J. Exp. Biomed. Sci. 2013, 19(3): 173~179
pISSN : 1738-3226

Review

Nucleotide-Binding Domain and Leucine-Rich Repeat Containing Receptor (NLR) and its Signaling Pathway

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Since the identification and characterization of toll-like receptors (TLR) in Drosophila, numerous scientific studies have examined the role of TLRs in host innate immunity. Recent studies have suggested a convergence of the nuclear factor kappa B (NF-κB) signaling and cytokine production regulated by the cytosolic elicitor known as NLRs (nucleotide-binding domain and leucine-rich repeat containing domain receptors) as a key modulator in inflammatory diseases. Among the NLRs, NOD1 and NOD2 have been intensively investigated for its role in inflammatory bowel disease (IBD). On the other hand, NLRs such as NLRP3, NLRP1, and NLRC4 (also known as IPAF) have been identified to form the inflammasome to activate downstream signaling molecules in response to pathogenic microbes. There is evidence to suggest that substantial crosstalk exists for the TLR and NLR signaling pathway in response to pathogen associated molecular pattern (PAMP). However, the substrate and the mechanistic role of NLRs are largely unknown in innate immune response. Understanding the signaling mechanisms by which NLRs recognize PAMP and other danger signals will shed light on elucidating the pathogenesis of various human inflammatory diseases such as IBD.

Key Words: NLRs, NOD1, NOD2, TLR, IBD, inflammasome, PAMP

INTRODUCTION

Humans have evolved a complex protective defense system to eradicate pathogenic microorganisms. To ensure efficient sensing and removal of harmful microbes, two distinct but overlapping immunologic mechanisms exist: innate immunity and adaptive immunity. Adaptive immunity is characterized by gene rearrangement of antigenic specific receptors and clonal selection of B lymphocytes and T lymphocytes. This process serves to generate a diverse repertoire of lymphocytes bearing antigen-specific receptors that can recognize any foreign antigen. In contrast, innate immunity serves as a first line of defense against microorganisms without somatic gene rearrangement. In innate immunity, germline-encoded innate immune receptors called pattern recognition receptors (PRRs) recognize evolutionarily conserved microbial structures known as pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLR) identified first in Drosophila were shown to bind to PAMPs and induce inflammatory responses. Since the discovery of lipopolysaccharide (LPS) as a canonical PAMP for TLR4, many other PAMPs have been identified. These include iE-DAP (NOD1 agonist, D-gamma-Glu-mDAP), MDP (NOD2 agonist, muramyldipeptide), flagellin (NLRC4 agonist) and microbial nucleic acids. These PAMPs are recognized by nucleotide-binding domain and leucine-rich repeat containing receptors (NLRs) also known as Nod-like receptors (nucleotide-binding oligomerization domain

*Received: June 12, 2013 / Revised: September 27, 2013
Accepted: September 27, 2013
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like receptors) that are involved in the formation of inflammasome. The recognition of PAMPs by PRRs triggers signaling pathways which results in recruitment of the inflammasome to promote the proinflammatory antimicrobial response. Recent studies have demonstrated a link between the activation of innate PRRs and activation of the adaptive immune response in response to invasive microorganisms. PRR is not only involved in sensing foreign pathogenic microorganisms but also endogenous molecular stress signals such as ATP, both of which induces the production of the pro-inflammatory cytokines such as nuclear factor-kappa B (NF-κB), pro-IL-1β, and pro-IL-18. There are three major classes of PRRs: TLRs, NLRs and RIG-1 (retinoid acid-inducible gene-1)-like receptors (RLRs). TLRs are a family of transmembrane proteins with the ligand binding domain protruding extracellularly or into endosomes. The NLRs are a family of cytosolic proteins that can detect microorganisms or microbial products present within the intracellular milieu. Among the NLRs, NOD1 (nucleotide-binding oligomerization domain containing 1) and NOD2 (nucleotide-binding oligomerization domain containing 2) have been studied the most extensively especially in the context of inflammatory bowel disease (IBD). Emerging evidence suggests the importance of NLRs for complementing the function of TLRs during host defense. Therefore, it is believed that the understanding of emerging PRR such as NLRs and its signaling mechanism will shed light on both the pathogenesis of microbial infection and host defense mechanisms. In this review, we will summarize our current understanding of NLR-mediated signaling mechanisms.

Characteristic features of the NLR proteins

The human NLR family is comprised of 22 genes and the murine NLR family is comprised of at least 33 genes (Ting et al., 2008). Many of the NLRs have been reported to sense pathogenic products and regulate cell signaling in the innate immune system. A variety of different names have been used to describe NLR including NACHT, CATERPILLER and NOD-like receptor (Martinon and Tschopp, 2004; Harton et al., 2002; Fritz et al., 2006). However, recent articles have adopted the nomenclature suggested by the Human Genome Organization (HUGO) Gene Nomenclature Committee which approved the nomenclature following consultation with over 100 scientists in the immunology community (Ting et al., 2008). This annotation system is based on the conserved nucleotide binding domain (NBD) and leucine rich repeat (LRR) domains. There are five families: NLRA, NLRB, NLRC, NLRP, and NLRX. CIITA (Class II, major histocompatibility complex, transactivator) is a member of the NLRA family. NAIP (NLR family, apoptosis inhibitory protein) is a member of the NLRB family. In the NLRC family, there are five members containing NOD1, NOD2, NLRC3 (NLR family, caspase activating and recruitment domain), NLRC4, and NLRC5. The NLRP is the largest families and includes the members NLRP1~NLRP14 (NLR family, pyrin domain). Finally, the NLRX family includes the member NLRX1 due to the lack of homology to the N-terminal domain. In this nomenclature system, human genes are written in upper case whereas murine gene orthologs are written in lower case with the first letter capitalized. Although NLRs are primarily expressed by immune cells such as macrophages and dendritic cells they can also be expressed by epithelial cells and mesothelial cells. The NLR family of proteins has three distinct structural domains. The N-terminal domain is responsible for interacting with a variety of structural domains such as CARD (recruitment domain), PYD (pyrin domain), acidic transactivating domain, or BIR (baculovirus inhibitor of apoptosis repeat). The central nucleotide-binding oligomerization (NBD) domain can either self-oligomerize or interact with agonists such as MDP or ATP upon activation (Mo et al., 2012). The C-terminal LRR domain recognizes and binds with PAMP or damage associated molecular pattern (DAMP). The signaling cascade induced by the N-terminal domain is important for downstream cellular regulation of inflammation. Basically, CARD is primarily involved in the inflammatory response and apoptotic cell death/survival through CARD-CARD interaction. PYD-

PYD homophilic interaction acts as a different type of regulation of downstream signaling which is homologous to CARD. Both CARD and PYD belong to the death domain superfamily which is involved in the activation of apoptotic cell death and inflammatory response.

NLR signaling

Binding of iE-DAP and MDP with NOD1 and NOD2 results in activation of NOD1/2 thus enabling NOD1/2 to recruit and in turn activate the serine/threonine kinase RIP2 (also known as RICK) through homophilic CARD-CARD interaction. Subsequently, RIP2 becomes activated in a K63-linked polyubiquitination at lysine 209 (K209) located at the kinase domain (Hasegawa et al., 2008). A recent report demonstrated that p62/SQSTM1 is involved in NOD2-mediated signaling and cytokine production through stabilizing NOD2 oligomerization (Park et al., 2013). This sequential K63-linked polyubiquitination of RIP2 mediates TAK1 kinase that is not dependent on kinase activity (Hasegawa et al., 2008), which activates the NF-κB complex and is also inhibited by the deubiquitinase A20. It is also known that NOD2 activates MAPK pathways that collaborate with NF-κB signaling pathway. NF-κB subsequently activates transcription of inflammatory cytokines and chemokines including TNF-α, IL-1β, IL-6 and MCP-1, which are important for stimulation and activation of additional effector cells during host defense (Fig. 1). However, the convergent targets of NLR signaling are not the same for all NLRs. Once PAMP is recognized by cognate NLR, the signaling modulators NF-κB and MAPKs link the inflammasome formation with caspase-1 which is one major end target of NLR signaling. As shown to be important in

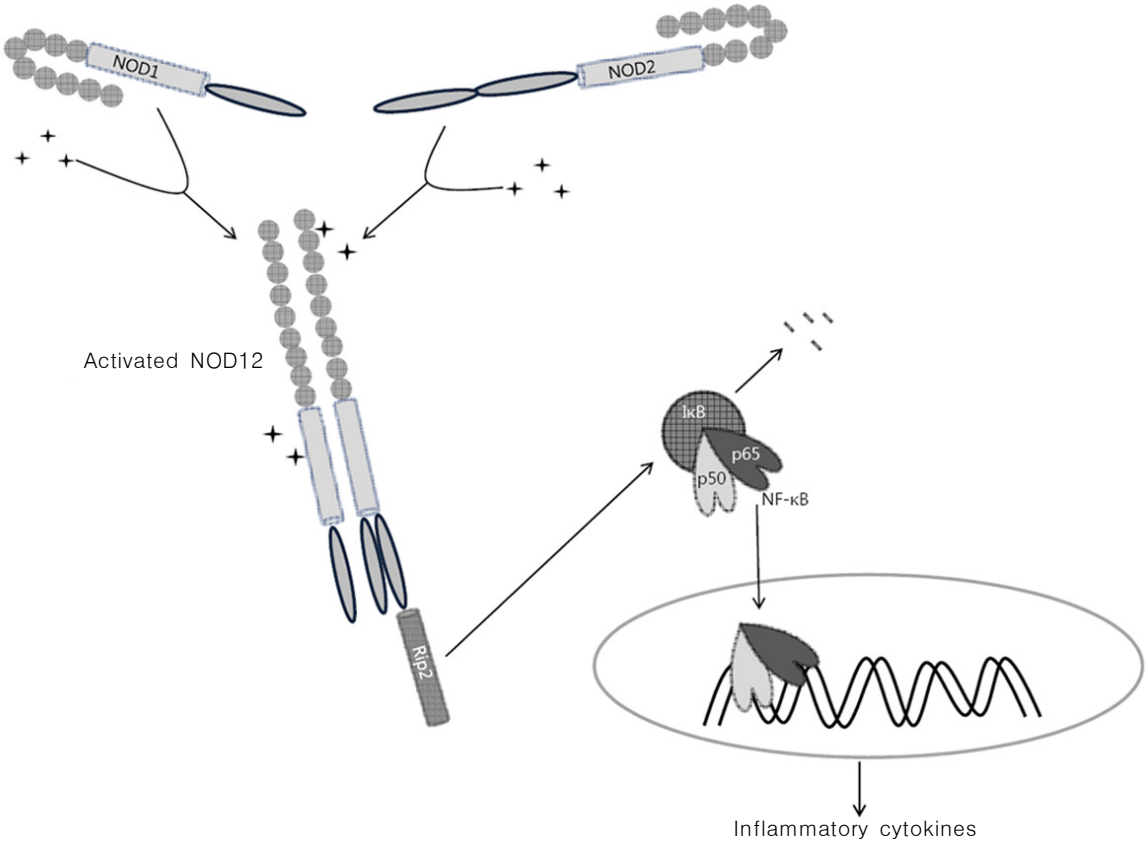

Fig. 1. In the absence of stimuli, NOD1 and NOD2 activate NF-κB via the serine-threonine kinase Rip2. The LRRs remain folded in a resting state, preventing oligomerization and subsequent activation. Once the ligand is sensed by NLRs such as NOD1 and NOD2, the NOD proteins unfold allowing self-oligomerization and interaction with Rip2 through homophilic CARD-CARD association. Rip2 promotes degradation of IκB and subsequent nuclear location of NF-κB which in turn induce expression of various inflammatory cytokines.

"NODosome" formation, polyubiquitination plays a crucial role in induction of downstream signaling. The E3 ubiquitin ligase and tumor necrosis factor receptor-associated factor 6 (TRAF6) is required for NOD2-mediated NF-κB activation whereas they are not required for NOD1 activation. However, TRAF2 and TRAF5 are essential for NOD1 downstream signaling (Hasegawa et al., 2008; Abbott et al., 2007), suggesting that additional substrate regulation exists during the production of inflammatory cytokines. In addition, many interacting partner proteins were shown to bind NOD2 and are involved in downstream NF-κB signaling events. These include ERBIN, GRIM-19, NLRC4 (also known as IPAF or CLAN), NLRP1, ATG16L1 and p62/SQSTM1 (Kufer et al., 2006; McDonald et al., 2005; Barnich et al., 2005; Damiano et al., 2004; Hsu et al., 2008; Travassos et al., 2010). The multimeric complexes of NOD2 are expected to function as a signaling platform such as the "NODosome" homologous to the "Inflammasome" (Tattoli et al., 2007). However, it remains to be elucidated whether these binding partners also regulates other NLR signaling events. Activation of NOD1 and NOD2 involves the activation of MAPKs (p38, ERK, JNK)(Park et al., 2007). Although it has been recently suggested that the CARD9 adaptor protein may be important in NOD2-RIP2 mediated activation of MAPKs downstream events (Hsu et al., 2007), the molecular pathway still remains unclear. In contrast to promoting NF-κB activation by NLRs, a negative regulatory role of NLR members has been reported including NLRP2 in macrophages and NLRP12/NLRC3 (Bruey et al., 2004; Lich et al., 2007; Conti et al., 2005) in T lymphocytes. However, the in vivo physiologic roles of these NLRs remain to be elucidated.

Biological responses to NOD1 and NOD2 signaling

Stimulation of NOD1-expressing epithelial cells with the agonist iE-DAP activates chemokines and proinflammatory molecules that are prerequisite for induction of innate immune responses. NOD1 stimulation in NOD1 knock-out (KO) mice did not induce TNF-γ and IFN-γ suggesting that the primary role of NOD1 is to induce the recruitment of immune cells (Masumoto et al., 2006). Mutations in the NOD1 gene have been associated with the development of asthma (Hysi et al., 2005). In addition, genetic variations in NOD1 and NOD2 are associated with Crohn's disease and contribute to the pathogenesis of inflammatory diseases in IBD patients (Hugot et al., 2001; McGovern et al., 2005). The NOD1 and NOD2 proteins initially were found through genomic database search for the protein homologous to the apoptotic regulator Apaf-1 (Inohara et al., 1999; Ogura et al., 2001).

Interleukin-1β Production and the Inflammasome

NLR can activate targets other than NF-κB and MAPKs. One important effector molecule activated by NLR signaling is ASC (apoptosis-associated speck-like protein containing caspase recruitment domain) whose CARD can recruit pro-caspase-1. Of the 22 members of the NLR family, only NLRP1, NLRP3, NLRC4, and NLRP6 or non-NLR AIM2 (absent in melanoma 2) have been identified to participate in caspase-1 activation. Caspase-1 activation is required for the cleavage of pro-IL-1β and pro-IL-18 into the biologically active forms. Some of these proteins such as NLRP2, NLRP7, NLRP12 and NLRC3 appear to negatively regulate functional effects of pathological responses. Recently, NLRP3, NLRP1, and NLRC4 were found to be involved in cell death pathways (Bruey et al., 2007; Suzuki et al., 2007). Many research articles have reported on the functional characterization of NLRs. For example, NLRP6 signaling which is dependent on caspase-1 dependent inflammasomal activation for cytokine release has been reported to be involved in the intestinal inflammation-related colon tumorigenesis (Elinav et al., 2011). There is evidence that the inflammasome contribute to host defense against microorganisms. But, the molecular mechanism by which NLRC4 and NLRP3 are activated remains largely unknown. ASC is an adaptor protein that

links NLRs to caspase-1 to form the inflammasome (Srinivasula et al., 2002). The inflammasome containing caspase-1 and NLRC4 is triggered to induce pyroptosis (caspase-1 dependent apoptosis), whereas the alternative inflammasome containing ASC, caspase-1 and NLRC4 mediates the release of IL-1β and IL-18 (Broz et al., 2010). These results suggest that NLR activation by microbial infections can operate through different NLRC4 -complex inflammasomes that exert alternative functions. A more detailed mechanism of the function of inflammasome complexes remains to be elucidated. Numerous receptors including TLRs and NLRs in common pathways converge to induce cytokine or chemokine production. The existence of multiple effects is to potentiate the inflammatory response to external infection. In fact, it is currently reported that synergic effects exist among NLRs and TLRs in response to TLRs and NOD2 agonists (Kobayashi et al., 2005).

CONCLUDING REMARKS

Since the identification of TLRs in the fruit fly, studies of cytoplasmic NLRs have yielded new insights into the mechanism of immune inflammatory responses. There is ample evidence of molecular crosstalk between NLRs and TLRs which are responsible for activation of adapter molecules to elicit the pro-inflammatory immune response. NOD1 and NOD2 sense evolutionarily conserved structures of microorganism to induce, MAPK, and NF-κB for stimulation of cytokine production. However, many questions remain unanswered including identification and understanding of signaling mechanism activated by NLRs in vivo. It is possible that other PRR engages NLRs and TLRs for the host defense. The study of NLR and TLR has yielded tremendous insights into understanding the immune system. We anticipate that as more discoveries unfold our view of immunological related diseases and its treatment will be impacted greatly.

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