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NOD-like receptor signaling beyond the inflammasome

Patrick J. Shaw¹, Mohamed Lamkanfi^{2,3}, and Thirumala-Devi Kanneganti^{1,*}

¹Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN, 38104, USA

²Department of Biochemistry, Ghent University, B-9000 Ghent, Belgium

³Department of Medical Protein Research, VIB, B-9000 Ghent, Belgium

Abstract

Recent years have witnessed a marked progress in our knowledge of Nod-like receptors (NLRs), intracellular sensors with central roles in innate and adaptive immunity. A majority of the research has focused on caspase-1 inflammasomes. However, several members of the mammalian NLR family exert important roles in immunity beyond inflammasome signaling. Here we highlight the emerging roles of several of these NLRs.

The NLR Family

The immune response is dependent on the recognition of evolutionary conserved microbial and self-derived danger signals by pattern recognition receptors (PRRs). Whereas the membrane-bound Toll-like receptors (TLRs) survey the extracellular milieu, members of the RIG-I-like receptors (RLRs) and NOD-like receptor (NLR) families have emerged as pivotal sensors of infection and stress in intracellular compartments. Mutations in several of the 22 human NLR genes are strongly linked to auto-immune and auto-inflammatory syndromes, highlighting the central roles of NLRs in the immune system. NLRs are characterized by a central NACHT domain and a stretch of carboxy-terminal leucine-rich repeat (LRR) motifs [1,2]. Important progress in understanding NLR signaling has been made in recent years, particularly for the NLRs mediating inflammasome activation. Here, we discuss CIITA, NLRP12, NLRX1 and NOD1/NOD2, the NLR proteins with emerging roles beyond the inflammasome (Figure 1 and 2).

CIITA

The MHC class II transactivator (CIITA) is expressed in macrophages, B cells, T cells and dendritic cells and can be regarded as the founding father of the NLR family [3,4]. It was initially identified as a critical transcription factor that is required for expression of MHC class II [5]. Notably, an alternative splicing mechanisms in macrophages and dendritic cells allows production of a CIITA variant that is equipped with an N-terminal caspase recruitment (CARD) domain. The CARD motif, which is absent from the CIITA form expressed in other immune cells, is believed to enhance MHC II gene expression in professional antigen-presenting cells [6]. The N-terminal CARD is followed by an acidic transactivation domain that is responsible for recruitment to the enhanceosome, a protein complex consisting of several nuclear factors including CBP, RFX5, NF-Y and CREB that binds *cis*-regulatory elements in the *MHC II* promoter [7]. CIITA serves as the master

*Address correspondence to: Thirumala-Devi Kanneganti, Department of Immunology, St Jude Children's Research Hospital, MS #351, 570, St. Jude Place, Suite E7004, Memphis TN 38105-2794. Tel: (901) 495-3634; Fax: (901) 495-5766; Thirumala-Devi.Kanneganti@StJude.org..

regulator of MHC II expression in this complex, driving transcription of *MHC II* and other genes involved in antigen presentation such as the invariant chain and *HLA-DM* genes [5].

CIITA is constitutively present in immune cells, but its expression is significantly elevated by IFN γ [5]. The crucial role of CIITA in mounting effective host defense mechanisms against viral and bacterial pathogens is highlighted by the linkage of loss-of-function mutations in CIITA to type II bare lymphocyte syndrome [8]. Patients with this severe hereditary disorder present with a defective T helper cell-dependent immune response that exposes them to repeated bacterial and/or viral infections to which they often succumb at an early age. The crucial role of CIITA in MHC II expression and the induction of T helper cell responses was confirmed in mice with a gene-targeted deletion in CIITA [9].

NLRP12

The expression of NLRP12 (also known as Monarch-1) is predominantly in cells of myeloid origin [10]. NLRP12 has been described as a negative regulator of TLR-driven NF- κ B activation [11]. The inhibitory function of NLRP12 requires ATP binding and hydrolysis in its central NACHT domain [12]. Notably, mutations in or around this domain are associated with hereditary periodic fever (HPF) syndromes resembling the auto-inflammatory disorders caused by gain-of-function mutations in NLRP3 [13]. However, NLRP12 mutants containing the disease-associated mutations failed to inhibit NF- κ B signaling, suggesting that the disease-associated NLRP12 variants represent loss-of-function mutations [13]. NLRP12 shuts down NF- κ B signaling by preventing hyper-phosphorylation of the TLR-adaptor IL-1 receptor associated kinase 1 (IRAK-1) and by enhancing degradation of the NF- κ B inducing kinase (NIK) [14]. In agreement with an inhibitory function, NLRP12 is downregulated following activation by TLR agonists, *M. tuberculosis*, TNF α or IFN γ , whereas expression of other NLRs is typically induced [14]. It is hypothesized that downregulation of NLRP12 expression allows for the induction of an appropriate immune response when microbial ligands are sensed in the cytosol. Once the infection has been cleared, NLRP12 expression levels are restored to prevent excessive inflammation and to maintain a quiescent phenotype in resting immune cells. To date, no endogenous or microbial ligands have been identified for NLRP12. However, its emerging role as a negative regulator of NF- κ B signaling may render NLRP12 an interesting novel therapeutic target for autoinflammatory disorders.

NLRX1

NLRX1 is highly conserved among species and the protein is ubiquitously expressed in immune and other cell types [15]. NLRX1 is the only NLR member with a mitochondrial localization. Indeed, NLRX1 contains a mitochondrial targeting sequence in the N-terminus [15,16]. However, the precise subcellular localization within mitochondria remains a matter of debate. In one study, NLRX1 was found in close proximity of the antiviral RLR adaptor protein MAVS/IPS-1/CARDIF, which is known to be associated with the mitochondrial outer membrane. Physical association between MAVS and NLRX1 prevented viral RNA-induced type I interferon responses, suggesting NLRX1 as a negative regulator of RLR signaling [15]. A second study contested the localization of NLRX1 to the outer mitochondrial membrane and showed that NLRX1 fully translocates to the mitochondrial matrix [16]. Accordingly, NLRX1 was found to amplify NF- κ B and JNK signaling by augmenting the production reactive oxygen species (ROS) in mitochondria [17]. Further studies will be required to resolve the precise localization of NLRX1 within mitochondria, and the generation of NLRX1-deficient mice may prove instrumental in determining the physiological role of this unique NLR family member.

NOD1/NOD2

NOD2 was the first susceptibility gene identified for Crohn's disease [18,19]. NOD2 mutations have also been linked to Blau syndrome [20,21], whereas mutations in the related NLR NOD1 underlie asthma and atopic eczema [22]. Moreover, NOD1 and NOD2 have been shown to be critical for host defense to bacterial infections in mice. NOD1-deficient mice are more susceptible to *H. pylori* [23], whereas NOD2-deficient mice are more susceptible to *Listeria monocytogenes* [24]. NOD1/NOD2-mediated signaling is also critical in the adaptive immune response to *Bacillus anthracis* [25].

The molecular functions of these NLRs are starting to emerge in detail. NOD1 and NOD2 act as cytosolic sensors of distinct peptidoglycan (PGN) fragments. NOD1 senses the PGN component *meso*-diaminopimelic acid, which is produced by all Gram-negative and some Gram-positive bacteria. On the other hand, NOD2 is activated by muramyl dipeptide, which is common to both Gram-negative and Gram-positive bacteria [26,27]. These PGN fragments can be released during bacterial cell division in the cytosol of infected immune cells, or may leak from lysosomes where PGN of phagocytosed bacteria is degraded by the host lysozyme(s). Once activated, NOD1 and NOD2 oligomerize and recruit the NF- κ B-activating kinase RIP2 (RICK, Ripk2) through homotypic CARD-CARD interactions involving their amino-terminal CARD motifs. RIP2 interacts with the regulatory NF- κ B subunit NEMO/IKK γ , triggering I κ B phosphorylation and NF- κ B activation. RIP2 is critical for NOD1- and NOD2-mediated NF- κ B activation because NOD1 and NOD2 signaling is abolished in RIP2 deficient cells [28]. The CARD-containing adaptor protein CARD9 was found to be important for the activation of p38 and JNK downstream of NOD2, although it was dispensable for NF- κ B activation [29].

Apart from their well-characterized role in RIP2-dependent NF- κ B activation, NOD1 and NOD2 were recently ascribed new roles. NOD2 was shown to facilitate IRF3 activation and the production of type I interferon induced by ssRNA and by respiratory syncytial virus (RSV) infection [30]. These NLRs were also shown to be critical for the autophagic response to invading bacteria independently of RIP2- and NF- κ B signaling [31]. Notably, Crohn's disease-associated mutations in NOD2 prevented the recruitment of the autophagy adaptor Atg16L1. Besides its role in autophagy, a T cell-intrinsic role for NOD2 in promoting type I immune responses to *Toxoplasma gondii* and during T cell-driven colitis was recently reported [32]. NOD2 was found to physically interact with c-Rel in T cells, resulting in the decreased nuclear accumulation of c-Rel and inhibition of IL-2 transcription. These observations emphasize the importance of NOD2 in immunity and offer alternative explanations for the mechanism by which NOD2 mutations trigger Crohn's disease.

Concluding Remarks

Despite marked progress in our knowledge of NLR signaling pathways in recent years, the functions and signaling pathways of a large subset of NLRs remains obscure. These NLRs may regulate several critical aspects of the immune response. A more detailed analysis of these NLRs will foster the development of improved therapeutic strategies to treat pathogens and chronic inflammatory diseases.

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Reference List

- [1]. Kanneganti TD, et al. *Immunity* 2007;27:549–559. [PubMed: 17967410]
- [2]. Ting JP, et al. *Immunity* 2008;28:285–287. [PubMed: 18341998]
- [3]. Ting JP, Trowsdale J. *Cell* 2002;109:S21–23. [PubMed: 11983150]
- [4]. Steimle V, et al. *J. Immunol* 2007;178:6677–6688. [PubMed: 17513710]
- [5]. Steimle V, et al. *Science* 1994;265:106–109. [PubMed: 8016643]
- [6]. Nickerson K, et al. *J. Biol. Chem* 2001;276:19089–19093. [PubMed: 11279191]
- [7]. LeibundGut-Landmann S, et al. *Eur J Immunol* 2004;34:1513–1525. [PubMed: 15162420]
- [8]. Reith W, Mach B. *Annu Rev Immunol* 2001;19:331–373. [PubMed: 11244040]
- [9]. Chang CH, et al. *Immunity* 1996;4:167–178. [PubMed: 8624807]
- [10]. Williams KL, et al. *J Immunol* 2003;170:5354–5358. [PubMed: 12759408]
- [11]. Lich JD, et al. *J Immunol* 2007;178:1256–1260. [PubMed: 17237370]
- [12]. Ye Z, et al. *Mol. Cell. Biol* 2008;28:1841–1850. [PubMed: 18160710]
- [13]. Jeru I, et al. *Proc. Natl. Acad. Sci* 2008;105:1614–1619. [PubMed: 18230725]
- [14]. Williams KL, et al. *J. Biol. Chem* 2005;280:39914–39924. [PubMed: 16203735]
- [15]. Moore CB, et al. *Nature* 2008;451:573–577. [PubMed: 18200010]
- [16]. Arnoult D, et al. *J. Cell. Sci* 2009;122:3161–3168. [PubMed: 19692591]
- [17]. Tattoli I, et al. *EMBO Rep* 2008;9:293–300. [PubMed: 18219313]
- [18]. Ogura Y, et al. *Nature* 2001;411:603–606. [PubMed: 11385577]
- [19]. Hugot JP, et al. *Nature* 2001;411:599–603. [PubMed: 11385576]
- [20]. Hugot JP, et al. *Nature* 1996;379:821–823. [PubMed: 8587604]
- [21]. Miceli-Richard C, et al. *Nat. Genet* 2001;29:19–20. [PubMed: 11528384]
- [22]. Geddes K, et al. *Nat. Rev. Drug. Discov* 2009;8:465–479. [PubMed: 19483708]
- [23]. Viala J, et al. *Nat. Immunol* 2004;5:1166–1174. [PubMed: 15489856]
- [24]. Kobayashi KS, et al. *Science* 2005;307:731–734. [PubMed: 15692051]
- [25]. Loving CL, et al. *Infect. Immun* 2009;77:4529–4537. [PubMed: 19620350]
- [26]. Chamaillard M, et al. *Proc. Natl. Acad. Sci* 2003;100:3455–3460. [PubMed: 12626759]
- [27]. Girardin SE, et al. *J. Biol. Chem* 2003;278:8869–8872. [PubMed: 12527755]
- [28]. Chin AI, et al. *Nature* 2002;416:190–194. [PubMed: 11894097]
- [29]. Hsu YM, et al. *Nat. Immunol* 2007;8:198–205. [PubMed: 17187069]
- [30]. Sabbah A, et al. *Nat. Immunol* 2009;10:1073–1080. [PubMed: 19701189]
- [31]. Travassos LH, et al. *Nat. Immunol.* 2009 Epub ahead of print.
- [32]. Shaw MH, et al. *Nat. Immunol* 2009;10:1267–1274. [PubMed: 19881508]

NLR	Structure	Function(s)
CTLA		Stimulate regulation of NF-κB expression
NLRP12		Regulation of inflammatory cytokines via NF-κB activation
NLRP1		Initiation of cell death via interaction with NLRP3
NOD1		Activation of NF-κB via NOD2, NOD3 activation
NOD2		Regulation of cytokine NF-κB signaling
NOD3		Regulation of cytokine NF-κB signaling
NOD4		Regulation of cytokine NF-κB signaling
NOD5		Regulation of cytokine NF-κB signaling
NOD6		Regulation of cytokine NF-κB signaling
NOD7		Regulation of cytokine NF-κB signaling
NOD8		Regulation of cytokine NF-κB signaling
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NOD98		Regulation of cytokine NF-κB signaling
NOD99		Regulation of cytokine NF-κB signaling
NOD100		Regulation of cytokine NF-κB signaling

Figure 1. The structure and primary functions of some of the NLR proteins

Figure 2. Summary of NLR functions beyond the inflammasome

NOD1 and *NOD2* recognize peptidoglycan fragments released either during bacterial division within the cytosol or by the lysosome during the degradation of phagocytosed bacteria. Activation of either *NOD1* or *NOD2* leads to the recruitment and activation of *RIP2*, which results in *MAPK* pathway and *NF-κB* activation. *NLRP12* acts to silence *MAPK* and *NF-κB* activation. These pathways are also activated by the recognition of bacterial components by *TLRs*. *NLRP12* inhibits the phosphorylation of interleukin-1 receptor-associated kinase 1 (*IRAK-1*), therefore blocking *MAPK* and *NF-κB* activation. *CIITA* is a transcriptional factor induced by *IFNγ* signaling pathways. *CIITA* binds to the enhanceosome and is the master regulator of *MHC II* expression. *NLRX1* function and localization is controversial and there are two potential mechanisms of action, which are not mutually exclusive. The first proposes that *NLRX1* is imported into the mitochondrial matrix by *TOM/TIM*. Once in the matrix, the mitochondrial leader sequence is cleaved from the N-terminal sequence and *NLRX1* interacts with the core II protein (*UQRC2*) of complex III of the mitochondrial respiratory chain. This interaction results in the production of *ROS*, which activate *JNK* and *NF-κB* signaling pathways. The second proposes that *NLRX1* is localized to the mitochondrial outer membrane, where it interacts with *MAVS*. This interaction prevents *MAVS* from interacting with *RIG-I* when it senses viral RNA. The resulting effect is the inhibition of immune responses induced by viral RNA recognition.