Toll-like Receptors and NOD-like Receptors in Innate Immune Defense during Pathogenic Infection

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In response to invading pathogens, the body immune system develops an immediate defense mechanism, i.e., innate immune response, which is detected in almost all living organisms including mammals, plants, insects, etc. Recent studies have identified numerous innate immune receptors that are able to recognize pathogen-associated molecular patterns and transduce the essential intracellular signaling cascades to mount early and successful host defenses against infectious challenge. Among innate immune receptors, we will focus on two important receptors, toll-like receptors (TLRs) and nucleotide binding oligomerization domain (Nod)-like receptors, and their major intracellular signaling pathways that culminate to activate innate immune effectors and inflammatory mediators during pathogen infection. In this review, we address the recent advances of understanding intracellular signaling mechanisms by which TLRs and NLRs activate host immune defense and inflammation. The role and regulatory mechanisms by which a subset of NLRs-associated inflammasome activation induce interleukin-1β secretion and their relevance with host defense will be also discussed. Both TLR- and NLR-mediated intracellular signaling networks serve crucial roles in mounting resistance to bacterial and viral infection through synthesis of immune mediators and antimicrobial chemicals during infection.

**Key Words:** Toll-like receptors, Nod-like receptors, Inflammasome, Host defense, Infection, Innate immunity

INTRODUCTION

Innate immune system is importantly required for the first line and immediate immune defense against infectious and dangerous stimuli. A number of germline-encoded innate receptors, pattern recognition receptors (PRRs), are responsible for the recognition of highly conserved microbial structures called PAMPs (pathogen-associated molecular patterns) or DAMPs (damage-associated molecular patterns) from a variety of microorganisms or modified self-antigens for induction of innate immune responses (1, 2).

Toll-like receptors (TLRs) are one of the best-characterized PRRs which signal through adaptor molecules including myeloid differentiation factor 88 (MyD88) and Toll/IL-1 receptor domain containing adaptor inducing interferon-β (TRIF) to culminate the activation of several important transcription factors, such as nuclear factor (NF)-xB, inter-
feron regulatory factors (IRFs), as well as mitogen-activated protein kinase (MAPK) pathway to trigger innate immune signaling (3, 4). The activation of innate immune signaling leads to the induction of proinflammatory cytokines, type I interferons (IFNs), and antimicrobial effector proteins (5, 6). Nucleotide binding oligomerization domain (Nod)-like receptor (NLR) constitute special type of PRRs, and play a pivotal role in detection of cytosolic microbial and danger components (7–9). Among NLRs, NOD1 and NOD2 are able to sense the peptidoglycan (PGN) fragments meso-DAP and muramyl dipeptide, respectively, to activate intracellular signaling pathways, NF-κB and MAPKs (8, 9).

In addition, other set of NLRs, i.e., pyrin molecules, can activate inflammasome complex to induce the maturation of specific proinflammatory cytokines, interleukin (IL)-1β and IL-18 through induction of caspase-1 activation (10).

In this review, we will discuss recent findings upon the PAMP and DAMP recognition by TLRs and NLRs, and their intracellular signal transduction pathways (Fig. 1).

Overview of TLRs and Their Intracellular Signaling Pathways

To date, at least 10 TLRs in humans and 12 in mice (TLR1 to TLR9 and TLR11 to TLR13) have been identified (11). TLR ligands have been identified for all TLRs except for human TLR10, mouse TLR12 and mouse TLR13 (11). Each TLR member can recognize distinct PAMPs, which consist of lipids, proteins, and nucleic acids (4). For example, TLR4 senses the lipopolysaccharide (LPS) of gram-negative bacteria, whereas TLR2/1 or TLR2/6 recognizes peptidoglycan and lipoproteins from various bacteria. Some TLRs (TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11) are located at the plasma membrane to recognize PAMPs from invading pathogens at cell surface, whereas other TLRs (TLR3, TLR7, TLR8 and TLR9) are found at the endosomal membrane inside the cells to mediate sensing nucleic acids. Each ligand and corresponding TLR in terms of the compartments in which TLR-ligands are found have been well-studied and discussed in previous reviews (4, 6, 11).

In structure, TLR members have leucine-rich-repeat motifs for binding of each ligand, a single transmembrane domain, and a cytoplasmic domain containing a TLR-IL-1 receptor (TIR) domain that interacts with several TIR-containing adaptor proteins including MyD88 or TRIF (12). MyD88 is a central adapter shared by most TLRs except TLR3. Upon TLR activation, MyD88 associates with TLR through TIR-TIR interactions and recruits the interleukin-1 receptor-associated kinase (IRAK) family members through its death domain. The IRAK proteins are serine/threonine kinases, and contain IRAK-1, IRAK-2, IRAK-4 and IRAK-M. Once IRAK-1 is phosphorylated during TLR signaling, TRAF6 is recruited to the proximal TLR complex and activates a complex containing TAK1 (transforming growth factor-β-activated kinase 1), TAB1 (transforming growth factor-β-activated protein kinase 1-binding protein 1) and TAB2. Formation of this TAK1 complex is an essential step for downstream intracellular activation of MAPK and NF-κB signaling pathways (13, 14).

MyD88-independent signaling involves the other adaptor TRIF, and results in the production of type I IFNs through phosphorylation and nuclear translocation of IRF3 by TRAF3 and TBK1/IKKi (15). Indeed, TRAF3 is an important ubiquitin ligase that can interact with both MyD88 and TRIF, with two different modes, i.e., degradative ubiquitination of TRAF3 in MyD88-dependent TLR signaling and noncanonical TRAF3 self-ubiquitination in TRIF-dependent signaling (16). Additional TIR domain-containing adaptor is TIR domain-containing adaptor protein/MyD88-adaptor-like (TIRAP/Mal), which is involved in the activation of MyD88-independent signaling pathways downstream of TLR4 (17, 18).

NOD1 and NOD2 and Their Intracellular Signaling Pathways

NLRs are crucial cytoplasmic sensors that contain a tripartite structure of the N-terminal protein-protein interaction domain (caspase recruitment domain (CARD), pyrin domain (PYD)), a centrally located NOD domain which is needed for self-oligomerization, and a C-terminal leucine-rich repeat (LRR) to detect PAMPs. Two NOD domain-
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Containing molecules, both NOD1 and NOD2, are well-studied NLRs that recognize distinct PGN fragments from bacterial cell walls (19, 20). NOD1 signaling is triggered by γ-D-glutamyl-meso-diaminopimelic acid (meso-DAP) from all Gram-negative bacteria and certain Gram-positive bacteria (21, 22), whereas NOD2 is activated by muramyl dipeptide, a common motif of both Gram-negative and Gram-positive bacteria (23, 24). Together with TLRs, NOD1 and NOD2 play key roles in innate and acquired immune responses through recognition of specific PAMPs and in production of proinflammatory mediators (25).

After engagement of NOD1 and NOD2 with their ligands, both receptors undergo signal transduction via recruitment and activation of the serine threonine kinase RICK (receptor-interacting protein kinase; RIP2 or CARDIAK), an essential adaptor protein for the activation of NF-κB and MAPK pathways (26–29). It was also shown that K63-linked polyubiquitination of RICK is important for the recruitment

Figure 1. Schematic diagrams of innate immune receptors. (Left) Toll-like receptor (TLR) signaling is activated by TLR ligands. TLRs which located in plasma membrane (TLR1, 2, 4, 5, 6, 11) activate NF-κB and MAPK (JNK, ERK, p38) signaling pathway via MyD88. Nucleic acid recognition by endosomal TLRs (TLR3, 7, 8, 9) induces production of type I interferon and proinflammatory cytokines via TRIF-IRF3 and MyD88-NF-κB signaling pathways, respectively. (Middle) NOD1 and NOD2 recognize γ-D-glutamyl-meso-diaminopimelic acid (meso-DAP) and muramyl dipeptide (MDP) from bacterial cell wall components, respectively. NOD-induced proinflammatory responses require MAPK and NF-κB activation through the recruitment of adaptor molecule CARD9 and RICK. (Right) Certain members of NOD-like receptors (NLRs) participate in the activation of inflammasome complex consisting of NLRs, procaspase-1 and ASC in the cytosol. Multiple ligands or stimulators including various pathogen-associated molecular patterns, damage-associated molecular patterns, and microbes, have been identified to activate inflammasome complex to produce mature IL-1β and IL-18 through activation of caspase-1.
of TAK1 and activation of IKK complexes, a critical step for downstream NF-κB activation in NOD1 and NOD2 signaling (30). Indeed, RICK induces K63-linked poly-ubiquitination of NEMO and RICK itself to promote recruitment of TAK1 complex, leading to TAK1-mediated phosphorylation and activation of the IKK complex and NF-κB signaling (31). Other adaptor XIAP, a member of the inhibitor of apoptosis protein (IAP) family, was also found to be important for NOD signaling through interaction with RICK via its BIR2 domain and for the NOD-dependent NF-κB signaling (32). Similar to TLR signaling, the E3 ligase TRAF6 is required for NOD2-dependent signaling, whereas TRAF2 and TRAF5 appear to be crucial for NOD1-mediated signaling (20). Both NOD1- and NOD2-mediated activation of NF-κB signaling results in production of proinflammatory cytokines and antimicrobial effectors, such as nitric oxide and antimicrobial peptides (33).

In NOD1 and NOD2 signaling, several key control points, i.e., RICK and TAK1, have been characterized in the activation of MAPK (p38, JNK, ERK) pathway (28, 34), albeit the molecular mechanisms have not been fully elucidated. Previous studies showed that RICK is a critical component of NOD1-dependent JNK activation and IL-8 secretion and that the kinase TAK1 is important for NOD1-induced JNK activation (34). Several lines of evidence showed that TAK1 is a central molecule for activation of NOD2-RICK signaling and NOD2-dependent MAPK activation in various cells (35, 36). The caspase-recruitment domain-containing protein CARD9, an essential adaptor for host defense to fungal infection, was found to be required for the activation of MAPK (p38 and JNK) and for inhibition of listerial infection and cytokine production (37). In addition, CARD9 showed an inducible association with both NOD2 and RICK (37). Moreover, secretion of IL-1β from intracellular pro-IL-1β stores is essentially required for MDP-mediated cytokine induction and MAPK activation in human macrophages, thus emphasizing the role of autocrine IL-1β loop in NOD signaling (38). Together, these studies indicate that the NOD1 and NOD2 signaling pathways activate complicated intracellular events leading to the production of proinflammatory cytokines through pathways that are mostly common to both receptors, i.e., RICK and TAK1-mediated signaling. The location and simplified intracellular signaling cascades of TLRs and NLRs (NOD1 and NOD2) are illustrated in Fig. 1.

NLR-mediated Inflammasome Complex Activation

Among other NLRs that contain N-terminal CARD domain, several NLR molecules including NLRP3 (also known as NALP3 or cryopyrin), NLRP1, NLRC4, NLRP6, NLRP7, NLRP12, and absence in melanoma 2 (AIM2), are also involved in the activation of the ‘inflammasome’ (20, 39, 40). The inflammasome is a large protein complex formed by a subset of NLR proteins and mediates the cleavage of inflammatory caspase (caspase-1 and caspase-11), which is required for the processing and secretion of the pro-inflammatory cytokines, IL-1β and IL-18 (40, 41). The exact mechanism of inflammasome activation remains still elusive, however, it is believed that the inflammasome-related NLRs (NLRP3, NLRP1, NLRC4, or AIM2) undergo conformational change after inflammasome stimuli, which results in the recruitment of and interaction with a common inflammasome-adaptor protein, ASC (PYCARD) and procaspase-1, leading to induction of autoactivation of caspase-1 (40, 42). The activation of caspase-1 subsequently induces the processing and maturation of IL-1β and IL-18, both of which are crucial cytokines for numerous effects on inflammation and induction of immune responses (40, 41). Here we will focus on major NLRs associated with inflammasome activation during pathogenic infection and sterile inflammation. The NLRs-associated inflammasome complex activation is shown in Fig. 1.

i. NLRC4 and inflammasome complex activation

NLRC4 (also known as IPAF, Card12 and CLAN) usually detect to two bacterial ligands in the cytosols, i.e., flagellin co-secreted with virulence factors (43, 44), and the rod protein, a component of the type III secretion system (45). While TLR5 senses flagellated bacteria in the cell membranes, NLRC4 is activated and recognizes flagellin that is delivered to the host cytosol via virulence factor secretion.
apparatus (the SPI1 type III secretion system (T3SS) and the Dot/Icm type IV secretion system (T4SS)) (46). During this process, a NLR family apoptosis inhibitory protein (NAIP) 5, a BIR-domain NLR protein, was found to be an essential component of the flagellin-NLRC4 pathway activation through a direct interaction with flagellin (47). In addition, another adaptor NAIP2 is required for NLRC4 activation by bacterial PrgJ, but not flagellin (48).

Earlier studies showed that NLRC4 is essential for the innate immune defense against Gram-negative bacterial infections by several pathogens including Salmonella enterica serovar Typhimurium (43, 44), Shigella flexneri (45), Legionella pneumophila (49, 50), and Pseudomonas aeruginosa (51, 52) leading to the secretion of proinflammatory cytokines and macrophage cell death (53). NLRC4 inflammasome complex activation by bacterial flagellin and T3SSs promotes caspase-1 activation and pyroptosis, a caspase-1-dependent programmed cell death (53, 54). Pyroptosis is thought to be not only important in removal of the intracellular replicative niche of pathogens, but also in re-exposure of the pathogens to extracellular immune responses (55).

It was of interest that the systemic inflammasome activation by NAIP5/NLRC4 inflammasome activation was independent of IL-1β or IL-18 release, but was dependent on an ‘eicosanoid storm’, a rapid pathological release of inflammatory lipid mediators, i.e., prostaglandins and leukotrienes, which were responsible for pathological effects of systemic inflammation and vascular fluid release by either flagellin or anthrax lethal toxin (56). Recent studies have also suggested that post-translational modification of NLRC4, i.e., phosphorylation of NLRC4 by PKCδ, is important in the activation of NLRC4 inflammasome (57). Notably, NLRC4 inflammasome activation seems to play a critical role in discrimination intestinal commensal from pathogenic bacteria, such as Salmonella and Pseudomonas, and play a major role in host defense against orogastric infection with intestinal pathogenic infection (54).

ii. NLRP3 and inflammasome complex activation

NLRP3 (also known as cryopyrin or NALP3) is one of the best characterized NLRs that are associated with inflammasome activation. Numerous ligands or stimuli including endogenous danger signals, pathogenic pore-forming toxins, particulates (silica, alum, uric acid crystals, and asbestos, etc) have been reported to assembly and activation of NLRP3 inflammasome complex (54, 58). Numerous pathogens including Staphylococcus aureus (59), Listeria monocytogenes (60, 61), Klebsiella pneumoniae (62), Neisseria gonorrhoeae (63), Candida albicans, (64, 65), Mycobacterium abscessus (66), Propionibacterium acnes (67), and influenza A virus (68, 69), activate NLRP3 inflammasome in a different context of cell types and status.

The structure of NLRP3 contains 3 domains: LRR, a central nucleotide domain termed the NACHT domain, and an N-terminal effector domain (pyrin domain [PYD]). ASC contains an N-terminal pyrin domain and a CARD (70, 71). For the molecular mechanisms by which NLRP3 inflammasome is activated, the first signal is provided by microbial stimuli such as TLR agonist stimulation to induce NF-κB signaling activation, and the expression of Nlpr3 and pro-IL-1β in macrophages as a priming signal for inflammasome activation (72–74). In the absence of microbial stimulation, Nlpr3 induction is also mediated via endogenous cytokines such TNF-α and IL-1β, and leads to the NLRP3 inflammasome activation in response to NLRP3 stimulators (75).

The second signal activating NLRP3 inflammasome is provided by three distinct mechanisms such as potassium efflux, lysosomal rupture and subsequent cleavage by released cathepsin, and the production of mitochondrial reactive oxygen species (ROS) (39, 58). During activation, the second common signal activation triggers NLRP3 oligomerization, and a homotypic interaction of pyrin domain of NLRP3 with the pyrin domain of ASC, followed by the recruitment of CARD of ASC and interaction with the CARD domain of pro-caspase-1, thus leading to caspase-1 activation (39, 58). However, the detailed molecular mechanisms for NLRP3 inflammasome complex activation should be elucidated in the future studies.

In addition, the other inflammatory caspase-11 activation was found to be involved in non-canonical NLRP3 inflammasome activation and inflammasome-triggered macro-
phage cell death triggered by clinically significant Gram-negative bacteria (76, 77). In addition, Gram-negative bacteria activate caspase-11 through TRIF-dependent type I IFN signaling, suggesting an important role of TLR in regulation of inflammasome activation (77).

iii. NLRP1 and inflammasome complex activation

NLRP1 is another member of the NLRs that are associated with inflammasome activation and release of the proinflammatory cytokines IL-1β and IL-18. NLRP1 inflammasome complex is composed of the NLRP1 receptor, the adaptor ASC, and precursor caspase-1 (78, 79), as mentioned above in a case with NLRP3 inflammasome. Earlier studies showed that the extremely polymorphic gene locus Nalp1b is associated with mouse susceptibility and macrophage death in response to lethal toxin from Bacillus anthracis (79). In addition, anthrax lethal toxin-induced lethality in rats showed a correlation with the certain polymorphisms within the N-terminal amino acids of the NLRP1 protein (80). Indeed, anthrax lethal toxin-mediated cleavage of rat NLRP1 induces inflammasome activation, maturation of IL-1β, and macrophage caspase-1-dependent cell death (81).

Recent studies for the role of NLRP1 in Toxoplasma gondii infection have shown that NLRP1 sequence is highly associated with rat strain differences in macrophage pyroptosis, IL-1β/IL-18 maturation, and inhibition of parasite proliferation against Toxoplasma (82). Silencing of Nlrp1 in pyroptosis-sensitive macrophages resulted in higher replication of parasites, whereas overexpression of the NLRP1 variant in pyroptosis-resistant cells led to controlling parasite replication through mediating sensitization of these macrophages (82).

iv. AIM2 and inflammasome complex activation

AIM2 is a cytosolic DNA sensor that forms AIM2 inflammasome activation to initiate antiviral and inflammatory responses. AIM2 binds to double-stranded DNA in various bacterial and viral infection in host cell cytoplasm (83–86). It contains an N-terminal pyrin domain (associated with ASC) and a C-terminal HIN200 domain (binds to DNA) for secretion of mature IL-1β and IL-18 through caspase-1 activation (40, 84). AIM2 is essential in regulation of caspase-1-dependent secretion of mature IL-1β and IL-18, as well as pyroptosis, in response to synthetic double-stranded DNA and various bacteria and virus, such as Francisella tularensis, vaccinia virus and mouse cytomegalovirus (86). In addition, AIM2 was found to be crucial in production of IL-18 and natural killer cell-dependent IFN-γ production, thus contributing in the early defense against mouse cytomegalovirus infection (86). Recent studies for Streptococcus pneumoniae-induced inflammasome activation have also shown that type I IFN signaling is essential for optimal activation of AIM2 inflammasome during infection (87).

In addition, several bacteria including Streptococcus pneumoniae (87) and Porphyromonas gingivalis (88) activate both NLRP3 and AIM2 inflammasome to induce maturation and secretion of IL-1β and IL-18. Interestingly, nonvirulent mycobacterial strain, such as Mycobacterium smegmatis activate AIM2 inflammasome through induction of IFN-β (89). However, ESX-1-deficient Mycobacterium tuberculosis attenuates the AIM2 inflammasome activation induced by either M. smegmatis or cytosolic double-stranded DNA (89). NLRP3 and AIM2 inflammasomes appear to share a large part of intracellular pathway for inflammasome activation. Recent studies have shown that cellular FLICE-inhibitory protein (c-FLIP), an inhibitor of caspase-8, is required for the activation of both NLRP3 and AIM2 inflammasomes for the generation of caspase-1 and active IL-1β (90). Upon triggering of inflammasome activation, both AIM2 and NLRP3 induce oligomerization, and recruit procaspase-1 through interaction with the common adaptor ASC (83). Moreover, both AIM2 and NLRP3 inflammasomes are able to induce apoptotic and pyroptotic cell death through activation of caspase-8 and -1 (74, 91). Future studies are urgently needed for clarifying the distinct molecular mechanisms by which each inflammasome sensor recognizes and activates the differential inflammasome complex within the cells.
CONCLUDING REMARKS

Tremendous advances have been achieved in our understanding of the roles, activation, and intracellular regulatory mechanisms upon innate immune activation. The roles and activating mechanisms of TLRs in various pathogenic infections are now well established. In addition, numerous NLRs and their ligands that trigger the activation of NLR-dependent innate and inflammatory responses have been identified. Both TLRs and NLRs activate common and distinct intracellular signaling pathways to combat invading pathogens and to induce inflammatory reaction during infection. As expected, a variety of pathogens can exploit and manipulate host innate signaling pathways by production of specific effectors and toxins, although this part has not been reviewed in detail in this review. It is also highlighted that a certain subset of NLRs induce inflammasomes, an intracellular protein platform that activates procaspase-1 to promote IL-1β and IL-18 maturation.

Enormous efforts have been made for the understanding of the activation and regulation of several inflammasomes in terms of host defense and inflammatory responses. It is also clear that many key questions including the exact molecular mechanisms of initiation of inflammasome complex activation remain unresolved. Moreover, our understanding for the interaction and cooperation between these innate receptors and other receptors are quite in its infancy and remain to be discovered in future studies. The efforts to discover the roles and intracellular signaling mechanisms induced by these innate receptors will provide innovative intervention into designing new preventive tools and therapeutics against infectious diseases and other inflammatory pathologies.

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