REVISTA DE INVESTIGACIÓN CLÍNICA

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Rev Inves Clin. 2018;70:18-28 IN-DEPTH REVIEW

NOD2: Activation During Bacterial and Viral Infections, Polymorphisms and Potential as Therapeutic Target

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ABSTRACT

Nucleotide-binding domain (NBD) leucine-rich repeat (LRR)-containing receptors or NLRs are a family of receptors that detect both, molecules associated to pathogens and alarmins, and are located mainly in the cytoplasm. *NOD2* belongs to the NLR family and is a dynamic receptor capable of interacting with multiple proteins and modulate immune responses in a stimuli-dependent manner. The experimental evidence shows that interaction between *NOD2* structural domains and the effector proteins shape the overall response against bacterial or viral infections. Other reports have focused on the importance of *NOD2* not only in infection but also in maintaining tissue homeostasis. However, not only protein interactions relate to function but also certain polymorphisms in the gene that encodes *NOD2* have been associated with inflammatory diseases, such as Crohn's disease. Here, we review the importance and general characteristics of *NOD2*, discussing its participation in infections caused by bacteria and viruses as well as its interaction with other pathogen recognition receptors or effectors to induce antibacterial and antiviral responses. Finally, the role of *NOD2* in chronic inflammatory conditions and its potential to be targeted therapeutically are examined.

Key words: *NOD2*. Inflammasome. Infection. Polymorphisms. Inflammatory disease.

INTRODUCTION

The repertoire of pathogen recognition receptors (PRRs) in the innate immune system is encoded in the germline. These PRRs are activated either by evolutionarily conserved pathogen-associated molecular patterns (PAMPs) or by endogenous damage-associated molecular patterns (DAMPs). PAMPs are molecules present in pathogens; in bacteria, they are found in the cell wall, flagella, lipoproteins, and

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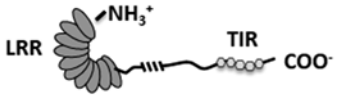
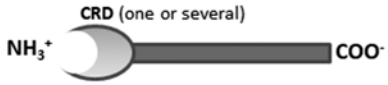
Received for publication: 08-07-2017
Approved for publication: 20-10-2017
doi: 10.24875/RIC.17002327

Figure 1. PRRs that participate in pathogen recognition and immune response. Pathogen recognition receptors (PRRs) are clustered into five families depending on their structural domains. Toll-like receptors (TLRs) are located in cell membrane and endosomes; they recognize a broad range of pathogens through their leucine rich-region (LRR) and signal by toll/interleukine-1 receptor domains. Retinoic acid-inducible gene-I-like receptors-like receptors (RLRs), such as RIG-I and MDA-5, are located in the cytoplasm and recognize motifs from pathogens such as dsRNA through its helicase domains (Hel-1 and Hel-2). C-type lectin receptors (CLRs) are transmembranal and include dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and C-type lectin domain family 5 member A (CLEC5a); these identify carbohydrates in a broad number of pathogens. ALRs include molecules such as absent in melanoma 2 (AIM2) are cytoplasmic and recognize mostly foreign dsDNA. Finally, the NLRs are mostly located in cytoplasm and mitochondria, and contain three domains, a variable N-terminal characterized by interacting with several effectors, a central nucleotide-binding domain (NBD), and most NLRs have a C-terminal LRR domain.

PRRs	Structure	Domains	Localization
TLRs		LRR, transmembranal, and TIR.	Transmembranal and Endosomal.
RLRs		CARDs, Hel-1, Hel-2, and CTD.	Cytoplasmic.
CLRs		CRDs or CRD-like domains.	Transmembranal.
ALRs		PYDs	Cytoplasmic.
NLRs		CARD, NBD, and LRR.	Cytoplasmic and Mitochondrial.

nucleic acids. Furthermore, some cell wall components in fungi, as well as nucleic acids and proteins in viruses, are recognized as PAMPs[1]. Unlike PAMPs, DAMPs are endogenous molecules released from cells under stress damage; these events are capable of initiating an inflammatory process. Some well-characterized DAMPs include DNA-binding proteins such as high-mobility group B1 proteins, heat-shock proteins, extracellular adenosine triphosphate (ATP), and uric acid crystals, among others.

PRRs are grouped into five families according to their structural domains (Fig. 1): (1) Toll-like receptors (TLRs), (2) retinoic acid-inducible gene-I-like receptors (RLRs), (3) C-type lectin receptors (CLRs), (4) absent in melanoma 2 (AIM2)-like receptors (ALRs), and (5) NOD-like receptors (NLRs)[2,3]. Following is a brief description of these PRRs.

TLRs are transmembrane glycoproteins that express an N-terminal ectodomain containing leucine-rich repeats (LRRs). Toll/interleukin-1 receptor (TIR) domain at their C-terminus allows signal transduction[4]. TLR interaction with its ligand enables TIR domain dimerization and triggers signal transduction through TIR-domain-containing adapter-inducing interferon-β or myeloid differentiation factor 88 (MyD88)[4,5]. This signaling activates the nuclear factor κB (NF-κB) and induces the transcription of genes related to proinflammatory cytokines.

RLRs are a family of receptors that include molecules of the RIG-I and MDA-5 (melanoma differentiation-associated gene-5)[6]. RIG-I and MDA-5 display two N-terminal caspase activation and recruitment domains (CARD domains) in tandem, a central DExD/H-box domain consisting of two helicase domains (Hel-1 and Hel-2), and a C-terminal regulatory domain[3,5,7]. These receptors are cytoplasmic and detect viral RNA both single-stranded (ssRNA) and double-stranded (dsRNA).

CLRs contain three types of receptors. Type I receptors are transmembrane and contain several carbohydrate recognition domains (CRDs) (e.g., CD205 or DEC205, and macrophage mannose receptor 1 or

MMR-1). Type II receptors are transmembrane proteins and typically contain only one CRD (e.g., DC-SIGN and Dectin-1 or -2). The third type has a soluble form and includes the mannose-binding lectin (MBL). In these CLRs, two conserved domains confer specificity. While EPN (Glu-Pro-Asn) motifs confer specificity to mannose, QPD (Gln-Pro-Asp) motifs have other CRDs[8].

ALRs are cytoplasmic and express an N-terminal pyrin domain (PYD) that binds to DNA. After binding, the PYD domain interacts with another PYD domain in the adapter protein ASC (apoptosis-associated speck-like protein that contains a CARD). Then, ASC recruits and activates procaspase 1, and releases caspase-1 that processes the inactive precursors of interleukin-1β (IL-1β) into the mature form. This process induces an inflammatory form of cell death called pyroptosis[5].

NLRs are receptors located mainly in the cytoplasm but also in mitochondria and express one of the four possible binding domains at the N-terminal. According to these domains, we identify four subfamilies: (1) NLRA (A for acidic transactivating domain, e.g., CIITA), (2) NLRB (B for BIR, or baculovirus inhibitor of apoptosis protein repeat, e.g., NAIP), (3) NLRC (C for CARD, e.g., *NOD2* or NLRC2), and (4) NLRP (P for PYD, e.g., NLRP3). In addition, these receptors contain an intermediate nucleotide-binding domain (NBD) also known as nucleotide oligomerization domain (NOD), which is necessary for binding and self-oligomerization. Finally, there is an LRR domain at the C-terminus[8]. The best-characterized function of NLRs is to sense PAMPs or DAMPs and activate the immune response.

NLRs

The family of NLRs consists of 22 receptors in humans and 34 in mice. In humans, only eight of these have been well-characterized (Fig. 2). NLRs express one of the three structural domains (CARD, PYD, or BIR motifs) at the N-terminal which are involved in protein-protein interactions. Importantly, an intermediate NBD allows ATP binding and oligomerization. The C-terminal region in NLRs contains an LRR-domain similar to the one present in TLRs[8]. The activation of some NLRs, such as NLRC4, NLRP1, NLRP3, NLRP6, NLRP7, and NLRP12, stimulates the inflammasome, which is a multimeric protein complex that mediates the activation of inflammatory caspases[9]. Inflammasome activation leads the processing of caspase-1 and -11 in mice and -4 and -5 in humans, which generates the active forms of IL-1β, IL-18, and IL-33. Besides the processing of cytokines, these caspases contribute to the cleavage of gasdermin-D, which induces a particular type of cell death termed pyroptosis[10].

Despite their central role in activating the inflammasome, NLRs have some other very important regulatory activities. For example, *NOD2* and NLRP3 regulate the signaling pathways of innate immunity, including the canonical and non-canonical pathways of NF-κB, mitogen-activated protein kinase (MAPK), and type I interferons (IFNs). They also regulate pathways that lead to the production of cytokines, chemokines, reactive oxygen species, and to the activation of ribonuclease L[11]. *NOD2* and NLRC4 along with its correceptor NAIP5, control the induction of autophagy and mitophagy[12]. Meanwhile, NLRP10 and NLRC5 regulate and modulate another NLRs or major histocompatibility complex (MHC) genes through some molecules such as Class II, MHC, transactivator (CIITA)[11,13]. Finally, NLRP2 and NLRP7 participate in embryonic/fetal development and uterine implantation, which are required to control the immune response[14].

It is undeniable that NLRs and their multiprotein complexes participate in inflammasome-dependent as well as inflammasome-independent pathways. However, there is increasing evidence of their importance as the central effector of the immune response. A novel connection between endoplasmic reticulum stress and triggering of the innate immunity mediated by NOD1 and *NOD2* has been described[15]. The present review focuses on the functions of one NLR, the *NOD2*, during bacterial and viral infections, as well as on polymorphisms associated with disease susceptibility during infections and other pathogenic conditions. Moreover, the potential of *NOD2* as a therapeutic target in the near future is exposed.

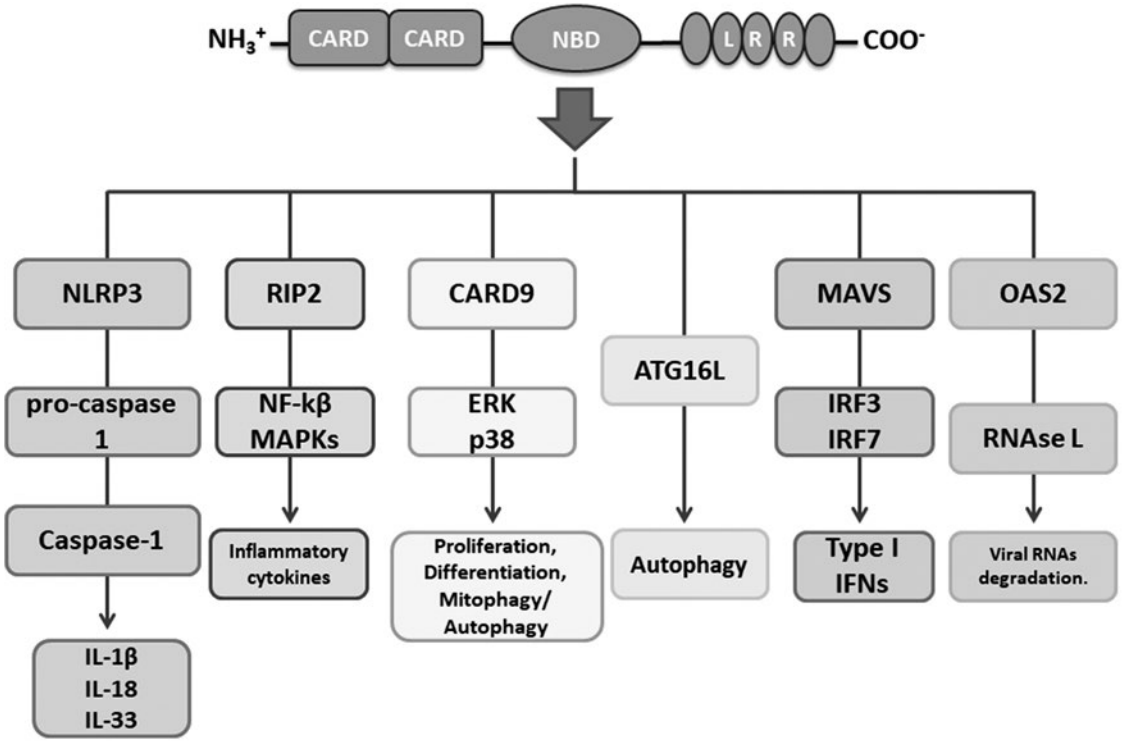
NOD2 DURING BACTERIAL AND VIRAL INFECTIONS

The *NOD2* receptor has a molecular weight of 110 kDa (1040 aa) (Fig. 3). It expresses two CARD domains in the N-terminus, a central NBD, and an LRR

Figure 2. NOD-like receptors (NLRs): General characteristics and functions for the eight best-characterized NLRs in humans. Some NLRs received a name according with the domain present in their amino-terminal, such as NLRCs that present a caspase-activation and recruitment domain (CARD) and NLRPs having with a Pyrin domain (PYD). Functions of these NLRs include the formation of inflammasome and nodosome (axis *NOD2*/RIP2), and some of them exhibit regulator activities.

NLRs	Structural Domains	Functions
NOD1	H_3N^+ CARD NBD L R R COO^-	Nodosome
NOD2	H_3N^+ CARD CARD NBD L R R COO^-	Nodosome
NLRP1	H_3N^+ PYD NBD L R R CARD COO^-	Inflammasome
NLRP3	H_3N^+ PYD NBD L R R COO^-	Inflammasome
NAIP	H_3N^+ BYR BYR BYR NBD L R R COO^-	Inflammasome
NLRP10	H_3N^+ PYD NBD COO^-	Regulator
NLRC5	H_3N^+ DD NBD L R R COO^-	Transcriptional activator
NLRX1	H_3N^+ X NBD L R R COO^-	Regulator
CIITA	H_3N^+ AD P/S/T NBD L R R COO^-	Transcriptional activator

Figure 3. Protein interactions established by the cytoplasmic receptor *NOD2* relevant for immune responses. *NOD2* is a very dynamic receptor; once it is activated in the cells, numerous interactions and responses are mediated. *NOD2* is involved in a broad range of cellular responses that include inflammasome regulation, production of proinflammatory cytokines, triggering autophagy, production of type I interferons, and other antiviral activities such the activation of RNAse L.



domain at the C-terminus[16]. *NOD2* is able to detect muramyl dipeptide (MDP), a molecular motif commonly expressed in the peptidoglycan (PGN) of Gram-negative and Gram-positive bacteria. This receptor is commonly expressed in cells that include Paneth intestinal cells, monocytes/macrophages (Mo/Mϕs), dendritic cells (DCs), and granulocytes.

NOD2 is predominantly located in the cell cytoplasm and is able to interact with multiple proteins (Fig. 3). Following increased expression of protein *in vitro*, *NOD2* becomes associated with the plasmatic membrane, an event that seems important to activate NF-κB[17]. Mϕs treated with MDP exhibit *NOD2* molecule in their acidic vesicles[1]. Several studies on Mϕs indicate that while DCs from animals and humans show that stimulation of *NOD2* by MDP induces a variety of responses that clearly differ from those triggered by TLRs, the activation of *NOD2* can, in fact, induce a two- to three-fold potentiation of the response mediated by TLRs[18].

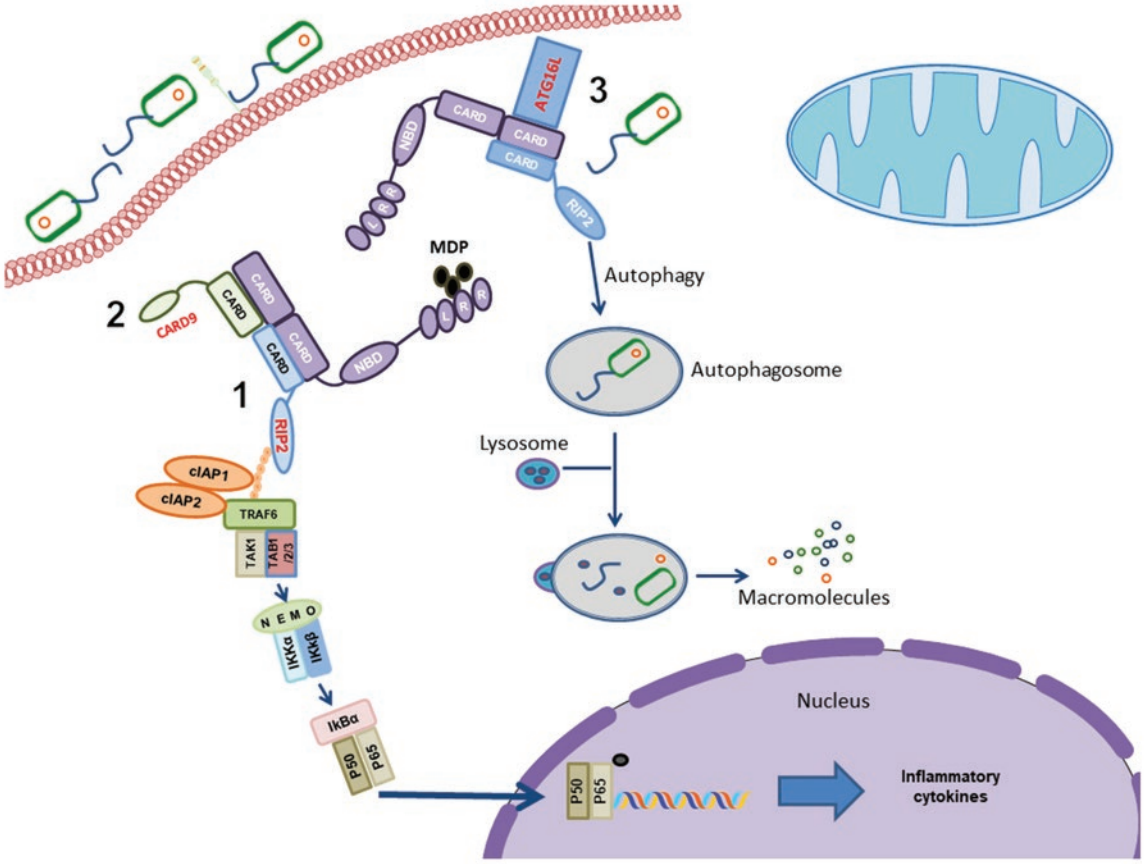
NOD2 enables those TLRs localized in the cytoplasmic membrane to complete or increase their response. This is particularly important in infections caused by intracellular pathogens[16]. For example, after the recognition of MDP by *NOD2*, and LPS by TLR4, the activation of both PRRs is synergistic and cytokine production is significantly potentiated[19]. An analysis of transcripts from cells infected with vacuolar pathogens showed a TLR response dependent on induction of MyD88. Meanwhile, in cells infected with intracellular pathogens, a *NOD2*- and interferon regulatory factor 3 (IRF3)-dependent responses were found[20].

Various authors have reported the involvement of *NOD2* in bacterial infections (Fig. 4). *NOD2* also participates in the expression of the inducible nitric oxide (NO) synthase to produce NO during *Mycobacterium tuberculosis* infections of human Mϕs[21]. *NOD2* mRNA levels increased in a rat model for *Staphylococcus aureus*-induced mastitis[22], while *NOD2* mRNA, as well as protein levels, increased in the central nervous system of a mouse model for pneumococcal meningitis[23]. *NOD2* has also been reported to participate in the immune response to periodontal pathogens[24] and contributes to the bone loss mediated by P*orphyromonas gingivalis*[25]. In cells infected with extracellular and intracellular bacteria, there is an increase in the expression and activation of *NOD2*, based on dependent and independent recruitment of receptor-interacting protein 2 (RIP2). Apart from this significant interaction of *NOD2* with RIP2, other non-canonical signaling pathways are also affected. Three of the most important interactions occurring with *NOD2* during infections are herein described.

The response of *NOD2* to MDP initiates a signaling cascade activating NF-κB and MAPK in a TLR-independent manner. Thus, *NOD2* senses MDP through its LRR domain which then induces the unfolding of the NBD domain, followed by self-oligomerization, and exposure of its CARD domains[1,16]. These events lead to the recruitment and binding of RIP2[26] by homophilic interactions through CARD-CARD[27]. The binding of *NOD2* and RIP2 culminates in the activation of NF-κB. RIP2 molecule recruits TNF receptor-associated factor 6-E3 ubiquitin-protein ligase, which subsequently triggers self-ubiquitination. The latter also has the ability to polyubiquitinate other proteins downstream from *NOD2*[28]. The kinase domain of RIP2 is associated with other E3 ubiquitin ligases such as cellular inhibitors of apoptosis (cIAP1/2). Both catalyze the ubiquitination of RIP2 at the Lys63 site (K63). The polyubiquitination of RIP2 recruits the transforming growth factor beta-activated kinase 1 complex (TAK1) (TAK1-TAB1/2/3), leading to the activation of the IkK kinases[29]. The phosphorylation and recruitment of the IkKβ kinase inhibit NF-κB through degradation of IKBs by the proteasome[1,27] (Fig. 4).

CARD9 is expressed predominantly in DCs and Mϕs; consequently, it is found in lymphoid organs and is an adapter protein with a CARD domain at the N-terminus and a "coiled-coil" domain at the C-terminus. It regulates signaling during fungal infections and is required in the immune response against intracellular pathogens[30]. In Mϕs and DCs treated with PGN, *NOD2* interacts with CARD9; this interaction activates p38 extracellular signal-regulated kinase (ERK)[1] and c-Jun N-terminal kinase (JNK), which activates the heterodimeric transcription factor activator protein 1[29]. The Mϕs of mice deficient in Card9$^{-/-}$ express defects in the activation of p38 and JNK kinases after a viral and bacterial infection, but not in NF-κB. Whereas the overexpression of CARD9 and *NOD2* normally occurs during infection with *Listeria monocytogenes*, in CARD9$^{-/-}$ mice cytokine production is deficient, and this bacterium cannot be eliminated[30].

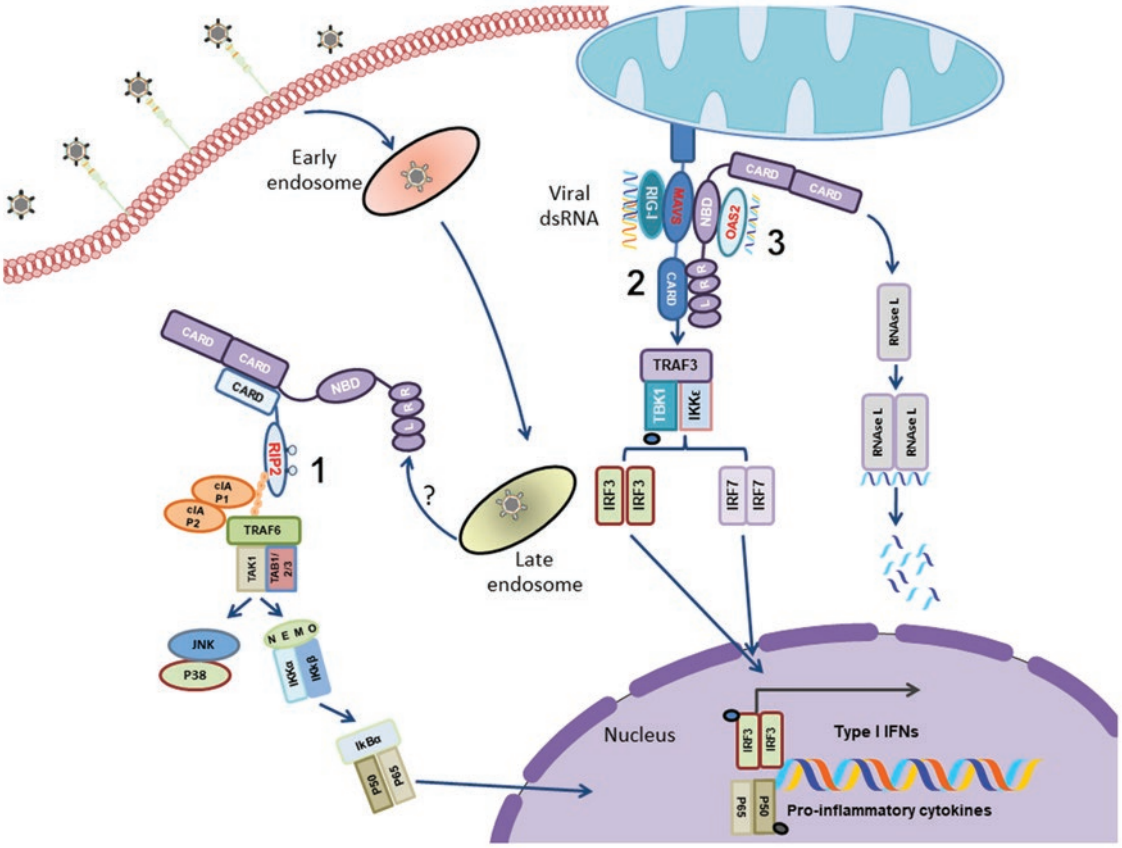
Figure 4. *NOD2*-activated pathways during infections with extracellular and intracellular bacteria. This figure shows a general overview of interactions established by *NOD2* through its structural domains. (1) The canonical pathway depends on *NOD2* interaction with RIP2 that leads downstream activation of NF-κB. (2) A non-canonical pathway on *NOD2* and CARD9 leads to the activation of AP-1. (3) Another non-canonical pathway that *NOD2* activates involves the interaction with the ATG16L protein. A final effect of signaling through these pathways is the production of proinflammatory cytokines and autophagy. RIP2: Receptor-interacting protein 2, NF-κB: Nuclear factor κB, CARD: Caspase activation and recruitment domains, AP-1: Activator protein 1.



Autophagy is a highly conserved catabolic process of eukaryotic cells used to recycle macromolecules[26]. This occurs in double-membrane vesicles that sequester damaged organelles, which are degraded afterward by the fusion of lysosomes to these vesicles. Membrane-recruited *NOD2* participates in the formation of autophagosome by interacting with the autophagy-related 16-like 1 protein complex (ATG16L1). During the bacterial invasion, ATG16L1 and *NOD2* lead bacteria elimination. Although it is known to be an NF-κB-independent mechanism, the involvement of RIP2 is still being debated[28,31] (Fig. 4). The Mϕs of TLR2$^{-/-}$, *NOD2*$^{-/-}$, and RIP2$^{-/-}$ mice are more susceptible to the infection with *L. monocytogenes*. In this infection model, the activation of the axis *NOD2*/RIP2 and the consequent induction of autophagy is dependent on the ATG16L protein, and the ERK-signaling pathway[31,32]. Thus, the protective response to *L. monocytogenes* depends on TLR2 and *NOD2* signaling. In infections with *Salmonella enterica* serovar typhimurium, the induction of autophagy by *NOD2* is necessary for the antigen presentation to take place in DCs[33].

NOD2 stimulation with viral ssRNA activates the interferon regulatory factors 3 and 7 (IRF3 and IRF7) and induces an antiviral response mediated by type I IFNs. This has raised new questions about molecular recognition and signaling by *NOD2* because there are no structural similarities between bacterial MDP and viral ssRNA or dsRNA motifs[34]. *NOD2* along with other NLRs, such as NOD1, might promote inflammation and facilitate antiviral response[35]. It is plausible that the activation of *NOD2* occurs following a direct interaction with viral genome or proteins during the viral replication cycle[36].

Figure 5. *NOD2* activation during viral infections. Distinct pathways involving *NOD2* are triggered by viruses and include: (1) The canonical MDP activation pathway involving *NOD2* and the kinase RIP2 with the subsequent activation of NF-κB, leading to inflammatory gene expression, (2) The second pathway involves ssRNA virus-induced activation of *NOD2*, which binds to MAVS, leading to IRF3/IRF7 activation and interferon gene expression, and (3) Another non-canonical pathway involves *NOD2* and OAS2 interaction which activates RNAse L that degrades viral RNA. MDP: Muramyl dipeptide, RIP2: Receptor-interacting protein 2, NF-κB: Nuclear factor κB, MAVS: Mitochondrial antiviral-signaling protein, IRF: Interferon regulatory factors, OAS2: 2'-5'-oligoadenylate synthetase type 2.



NOD2 activation has been demonstrated in several infections with viruses (Fig. 5) that have an ssRNA genome such as vesicular stomatitis virus, respiratory syncytial virus (RSV), and parainfluenza virus 3, and also for some viruses with DNA genomes such as human cytomegalovirus. In these infections, there is an increased expression of *NOD2* and type I IFNs in human bronchial epithelial cells, Mϕs, and embryonic fibroblasts[36,37]. In Mϕs and epithelial cells *NOD2* binds to other proteins apart from RIP2 including CARD9, ATG16L, mitochondrial antiviral-signaling protein (MAVS), and 2'-5'-oligoadenylate synthetase type 2 (OAS2)[16] (Fig. 5).

A study of neutrophils from individuals exposed to human immunodeficiency virus revealed that the hyporesponse of *NOD2* and other effectors are related to the maintenance of seronegativity[38]. Recent evidence suggests that leukotriene B4 directly impacts on the *NOD2* pathway enhancing the immune response against influenza A virus (IAV)[39]. On the other hand, hepatitis E virus exhibits an intrinsic ability to counteract the activity of *NOD2* and other PRRs[40].

During viral infections, an activation of the canonic axis *NOD2*/RIP2 also occurs. In an infection model of *NOD2*$^{-/-}$ and RIP2$^{-/-}$ mice with IAV, these become hypersensitive to the infection. The analysis of individual of RIP2$^{-/-}$ cells showed that this is due to the induction of mitophagy (mitochondrial autophagy), a phenomenon that causes an increase in superoxide production and leads to mitochondrial damage. This causes a strong activation of the NLRP3 inflammasome, resulting in increased production of IL-18. The RIP2 protein regulates mitophagy through

phosphorylation of the Unc-51-like kinase 1. This model demonstrates that *NOD2* and RIP2 downregulate the activation of the NLRP3 inflammasome and production of IL-18 through ULK-1[41].

In addition, during viral infections, *NOD2* can also be relocated to the mitochondria by its interaction with the MAVS protein through its LRR and NOD domains. This interaction triggers the nuclear translocation of IRF3 and induces the production of type I IFNs[26,28] (Fig. 5). The activation mechanism involves macromolecular complexes consisting of RIP2 and TRAF3[28,29]. In fact, depletion of *NOD2* abates the expression of type I IFNs. Viral ssRNAs may activate the *NOD2* receptor, causing activation of the MAVS protein in the mitochondria. MAVS phosphorylates IRF3/IRF7, two factors that form homodimers and translocate to the nucleus[26,36]. Moreover, the interaction of *NOD2* and the adapter protein MAVS regulate the production of type I IFN and increase expression of *NOD2 de novo*. It is possible that *NOD2* interacts with the MAVS-RIG-I/MDA-5 complex associated with viral RNA[11].

In a murine model of infection with RSV, the activation of *NOD2* allows its relocation to the mitochondria and increases *NOD2* mRNA expression coinciding with the increased expression of RIG-I and TLR3, suggesting the synergic role of the latter two receptors in the antiviral response[37]. Proteomic analysis revealed that *NOD2* interacts with OAS2 in the THP-1 cell line[42]. The OAS2 molecule is necessary for the activation of RNase L, which degrades viral and cellular RNA limiting viral replication[14,51]. The interaction between *NOD2* and OAS2 takes place during cellular response to dsRNA and synthetic ligands such as poly (I:C), which mimic viral RNAs. Data suggest that in some viral infections, *NOD2* possibly participates in inducing the expression of type I IFNs through a mechanism independent of RLRs[26]. Clearly, *NOD2* is a dynamic cytoplasmic receptor exhibiting an exceptional plasticity, whose cellular activity depends on the adapter molecules recruited in each response[36,37].

NOD2 POLYMORPHISMS INVOLVED IN HEALTH AND DISEASE

NOD2 deficiency in mice has been associated with chronic inflammatory disease. In humans, a mutation in the *CARD15* gene (in the LRR region of *NOD2*) has been associated with chronic bowel inflammation in Crohn's disease (CD). Moreover, polymorphisms in this receptor are associated with various diseases such as Blau syndrome, arthritis, atopic dermatitis, sarcoidosis, and possibly asthma[43,44].

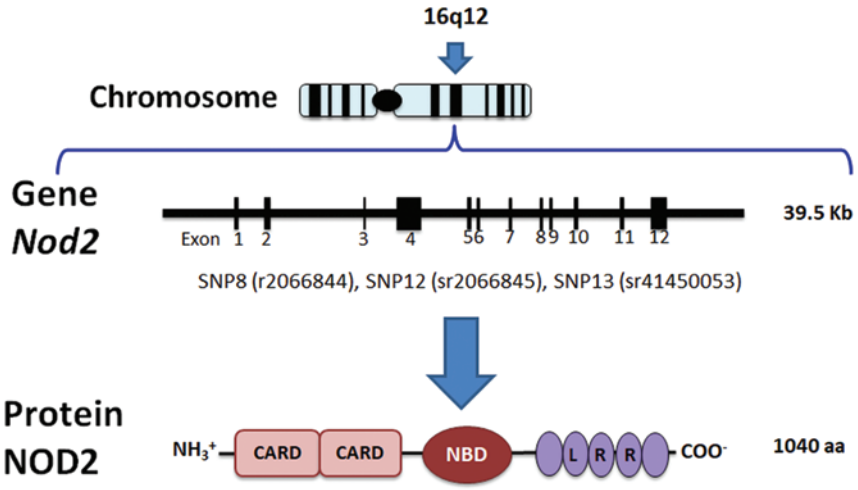
The gene encoding *NOD2* is located in the "q" region of chromosome 16 and is highly polymorphic (Fig. 6), with at least 660 single nucleotide polymorphisms (SNPs) described with alleles varying among individual populations and across geographical locations. There are three main polymorphisms in *NOD2* frequently associated with disease, SNP8 (rs2066844), SNP12 (rs2066845), and SNP13 (rs41450053)[45]. Individuals that are heterozygous for any of these SNPs show a 2- to 4-fold greater risk of developing CD, while those homozygous for these SNPs exhibit an almost 20-fold greater risk of developing this disease[46,47].

SNPs 8, 12, and 13 in the *NOD2* gene are located in exons 4, 8, and 11, respectively. Whereas there is only an amino acid change in SNP8 and 12, a frameshift takes place in SNP13, leading to the emergence of a truncated protein[48]. Some polymorphisms in the region coding for *NOD2/CARD15* increase the risk of developing CD up to 17.1-fold in either homozygous or heterozygous individuals[49]. Mutations in *NOD2/CARD15* decrease activity in the pathway, leading to inhibition of NF-κB. This causes the over-reactivation of this protein complex and the proinflammatory symptoms observed in CD[50].

In addition to CD, these polymorphisms in *NOD2* have been implicated in other ailments such as anquilosante spondylitis, arthritis, and cancer[50]. Moreover, *NOD2* (rs8057431) seems to be implicated in susceptibility to *Mycobacterium leprae* in diverse populations[51]. Furthermore, the SNP13 in *NOD2* has been implicated in septic shock following transplantation with stem cells[52].

Strikingly, *NOD2* sensing activity of commensals has been shown to be indispensable to aid in the right sorting of antimicrobial peptides to the intracellular compartments of the Paneth cells and also for the correct establishment of symbiosis[53]. Thus, in CD associated with *NOD2* deficiencies, the ability to respond to the MDP present in commensal and pathogenic bacteria is clearly altered[54].

Figure 6. Chromosomal location, gene, and protein characteristics for *nod2* and *NOD2*. The gene encoding for *NOD2* protein is located in the q arm of chromosome 16. The gene is constituted by multiple exons and introns distributed along 39.5 Kb. The protein contains 1040 aa.



IS NOD2 A SUITABLE THERAPEUTIC TARGET?

Current data on NLRs suggest that these molecules hold a central role in the innate immune response as well as in regulating proinflammatory pathways. In 2008, for example, two independent studies reported that NLRP3 is a chief sensor in response to aluminum salts[55,56], which are the lone approved adjuvant for human vaccines. This represents the first insight into the action mechanisms for these salts. Stimulation of *NOD2* by MDP triggers a wide repertoire of transcripts *in vitro*, including those encoding for chemokines, proinflammatory cytokines, antimicrobial peptides, and adhesion molecules[57]. Several authors have demonstrated that resistance to viruses is conferred by MDP alone or in combination with other agents. As MDP signaling is mostly carried out through *NOD2*, this NLR has been envisioned as a potential therapeutic target[58].

Considering that *NOD2*-mediated inflammation exacerbates some ailments, it could be a suitable therapeutic target through its downregulation. An important point of intervention is through the RIP2 molecule, which as mentioned is downstream of the *NOD2* signaling pathway. RIP2 activity can be effectively impeded by existing pharmaceutical type II kinase inhibitors[59].

CONCLUDING REMARKS

NOD2, a PRR, exhibits critical activities during bacterial and viral infections. *NOD2* is able to synergize with TLRs and has an important role in the activation of NF-κB. Moreover, due to its dynamic nature, *NOD2* can determine multiple cell responses not only in infectious diseases but also in health. Therefore, this molecule constitutes an important target for pathogen evasion mechanisms and is also a potential target to be intervened therapeutically in the future.

ACKNOWLEDGMENTS

The authors thank Allen Larsen and Jesús M Torres Flores for critical and helpful comments on this manuscript.

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