 receptor (IL-1R), IL-18 receptor (IL-18R) and plant R are vital to the induction of acute inflammation as well as various adaptive immune responses upon invasion of microorganisms. These receptors share a common cytoplasmic domain called the TIR (TLR/IL-1R/plant R) domain and the signaling cascade involving the TIR domain is conserved from invertebrate to vertebrate.

The engagement of TIR domain containing receptors initiates their signaling through several intermediate proteins including serine-threonine kinase IL-1 receptor associated kinases (IRAKs). The IRAK family has four members and the newest member, IRAK-4, is indispensable to the TIR-mediated signaling pathway. The improper regulation of TIR receptor signaling leads to the development of such severe inflammatory diseases as sepsis, asthma, rheumatoid arthritis and even cancer. Therefore, it is very important to determine precisely the implications of TIR signaling in those inflammatory diseases for appropriate medical treatment and drug development. As IRAK-4 is the critical molecule for TIR-mediated signaling, it is a promising therapeutic target for many inflammatory diseases. In this review, we discuss the functions of the IRAK family members with focus on IRAK-4, to seek the possibility of yielding new therapeutic strategies.

Keywords: IRAK-1, IRAK-2, IRAK-M, IRAK-4, TIR domain, innate immunity

INTRODUCTION

Toll-like receptors (TLRs) are the key components for inducing acute inflammation followed by various adaptive immune responses because they have the ability to recognize specific pathogen-associated molecular patterns (PAMPs) [1-3]. The structures of the TLRs are evolutionarily conserved with that of *Drosophila melanogaster* Toll. Toll was originally found to play an important role in dorsoventral axis formation in embryogenesis; however, recently, its role in anti-fungal immune response in adulthood has been widely reported [2]. Toll initiates its signal through the adaptor molecule Tube and dMyD88, and this is followed by activation of the serine-threonine kinase Pelle [4]. Pelle then leads to the phosphorylation of Cactus, which binds to the transcription factor Dorsal, an NF- κ B/Rel family protein. Released from Cactus, Dorsal freely translocates to the nucleus to induce the expression of target genes involved in dorsoventral polarity or anti-fungal immune response [5]. The signaling pathway of Toll is highly conserved with that of mammalian TLR. A human homologue of Pelle has been identified as a member of the interleukin-1 receptor (IL-1R) associated kinase (IRAK) family [6]. IRAKs are membrane proximal putative serine-threonine kinases. The name IRAK was given because it was initially found to be associated with the cytoplasmic domain of IL-1R. However, later, it was implicated that IL-1R, IL-18 receptor (IL-18R) and TLRs share the same homology in their intracellular domains

called the TIR (TLR/IL-1R/plant R) domain and that the IRAK family is critically involved in TIR domain containing receptor signaling [7]. The engagement of TIR domain containing receptors initiates the activation of downstream transcription factors such as NF- κ B [8] and AP-1 to induce the transcription of various pro-inflammatory genes. Immediately after the stimulation of the IL-1R/TLR superfamily, receptors recruit cytoplasmic adaptor proteins, mainly MyD88 [9], for signal transduction. Aside from MyD88, MyD88 adaptor-like proteins (Mal/TIRAP) [10, 11], TIR domain containing adaptor proteins inducing interferon-beta (TRIF/TICAM-1) [12] and TRIF-related adaptor molecules (TRAM/TIRP/TICAM-2) [13] are also involved in specific TLR signaling. The receptor-adaptor complexes then recruit tumor necrosis factor (TNF) receptor associated factor 6 (TRAF6) in order to transmit signals from receptors to downstream transcription factors [14]. In this regard, IRAKs are considered to be very important factors for mediating the signals from the receptor-adaptor complexes to TRAF6.

To date, extensive progress has been made in understanding the importance of the IRAK family members in TIR containing receptor signaling. Recently, IRAK-4, the newest IRAK member, was shown to be indispensable to the IL-1R/TLR/IL-18R signaling pathway by gene targeting study with mice [15]. Moreover, it has been reported that patients genetically deficient in IRAK-4 expression suffer from severe bacterial infection [16]. Therefore, from a clinical point of view, it is important to elucidate how IRAK-4 and other IRAKs function to regulate the signaling cascade of TIR-containing receptors, which are critical for innate immune responses as well as adaptive immunity.

*Address correspondence to this author at the Laboratory for Cell Signaling, RIKEN Research Center for Allergy and Immunology, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan; Tel: +81-45-503-7037; Fax: +81-45-503-7036; E-mail: saito@rcai.riken.jp

STRUCTURE OF IRAK FAMILY

To date, four distinct members of the IRAK family have been reported: IRAK-1 [6], -2 [17], -M [18] and -4 [19]. All the IRAKs contain the conserved death domain at their N-terminus and the kinase domain at the central part of their structure (Fig. 1). The death domain has been described initially as a region in the cytoplasmic tail of TNF receptor 1 [20], which is involved in TNF-mediated cell death signaling. The reason why it is called the death domain is because proteins with this domain are usually involved in programmed cell death. In most of these proteins, the death domain is located at the extreme C-terminus. However, the death domains of MyD88 and IRAKs are located at their N-terminus and are mainly involved in protein-protein interactions for transmitting signals to induce inflammatory responses. Recent studies have suggested that MyD88 is also involved in TLR-induced cell death [21, 22]. There are many conserved regions in the catalytic domain of serine/threonine kinases. The N-terminus of the catalytic domain contains a glycine-rich stretch of residues in the vicinity of a lysine residue, which is involved in ATP binding. At the central part is a conserved aspartic acid residue that is important for the catalytic activity of the enzyme. The structure of the *Drosophila* protein kinase Pelle resembles those of the IRAK members, although it has been revealed that the closest human homologue of Pelle is IRAK-4. Despite their structural similarities, however, only IRAK-1 and IRAK-4 are active kinases, whereas the other two are inactive because they lack the catalytically active aspartic acid residue in the kinase domain. Although the exact functions of the IRAK proteins remain unclear, each of them seems to play divergent roles in regulating the TIR signaling cascade in host defense against microorganisms.

FUNCTIONAL ANALYSIS OF IRAK FAMILY MEMBERS

1) IRAK-1

IRAK-1 is the first prototypic IRAK family member to be described; it was reported by Martin *et al.* in 1994 [23]

and cloned by Cao *et al.* in 1996 [6]. IRAK-1 is ubiquitously expressed and has auto- and cross-phosphorylation kinase activities, similar to Pelle. Upon receptor engagement, IRAK-1 is recruited to the receptor-MyD88 complex where it is rapidly activated by phosphorylation. Hyperphosphorylated IRAK-1 mediates the recruitment of TRAF6 to the receptor complex and then the IRAK-1-TRAF6 complex leaves the receptor complex to translocate to the membrane where it interacts with TGF-beta (transforming growth factor-beta)-activated protein kinase (TAK1, a member of the MAP kinase kinase kinase (MAPKKK) family [24]) along with TAK1-binding protein-1 (TAB1) [25], TAB2 [26] and TAB3 [27] molecules. TAK1 and TAB2 are phosphorylated on the membrane, which facilitates the formation of the TRAF6-TAK1-TAB1-TAB2 complex and its translocation from the membrane to the cytosol for TAK1 activation [28], which subsequently leads to the activation of I B kinase (IKK). TAK1 also activates mitogen-activated protein kinases (MAPKs) and c-Jun NH2-terminal kinase (JNK). After the TRAF6-TAK1-TAB1-TAB2 complex translocates to cytosol, IRAK-1 is eventually ubiquitinated. After ubiquitination, IRAK-1 is transferred into the proteasome where it is degraded. IRAK-1 has dispensable kinase activity because the IL-1-induced NF- B activation can still be driven by a kinase-inactive mutant [29]. IRAK-1-deficient mice and cells show diminished levels of cytokine production in response to IL-1, IL-18 and LPS, although the inhibitory effects are partial [30]. Moreover, the kinase-inactive mutant IRAK-1 is still phosphorylated in response to IL-1 stimulation when it is transfected into IRAK-1-deficient cells, suggesting that there must be an IRAK-1 kinase in the pathway. Indeed, it is now believed that IRAK-4 is the IRAK-1 kinase and the characteristics of IRAK-4 are described in the following section.

The human *IRAK1* gene encodes three isoforms by alternative splicing, namely, IRAK-1 [6], IRAK-1b [31], and IRAK-1-S [32]. IRAK-1b is produced by the use of an alternative 5'-acceptor splicing site defined by a sequence within exon 12 of IRAK-1. In contrast to the full-length isoform, IRAK-1b displays no change in its protein level

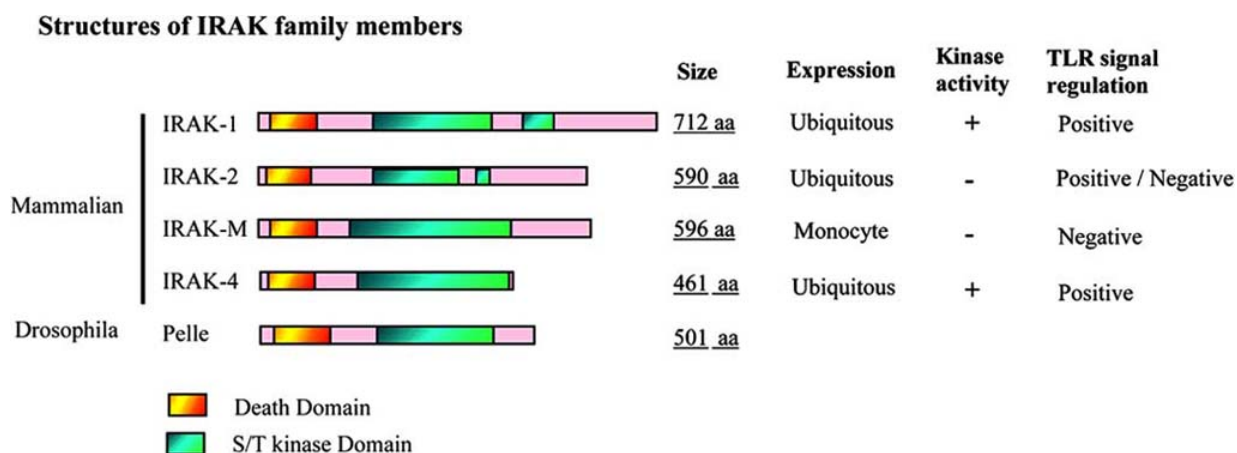


Fig. (1). Structures of IRAK family members

Each IRAK family member has a death domain (DD) at its N-terminus for interaction with other DD-containing proteins. The central-terminal domains share the same homology with a serine-threonine kinase.

following IL-1 stimulation. The kinase function of IRAK-1b is inactive due to partial deletion of the kinase domain, although it can activate NF- κ B. On the other hand, IRAK-1-S is the splicing variant of IRAK-1, and is 271 nucleotides shorter than the original one. Although IRAK-1-S lacks the TRAF6 binding domain, the overexpression of IRAK-1-S in cultured cells results in NF- κ B activation. IRAK-1-S is functionally active and highly stable upon IL-1 stimulation. The functional significance of the presence of the alternative splicing variants of IRAK1 needs to be investigated.

Recently, the possibility that IRAK-1 may function to regulate other signaling cascades that lead to mainly NF- κ B activation has been raised. One of the TNF receptor family members, nerve growth factor (NGF), may require the function of IRAK-1 in its signaling pathway. Mamidipudi *et al.* have shown that NGF-induced NF- κ B activation and cell survival is dependent on IRAK-1 [33]. They have also demonstrated that the NGF-induced association of IRAK-1 with atypical protein kinase C ι (PKC ι) and the recruitment of IRAK-1 to the receptor are dependent on the PKC ι [34]. Another pathway involving IRAK-1 is the latent membrane protein 1 (LMP1) signaling pathway [35]. The Epstein Barr virus LMP1 is known to activate the I κ B kinase complex and the JNK pathway [35]. Luft *et al.* have shown that LMP1 activates NF- κ B by interacting with TRAF6 and IRAK-1, whereas IRAK-4 is not required in the LMP1-induced NF- κ B activation [36]. Wan *et al.* have also confirmed that LMP1 induces IRAK-1 phosphorylation although it does not require IRAK-4 [37]. Thus, IRAK-1, which was first reported to play a critical role in the IL-1 signaling cascade, is clearly required for such signaling pathways as TLR, IL-18, NGF, and LMP1.

2) IRAK-2

Similar to IRAK-1, IRAK-2 is ubiquitously expressed. However, in contrast to IRAK-1, IRAK-2 is catalytically inactive due to the lack of critical amino acid residues within its kinase domain. Nevertheless, IRAK-2 can reconstitute the IL-1 response in a 293 mutant cell line lacking IRAK-1 with an unknown mechanism. Moreover, in contrast to IRAK-1, the IRAK-2 protein level remains constant after LPS treatment. Detailed analyses of the murine *irak-2* genome have been performed and four isoforms of IRAK-2 (IRAK-2a, IRAK-2b, IRAK-2c and IRAK-2d) have been found to be generated by alternative splicing at the 5' end of the gene [38]. Each splicing variant shows a different structure in the N-terminal death domain and/or the inter-domain. When over-expressed, IRAK-2a and IRAK-2b, which have putative NF- κ B binding sites, can augment the induction of NF- κ B activation by LPS stimulation. Interestingly, however, IRAK-2c and IRAK-2d seem to have inhibitory functions in LPS-induced signaling. The induction of IRAK-2c or IRAK-2d by LPS stimulation may represent a negative feedback machinery in the subsequent TLR signaling pathways.

3) IRAK-M

IRAK-M expression is restricted mainly in cells of monomyeloid origin, such as monocytes and macrophages. Similar to IRAK-2, IRAK-M is catalytically inactive. Gene

targeting study has revealed that IRAK-M-deficient mice and the cells derived from these mice exhibited increased cytokine production upon IL-1/TLR stimulation and increased inflammatory response to bacterial infection [39]. Interestingly, it has been reported that IRAK-M negatively regulates TLR signaling by inhibiting the dissociation of IRAK-4 and IRAK-1 from the complex with MyD88 after TLR engagement [39]. This process is an indispensable step for transmitting signals downstream. Thus, IRAK-M is shown to be a critical negative regulator of TLR signaling.

4) IRAK-4

The *irak-4* gene was first cloned by analysis of a human V beta gene subfamily [40]. Later, it was also cloned by SEREX (serologic analysis of recombinant cDNA expression libraries) screening of renal tumors [41]. REN64 is a 460 amino acid protein that is strongly expressed in kidney. The expression of REN64 in other tissues, such as lung, testis, small intestine, breast, liver, and placenta, was confirmed by RT-PCR. By searching database for IRAK-like sequences and PCR of a universal cDNA library, Li *et al.* found a Pelle-related cDNA that is 98% identical to REN64 cDNA. The predicted human IRAK-4 protein is 84% identical to the mouse IRAK-4 protein. Similar to IRAK-1, IRAK-2, and IRAK-M, IRAK-4 has an N-terminal death domain and a central kinase domain. Unlike the three IRAKs, however, IRAK-4 has a short C-terminal domain that is most similar to that of *Drosophila* Pelle. Li *et al.* have shown that IRAK-4 is ubiquitously expressed in all tissues [19]. As it has been suggested that IRAK-4 may be a true mammalian homologue of *Drosophila* Pelle, we have made IRAK-4-deficient mice using the gene targeting method. From knockout mice analyses, we have found that only IRAK-4 is the indispensable positive regulator of IR-mediated signals among the IRAK family members [15]. IRAK-4-deficient mice are completely resistant to a lethal dose of LPS. All responses to IL-1, IL-18 and ligands of various TLRs are significantly abolished in macrophages and embryonic fibroblasts (MEFs) from IRAK-4-deficient mice. Moreover, IRAK-4-deficient mice challenged with lymphocytic choriomeningitis virus show reduced production of gamma-interferon by natural killer cells. Infection with *Staphylococcus aureus* produces a lethal phenotype in all the mutant mice but not in wild-type mice. Although it is not clear how IRAK-4 regulates the signaling cascade, these observations strongly indicate that IRAK-4 is a key regulator of TIR signal transduction and plays a crucial role in innate immune response [42].

MOLECULES ASSOCIATED WITH IRAKS

Aside from TRAF6 and MyD88, the IRAK family members are known to interact with several molecules involved in TIR signal transduction. Studies of these IRAK-associated molecules will reveal the detailed mechanism involved in IRAK-mediated signal transduction.

(i) Tollip

Tollip was first identified as an intermediate adaptor protein associated with IRAK-1 [43] in IL-1R signaling, and is now known to be associated with various TLR receptors,

including TLR2 and TLR4 [44]. Tollip is a substrate for IRAK-1 and is known to be phosphorylated by IRAK-1 upon receptor engagement. Tollip suppresses the kinase activity of IRAK-1 after its activation. The negative regulation of TLR signaling by Tollip is limited to the production of proinflammatory mediators during inflammation and infection. Although the precise mechanism involved in the suppression of IRAK-1 activation by Tollip is not clear yet, one suppression mechanism has been surmised, namely, IRAK-1 ubiquitination is controlled by the novel Tollip-interacting protein, Tom1 [45]. Tom1 is a VHS domain containing protein and is associated with Tollip. A VHS domain containing protein is capable of binding to ubiquitin and functions as a component of the sorting machinery for ubiquitinated proteins in the endosome [46]. Tollip may negatively regulate the TIR signaling by inducing IRAK-1 degradation.

(ii) Pellinos1, 2 and 3

Recently, a novel Pelle-interacting protein named Pellino was identified in *Drosophila*. The mammalian counterparts of Pellino were named Pellinos 1 [47], 2 [48] and 3 [49]. All the three proteins are required for NF- κ B activation in response to IL-1, probably through interaction with IRAK-4, IRAK-1, and TRAF6. The Pellino-IRAK-1-IRAK-4-TRAF6 signaling complex is likely to function between the IL-1 receptor complex and the TAK1 complex in the IL-1 signaling pathway [47].

(iii) Btk

It is known that Btk is activated by LPS. The interactions of Btk with MyD88, Mal, and IRAK-1 have been observed also after LPS stimulation in the THP-1 human monocyte cell line. The expression of dominant negative Btk inhibits LPS-induced NF- κ B-dependent reporter gene expression in a number of cell lines. However, the overexpression of wild-type Btk does not induce any NF- κ B responses. Thus, as a TIR domain binding protein, Btk can affect NF- κ B activation in the TIR-mediated signaling cascade [50].

(iv) SIGIRR

SIGIRR [51] is a single immunoglobulin domain containing IL-1 receptor related protein that is ubiquitously expressed except in macrophages. It is a transmembrane protein containing a TIR domain in its cytoplasmic region. Its ligand has not been identified so far. It has been shown that SIGIRR fails to bind to known IL-1 family members. When co-expressed with IL-1R, SIGIRR exerts no effect on the binding of IL-1 and on the subsequent NF- κ B activation. Therefore, the ligand stimulation of SIGIRR may be critical for its function. SIGIRR-deficient mice have been shown to be hyper-responsive to LPS, CpG-ODN, and IL-1 but not to TNF, indicating specific suppressive function in TIR signaling as TNF is known not to be involved in TIR-mediated signals. No functional difference has been found between SIGIRR-deficient and wild-type macrophages, consistent with the lack of expression of SIGIRR in macrophages. SIGIRR may work as a negative regulator by preventing signal transduction from the IRAK-1/TRAF6 complex because it can associate with both IRAK-1 and

TRAF6, although the precise inhibitory mechanisms of SIGIRR have to be clarified [52]. It is also possible that SIGIRR launches a negative signal.

(v) MyD88s

MyD88s is the splice variant of murine MyD88. MyD88s lacks the exon 3 region of MyD88. Whereas MyD88 is mainly expressed in monocytes, MyD88s is strongly expressed in spleen. However, both MyD88 and MyD88s are ubiquitously expressed in all tissues. It has been shown that IRAK-4 recruitment to the receptor complexes is prevented by MyD88s [53]. This would be the mechanism how MyD88s suppresses IRAK-1 phosphorylation and subsequent downstream events including NF- κ B activation. Thus, MyD88s may play a negative regulatory role in TIR signaling under physiological conditions [53].

(vi) SOCS-1

The suppressor of the cytokine signaling (SOCS) protein family consists of cytokine-inducible proteins that attenuate cytokine signal transduction in the negative feedback loop. SOCS proteins are characterized by a central SRC-homology 2 (SH2) domain, an amino-terminal domain of divergent sequence and a carboxy-terminal conserved SOCS-box domain [54]. It has been shown that SOCS-1-deficient mice are more sensitive to LPS-induced lethal effects than the wild-type littermates [55]. SOCS-1 can suppress TLR4 signaling by interacting directly or indirectly with IRAKs. The C-terminal SOCS box of SOCS-1, homologous to the F-box, has been reported to act as a bridge between specific substrate-binding domains and a large family of E3 ubiquitin protein ligases [56]. In this regard, SOCS-1 protein regulates protein turnover by targeting proteins for polyubiquitination and degradation. It is known that IRAK-1 is phosphorylated upon ligand stimulation and the phosphorylated IRAK-1 is ubiquitinated and degraded eventually [57]. Therefore, it is possible that SOCS-1 enhances the ubiquitination and degradation of IRAK-1. The inhibitory effect of SOCS-1 protein seems to be mediated by a combination of two mechanisms. One is the inhibitory effect on the specific interaction between IRAKs and other signaling molecules, and the other is the downregulation of the expression of IRAKs by promoting the ubiquitin-mediated degradation of IRAKs.

EACH IRAK MOLECULE POSITIVELY AND NEGATIVELY REGULATES TIR SIGNALING PATHWAYS

Positive Regulation

It is of no doubt that IRAK-4 is the critical component for TIR signaling. Other IRAK members also regulate the TIR signaling cascade. It has been suggested that IRAK-4 functions downstream of MyD88 and Mal and upstream of TRAF6 in the TIR signaling pathway. Immunoprecipitation analyses have revealed that IRAK-4 interacts with only IRAK-1 and not with other IRAK family members. IRAK-4 can interact with MyD88 and TRAF6 in an *in vitro* overexpression system [19]. The above observations have been confirmed in endogenous molecules. Endogenous IRAK-4 can associate with nonphosphorylated IRAK-1 and

TRAF6 upon ligand stimulation. IRAK-4 regulates IRAK-1 phosphorylation to trigger a cascade of downstream events, suggesting that IRAK-4 functions upstream of other IRAKs [19]. Indeed, IRAK-4 phosphorylates IRAK-1 at Thr 387 for its activation [19]. On the other hand, it has been reported that the kinase-inactive mutant of IRAK-4, similar to the wild-type IRAK-4 construct, is able to restore the IL-1-mediated signaling in terms of NF- κ B activation and IL-8 gene expression in human IRAK-4-deficient cells. It is still controversial whether IRAK-4 kinase activity itself is indeed important for its signaling function or not. We have shown that the kinase activity of IRAK-4 is critical for the maximal induction of inflammatory cytokines. By contrast, Qin *et al.* have reported that the kinase activity of IRAK-4 is dispensable for its function [58]. Although the reasons for this discrepancy are unclear, it is surmised that the difference in experimental system may be one of the factors contributing to it. On the other hand, the kinase domain of IRAK-4 is essential for the formation of the receptor-adaptor complex, which is an indispensable step for transmitting signals to downstream molecules. Altogether, the results suggest that IRAK-4 is at least required as an adaptor for the efficient recruitment of IRAK-1 to the IL-1 receptor complex [58]. IRAK-4 is a true mammalian homologue of *Drosophila* Pelle, and can transmit signals from the receptor complex to

TRAF6 without IRAK-1, as revealed by IRAK-4 knockout studies. Among the IRAK family members, IRAK-4 is the most receptor-proximal kinase. Therefore, IRAK-4 null mutation can affect all other IRAK family members that function downstream of IRAK-4 in the TIR signaling pathway. This makes IRAK-4 a very special member of the IRAK family, as it is the key molecule in the TIR domain containing receptor signaling (Fig. 2).

Despite the fact that IRAK-4 is the critical protein for the TIR signaling cascade, IRAK-1, upon association with IRAK-4, is also an important component of the TIR signaling. The overexpression of IRAK-1 in 293 cells results in the strong induction of NF- κ B activity, whereas IRAK-4 overexpression induces only the weak activation of NF- κ B. Furthermore, IRAK-1-deficient cells induced the reduction, albeit incompletely, of IL-1 response [59]. Therefore, IRAK-1 is a positive regulator of IRAK-4.

Negative Regulation

The TIR signaling cascade is also negatively regulated to control inflammatory responses. One major mechanism for the negative regulation involves IRAK-1 degradation, although the detailed mechanism is not well understood. This degradation process is expected to play a critical role in the

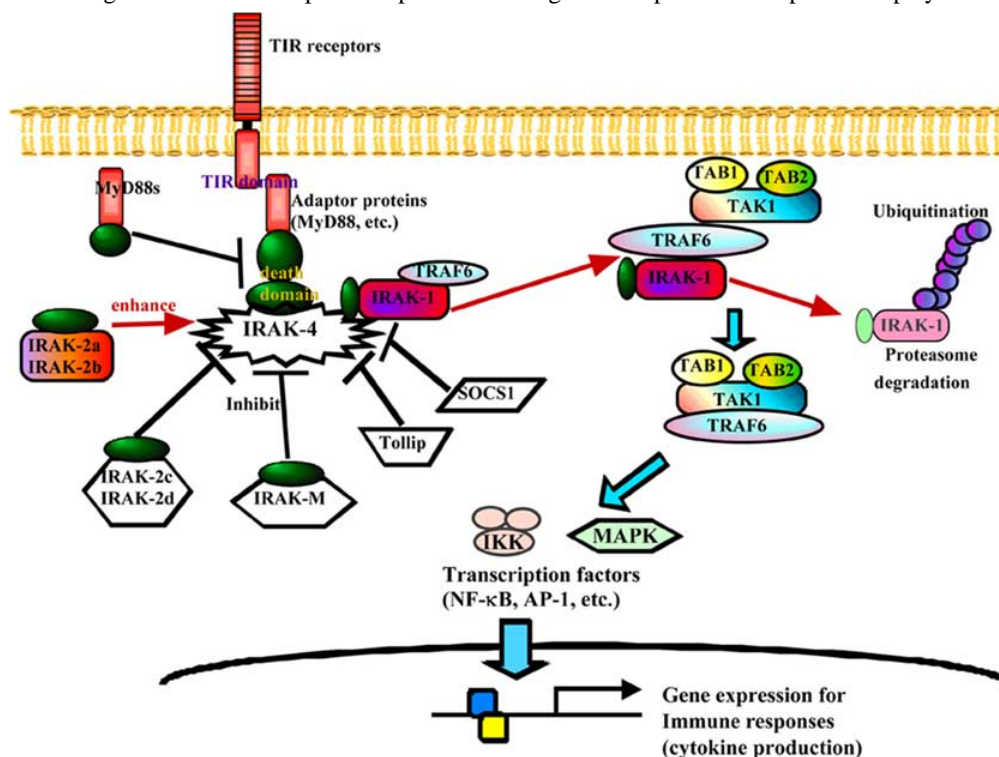


Fig. (2). Role of IRAK family members in TIR domain containing receptor signaling

Upon receptor engagement, adaptor proteins such as MyD88 are first recruited to the receptor through the TIR domain. Then, a receptor associated adaptor protein (MyD88) recruits IRAK-4 by interacting through the death domain. During this step, MyD88s, an alternative splicing form of MyD88, IRAK-M, IRAK-2c and IRAK-2d negatively regulate this TIR signaling whereas IRAK-2a and IRAK-2b enhance its signaling. IRAK-4 activates IRAK-1 in order to form the IRAK-1/TRAF6/TAK1 complex, which finally leads to activation of the I B kinase (IKK) complex. Thereafter, IRAK-1 is ubiquitinated and degraded in order to diminish the signaling. The IKK complex phosphorylates I B, resulting in the nuclear translocation of NF- κ B that induces the gene expression of inflammatory cytokines. TRAF6 also mediates MAPK activation, leading to the activation of AP-1 transcription factors. Unlike other IRAKs, IRAK-4 deficiency results in the complete inhibition of TIR signal transduction.

negative feedback loop by regulating the signal strength from the receptors. Recently, it has been reported that the inhibition of IRAK-1 degradation by proteasome inhibitors leads to significant enhancement of LPS-induced cytokine production by macrophages [60]. Aside from IRAK-1 degradation, IRAK-M and the spliced variants of IRAK-2 have been implicated to play roles in negative regulation [38, 39]. The conformational change of IRAK-1/IRAK-4 results in reduced affinity for the TLR signaling complex, and IRAK-1/IRAK-4 are released to induce the activation of downstream molecules. In the presence of such inhibitory IRAKs as IRAK-2c, IRAK-2d and IRAK-M, TLR stimulation by PAMPs results in the recruitment of not only IRAK-1/IRAK-4 but also the inhibitory IRAKs to the signaling complex. These associations with the inhibitory IRAKs are perceived to inhibit the release of IRAK-1/IRAK-4 from the TLR signaling complex by either inhibiting the phosphorylation of IRAK-1/IRAK-4 or stabilizing the TLR/MyD88/IRAK-1/IRAK-4 complex, resulting in the interruption of downstream signaling [38, 39].

Another important mechanism for the regulation of immune response is programmed cell death (PCD). The induction of PCD in host cells is a common strategy for getting rid of infected cells for host defense. The infected cells must be omitted after appropriate immune responses; otherwise, severe immune responses would harm the host. Not only known death receptors such as Fas or TNFR, but also TLRs induce cell death upon stimulation under certain circumstances [21]. In TLR signaling, MyD88 mediates cell death along with IRAK-2, but not with IRAK-1 or TRAF6. The signal from MyD88/IRAK-2 potentially targets the Fas-associated death domain protein (FADD)/caspase-8 apoptotic pathway, whereas IRAK-1 and TRAF6 are quite important in the anti-apoptotic signaling, which requires NF- κ B activation [61]. After LPS treatment, MyD88 and IRAK family members are recruited to the plasma membrane, and sequentially two different TLR4 pathways are activated. One is the survival pathway that leads to NF- κ B activation via the TRAF6/IKK complex. The other is a death pathway that induces the activation of the caspase cascade via FADD/caspase-8. The balance of these two pathways may determine the induction of PCD. Indeed, the bifurcation of survival and death pathways depends on MyD88 and IRAK family members [61].

IMPORTANCE OF IRAK-4 FROM A CLINICAL POINT OF VIEW

The first study of human patients who have inherited IRAK-4 deficiency was that conducted by Picard *et al.* in 2003 [16]. In that study, genetic screening was performed on three child patients who recurrently developed pyogenic bacterial infections, mostly caused by *Staphylococcus aureus* and *Streptococcus pneumoniae*. They found that a homozygous deletion of the IRAK-4 gene resulted in recurrent life-threatening infections due to the lack of innate immunity. Lymphocytes and fibroblast cells from those patients did not activate NF- κ B or MAPK, and failed to induce cytokine production. Some patients carried the C-to-T transition at nucleotide 877 in exon 8 of the IRAK-4 gene, resulting in a glutamine-to-stop substitution at codon 293 (Q293X). No IRAK-4 mRNA or protein was detected in any

of the patients. They showed similar immune deficiency against pyogenic bacterial infections similar to the first described patient [16]. In another report of IRAK-4-deficient patients, one patient having two distinct *IRAK-4* mutations was identified; a point mutation (C877T) on one allele and a two-nucleotide AC deletion (620 and 621 deletions in cDNA) on the other allele inherited from phenotypically normal parents. Both mutations resulted in no expression of the IRAK-4 protein. Cells from that patient were hyporesponsive to LPS and IL-1, phenotypically resembling IRAK-4-deficient mice. Failure to express wild-type IRAK-4 resulted in profound defects of inflammatory responses, as seen in that patient [61]. In addition, a recent report [62] has indicated that one of the above-mentioned patients is unable to sustain antibody responses to polysaccharides, protein antigens or a neoantigen-bacteriophage, and suggested that IRAK-4 is also important in not only innate immunity but also the consequently acquired immune responses. So far, IRAK-4 is the only molecule the deletion of which has been reported to result in severe infection in human among the IRAK family, the TRAF family, and adaptor molecules such as MyD88 involved in TIR signaling.

IRAKS AS A NEW DRUG TARGET FOR INFLAMMATORY AND INFECTIOUS DISEASES

Judging from the studies of human patients having the IRAK-4 null mutation as well as of IRAK-4-deficient mice, it is suggested that IRAK-4 is a critical therapeutic target for alleviating disorders in innate immunity, particularly for TIR-mediated inflammatory diseases. The TIR-mediated inflammatory diseases include sepsis, human systemic lupus erythematosus, rheumatoid arthritis (RA), cardioditis, and gout, to name a few. RA is a systemic, chronic, inflammatory disorder and its apparent symptoms appear most frequently in the joints. The high production of various proinflammatory cytokines, including IL-1, IL-6, and TNF- α , is detected in the joints of RA patients [63]. These cytokines play important roles in the development of RA because they can induce inflammation and promote synovial cell growth. Among these cytokines, IL-1 is one of the crucial mediators of RA, because the injection of IL-1 into healthy rabbit joints causes severe arthritis [64] and the inhibition of IL-1 in joints ameliorates RA [65, 66]. It is also known that dilated cardiomyopathy, which is the most common cause of heart failure in young patients, is also an IL-1-related autoimmune disease. By blocking IL-1 signaling, this autoimmune myocarditis was cured in a mouse model [67]. IL-18 is also involved in the developing autoimmune as a TIR-mediated signal transducer. IL-18, formerly known as an IFN- γ -inducing factor, has the ability to induce IFN- γ production, leading to T helper lymphocyte (Th1) cell activation. The lupus-like autoimmune syndrome is characterized by progressive lymphadenopathy and autoantibody production. Cells from MRL/lpr (lpr) mice with the lupus-like autoimmune syndrome express high levels of IL-18 and are hypersensitive to IL-18. The *in vivo* inhibition of IL-18 resulted in the curing of the pathogenesis in this animal model [68]. Young lpr mice that can produce autoantibodies against murine IL-18 exhibit a significant reduction in spontaneous lymphoproliferation and IFN- γ production. These studies support the concept that

IL-18 plays a major role in the pathogenesis of the autoimmune syndrome in lpr mice. It has been suggested also that the reduction of IL-18 activity may be one of the therapeutic strategies for autoimmune diseases. Finally, it has been reported that the host-derived DNA itself can drive autoimmune diseases via TLR9 [69]. Autoreactive B cells are present in the lymphoid tissues of healthy individuals, but remain quiescent under normal conditions. When homeostasis is perturbed, the formation of self-reactive antibodies can lead to serious pathological consequences. The activation of these autoreactive B cells requires the synergistic engagement of the antigen receptor and TLR9. This finding establishes the critical linkage between the innate and adaptive immune systems in the development of systemic autoimmune diseases.

From these observations, we have confirmed the importance of determining precisely the implications of TIR signal transduction in inflammatory diseases for the development of appropriate therapeutic strategies. Given the critical and universal roles of IRAKs as signaling molecules in TIR-mediated signals, the functional interference of IRAKs would be one of the therapeutic strategies. The observation that various IRAK-deficient mice seem to be healthy and grow normally indicates that IRAK antagonists may be applicable to various inflammatory and infectious diseases. As IRAK-4 is the most critical IRAK for TIR mediated signals, one promising approach is to reduce the IRAK-4 expression level by using small compound inhibitors that can block both the kinase activity of IRAK-4 and its recruitment to the receptor complex. The elucidation of the crystal structure of IRAK-4 will also facilitate the design of IRAK-4 drugs. Indeed, after the crystal structure of TRAF6 was elucidated, synthetic peptides that corresponded to the binding site between IRAK-1 and TRAF6 were successfully produced in order to block NF- κ B activation after ligand treatment [70]. From these points of view, an understanding of the role of IRAK-4 in innate and acquired immunity and its structure will enable us to design novel strategies for therapeutic intervention in human infectious and autoimmune diseases.

ACKNOWLEDGEMENTS

We would like to thank H. Yamaguchi for secretarial assistance. This work was supported in part by a Grant-in-Aid for Priority Area Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (T. S. and N. S.), and by Grants-in-Aid from Kowa Life Science Foundation (N. S.), the Sumitomo Foundation (N. S.), and the Tokyo Biochemical Research Foundation (N. S.).

REFERENCES

- Akira, S., Takeda, K., Kaisho, T. *Nat. Immunol.*, **2001**, *2*, 675-680.
- Wasserman, S. A. *Curr. Opin. Genet. Dev.*, **2000**, *10*, 497-502.
- Akira, S. *Curr. Opin. Immunol.*, **2003**, *15*, 238.
- Shelton, C. A., Wasserman, S. A. *Cell*, **1993**, *72*, 515-525.
- Belvin, M. P., Anderson, K. V. *Annu. Rev. Cell Dev. Biol.*, **1996**, *12*, 393-416.
- Cao, Z., Henzel, W. J., Gao, X. *Science*, **1996**, *271*, 1128-1131.
- O'Neill, L. *Biochem. Soc. Trans.*, **2000**, *28*, 557-563.
- Karin, M. *J. Biol. Chem.*, **1999**, *274*, 27339-27342.
- Adachi, O., Kawai, T., Takeda, K., Matsumoto, M., Tsutsui, H., Sakagami, M., Nakanishi, K., Akira, S. *Immunity*, **1998**, *9*, 143-150.
- Fitzgerald, K. A., Palsson-McDermott, E. M., Bowie, A. G., Jefferies, C. A., Mansell, A. S., Brady, G., Brint, E., Dunne, A., Gray, P., Harte, M. T., McMurray, D., Smith, D. E., Sims, J. E., Bird, T. A., O'Neill, L. A. *Nature*, **2001**, *413*, 78-83.
- Hornig, T., Barton, G. M., Medzhitov, R. *Nat. Immunol.*, **2001**, *2*, 835-841.
- Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K., Akira, S. *Science*, **2003**, *301*, 640-643.
- Oshiumi, H., Sasai, M., Shida, K., Fujita, T., Matsumoto, M., Seya, T. *J. Biol. Chem.*, **2003**, *278*, 49751-49762.
- Cao, Z., Xiong, J., Takeuchi, M., Kurama, T., Goeddel, D. V. *Nature*, **1996**, *383*, 443-446.
- Suzuki, N., Suzuki, S., Duncan, G. S., Millar, D. G., Wada, T., Mirtsos, C., Takada, H., Wakeham, A., Itie, A., Li, S., Penninger, J. M., Wesche, H., Ohashi, P. S., Mak, T. W., Yeh, W. C. *Nature*, **2002**, *416*, 750-756.
- Picard, C., Puel, A., Bonnet, M., Ku, C. L., Bustamante, J., Yang, K., Soudais, C., Dupuis, S., Feinberg, J., Fieschi, C., Elbim, C., Hitchcock, R., Lammass, D., Davies, G., Al-Ghoni, A., Al-Rayes, H., Al-Jumaah, S., Al-Hajjar, S., Al-Mohsen, I. Z., Frayha, H. H., Rucker, R., Hawn, T. R., Aderem, A., Tufenkeji, H., Haraguchi, S., Day, N. K., Good, R. A., Gougerot-Pocidal, M. A., Ozinsky, A., Casanova, J. L. *Science*, **2003**, *299*, 2076-2079.
- Muzio, M., Ni, J., Feng, P., Dixit, V. M. *Science*, **1997**, *278*, 1612-1615.
- Wesche, H., Gao, X., Li, X., Kirschning, C. J., Stark, G. R., Cao, Z. *J. Biol. Chem.*, **1999**, *274*, 19403-19410.
- Li, S., Strelow, A., Fontana, E. J., Wesche, H. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 5567-5572.
- Tartaglia, L. A., Ayres, T. M., Wong, G. H., Goeddel, D. V. *Cell*, **1993**, *74*, 845-853.
- Aliprantis, A. O., Yang, R. B., Mark, M. R., Suggett, S., Devaux, B., Radolf, J. D., Klimpel, G. R., Godowski, P., Zychlinsky, A. *Science*, **1999**, *285*, 736-739.
- Aliprantis, A. O., Yang, R. B., Weiss, D. S., Godowski, P., Zychlinsky, A. *EMBO J.*, **2000**, *19*, 3325-3336.
- Martin, M., Bol, G. F., Eriksson, A., Resch, K., Brigelius-Flohe, R. *Eur. J. Immunol.*, **1994**, *24*, 1566-1571.
- Yamaguchi, K., Shirakabe, K., Shibuya, H., Irie, K., Oishi, I., Ueno, N., Taniguchi, T., Nishida, E., Matsumoto, K. *Science*, **1995**, *270*, 2008-2011.
- Shibuya, H., Yamaguchi, K., Shirakabe, K., Tonegawa, A., Gotoh, Y., Ueno, N., Irie, K., Nishida, E., Matsumoto, K. *Science*, **1996**, *272*, 1179-1182.
- Takaesu, G., Kishida, S., Hiyama, A., Yamaguchi, K., Shibuya, H., Irie, K., Ninomiya-Tsuji, J., Matsumoto, K. *Mol. Cell*, **2000**, *5*, 649-658.
- Cheung, P. C., Nebreda, A. R., Cohen, P. *Biochem. J.*, **2004**, *378*, 27-34.
- Jiang, Z., Ninomiya-Tsuji, J., Qian, Y., Matsumoto, K., Li, X. *Mol. Cell Biol.*, **2002**, *22*, 7158-7167.
- Li, X., Commane, M., Jiang, Z., Stark, G. R. *Proc. Natl. Acad. Sci. U S A*, **2001**, *98*, 4461-4465.
- Swantek, J. L., Tsen, M. F., Cobb, M. H., Thomas, J. A. *J. Immunol.*, **2000**, *164*, 4301-4306.
- Jensen, L. E., Whitehead, A. S. *J. Biol. Chem.*, **2001**, *276*, 29037-29044.
- Yanagisawa, K., Tago, K., Hayakawa, M., Ohki, M., Iwahana, H., Tominaga, S. *Biochem. J.*, **2003**, *370*, 159-166.
- Mamidipudi, V., Li, X., Wooten, M. W. *J. Biol. Chem.*, **2002**, *277*, 28010-28018.
- Mamidipudi, V., Lin, C., Seibenhener, M. L., Wooten, M. W., Li, X. *J. Biol. Chem.*, **2004**, *279*, 4161-4165.
- Cahir-McFarland, E. D., Davidson, D. M., Schauer, S. L., Duong, J., Kieff, E. *Proc. Natl. Acad. Sci. U S A*, **2000**, *97*, 6055-6060.
- Luftig, M., Prinarakis, E., Yasui, T., Tschritzis, T., Cahir-McFarland, E., Inoue, J., Nakano, H., Mak, T. W., Yeh, W. C., Li, X., Akira, S., Suzuki, N., Suzuki, S., Mosialos, G., Kieff, E. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 15595-15600.
- Wan, J., Sun, L., Mendoza, J. W., Chui, Y. L., Huang, D. P., Chen, Z. J., Suzuki, N., Suzuki, S., Yeh, W. C., Akira, S., Matsumoto, K., Liu, Z. G., Wu, Z. *Mol. Cell Biol.*, **2004**, *24*, 192-199.

- [38] Hardy, M. P., O'Neill, L. A. *J. Biol. Chem.*, **2004**.
- [39] Kobayashi, K., Hernandez, L. D., Galan, J. E., Janeway, C. A. Jr., Medzhitov, R., Flavell, R. A. *Cell*, **2002**, *110*, 191-202.
- [40] Siu, G., Strauss, E. C., Lai, E., Hood, L. E. *J. Exp. Med.*, **1986**, *164*, 1600-1614.
- [41] Scanlan, M. J., Gordan, J. D., Williamson, B., Stockert, E., Bander, N. H., Jongeneel, V., Gure, A. O., Jager, D., Jager, E., Knuth, A., Chen, Y. T., Old, L. J. *Int. J. Cancer*, **1999**, *83*, 456-464.
- [42] Suzuki, N., Suzuki, S., Yeh, W. C. *Trends Immunol.*, **2002**, *23*, 503-506.
- [43] Burns, K., Clatworthy, J., Martin, L., Martinon, F., Plumpton, C., Maschera, B., Lewis, A., Ray, K., Tschopp, J., Volpe, F. *Nat. Cell Biol.*, **2000**, *2*, 346-351.
- [44] Zhang, G., Ghosh, S. *J. Biol. Chem.*, **2002**, *277*, 7059-7065.
- [45] Yamakami, M., Yoshimori, T., Yokosawa, H. *J. Biol. Chem.*, **2003**, *278*, 52865-52872.
- [46] Polo, S., Sigismund, S., Faretta, M., Guidi, M., Capua, M. R., Bossi, G., Chen, H., De Camilli, P., Di Fiore, P. P. *Nature*, **2002**, *416*, 451-455.
- [47] Jiang, Z., Johnson, H. J., Nie, H., Qin, J., Bird, T. A., Li, X. *J. Biol. Chem.*, **2003**, *278*, 10952-10956.
- [48] Jensen, L. E., Whitehead, A. S. *FEBS Lett.*, **2003**, *545*, 199-202.
- [49] Jensen, L. E., Whitehead, A. S. *J. Immunol.*, **2003**, *171*, 1500-1506.
- [50] Jefferies, C. A., Doyle, S., Brunner, C., Dunne, A., Brint, E., Wietek, C., Walch, E., Wirth, T., O'Neill, L. A. *J. Biol. Chem.*, **2003**, *278*, 26258-26264.
- [51] Thomassen, E., Renshaw, B. R., Sims, J. E. *Cytokine*, **1999**, *11*, 389-399.
- [52] Wald, D., Qin, J., Zhao, Z., Qian, Y., Naramura, M., Tian, L., Towne, J., Sims, J. E., Stark, G. R., Li, X. *Nat. Immunol.*, **2003**, *4*, 920-927.
- [53] Burns, K., Janssens, S., Brissoni, B., Olivos, N., Beyaert, R., Tschopp, J. *J. Exp. Med.*, **2003**, *197*, 263-268.
- [54] Alexander, W. S. *Nat. Rev. Immunol.*, **2002**, *2*, 410-416.
- [55] Kinjyo, I., Hanada, T., Inagaki-Ohara, K., Mori, H., Aki, D., Ohishi, M., Yoshida, H., Kubo, M., Yoshimura, A. *Immunity*, **2002**, *17*, 583-591.
- [56] Kile, B. T., Schulman, B. A., Alexander, W. S., Nicola, N. A., Martin, H. M., Hilton, D. J. *Trends Biochem. Sci.*, **2002**, *27*, 235-241.
- [57] Yamin, T. T., Miller, D. K. *J. Biol. Chem.*, **1997**, *272*, 21540-21547.
- [58] Qin, J., Jiang, Z., Qian, Y., Casanova, J. L., Li, X. *J. Biol. Chem.*, **2004**, *279*, 26748-26753.
- [59] Thomas, J. A., Allen, J. L., Tsen, M., Dubnicoff, T., Danao, J., Liao, X. C., Cao, Z., Wasserman, S. A. *J. Immunol.*, **1999**, *163*, 978-984.
- [60] Cuschieri, J., Gourlay, D., Garcia, I., Jelacic, S., Maier, R. V. *Cell Immunol.*, **2004**, *227*, 140-147.
- [61] Ruckdeschel, K., Mannel, O., Schrottner, P. *J. Immunol.*, **2002**, *168*, 4601-4611.
- [62] Day, N., Tangsinmankong, N., Ochs, H., Rucker, R., Picard, C., Casanova, J. L., Haraguchi, S., Good, R. *J. Pediatr.*, **2004**, *144*, 524-526.
- [63] Feldmann, M., Brennan, F. M., Maini, R. N. *Annu. Rev. Immunol.*, **1996**, *14*, 397-440.
- [64] Pettipher, E. R., Higgs, G. A., Henderson, B. *Proc. Natl. Acad. Sci. USA*, **1986**, *83*, 8749-8753.
- [65] Makarov, S. S., Olsen, J. C., Johnston, W. N., Anderle, S. K., Brown, R. R., Baldwin, A. S., Jr., Haskill, J. S., Schwab, J. H. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 402-406.
- [66] Arend, W. P., Gabay, C. *Arthritis Res.*, **2000**, *2*, 245-248.
- [67] Eriksson, U., Kurrer, M. O., Sonderegger, I., Iezzi, G., Tafuri, A., Hunziker, L., Suzuki, S., Bachmaier, K., Bingisser, R. M., Penninger, J. M., Kopf, M. *J. Exp. Med.*, **2003**, *197*, 323-331.
- [68] Bossu, P., Neumann, D., Del Giudice, E., Ciaramella, A., Gloaguen, I., Fantuzzi, G., Dinarello, C. A., Di Carlo, E., Musiani, P., Meroni, P. L., Caselli, G., Ruggiero, P., Boraschi, D. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 14181-14186.
- [69] Vinuesa, C. G., Goodnow, C. C. *Nature*, **2002**, *416*, 595-598.
- [70] Janssens, S., Beyaert, R. *Mol. Cell*, **2003**, *11*, 293-302.