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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.

Keywords: TLR; Negative regulation; Degradation; Deubiquitination

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 IκB kinase (IKK-*i*, 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].

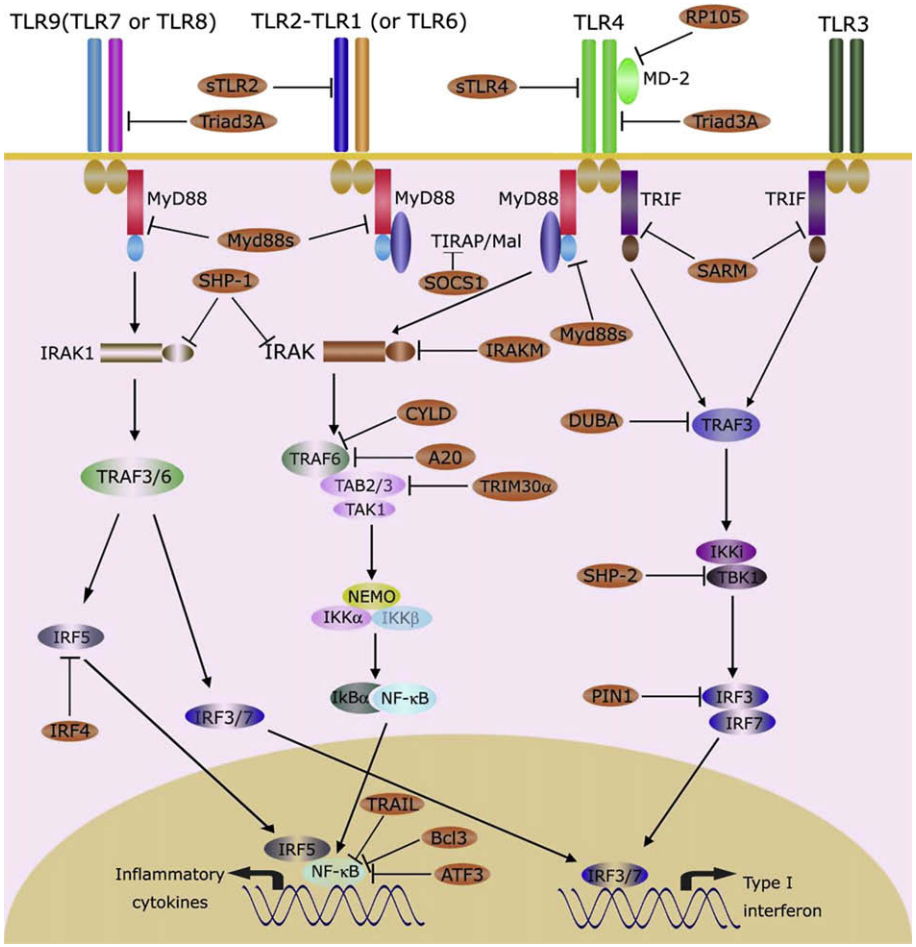

Fig. 1 The negative regulators of TLR signaling pathways. The negative regulators were marked using the brown color around their target proteins.