Toll-like Receptors and Type I Interferons

MINIREVIEW
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Toll-like receptors (TLRs) are key molecules of the innate immune systems, which detect conserved structures found in a broad range of pathogens and trigger innate immune responses. A subset of TLRs recognizes viral components and induces antiviral responses. Whereas TLR4 recognizes viral components at the cell surface, TLR3, TLR7, TLR8, and TLR9 recognize viral nucleic acids on endosomal membrane. After ligand recognition, these members activate their intrinsic signaling pathways and induce type I interferon. In this review, we discuss the recent findings of the viral recognition by TLRs and their signaling pathways.

Type I interferon (IFN), which was first discovered by Isaacs and Lindenmann in 1957 (1), derives its name from a function to “interfere” in viral replication. Type I IFNs are encoded by and Lindenmann in 1957 (1), derives its name from a function to “interfere” in viral replication. Type I IFNs are encoded by

These receptors recognize conserved molecular patterns characteristic of microorganisms, which are not generated by the host and are essential for microbial survival. The pattern recognition receptors involved in the induction of type I IFNs are divided into two categories: Toll-like receptors (TLRs) and RIG-I-like helicases (RLHs). RLHs are expressed ubiquitously and are localized in the cytosol, where they recognize dsRNA produced upon viral infection. On the other hand, TLRs are located on cell surfaces or in endosomes, where they detect viral components or viral nucleic acid (3). Here we review the induction of type I IFNs by TLRs, especially focusing on their signaling pathways.

Toll-like Receptors

TLRs, a family of evolutionarily conserved pathogen recognition receptors, play a pivotal role in innate immunity. To date, the TLR family consists of 13 mammalian members. The cytoplasmic portions of TLRs show high similarity to that of the interleukin-1 receptor (IL-1R) family and are now called the Toll/IL-1 receptor (TIR) domain. A TIR domain is required to initiate intracellular signaling. The extracellular regions of TLRs and IL-1R are markedly different. Whereas IL-1R possesses an Ig-like domain, TLRs contain leucine-rich repeats in their extracellular domains. TLRs are pattern recognition receptors that sense a wide range of microorganisms, such as bacteria, fungi, protozoa, and viruses. Each TLR has its own intrinsic signaling pathway and induces specific biological responses against microorganisms such as dendritic cell maturation, cytokine production, and the development of adaptive immunity (2).

TLR-mediated Type I IFN Signaling Pathways

The activation of TLR signaling pathways originates from the cytoplasmic TIR domains. Each TLR mediates distinctive responses in association with a different combination of four TIR domain-containing adapters (MyD88, TIRAP/MAL, TRIF, and TRAM) through the homophilic interaction of TIR domains (2). The association of TLRs with MyD88, which is utilized by all TLRs except TLR3, recruits IL-1R-associated kinase (IRAK)-1 and IRAK-4 and tumor necrosis factor receptor-associated factor-associated factor 6 (TRAF6). IRAK-1 and TRAF6 then dissociate from this receptor complex and associate with another complex composed of transforming growth factor-β-activated kinase (TAK1) and TAK1-binding proteins 1 (Tab1) and 2 (Tab2). This complex formation leads to the activation of TAK1, which in turn activates the transcription factors nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) through the canonical IκB kinase (IKK) complex and the mitogen-activated protein kinase pathway, respectively. The kinase activity of the IKK complex is modulated by its IKKγ subunit, the transcription factor NF-κB essential modulator (NEMO). NF-κB activates multiple proinflammatory cytokine genes, including tumor necrosis factor α, IL-6, and IL-1β. In addition to this common pathway, called a MyD88-dependent pathway (Fig. 1),
some TLR family members specifically involved in virus recognition have unique signaling pathways to induce type I IFNs (2).

**TLR3—**TLR3 recognizes a synthetic analog of viral dsRNA, polyinosinic acid-cytidylic acid (poly(I:C)), and viral dsRNAs derived from dsRNA viruses such as reovirus or ssRNA viruses such as West Nile virus, respiratory syncytial virus, and encephalomyocarditis virus (2). Although TLR3 is expressed on the cell surface of fibroblasts, it localizes to endosomes in conventional dendritic cells (cDCs), requiring acidification of vesicles for its signaling (4). The three-dimensional structure of human TLR3 leucine-rich repeat motifs demonstrated direct binding of the TLR3 ectodomain to poly(I:C) (5). TLR3 interacts with CD14 and c-Src for ligand uptake and signal transduction (6, 7).

TLR3 signaling activates the transcription factors interferon regulatory factor 3 (IRF3) and NF-κB via the adapter molecule TRIF (8, 9) (Fig. 1). TRIF interacts with noncanonical IKKs TBK1 (also called NAK or T2K) and IKKζ (also called IKKe) through TRAF3 and NAK-associated protein 1 (NAP1), which mediate phosphorylation of IRF3 (10–13). Activated IRF3 translocates into the nucleus and induces expression of IFN-β (14, 15). In addition to TBK1/IKKζ, phosphatidylinositol 3-kinase and its downstream kinase Akt are necessary for full activation of IRF3 (16). TRAF3 interacts with TRIF and negatively regulates TRIF-mediated signaling pathways (17, 18). Furthermore, IRF3 activation is negatively regulated by the peptidyl-prolyl isomerase Pin1 (19).

Receptor-interacting protein-1 (RIP1) binds the C terminus of TRIF via a Rip homotypic interaction motif and mediates the TLR3-mediated NF-κB pathwys but not IRF3 activation (20). TRAF6 binds the N terminus of TRIF and cooperatively activates NF-κB (21). Although NF-κB activation by poly(I:C) is abrogated in TRAF6-deficient fibroblasts, TRIF-mediated signaling pathways were not impaired in TRAF6-deficient macrophages (22).

TLR3 signaling was shown to promote cross-priming of T cells, a process that is necessary for the induction of virus-specific T cell responses. In mice, plasmacytoid DCs (pDCs) and CD8+ DCs appear to be the major adenomatous polyposis coli subtypes involved in priming antiviral cytotoxic T lymphocyte. When CD8+ DCs expressing high levels of TLR3 phagocytize the apoptotic bodies of virus-infected or dsRNA-loaded cells, the dsRNA in the apoptotic bodies is recognized by TLR3, triggering the maturation of immature CD8+ DCs that are required for the subsequent induction of antigen-specific CD4+ and CD8+ T cell responses. In addition, type I interferons released from virus-infected cells facilitate cross-priming (23). In contrast to RLHs, plasmacytoid receptors for dsRNA, TLR3 plays a crucial role in the cross-priming of cytotoxic T lymphocytes against viruses that do not directly infect DCs.

The functions of TLR3 have been elucidated in actual viral infections. TLR3-deficient mice showed susceptibility to mouse cytomegalovirus infection because of reduced interferon production (24). However, TLR3-deficient mice were resistant to West Nile virus infection. This ssRNA flavivirus induces inflammatory responses in a TLR3-dependent manner that trigger a breakdown of the blood-brain barrier, which subsequently results in enhanced brain infection (25). These results are quite interesting, revealing that TLR3-mediated inflammatory responses to West Nile virus contribute to pathogenesis rather than to protection.

**TLR4—**TLR4, the first mammalian homologue of the Drosoiphila Toll protein (26) recognizes lipopolysaccharide (LPS), which is a cell wall component of Gram-negative bacteria (27, 28). TLR4 recognizes not only bacterial components but also viral proteins, such as the fusion (F) protein from respiratory syncytial virus and the envelope protein of mouse mammary tumor virus (29, 30). TLR4-mutated C3H/HeJ mice are sensitive to respiratory syncytial virus infection (31). In addition to proinflammatory signals, TLR4 possesses TRIF-mediated signaling pathways (8, 9) (Fig. 1). Unlike TLR3, this pathway requires an additional adapter molecule, TRAM.
TLR9 (38). TLR9-deficient mice were also shown to be susceptible to mouse cytomegalovirus infection, suggesting that TLR9 induces antiviral responses by sensing the CpG-containing DNA of DNA viruses (24, 39, 40).

TLR7 and TLR8 are structurally highly conserved proteins (2). The synthetic imidazoquinoline-like molecules imiquimod (R837) and resiquimod (R848) have potent antiviral activities and are used clinically for the treatment of viral infections. Murine TLR7 and human TLR7 and TLR8 recognize imidazoquinoline compounds (41, 42). Furthermore, murine TLR7 has been shown to recognize guanosine analogs such as loxoribine, which has antiviral and anti-tumor activities (2). Recently, TLR7 and human TLR8 have been shown to recognize guanosine- or uridine-rich ssRNA from viruses such as human immuno- 

deficiency virus, vesicular stomatitis virus, and influenza virus (43, 44).

The induction of type I IFNs by TLR7 and TLR9 depends entirely on MyD88 in pDCs (45) (Fig. 2). IRF7 is a transcription factor that is structurally related to IRF3 and is expressed constitutively in pDCs. IRF7 forms a signaling complex with MyD88 and TRAF6 in the cytoplasm (46, 47). In this complex, TRAF6 activates IRF7 through its ubiquitin E3 ligase activity (46). After ligand stimulation, IRF7 is activated by its phosphorylation in TBK1/IKKα-independent manner and translocates into the nucleus to induce the expression of type I IFNs (46–48). Mouse pDCs lacking IRAK-4 fail to produce both inflammatory cytokines and IFNs-α (47). Human TLR7-, TLR8-, and TLR9-mediated induction of IFN-α/β and IFN-λ was also IRAK-4-dependent (49). In mice, IRAK-1 has been shown to serve as an IRF7 kinase. IRF7 activation by TLR9 ligand is impaired in IRAK-1-deficient mice, despite normal NF-κB activation. IRAK-1, but not IRAK-4, can directly bind and phosphorylate IRF7; thus, IRAK-1 specifically mediates IFN-α induction downstream of MyD88 and IRAK-4 (50). In addition to IRAKs, IKKα has been shown to be essential for the phosphorylation of IRF7, suggesting that the IRAK-4/IRAK-1/IKKα kinase cascade might lead to the full activation of IRF7 (51).

To elucidate the mechanism of IRF7 phosphorylation more precisely, analysis of mice expressing kinase-negative mutants of these molecules will be necessary in the near future. Recently, TRAF3 and osteopontin have also been shown to be involved in this signaling pathway (10, 11, 52).

The TLR9 ligands, CpG DNA, are divided into at least three distinct classes with different biological effects (53). Conventional CpG DNA, also called K/B-type CpG DNA, has a potency to activate B cells and induces inflammatory cytokines from macrophages. D/A-type CpG DNA shows a weaker activity on
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B cells or macrophages but induces robust production of type I IFNs from pDCs. In addition, C-type CpG DNA mediates the induction of type I IFNs, as well as B cell activation, but its potency in both effects is moderate (54). It remains unknown how different TLR9 agonists lead to such distinct outcomes. The importance of prolonged localization of a TLR9 ligand in an endosome has been suggested (55). K/B-type CpG DNA is rapidly transferred and degraded in the lysosome, whereas D/A-type CpG DNA is retained in the endosomes of pDCs. These results suggest that the endosomal retention of ligands in pDCs probably provides a platform for the interactions of signal-transducing molecules such as MyD88 and IRAF. However, this hypothesis does not fully explain the ligand specificity. If the endosomal retention of a ligand enhances its signaling, it is easy to assume that D/A-type CpG DNA induces robust production of inflammatory cytokines as well as IFN-α. As is generally known, D/A-type CpG DNA shows lower efficacy in the induction of proinflammatory cytokines than K/B-type CpG DNA. Furthermore, K/B-type CpG DNA even induces a certain amount of IFN-α from pDCs at lower concentrations (45). In IRAK-1-deficient pDCs, both K/B-type- and A/D-type-mediated IFN-α induction was severely impaired (50), suggesting that an additional mechanism exists to determine the distinct response to each type of CpG DNA.

K/B-type CpG DNA-mediated IFN-β Induction in cDCs—Granulocyte macrophage-colony-stimulating factor-induced DCs produce a small amount of IFN-β, but not IFN-α, in response to K/B-type CpG DNA (45). Neither IRF7 nor IRF3 is essential for this signaling pathway (56) (Fig. 2). A recent report showed that IRF1 specifically participates in induction of the IFN-β gene in this signaling pathway. IRF1 is induced by IFN-γ stimulation. After ligand stimulation, IRF1 interacts with MyD88, is activated by an unknown mechanism, and translocates into the nucleus to induce the expression of IFN-β, inducible nitric-oxide synthase, and IL-12 p35 (56). Furthermore, IRF1 is not essential for the TRIF-dependent pathway or the TLR9-mediated pathway in pDCs. Thus, the induction of type I IFNs by CpG DNA is quite distinct between cDCs and pDCs.

Conclusion and Future Perspectives

The study of innate immunity has progressed rapidly over the last decade. TLR family members, which were initially considered to be receptors for bacterial components, have been shown to be involved in viral recognition and subsequent induction of type I IFNs, which are the most important antiviral agents. Simultaneously, pDCs have been identified as professional interferon-producing cells, which play crucial roles during viral infection. It has become clear that TLRs are the major receptors for the initiation of type I IFN production in pDCs (57). In the process of these studies, evidence has been obtained that non-professional interferon-producing cells, such as cDCs and epithelial cells, can produce type I IFNs in a TLR-independent manner. All of these results led to the discovery of RLH family members, the functions and signaling pathways of which are currently among the hottest topics in immunology. Although antiviral responses have been well clarified at the cellular level, it remains unknown when, how, and in what kind of cells TLR and RLH systems are used to exclude viruses. It will be necessary to examine the functions of TLRs and RLHs dynamically by using various viral infection models in the future.

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