Published in final edited form as:

Trends Immunol. 2017 October ; 38(10): 758–767. doi:10.1016/j.it.2017.07.004.

NOD1 and NOD2: beyond peptidoglycan sensing

A. Marijke Kestra-Gounder¹ and Renée M. Tsois^{2,*}

¹University of Colorado Health Sciences Center, Anschutz Medical Campus, Aurora, CO, USA

²University of California at Davis, School of Medicine, Davis, CA USA

Abstract

NOD1 and NOD2 are pattern recognition receptors of the innate immune system with well-established roles in sensing fragments of bacterial peptidoglycan. In addition to their role as microbial sensors, recent evidence indicates that NODs can also recognize a broader array of danger signals. Indeed, recent work has expanded the roles of NOD1 and NOD2 to encompass not only sensing of infections with viruses and parasites but also perceiving perturbations of cellular processes such as regulation of the actin cytoskeleton and maintenance of endoplasmic reticulum homeostasis. This review will comment on recent progress and point out emerging questions in these areas.

Discovery of NOD receptors as peptidoglycan sensors

The innate immune system is the first line of defense that detects danger-associated molecular patterns (referred to collectively as DAMPs) by several families of pattern recognition receptors (PRRs). Most PRRs recognize pathogen specific molecules such as LPS, flagellin, RNA, DNA or peptidoglycan, also known as microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) [1, 2]. Some PRRs, such as NLRP3, sense DAMPs that are byproducts of pathogen invasion or sterile cellular damage, such as uric acid crystals, reactive oxygen species (ROS), extracellular ATP release, ER stress and mitochondrial damage [3–6]. Damage to the host or interference with metabolic pathways results in the release of certain danger signals that are not present under homeostatic conditions, such as potassium efflux, mtDNA and ROS [5–9]. Among PRRs, nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are a family of intracellular receptors that are characterized by three motifs consisting of a C-terminal leucine rich repeat (LRR) domain, a central nucleotide-binding domain (NBD domain) and a variable N-terminal effector domain [10]. The NLR family is further divided into four subfamilies on the basis of their N-terminal region. NOD1 (CARD4) and NOD2 (CARD15) are both members of the NLRC family and contain one and two N-terminal caspase recruitment domains (CARD), respectively. NOD1 and NOD2 are the first of the NLRs to be identified

Contact information: Medical Microbiology & Immunology, University of California, Davis, One Shields Avenue, Davis CA 96716, Phone: 530-754-8497.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and they were initially shown to detect specific structures within bacterial peptidoglycan [11, 12]. NOD1 recognizes γ -D-glutamyl-mesodiaminopimelic acid (iE-DAP) from Gram-negative bacteria and certain Gram-positive bacteria and NOD2 recognizes muramyl dipeptide (MDP) structures from both Gram-positive and Gram-negative bacteria [13–16]. Upon activation, NOD1 and NOD2 oligomerize and interact with the scaffolding kinase receptor-interacting protein (RIP2) to activate NF- κ B and MAPK pathways [12, 17, 18]. The importance of NOD2 in inflammatory diseases is evident by the association of mutations in NOD2 and the increased risk for developing Crohn's disease (CD) [19, 20]. Since then three major CD-associated mutations, R702W, G908R and L1007fsinsC (all located in the LRR domain), and multiple rare variants have been found to be deficient in their ability to sense peptidoglycan [15, 16, 21]. Mutations in NOD2 have also been associated with other inflammatory diseases such as Blau syndrome and early-onset sarcoidosis (EOS) [22, 23]. In contrast, the most common Blau syndrome and EOS mutations, R334Q-W and L469F (located in the NBD domain), promoted peptidoglycan-independent activation of NF- κ B [16, 24]. This difference may be caused by the distinct roles of the three domains in the modulation of NOD2 activation [25]. An insertion-deletion polymorphism (ND(1)+32656) in NOD1 is strongly associated with asthma, a familial inflammatory disease of the airways of the lung [26]. The same mutation was also significantly associated with early-onset inflammatory bowel disease [27]. Genetic variations in NOD1 have also been associated with Behcet's Disease and sarcoidosis [28, 29]. Altogether, these initial results clearly demonstrated the role of NODs in recognition of microbe-derived DAMPs, however as we review here, recent evidence indicates that they can also participate in recognition of DAMPs originating from cellular damage. These studies may influence our understanding of the role of NODs in the pathogenesis of the diseases mentioned above.

Sensing of rho GTPase activation state by NOD1 and NOD2

Increasing evidence suggests that NOD1 and NOD2, besides the recognition of peptidoglycan, can also sense damage-induced processes inflicted by pathogens [30–33]. For example, the effector proteins IpgB2 and OspB of *Shigella flexneri* induce NF- κ B activation in a peptidoglycan-independent, NOD1-dependent manner through the interaction of NOD1 with GEF-H1, a guanidine exchange factor for the small Rho GTPase RhoA (Fig. 1) [30]. The *Salmonella enterica* serotype Typhimurium E3 ubiquitin ligase effector SspH2 ubiquitinates and modifies NOD1 in the absence of peptidoglycan. The interaction between SspH2 and NOD1 required SGT1, a NLR cochaperone protein, resulting in a significant increase in IL-8 secretion [33–35]. Two more effector proteins of *S. Typhimurium*, SipA and SopE, were found to activate the NOD1/2 signaling pathway [31, 32]. SopE is a GEF for the small Rho GTPases Rac1 and Cdc42. Constitutively active Rac1 and Cdc42 were identified as components of the NOD1 protein complex at the cell membrane required for signaling in the absence of peptidoglycan and bacterial effector proteins. (Fig. 1). Similarly, *E.coli*'s cytotoxic necrotizing factor 1 (CNF1) activates the small Rho GTPase Rac2, which then interacts with RIP2 to drive an inflammatory response [36]. The mechanism by which peptidoglycan-independent activation of NOD1 and NOD2 occurs is largely unknown. A common mechanistic link between activation of NOD1 and NOD2 by both peptidoglycan and bacterial effectors is cytoskeletal dynamics. Disruption of the actin cytoskeleton with

cytochalasin D increased NOD1 and NOD2-mediated NF- κ B activation in the absence of peptidoglycan (Fig. 1), suggesting that alterations to the cytoskeleton are responsible for the activation of NOD1 and NOD2 [37–39]. A dominant negative Rac1 inhibited iE-DAP induced NF- κ B activation, indicating a role for the Rho GTPases in peptidoglycan signaling [32]. In addition, GEF-H1 interaction with NOD1 is required for the detection of iE-DAP fragments [30]. Further, GEF-H1 also promotes MDP-induced NOD2 activation [40]. Rac1 and Rac2 mutated in the C-terminus to prevent membrane insertion of the small GTPases were unable to induce an inflammatory response, suggesting that localization of NOD1 and NOD2 to the cell membrane is essential for formation of a functional protein complex. Further evidence for the involvement of actin cytoskeletal dynamics and membrane targeting in NOD signaling comes from studies of *S. flexneri* infection, in which NOD1 is recruited to the membrane at the site of bacterial entry. [38]. Additionally, stimulation of epithelial cells with peptidoglycan resulted in the membrane targeting of NOD2, which is required for NF- κ B activation [41]. In monocytes treated with MDP, NOD2 is recruited to membrane ruffles by a mechanism requiring Rac1 and Rho GEF7 [42]. NOD2 and NOD1 recruit the autophagy protein ATG16L1 to the plasma membrane to direct autophagy of the invading *S. flexneri* [43]. Furthermore, the cofilin phosphatase SSH1, which acts downstream of the small Rho GTPase RhoA, forms a complex with NOD1 at F-actin rich domains at the cell membrane and is needed for NOD1 induced IL-8 production. Vimentin, a cytoskeletal protein, interacts with NOD2 at the cell membrane to regulate NF- κ B activation and autophagy [44]. These data suggest that recruitment of NOD1 and NOD2 to the cell membrane results from alteration in the cytoskeleton caused by invading bacteria, peptidoglycan fragments, or secreted effector proteins. Cytoskeletal dynamics are controlled by a balance between the active GTP-bound and inactive GDP-bound forms of small Rho GTPases [45]. Recent work has shown that bacteria-induced changes to the Rho GTPases RhoA, RhoB and RhoC activate the pyrin inflammasome [46]. The active forms of Rac1 and Cdc42 can activate NF- κ B and recent evidence suggests that the active forms are sensed by NOD1 and NOD2 and these changes affect the signaling outcome [32, 47]. Thus, the picture emerging from these studies suggests that perturbation of cytoskeletal remodeling and Rho GTPase activity affects NOD1 and NOD2 activation and innate immune responses.

There are interesting similarities in the activation and regulation of mammalian, insect and plant NLRs. In plants, host sensing of microbial DAMPs activates rapid defense responses such as calcium influx, production of ROS, induction of defense-related genes, and production of antimicrobial peptides [48]. The small Rac/Rop GTPases are involved in plant innate immunity by regulating ROS production, inducing the hypersensitive response, and activating MAPK6 [49]. The rice GTPase OsRac1 interacts directly with Pit, an NLR protein that induces immune resistance towards fungi, at the plasma membrane, resulting in ROS production and activation of the hypersensitive response [50]. Additionally, the innate immune receptor CERK1 senses fungal chitin at the plasma membrane where it forms a protein complex with Rac1, HSP90 and Hop, similar to the NOD1 protein complex [32, 51, 52]. Furthermore, the chaperone SGT1, important for NOD signaling, was found in a complex with HSP90 and OsRac1 in the rice plant [34, 35, 53]. This type of immune surveillance system in plants has been termed effector-triggered immunity (ETI). In ETI the host is, in addition to the PRR system, able to monitor pathogen-induced pathways and

disruption of cellular homeostasis [48, 54]. Similarly, in *Drosophila melanogaster* the small Rho GTPase Rac2 is activated by the *E. coli* CNF1 toxin, thereby inducing the IMD/relish pathway, homologues of the mammalian Rip1/Rip2/NF- κ B pathway [36]. Together, these observations suggest that the NOD proteins are not only intracellular peptidoglycan recognition receptors but are also similar to plant NLRs that can sense perturbations of host cell homeostasis. There is some evidence suggesting activation of NOD1 and NOD2 that is not linked to perturbations of the actin cytoskeleton. The *S. Typhimurium* effector protein SipA has an actin binding domain to induce actin rearrangement that facilitates the invasion of *S. Typhimurium* [55]. However, the actin binding domain was shown not to be essential for the induction of NF- κ B activation and intestinal inflammation [31, 56]. This finding suggests that SipA activates the NOD1/2 signaling pathway independently of actin rearrangement [31]. These results do not, however, exclude the possibility that SipA can activate a small GTPase that is sensed by NOD1 and/or NOD2. Mutation studies in Rac2 indicated that introduction of a F37A mutation in a constitutive active form of Rac2 blocked actin rearrangements, but was still able to induce production of antimicrobial peptides, indicating that an active Rho GTPase can still activate NF- κ B without rearranging the actin cytoskeleton [36].

Roles of NOD1 and NOD2 in viral and parasitic infections

Studies of infection by viruses and parasites have provided evidence for additional peptidoglycan-independent roles of NOD1 and NOD2 (Table 1). During respiratory syncytial virus (RSV) infection, NOD2, but not NOD1, can sense single stranded RNA (ssRNA) from the virus, and interacts with MAVS (mitochondrial antiviral signaling), leading to activation of interferon regulatory factor-3 (IRF3) and expression of type I interferon [57]. NOD2 is also required for production of type I IFNs after infection with two other ssRNA viruses, vesicular stomatitis virus and influenza virus [57], suggesting that direct sensing of ssRNA by NOD2 activates MAVS. NOD2 interacts via its NBD and LRR with MAVS, leading to formation of a complex with TBK1 and IKK α (Figure 2), which would enable phosphorylation and activation of IRF3 by TBK1 [58]. Sensing of ssRNA by NOD2 is an essential part of the antiviral response during influenza A infection, as mice deficient for *Nod2* or its downstream signaling components are highly susceptible to and exhibit increased immunopathology during infection [57, 59].

Interestingly, NOD2 activation not only drives of type I IFN expression, type I IFN also feeds back to increase expression of both *NOD2* and *NOD1* [60]. This enhanced NOD1/2 expression in the context of viral infection may have negative consequences for subsequent bacterial infections in that it amplifies the inflammatory response to peptidoglycan-derived ligands [60]. This interaction may explain in part how a preceding viral infection can predispose to more severe bacterial infection. In the context of viral infection, however, pre-stimulation of NOD2 with bacterial ligands may be beneficial, since MDP treatment of dendritic cells improved their ability to prime virus-specific CD8 T cells in the context of influenza A virus infection [59]. While the NOD2-activating viruses discussed above all have ssRNA genomes, it is interesting to note that both NOD1 and NOD2 have been implicated in the response to human cytomegalovirus (HCMV), which has a double-stranded

DNA genome [61, 62]. This raises the question of the stimulus sensed by NOD1 and NOD2 during HCMV infection.

A second group of pathogens lacking peptidoglycan, for which NOD1 and NOD2 have been implicated in the inflammatory response is protozoan parasites. Compared to viral and bacterial infections, less is known mechanistically about the specific activators of NOD1/NOD2 signaling during parasite infection. In a model of malaria utilizing *Plasmodium berghei*, mice deficient for NOD1 and NOD2 exhibited decreased inflammatory cytokine responses, but no difference in control of the parasite or development of lethal disease [63]. Work in a cellular model suggests that an unidentified ligand from *P. falciparum*-infected erythrocytes, that co-purifies with the parasite product hemozoin, stimulates NOD2 in a RIP2/CARD9-dependent manner to drive nitric oxide production by macrophages, however the identity of the stimulatory ligand and its mechanism of NOD2 activation remains to be determined [64]. In the case of infection with *Trypanosoma cruzi*, mice deficient for NOD1, but not for NOD2, succumbed more rapidly to parasite infection and failed to control parasite replication in the spleen and heart [65]. This enhanced susceptibility to infection correlated with a reduction of macrophage inflammatory responses to *T. cruzi* infection. However, neither a ligand nor a parasite-induced process has been linked to this activation.

NOD1/2 sensing of endoplasmic reticulum (ER) stress

It has long been known that viruses must subvert the function of the endoplasmic reticulum to reproduce in the cell, and more recently, it has been recognized that bacteria and parasites causing intracellular infection also co-opt the function of the early secretory pathway to promote their growth [66]. The resulting disruption of ER function elicits a homeostatic host response known as the unfolded protein response (UPR), that attempts to resolve the dysfunction by increasing function of the protein folding and degradation machinery, transiently reducing protein translation, and inducing ER repair mechanisms such as autophagy [67–69]. Signaling pathways of the UPR are also linked to inflammation, and pathways of the UPR have been linked to inflammation via activation of NF- κ B and IRF3, that in turn drive the expression of proinflammatory cytokines [70].

Recent findings have implicated NOD1 and NOD2 in ER stress-driven inflammation, by linking activation of the IRE1 α pathway of the UPR to NF- κ B activation [71]. Activation of IRE1 α led to a TRAF2-dependent activation of NOD1 and NOD2 to initiate this inflammatory branch of the UPR, which also required the adaptor protein RIP2 (Figure 3). The IRE1 α -TRAF2-NOD1/2-RIP2 pathway could be activated by two bacterial pathogens that replicate intracellularly in association with the endoplasmic reticulum, *Brucella abortus* and *Chlamydia muridarum*, or by chemical inducers of the UPR such as dithiothreitol (DTT), which induces protein misfolding by oxidizing disulfide bonds, and thapsigargin, which blocks uptake of calcium into the endoplasmic reticulum.

The link between ER stress and NOD1/NOD2 signaling may also be of relevance for other pathologies in which NOD1 and or NOD2 have been implicated in the inflammatory response, such as infection with HCMV infection, which is known to modulate the function of the endoplasmic reticulum for its replication, and induces the unfolded protein response

(UPR) [72]. Similarly, the role of NOD2 in sensing influenza virus infection (discussed above) could be related to activation of IRE1 α -induced inflammatory responses [71], because influenza virus is known to trigger ER stress [73]. Further, in the case of malaria, the liver stage of *P. berghei* can elicit the unfolded protein response in hepatocytes to promote its intracellular replication [74]. While this latter study did not assess hepatic inflammation, it is tempting to speculate that the role of NOD1/NOD2 in malaria parasite-induced inflammation could be linked to induction of the IRE1 pathway.

This role of NOD1 and NOD2 in UPR-induced inflammation has broad implications for understanding non-communicable diseases as well, because ER stress factors prominently in several inflammatory pathologies, including diabetes ([75]; see below), atherosclerosis [76], and Crohn's disease (CD) [77]. The link between the IRE1 α pathway of the UPR and NOD1/2 is of particular interest for CD, since this inflammatory pathology has been linked to ER stress-mediated dysfunction of Paneth cells (reviewed in [78, 79]). Interestingly, two proteins affected by CD-associated risk alleles, ATG16L1, required for autophagy, and XBP1, a regulator of the unfolded protein response, alleviate ER stress and reduce activation of the IRE1 α pathway. Consistent with our model (Fig. 3), ATG16L1 and XBP1 suppress IRE1 α /NOD1/NOD2 signaling in an autophagy-independent manner [80]. In this scenario, a failure to regulate ER stress-induced NOD1/NOD2 signaling may predispose to intestinal inflammation. This idea is consistent with a recent report linking hyperactivation of the IRE1 α pathway in the context of defective ATG16L1 or XBP1 function to colitis [82], suggesting that downstream signaling via NOD1/NOD2 could also be important in this context.

NOD1/2 signaling and diabetes

Type 1 diabetes results from the destruction of pancreatic beta cells induced by ER stress, leading to insulin deficiency and hyperglycemia. Treatment of Akita mice, a mouse model for Type 1b diabetes, with KIRA6 showed that inhibition of IRE1 α resulted in the preservation of pancreatic beta cells, increased insulin secretion and reduction of hyperglycemia, indicating the importance of the IRE1 α arm of the UPR [82]. In Type 2 diabetes, ER stress is a major hallmark in the development of insulin resistance, demonstrated by the observation that mice deficient in XBP-1 develop diet-induced insulin resistance [83]. Obesity is a major cause of developing insulin resistance and T2D. Saturated fatty acids (SFAs) in the blood are involved in the induction of a chronic inflammatory response that will damage the body over time. Interestingly, SFAs induce ER stress by depleting ER calcium resulting in the activation of the UPR [84]. Furthermore, SFAs induce an inflammatory response and insulin resistance that is partially dependent on NOD1, however the exact mechanism remains to be elucidated [85]. The recent finding that NOD1 and NOD2 can sense ER stress linking it to inflammation, suggests that SFAs activate the UPR that is sensed by NOD1 to induce an inflammatory response in a peptidoglycan-independent manner [71].

Concluding remarks and future perspectives

Recent work has expanded our view of NOD1 and NOD2 function beyond their initially identified role in sensing peptidoglycan fragments to encompass such diverse stimuli as viral infection, manipulation of the actin cytoskeleton, and endoplasmic reticulum stress. Each of these functions appear to occur at differing cellular locations and to require overlapping sets of NOD-interacting proteins, which raises the question of whether there is a common underlying cellular signal generated by these diverse processes that results in NOD1 and NOD2 activation (see Outstanding Questions). In particular, a better understanding of the links between NOD1/2 signaling and ER stress-induced inflammation will be important, as it will reveal whether this pathway can be targeted for ER-stress mediated inflammatory diseases such as inflammatory bowel disease, cardiovascular disease and diabetes.

References

1. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol.* 2001; 2(8):675–80. [PubMed: 11477402]
2. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol.* 1989; 54(Pt 1):1–13.
3. Mariathasan S, et al. Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature.* 2006; 440(7081):228–32. [PubMed: 16407890]
4. Martinon F, et al. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature.* 2006; 440(7081):237–41. [PubMed: 16407889]
5. Dostert C, et al. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science.* 2008; 320(5876):674–7. [PubMed: 18403674]
6. Wolf AJ, et al. Hexokinase Is an Innate Immune Receptor for the Detection of Bacterial Peptidoglycan. *Cell.* 2016; 166(3):624–36. [PubMed: 27374331]
7. Perregaux D, Gabel CA. Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. *J Biol Chem.* 1994; 269(21):15195–203. [PubMed: 8195155]
8. Petrilli V, et al. Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ.* 2007; 14(9):1583–9. [PubMed: 17599094]
9. Munoz-Planillo R, et al. K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity.* 2013; 38(6):1142–53. [PubMed: 23809161]
10. Inohara N, Nunez G. NODs: intracellular proteins involved in inflammation and apoptosis. *Nat Rev Immunol.* 2003; 3(5):371–82. [PubMed: 12766759]
11. Inohara N, et al. Human Nod1 confers responsiveness to bacterial lipopolysaccharides. *J Biol Chem.* 2001; 276(4):2551–4. [PubMed: 11058605]
12. Ogura Y, et al. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J Biol Chem.* 2001; 276(7):4812–8. [PubMed: 11087742]
13. Chamaillard M, et al. An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nat Immunol.* 2003; 4(7):702–7. [PubMed: 12796777]
14. Girardin SE, et al. Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. *Science.* 2003; 300(5625):1584–7. [PubMed: 12791997]
15. Girardin SE, et al. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem.* 2003; 278(11):8869–72. [PubMed: 12527755]
16. Inohara N, et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem.* 2003; 278(8):5509–12. [PubMed: 12514169]
17. Inohara N, et al. Nod1, an Apaf-1-like activator of caspase-9 and nuclear factor-kappaB. *J Biol Chem.* 1999; 274(21):14560–7. [PubMed: 10329646]

18. Bertin J, et al. Human CARD 4 protein is a novel CED-4/Apaf-1 cell death family member that activates NF-kappaB. *J Biol Chem.* 1999; 274(19):12955–8. [PubMed: 10224040]
19. Hugot JP, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature.* 2001; 411(6837):599–603. [PubMed: 11385576]
20. Ogura Y, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature.* 2001; 411(6837):603–6. [PubMed: 11385577]
21. Kim YG, et al. Cutting edge: Crohn's disease-associated Nod2 mutation limits production of proinflammatory cytokines to protect the host from *Enterococcus faecalis*-induced lethality. *J Immunol.* 2011; 187(6):2849–52. [PubMed: 21849681]
22. Miceli-Richard C, et al. CARD15 mutations in Blau syndrome. *Nat Genet.* 2001; 29(1):19–20. [PubMed: 11528384]
23. Kanazawa N, et al. Early-onset sarcoidosis and CARD15 mutations with constitutive nuclear factor-kappaB activation: common genetic etiology with Blau syndrome. *Blood.* 2005; 105(3):1195–7. [PubMed: 15459013]
24. Chamaillard M, et al. Gene-environment interaction modulated by allelic heterogeneity in inflammatory diseases. *Proc Natl Acad Sci U S A.* 2003; 100(6):3455–60. [PubMed: 12626759]
25. Tanabe T, et al. Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. *EMBO J.* 2004; 23(7):1587–97. [PubMed: 15044951]
26. Hysi P, et al. NOD1 variation, immunoglobulin E and asthma. *Hum Mol Genet.* 2005; 14(7):935–41. [PubMed: 15718249]
27. McGovern DP, et al. Association between a complex insertion/deletion polymorphism in NOD1 (CARD4) and susceptibility to inflammatory bowel disease. *Hum Mol Genet.* 2005; 14(10):1245–50. [PubMed: 15790594]
28. Li L, et al. Genetic Variations of NLR family genes in Behcet's Disease. *Sci Rep.* 2016; 6:20098. [PubMed: 26833430]
29. Tanabe T, et al. Sarcoidosis and NOD1 variation with impaired recognition of intracellular *Propionibacterium acnes*. *Biochim Biophys Acta.* 2006; 1762(9):794–801. [PubMed: 16935475]
30. Fukazawa A, et al. GEF-H1 mediated control of NOD1 dependent NF-kappaB activation by *Shigella* effectors. *PLoS Pathog.* 2008; 4(11):e1000228. [PubMed: 19043560]
31. Keestra AM, et al. A *Salmonella* virulence factor activates the NOD1/NOD2 signaling pathway. *MBio.* 2011; 2(6)
32. Keestra AM, et al. Manipulation of small Rho GTPases is a pathogen-induced process detected by NOD1. *Nature.* 2013; 496(7444):233–7. [PubMed: 23542589]
33. Bhavsar AP, et al. The *Salmonella* type III effector SspH2 specifically exploits the NLR cochaperone activity of SGT1 to subvert immunity. *PLoS Pathog.* 2013; 9(7):e1003518. [PubMed: 23935490]
34. Mayor A, et al. A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses. *Nat Immunol.* 2007; 8(5):497–503. [PubMed: 17435760]
35. da Silva Correia J, et al. SGT1 is essential for Nod1 activation. *Proc Natl Acad Sci U S A.* 2007; 104(16):6764–9. [PubMed: 17420470]
36. Boyer L, et al. Pathogen-derived effectors trigger protective immunity via activation of the Rac2 enzyme and the IMD or Rip kinase signaling pathway. *Immunity.* 2011; 35(4):536–49. [PubMed: 22018470]
37. Legrand-Poels S, et al. Modulation of Nod2-dependent NF-kappaB signaling by the actin cytoskeleton. *J Cell Sci.* 2007; 120(Pt 7):1299–310. [PubMed: 17356065]
38. Kufer TA, et al. The pattern-recognition molecule Nod1 is localized at the plasma membrane at sites of bacterial interaction. *Cell Microbiol.* 2008; 10(2):477–86. [PubMed: 17970764]
39. Uehara A, et al. Mesodiaminopimelic acid and meso-lanthionine, amino acids specific to bacterial peptidoglycans, activate human epithelial cells through NOD1. *J Immunol.* 2006; 177(3):1796–804. [PubMed: 16849490]
40. Zhao Y, et al. Control of NOD2 and Rip2-dependent innate immune activation by GEF-H1. *Inflamm Bowel Dis.* 2012; 18(4):603–12. [PubMed: 21887730]

41. Barnich N, et al. Membrane recruitment of NOD2 in intestinal epithelial cells is essential for nuclear factor- κ B activation in muramyl dipeptide recognition. *J Cell Biol.* 2005; 170(1): 21–6. [PubMed: 15998797]
42. Eitel J, et al. Beta-PIX and Rac1 GTPase mediate trafficking and negative regulation of NOD2. *J Immunol.* 2008; 181(4):2664–71. [PubMed: 18684957]
43. Travassos LH, et al. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat Immunol.* 2010; 11(1):55–62. [PubMed: 19898471]
44. Stevens C, et al. The intermediate filament protein, vimentin, is a regulator of NOD2 activity. *Gut.* 2013; 62(5):695–707. [PubMed: 22684479]
45. Narumiya S. The small GTPase Rho: cellular functions and signal transduction. *J Biochem.* 1996; 120(2):215–28. [PubMed: 8889802]
46. Xu H, et al. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature.* 2014; 513(7517):237–41. [PubMed: 24919149]
47. Perona R, et al. Activation of the nuclear factor- κ B by Rho, CDC42, and Rac-1 proteins. *Genes Dev.* 1997; 11(4):463–75. [PubMed: 9042860]
48. Jones JD, Dangl JL. The plant immune system. *Nature.* 2006; 444(7117):323–9. [PubMed: 17108957]
49. Kawano Y, Kaneko-Kawano T, Shimamoto K. Rho family GTPase-dependent immunity in plants and animals. *Front Plant Sci.* 2014; 5:522. [PubMed: 25352853]
50. Kawano Y, et al. Activation of a Rac GTPase by the NLR family disease resistance protein Pit plays a critical role in rice innate immunity. *Cell Host Microbe.* 2010; 7(5):362–75. [PubMed: 20478538]
51. Chen L, et al. The Hop/Sti1-Hsp90 chaperone complex facilitates the maturation and transport of a PAMP receptor in rice innate immunity. *Cell Host Microbe.* 2010; 7(3):185–96. [PubMed: 20227662]
52. Akamatsu A, et al. An OsCEBiP/OsCERK1-OsRacGEF1-OsRac1 module is an essential early component of chitin-induced rice immunity. *Cell Host Microbe.* 2013; 13(4):465–76. [PubMed: 23601108]
53. Nakashima A, et al. RACK1 functions in rice innate immunity by interacting with the Rac1 immune complex. *Plant Cell.* 2008; 20(8):2265–79. [PubMed: 18723578]
54. Vance RE, Isberg RR, Portnoy DA. Patterns of pathogenesis: discrimination of pathogenic and nonpathogenic microbes by the innate immune system. *Cell Host Microbe.* 2009; 6(1):10–21. [PubMed: 19616762]
55. Zhou D, Mooseker MS, Galan JE. Role of the *S. typhimurium* actin-binding protein SipA in bacterial internalization. *Science.* 1999; 283(5410):2092–5. [PubMed: 10092234]
56. Li D, et al. The actin-polymerizing activity of SipA is not essential for *Salmonella enterica* serovar Typhimurium-induced mucosal inflammation. *Infect Immun.* 2013; 81(5):1541–9. [PubMed: 23439302]
57. Sabbah A, et al. Activation of innate immune antiviral responses by Nod2. *Nat Immunol.* 2009; 10(10):1073–80. [PubMed: 19701189]
58. Liu S, et al. Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. *Science.* 2015; 347(6227):aaa2630.
59. Lupfer C, Thomas PG, Kanneganti TD. Nucleotide oligomerization and binding domain 2-dependent dendritic cell activation is necessary for innate immunity and optimal CD8⁺ T Cell responses to influenza A virus infection. *J Virol.* 2014; 88(16):8946–55. [PubMed: 24872587]
60. Kim YG, et al. Viral infection augments Nod1/2 signaling to potentiate lethality associated with secondary bacterial infections. *Cell Host Microbe.* 2011; 9(6):496–507. [PubMed: 21669398]
61. Fan YH, et al. Role of nucleotide-binding oligomerization domain 1 (NOD1) and its variants in human cytomegalovirus control in vitro and in vivo. *Proc Natl Acad Sci U S A.* 2016; 113(48):E7818–E7827. [PubMed: 27856764]
62. Kapoor A, Fan YH, Arav-Boger R. Bacterial Muramyl Dipeptide (MDP) Restricts Human Cytomegalovirus Replication via an IFN- β -Dependent Pathway. *Sci Rep.* 2016; 6:20295. [PubMed: 26830977]

63. Finney CA, et al. Disruption of Nod-like receptors alters inflammatory response to infection but does not confer protection in experimental cerebral malaria. *Am J Trop Med Hyg.* 2009; 80(5): 718–22. [PubMed: 19407112]
64. Corbett Y, et al. Involvement of Nod2 in the innate immune response elicited by malarial pigment hemozoin. *Microbes Infect.* 2015; 17(3):184–94. [PubMed: 25462568]
65. Silva GK, et al. Cutting edge: nucleotide-binding oligomerization domain 1-dependent responses account for murine resistance against *Trypanosoma cruzi* infection. *J Immunol.* 2010; 184(3): 1148–52. [PubMed: 20042586]
66. Celli J, Tsolis RM. Bacteria, the endoplasmic reticulum and the unfolded protein response: friends or foes? *Nat Rev Microbiol.* 2015; 13(2):71–82. [PubMed: 25534809]
67. Bernales S, McDonald KL, Walter P. Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. *PLoS Biol.* 2006; 4(12):e423. [PubMed: 17132049]
68. Ding WX, et al. Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival. *J Biol Chem.* 2007; 282(7):4702–10. [PubMed: 17135238]
69. Ogata M, et al. Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol.* 2006; 26(24):9220–31. [PubMed: 17030611]
70. Janssens S, Pulendran B, Lambrecht BN. Emerging functions of the unfolded protein response in immunity. *Nat Immunol.* 2014; 15(10):910–9. [PubMed: 25232821]
71. Keestra-Gounder AM, et al. NOD1 and NOD2 signalling links ER stress with inflammation. *Nature.* 2016; 532(7599):394–7. [PubMed: 27007849]
72. Nakamura H, et al. Human cytomegalovirus induces apoptosis in neural stem/progenitor cells derived from induced pluripotent stem cells by generating mitochondrial dysfunction and endoplasmic reticulum stress. *Herpesviridae.* 2013; 4(1):2. [PubMed: 24144363]
73. Roberson EC, et al. Influenza induces endoplasmic reticulum stress, caspase-12-dependent apoptosis, and c-Jun N-terminal kinase-mediated transforming growth factor-beta release in lung epithelial cells. *Am J Respir Cell Mol Biol.* 2012; 46(5):573–81. [PubMed: 21799120]
74. Inacio P, et al. Parasite-induced ER stress response in hepatocytes facilitates *Plasmodium* liver stage infection. *EMBO Rep.* 2015; 16(8):955–64. [PubMed: 26113366]
75. Montane J, Cadavez L, Novials A. Stress and the inflammatory process: a major cause of pancreatic cell death in type 2 diabetes. *Diabetes Metab Syndr Obes.* 2014; 7:25–34. [PubMed: 24520198]
76. McAlpine CS, Werstuck GH. The development and progression of atherosclerosis: evidence supporting a role for endoplasmic reticulum (ER) stress signaling. *Cardiovasc Hematol Disord Drug Targets.* 2013; 13(2):158–64. [PubMed: 23988003]
77. Kaser A, et al. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell.* 2008; 134(5):743–56. [PubMed: 18775308]
78. Kaser A, Blumberg RS. ATG16L1 Crohn's disease risk stresses the endoplasmic reticulum of Paneth cells. *Gut.* 2014; 63(7):1038–9. [PubMed: 24304670]
79. Hosomi S, Kaser A, Blumberg RS. Role of endoplasmic reticulum stress and autophagy as interlinking pathways in the pathogenesis of inflammatory bowel disease. *Curr Opin Gastroenterol.* 2015; 31(1):81–8. [PubMed: 25426970]
80. Sorbara MT, et al. The protein ATG16L1 suppresses inflammatory cytokines induced by the intracellular sensors Nod1 and Nod2 in an autophagy-independent manner. *Immunity.* 2013; 39(5): 858–73. [PubMed: 24238340]
81. Tschurtschenthaler M, et al. Defective ATG16L1-mediated removal of IRE1alpha drives Crohn's disease-like ileitis. *J Exp Med.* 2017; 214(2):401–422. [PubMed: 28082357]
82. Ghosh R, et al. Allosteric inhibition of the IRE1alpha RNase preserves cell viability and function during endoplasmic reticulum stress. *Cell.* 2014; 158(3):534–48. [PubMed: 25018104]
83. Ozcan U, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science.* 2004; 306(5695):457–61. [PubMed: 15486293]
84. Cunha DA, et al. Initiation and execution of lipotoxic ER stress in pancreatic beta-cells. *J Cell Sci.* 2008; 121(Pt 14):2308–18. [PubMed: 18559892]

85. Zhou YJ, et al. Saturated fatty acid induces insulin resistance partially through nucleotide-binding oligomerization domain 1 signaling pathway in adipocytes. *Chin Med Sci J.* 2013; 28(4):211–7. [PubMed: 24382222]
86. Vegna S, et al. NOD1 Participates in the Innate Immune Response Triggered by Hepatitis C Virus Polymerase. *J Virol.* 2016; 90(13):6022–35. [PubMed: 27099311]
87. Lencer WI, et al. Innate immunity at mucosal surfaces: the IRE1-RIDD-RIG-I pathway. *Trends Immunol.* 2015; 36(7):401–9. [PubMed: 26093676]

Trends Box

NOD1 and NOD2 receptors participate in peptidoglycan-dependent and – independent inflammatory pathways.

Excessive activation of small Rho GTPases and perturbations of cytoskeletal remodeling is a danger signal detected by NOD1 and NOD2.

Endoplasmic Reticulum (ER) stress activates a danger signal that is sensed by NOD1 and NOD2 to produce an inflammatory response.

Peptidoglycan-free pathogens such as viruses and parasites induce inflammatory responses dependent on NOD1 and NOD2.

Emerging roles for NOD1 and NOD2 in ER stress-driven chronic inflammatory diseases such as Crohn's Disease, obesity and Type 2 Diabetes.

Outstanding Questions

What is the mechanism downstream of ER stress induced NOD activation? Are NODs recruited to the ER membrane to induce downstream signaling? What is the role of TRAF2? Does ER stress trigger the interaction of TRAF2 with NOD1 and NOD2? Does ER stress release certain danger signals that are not present under homeostatic conditions that activate NOD1 and NOD2?

Is the nodosome composition downstream of ER stress signaling similar to the nodosome complex at the cell membrane?

Is ER stress-induced NOD1/NOD2 activation important in chronic inflammatory diseases such as Crohn's Disease and Type 2 Diabetes? And can inhibition of the IRE1a-TRAF2-NOD1/2-Rip2K pathway be exploited for the treatment of these ER stress driven inflammatory diseases?

Is there a common mechanism that links peptidoglycan recognition, Rho GTPase activity, ER stress and virus-induced activation of NOD1 and NOD2 signaling?

Figure 1. NOD1/NOD2 pathways involved in sensing rho GTPase modulation

Bacterial effector proteins from *S. Typhimurium* and *S. flexneri* and disruption of the cytoskeleton by cytochalasin D induce the NOD1/2 signaling pathway and this is dependent on the activation of small Rho GTPases. Rho GTPases have also been shown to act downstream of NOD1/2 activation by peptidoglycan fragments. Downstream signaling involves RIP2 to induce activation of NF-κB and the transcription of pro-inflammatory genes.

Figure 2. Role of NOD2 in sensing infection with ssRNA viruses

Binding of ssRNA to NOD2 triggers its association with MAVS, which promotes formation of a complex with TBK1 and IKK α . The resulting phosphorylation and activation of IRF3 by TBK1 leads to expression of antiviral type I interferon.

Figure 3. NOD1/2 pathways involved in sensing ER stress

Induction of ER stress with the chemical inducers DTT and Thapsigargin or infection with *Brucella abortus* induce the IRE1 pathway of the unfolded protein response, which leads to activation of NF- κ B via a process dependent on TRAF2, NOD1/2 and RIP2. IRE1 activation can also lead to activation of IRF3, via its ribonuclease function [87].

Table 1

Viral and parasitic infections for which NOD1 or NOD2 have been implicated in the immune response

Viruses			
Respiratory syncytial virus	NOD2	Activation of type I interferon via detection of ssRNA	[57]
Vesicular stomatitis virus	NOD2	Activation of type I interferon via detection of ssRNA	[57]
Parainfluenza	NOD2	Activation of type I interferon via detection of ssRNA	[57]
Influenza A	NOD2	Activation of type I interferon via detection of ssRNA	[57, 59]
Human cytomegalovirus	NOD1 NOD2	Restriction of viral replication	[61, 62]
Hepatitis C virus	NOD1	Independent of MAVS; likely dsRNA produced by NS5B polymerase	[86]
Parasites			
<i>Plasmodium berghei</i> and <i>P. falciparum</i>	NOD1 NOD2	Induction of IL-1 β , KC, IFN γ Hemozoin-mediated induction of NOS	[63, 64]
<i>Trypanosoma cruzi</i>	NOD1	NOD1-KO mice are highly susceptible to infection	[65]