Review

Negative regulation of Toll-like receptor signaling pathway

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Abstract
TLRs are primary sensors of invading pathogens, recognizing conserved microbial molecules and activating signaling pathways that are pivotal to innate and adaptive immune responses. However, a TLR signaling pathway must be tightly controlled because its excessive activation can contribute to the pathogenesis of many human diseases. This review provides a summary of the different mechanisms that are involved in the negative regulation of TLR signaling pathways.

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1. Introduction
Toll-like receptors (TLRs), which are broadly distributed on cells of the immune system, function as primary sensors of invading pathogens, recognizing conserved microbial molecules (PAMP, pathogen-associated molecular patterns). They are evolutionarily conserved from the worm Caenorhabditis elegans to mammals. To date, 12 members of the TLR family have been identified in mammals. TLR family members are characterized structurally by the presence of a leucine-rich repeat (LRR) domain in their extracellular domain and a Toll/interleukin-1 (IL-1) receptor (TIR) domain in their intracellular domain. These are essential for provoking the innate response and enhancing adaptive immunity against pathogens [1].

2. TLR signaling pathways
Stimulation of TLRs triggers the activation of signaling cascades, leading to the induction of immune and pro-inflammatory genes. After ligand binding, TLRs dimerize and undergo conformational changes. This is followed by recruitment to the receptor of TIR-domain-containing adaptors including myeloid differentiation primary-response protein 88 (MyD88) and TIR-domain-containing adaptor protein-inducing IFN-β (TRIF), which are responsible for the activation of distinct signaling pathways.

MyD88 is critical for the signaling from all TLRs except TLR3. Upon stimulation, MyD88 associates with the cytoplasmic portion of TLRs and then recruits IL-1R-associated kinase 4 (IRAK-4) and IRAK-1 through a homophilic interaction of the death domains. After IRAK-1 associates with MyD88, it is phosphorylated by the activated IRAK-4 and subsequently associates with TNFR-associated factor 6 (TRAF6), which acts as an ubiquitin-protein ligase (E3). Subsequently, TRAF6, together with an E2 ubiquitin ligase complex of UBC13 and UEV1A, catalyzes the formation of the K63-linked polyubiquitin chain on TRAF6 itself and IKK-γ/NF-κB essential modulator (NEMO). This ubiquitination activates a complex composed of TGF-β-activated kinase 1 (TAK1) and the TAK1 binding proteins, TAB1, TAB2, and TAB3. TAK1 then phosphorylates IKK-β and MAP kinase kinase 6 (MKK6), which modulates the activation of NF-κB and MAP kinases, resulting in induction of genes involved in inflammatory responses.

On the other hand, TRIF activates TRAF-family-member-associated NF-κB activator (TANK) binding kinase 1

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(TBK1; also known as NAK or T2K) via TRAF3. TBK1 comprises a family including inducible IkB kinase (IKK-ι, also known as IKK-ε) and these kinases directly phosphorylate IFN-regulatory factor-3 (IRF-3) and IRF-7. The phosphorylated IRF3 and IRF7, in turn, form homodimers or heterodimers, translocate into the nucleus and induce the expression of type I IFN as well as IFN-inducible gene [1,2].

However, excessive activation of the TLR signaling pathway contributes to pathogenesis of autoimmune, chronic inflammatory and infectious diseases [3]. TLR signaling and subsequent functions therefore must be under tight negative regulation to maintain immune balance. It has been reported that negative regulation of TLRs can be achieved at multiple levels (Fig. 1).

3. Negative regulation of TLR signaling

3.1. Negative regulation of TLR signaling pathways by degradation

In general, degradation or destabilization of signal transduction factors is one of the principal mechanisms that reduces or terminates the activation of signaling pathways. This type of mechanism occurs directly or indirectly during negative regulation of TLR-mediated immune responses.

The conjugation of ubiquitin molecules, the 76-amino-acid peptides, to protein substrates has long been known as a mechanism that targets proteins for degradation by the 26S proteasome. Not surprisingly, this kind of mechanism has been used in the regulation of TLR signaling pathways by some negative regulators. One of the best known is the ubiquitin-modifying enzyme Triad domain-containing protein 3 (triad3A). Tsung-Hsien Chuang and his colleagues described the manner in which a RING finger protein, Triad3A, acts as an E3 ubiquitin-protein ligase and enhances ubiquitination and proteolytic degradation of certain TLRs. They used the TIR domain of TLR9 as bait to screen a leukocyte cDNA library in the yeast two-hybrid system and identified Triad3A as a TLR9-interacting protein. Indeed, Triad3 interacted with TLR3, TLR4, TLR5 and TLR9 but not with TLR2. Triad3A overexpression promoted substantial degradation of TLR4 and TLR9 but did not affect TLR2 expression. The degradation was blocked by treatment of cells with the proteasome inhibitor lactacystin but not by the lysosomotropic agent or the lysosomal protease inhibitor. Triad3A overexpression induced a decrease in TLR4 and TLR9 signaling but did not affect TLR2 signaling. Conversely, a reduction in endogenous Triad3A by small interfering RNA increased TLR expression and enhanced TLR activation [4].

Fig. 1 The negative regulators of TLR signaling pathways. The negative regulators were marked using the brown color around their target proteins.
Another good example came from the research on Suppressor of cytokine signaling (SOCS)-1. There are four cytosolic TIR-containing adaptor proteins used by TLRs for signal transduction. MyD88-adaptor like (Mal) is required for signaling by TLR2 and TLR4, serving as a bridge to recruit MyD88. SOCS-1 was first identified as a negative regulator involved in the JAK–STAT signaling pathway. In fact, macrophages from SOCS-1 deficient mice developed enhanced production of inflammatory cytokines such as IL-6 and TNF in response to LPS. In addition, Socs1−/− Ifng−/− mice were hypersensitive to LPS-induced shock as compared to wild-type mice, indicating that SOCS-1 might also be involved in a TLR signaling pathway. Indeed, after TLR2 and TLR4 stimulation, Mal undergoes TLR-induced tyrosine phosphorylation mediated by Bruton’s tyrosine kinase (Btk). Phosphorylated Mal can be recognized by the SH2 domain of SOCS-1. SOCS-1 interacts with Mal and induces its polyubiquitination and subsequent proteasomal degradation. Therefore, SOCS-1 is a negative regulator of TLR2 and TLR4 signaling pathways [5].

Another example of this appeared in a study demonstrating that the turnover of IRF3 is under strict control by the prolyl isomerase (Pin)1. Exogenous expression of Pin1 suppressed TLR3 mediated, IRF3-dependent transcriptional activation. Expression of Pin1-specific shRNA enhanced IRF3-dependent transcriptional activation and increased the production of IFN-β. Culture supernatants of poly(I)−poly(C)-stimulated 293-TLR3 cells expressing Pin1-specific shRNA exhibited much greater antiviral activity than did supernatants of cells expressing control shRNA. Furthermore, Pin1−/− mice produced more IFN-β in response to dsRNA stimulation. As a matter of fact, Pin1 is a peptidyl-prolyl isomerase that, via its WW domain, recognizes phosphorylated serine or threonine residues followed by proline and then catalyzes a conformational change of the bound substrate in a phosphorylation-dependent way. By this mechanism, after stimulation with double-stranded RNA, induced phosphorylation of the Ser339–Pro340 motif of IRF3 leads to its interaction with Pin1 and finally to polyubiquitination and then proteasome-dependent degradation of IRF3. However, it does not directly catalyze the ubiquitination or subsequent proteasome-dependent degradation of its substrates. Further understanding of this regulation requires the characterization of the associated ubiquitin ligase [6].

Of course, the ubiquitin-proteasome pathway is not the only one used by negative regulators. We identified Tripartite-motif protein (Trim) 30α as a negative regulator of TLR-mediated NF-κB activation that acts by targeting TAB2 and TAB3 for degradation. However, although TRIM30α has a RING finger, which is often indicative of E3 ubiquitin ligase activity, neither lactacystin nor MG132, inhibitors of the ubiquitin-proteasome pathway, was able to block the downregulation of TAB2 by TRIM30α. Therefore, the TRIM30α-stimulated degradation of TAB2 and TAB3 is independent of the ubiquitin-proteasome pathway. We then found that downregulation of TAB2 is blocked by NH4Cl and chloroquine, both inhibitors of lysosomal protein degradation, and determined that TRIM30α localized together with TAB2 in lysosomes but not in early endosomes or in the Golgi apparatus. Moreover, overexpression of Trim30α in J774 resulted in decreased production of TLR-induced IL-6 and TNF. In vivo studies showed that transfected or transgenic mice overexpressing TRIM30α were more resistant to endotoxic shock. Consistent with that, in vivo ‘knockdown’ of TRIM30α mRNA by small interfering RNA impaired lipopolysaccharide-induced tolerance. In addition, expression of TRIM30α depended upon NF-κB activation. Thus, Trim30α is part of a previously unknown negative feedback loop used to constrain TLR-mediated inflammation [7].

3.2. Negative regulation of TLR signaling pathways by deubiquitination

We have discussed above the fact that ubiquitination plays a very important role in innate immunity by tagging proteins for degradation. However, this is only part of the story. As we know, besides degradative molecular functions, ubiquitination mediates many others. It is used as a fundamental mechanism of regulation of signal transduction by innate immunity. K63-linked ubiquitin chains may have the ability to facilitate protein–protein interactions in the recruitment and activation of downstream signaling molecules. Similar to phosphorylation, ubiquitination is a reversible process. It is regulated both by ubiquitinating enzymes and de-ubiquitinating enzymes. Recent research provides increasing numbers of examples proving the importance of de-ubiquitinating enzymes in regulation of TLR signaling pathways.

A20 was the first deubiquitination enzyme found to be involved in innate immune regulation. A20 was originally discovered as a TNF-induced zinc-finger protein that could inhibit TNF-mediated NF-κB activation. Interestingly, it is a dual-function enzyme: firstly, it operates as a de-ubiquitinating enzyme, removing the K63-linked polyubiquitin chain from RIP1; and secondly, it operates as an E3 ligase, promoting K48-linked polyubiquitination of RIP1 and thereby targeting it for degradation [8]. However, mice doubly deficient in either A20 and TNF or A20 and TNF receptor 1 developed spontaneous inflammation, indicating that A20 is also critical for the regulation of TNF-independent signals in vivo. Indeed, when stimulated with LPS, A20-deficient BMDMs produced more TNF, IL-6 and nitric oxide than wild-type BMDMs. In vivo data indicated that A20 could protect mice from endotoxic shock by restriction of innate immune cell responses to LPS. Indeed, the N-terminal conserved OTU-like domain of the A20 protein could remove K63-linked polyubiquitin chains from TRAF6, thereby terminating NF-κB activation induced by TLR ligation [9].

Another important de-ubiquitinating enzyme involved in the negative regulation of a TLR signaling pathway is de-ubiquitinating enzyme A (DUBA). Both DUBA and A20 belong to a subfamily of 14 DUBs characterized by an ovarian tumor (OTU) domain. Knock down of DUBA by siRNA increases TLR3-induced production of type I interferon, whereas ectopic expression of DUBA has the converse effect. In fact, DUBA physically interacts with TRAF3 and inhibits
the self-ubiquitination of TRAF3. TRAF3 is an E3 ubiquitin ligase that preferentially assembles lysine-63-linked polyubiquitin chains. It is essential for type I interferon production. DUBA selectively cleaves the lysine-63-linked polyubiquitin chains on TRAF3, resulting in its dissociation from the downstream signaling complex containing TANK-binding kinase 1. Therefore, DUBA is a negative regulator of innate immune responses [10].

The cylindromatosis protein (CYLD) was originally discovered as a tumor suppressor. It belongs to the ubiquitin-specific protease (USP) family of deubiquitination enzymes, containing a classical ubiquitin C-terminal hydrolase activity for the cleavage of polyubiquitin chains. It has been shown that CYLD can regulate diverse biological functions by targeting multiple signaling molecules, such as members of the TRAF family, NEMO, Bcl3, TAK1, RIP1, etc. With regard to innate immunity, it was identified as a negative regulator of the TLR2 signaling pathway. ‘Knockdown’ of endogenous CYLD expression by interfering RNA led to increased production of pro-inflammatory cytokines. Furthermore, CYLD negatively regulated TLR-mediated immune and inflammatory responses by inhibiting activation of TRAF6 and TRAF7. It has been noted that this function might be dependent upon a deubiquitination-dependent mechanism [11].

3.3. Negative regulation of TLR signaling pathways by competition

Toll-like receptors discriminate between different pathogen-associated molecules and activate signaling cascades that lead to immune responses. The specificity of Toll-like receptor signaling occurs by means of adaptor proteins containing Toll/interleukin-1 receptor (TIR) domains, known as MyD88, MAL, TRIF, TRAM and Sterile alpha and Armadillo motif containing protein (SARM). Unlike the other four that have activating functions, SARM is a negative regulator of TRIF-dependent Toll-like receptor signaling. Expression of SARM blocks gene induction ‘downstream’ of TRIF but not of MyD88 and ‘knockdown’ of endogenous SARM expression by interfering RNA leads to enhanced TRIF-dependent cytokine and chemokine induction. SARM and TRIF were found to interact when overexpressed, and in a key experiment, the interaction was shown to be enhanced by LPS after 15 min of treatment. The interaction between SARM and TRIF was also demonstrated with endogenously expressed proteins. The complex of SARM and TRIF in effect limits TRIF signaling by preventing it from being involved in TIR interactions with other adaptors or TLRs. SARM functions as a negative regulator of TLR signaling, specifically as a negative regulator of TRIF-dependent signaling [12].

Two members of the IFN-regulatory factor (IRF) family of transcription factors, IRF-5 and IRF-7, interact with MyD88 and induce pro-inflammatory cytokines and type I IFNs, respectively. IRF-4 also interacts with MyD88 and IRF-4 competes with IRF-5, but not with IRF-7, for MyD88 interaction. The TRIF-dependent induction of pro-inflammatory cytokines is markedly enhanced in peritoneal macrophages from mice deficient in the Irf4 gene, whereas the induction is inhibited by the ectopic expression of IRF-4 in a macrophage cell line. The critical function of IRF-4 in TLR signaling in vivo is underscored by the observation that IRF-4 deficient mice shows hypersensitivity to DNA-induced shock, with elevated serum pro-inflammatory cytokine levels. Thus, IRF-4 is seen to be a negative regulator of TLR signaling [13].

Radioprotective 105 (RP105) is a specific homolog of TLR4. Like the TLRs, RP105 has a conserved extracellular leucine-rich repeat domain and a TLR-like pattern of juxtamembrane cysteines. However, it does not have a Toll-IL-1 receptor domain, containing a mere 6–11 intracytoplasmic amino acids instead. RP105 is expressed on human and mouse macrophages and DCs, just as is TLR4. RP105 expression specifically inhibits TLR4-driven NF-κB activation by HEK 293 cells. DCs from RP105-deficient mice produced significantly higher concentrations of pro-inflammatory cytokines after stimulation with purified Escherichia coli LPS than did DCs from wild-type controls. High-dose LPS challenge led to significant acceleration and amplification of endotoxicity in RP105-deficient mice. In fact, RP105 and its helper molecule, MD-1, have a physical association with TLR4/MD2, and this association inhibits LPS–TLR4/MD2 complex formation. Given the evidence that no direct interactions between LPS and RP105-MD-1 were demonstrated in this system, RP105-MD-1 is thought to inhibit the TLR4 signaling pathway by blocking the binding of the microbial ligand LPS [14].

Alternative splicing is a mechanism that allows individual genes to express multiple mRNAs that encode proteins with diverse and even antagonistic functions. Indeed, several splice variants have been described as involved in the negative regulation of TLR signaling pathways by means of competing with the various adaptors and transcription factors for binding sites. One such example is MyD88s, an essential adaptor used by all TLRs except TLR3. It is composed of three main domains: the N-terminal death domain, the intermediate domain and the TIR domain. MyD88s, which lacks the intermediate domain, has been described as a dominant-negative inhibitor of TLR signaling pathways. Overexpression of MyD88s can inhibit LPS-induced NF-κB activation. In fact, the presence of MyD88s favors the formation of MyD88s-MyD88 heterodimers, which can still recruit IRAK-1 but cannot phosphorylate it [15]. Another team showed that IRAK-4 is a similar participant. MyD88 strongly interacts with IRAK-4, and that this interaction is essential for the phosphorylation of IRAK-1. However, MyD88s were not able to interact with IRAK-4, resulting in inhibition of the phosphorylation of IRAK-1 and the subsequent signaling pathway [16].

3.4. Other mechanisms involved in the negative regulation of TLR signaling pathways

There are many other negative regulators that use different mechanisms to control TLRs signaling pathways. Some of the better established ones are described below.

TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) was originally identified through its homology to TNF,
FasL, and other members of the TNF superfamily. Although four kinds of TRAIL-Rs have been found in humans, only one full-length TRAIL-R has been reported in mice. TRAIL-R<sup>−/−</sup> mice develop normal lymphocyte populations but possess enhanced innate immune responses. TRAIL-R-deficient macrophages and dendritic cells have been found to exhibit elevated levels of cytokine production after TLR2, TLR3, and TLR4 stimulation when compared to cells isolated from wide-type littersmates. Furthermore, when TRAIL-R was exogenously expressed in TRAIL-R<sup>−/−</sup> bone-marrow-derived macrophages by retrovirus, the level of IL-12 production after LPS treatment became lower. The mechanism for this involved IκB-α. IκB-α sequestered NF-κB dimers present in the cytoplasm in an inactive form. Activation of NF-κB involves the phosphorylation and proteolysis of the IκB proteins and the concomitant release and nuclear translocation of the NF-κB factors. When macrophages or dendritic cells from TRAIL-R<sup>−/−</sup> animals and wild-type littersmates were treated with LPS, there were no significant differences in IκB-α phosphorylation and degradation in the first hour. However, at later time points (4 and 8 h post stimulation), when IκB-α protein was reexpressed in the wild-type cells, it could not be detected in TRAIL-R<sup>−/−</sup> cells. As no difference in IκB-α mRNA levels was observed between wild-type and knockout cells, TRAIL-R most likely negatively regulated the TLR signaling pathway by stabilization of IκB-α [17].

B cell leukemia (Bcl)-3 interacts with NF-κB family member p50 and belongs to the IκB family. Macrophages and dendritic cells isolated from Bcl-3-deficient mice show cytokine production enhanced over that in wild-type cells in response to several LPS ligands. Bc13−/− macrophages pre-treated with LPS lack the ability to achieve the tolerance seen in wild-type cells, whose function is characterized by reduced cytokine gene expression upon restimulation. Furthermore, when bone-marrow chimeric mice were used to study TLR tolerance in vivo, it was found that LPS pretreatment of mice receiving wild-type bone-marrow protected them from septic shock while the vast majority of mice that received Bc13<sup>−/−</sup> bone marrow died. The negative regulation of a TLR signaling pathway by Bcl3 involves its association with p50. In unstimulated wild-type macrophages, p50 homodimers occupied the NF-κB DNA binding sites, inducing the polyubiquitination and degradation of p50, and allowing it to be replaced by transcription active dimmers of c-Rel and p65 after stimulation with LPS. However, Bc13 is able to prolong the half time of DNA-bound p50 homodimers by effective inhibition of its polyubiquitination and degradation. The enhanced p50 homodimer occupancy thus controls LPS response. Taken together, the data show that the negative regulation of TLR signaling is dependent upon the coordinated action of both the inhibitor p50 and its stabilizer, Bcl-3 [18].

IRAK-M belongs to the IRAK family. Although it has 12 serine/threonine kinase subdomains, IRAK-M lacks the catalytically active aspartate, so it does not have the kinase activity seen in family members IRAK-1 and IRAK-4. Interestingly, while IRAK-1, IRAK-2 and IRAK-4 are expressed ubiquitously, IRAK-M is mainly expressed in monocytes/macrophages. Compared to wild-type cells, IRAK-M-deficient macrophages show elevated production of cytokines in response to TLR ligands, especially in regard to CpG DNA. In CpG DNA treated IRAK-M-deficient macrophages, phosphorylation of JNK, p38, and ERK1/2 was enhanced with more rapid phosphorylation and degradation of IκBζ. Furthermore, IRAK-M-deficient macrophages showed a lack of endotoxin tolerance, and the cytokine levels produced upon LPS restimulation were not decreased as much as those seen in restimulated wild-type macrophages. IRAK-M negatively regulated the TLR pathway associated with IRAK-1. Over-expression of IRAK-M blocked the formation of IRAK-1/ TRAF6 complex. Surprisingly, instead of competition for the same binding site as found with some other negative regulators, IRAK-M inhibited the dissociation of IRAK-1 from MyD88 after IRAK activation, for it increased the affinity of both phosphorylated and unphosphorylated forms of IRAK for MyD88 [19].

Activating transcription factor-3 (ATF3) is a member of the ATF/CREB family of bZip transcription factors that binds to the consensus CRE, and it might play a role both as a transcriptional activator or repressor depending upon the cell type and stimulus involved. Compared to wild-type cells, ATF3-deficient bone-marrow-derived macrophages show elevated production of cytokines, such as IL-12p40, IL-6 and TNF, in response to LPS. After receiving LPS intraperitoneally, ATF3-deficient mice exhibited serum levels of IL-12p40, IL-6 and TNF significantly higher than did wild-type mice. In vivo endotoxic shock experiments proved that ATF3 could protect mice from LPS-induced endotoxic shock. The mechanism of ATF3 exertion of its negative regulation function was identified. Acetylation and deacetylation of histones is very important for transcription by chromatin remodeling; while acetylation says ‘go’ to the transcription factors, deacetylation says ‘stop’. ATF3 can interact with histone deacetylase HDAC1 in a LPS-induced pattern. ATF3 binds to the promoters and recruits HDAC1, resulting in altered chromatin structure to limit access to transcription factors (such as NF-κB) [20]. Recent research demonstrated ATF3 as a negative regulator not only in the TLR4-stimulated inflammatory response, but also in TLR2/6 heterodimer, TLR3, TLR5, TLR7, and TLR9 signaling pathways [21].

Soluble decoy TLRs (sTLR) are currently the only described extracellular negative regulators of TLR signaling pathways. Although there is only one copy of the TLR4 gene, several mRNA products have been detected to be TLR4 isoforms. Interestingly one of them encodes a 122-aa protein, the first 86-aa of which are identical with the extracellular domain of TLR4, while the remaining 36-aa share 70% homology with the N-terminal region of phosphatidylinositol 3-kinase (PI3 K). It is a soluble protein and thus was later termed as sTLR. Overexpression of sTLR4 in RAW264.7 resulted in decreased NF-κB activation in response to LPS treatment. The exact mechanism remains unknown. Perhaps, sTLR4 terminates TLR4 signaling by breaking the interaction between TLR4 and other co-receptor complexes [22]. Another member of the sTLR family is sTLR2. It was found in human blood...
monocytes as the post-translational modification of the transmembrane receptor. In the presence of sTLR2, lower IL-8 and TNF-A was detected. The mechanism may be associated with the fact that sTLR2 could interact with sCD14, but it needs to be further investigated [23].

DC-SIGN is a C-type lectin expressed by DCs and known for its interaction with HIV-1. Indeed, DC-sign functions as a receptor exclusive of this relationship. It is a pattern recognition receptor that is able to interact with a large array of pathogens. It has been proved that DC-SIGN is an important receptor on DCs for viable \textit{Mycobacteria tuberculosis} and that this interaction is mediated by ManLAM, a cell wall component abundantly expressed by \textit{Mycobacteria tuberculosis}. Binding of ManLAM to DC-SIGN impairs LPS-induced DC maturation, allowing a marked increase of the immunosuppressive cytokine IL-10 [24]. The exact mechanism is unknown. However, a recent study demonstrated the modulation of TLR signaling by Raf-1-dependent acetylation of NF-κB. Interaction of DC-SIGN with ManLAM triggers the activation of serine and threonine kinase Raf-1, leading to subsequent acetylation of the p65 unit of NF-κB, and so enhances the production of IL-10, thereby inducing pathogen-specific anti-inflammatory cytokine responses. Interestingly, Raf-1 cannot induce NF-κB activation by itself. It functions only after TLR signaling has activated NF-κB, indicating that it was just a new pathway that was identified [25].

TNFAIP8-like (TIPE) 2, containing a putative DED-like domain identical to other known DED sequences, is a recently identified protein sharing a high degree of homology with TNFAIP8, and able to inhibit caspase-mediated apoptosis. Bone-marrow-derived TIPE2-deficient macrophages produce more IL-6, IL-12 and TNF-α than wild-type cells in response to LPS. Consistently, knock down of TIPE2 expression in RAW264.7 enhances the production of IL-6 in response to several TLR ligands. Furthermore, TIPE2-deficient mice are hypersensitive to LPS-induced septic shock. The mechanism may be associated with caspase-8 as inhibiting caspase-8 significantly blocks the hyper-responsiveness of TIPE2-deficient cells. Actually, TIPE2 can interact with caspase-8 in both resting and LPS-stimulated RAW264.7. However, the importance of this interaction should be further investigated [26].

Both the Src homology 2 domain-containing protein tyrosine phosphatase (SHP)-1 and SHP-2 are intracellular tyrosine phosphatases, containing two SH2 domains at the N terminus, followed by a catalytic domain. Interestingly, SHP-1 and SHP-2 regulate TLR-mediated production of pro-inflammatory cytokines and type I interferon in different ways. SHP-2 specifically inhibits TRIF-dependent TLR3 and TLR4 signal transduction, including the production of type I interferon and pro-inflammatory cytokine. Endogenous interaction between SHP-2 and TBK1 after TLR3 activation has been identified in RAW264.7 macrophages and the N-terminal domain of TBK1 is essential for this interaction. Actually, this interaction inhibits phosphorylation of the substrate by TBK1 and thus suppresses subsequent cytokine production [27]. Strikingly, the case surrounding SHP-1 is very complicated. While SHP-1 inhibits TLR-mediated production of pro-inflammatory cytokines by suppressing activation of NF-κB and MAP kinases, it promotes production of type I IFN at the same time. Interestingly, the mechanism in both cases appears to be the interaction between SHP-1 and IRAK-1, by which course the kinase activity of IRAK-1 is inhibited. Based upon the fact that the kinase activity is required for pro-inflammatory cytokine induction and acts against type I IFN production, SHP-1 appears as a negative regulator of pro-inflammatory cytokine production and a positive regulator of type I IFN production in response to TLR by inhibiting the kinase IRAK-1 [28].

4. Conclusions

TLRs are the best-known sensors of the invasion of pathogenic microorganisms. They are critical for the development of innate immunity to pathogens. However, the strength and the duration of the activation of TLR signaling pathways must be tightly controlled for overactivation of TLRs can be dangerous to the host. Too much inflammation can cause autoimmunity, chronic inflammatory and infectious disease such as sepsis, EAE, SLE, etc. The balance is maintained by multiple negative regulators and the regulation is very precise.

The expression of most negative regulators can be induced by the activation of TLRs and uses a mode of negative feedback to terminate TLRs activation. However, there are also some constitutively expressed factors that could possibly exert their functions only when TLRs are overactivated. For example, Pin1 specifically recognizes phosphorylated IRF3 and then controls the termination of IRF3-dependent transcriptional activation. Each negative regulator has its own specific target protein and the exact mechanism may differ from one to another. There exist many kinds of mechanisms, but degradation, deubiquitination, and competition are most frequently observed. The negative regulators cannot compensate for each other for deficiency in any one of them leads to fatal disease.

The regulation of TLR signaling pathways constitutes a complex network. Although it has been studied for many years, additional new components and regulatory aspects of known components continue to be revealed. We trust that additions to the body of information concerning this regulation may provide added possibilities for therapeutic manipulation of these pathways and thus for more effective treatment of human disease involving TLRs.

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