# Signalling pathways and molecular interactions of NOD1 and NOD2

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Abstract | The NOD (nucleotide-binding oligomerization domain) proteins NOD1 and NOD2 have important roles in innate immunity as sensors of microbial components derived from bacterial peptidoglycan. The importance of these molecules is underscored by the fact that mutations in the gene that encodes NOD2 occur in a subpopulation of patients with Crohn's disease, and NOD1 has also been shown to participate in host defence against infection with *Helicobacter pylori*. Here, we focus on the molecular interactions between these NOD proteins and other intracellular molecules to elucidate the mechanisms by which NOD1 and NOD2 contribute to the maintenance of mucosal homeostasis and the induction of mucosal inflammation.

LRR domain
(leucine-rich-repeat domain). A domain that mediates the detection of ligands derived from microbial components. The LRR domain of NLRs (NACHT-LRR proteins) is similar to that of Toll-like receptors. It consists of leucine-rich amino-acid strands forming a peptide loop. The loops occur as tandem repeats that, together, form a coil or 'solenoid' and contain constant sequences, as well as unique 'insertions' or variable residues for each ligand.

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In the past few years, intensive study of Toll-like receptors (TLRs) has taught us that innate immune responses can be initiated by a system of structurally related proteins that function as receptors for specific microbial components[1]. This model of host recognition and response, however, is not restricted to TLRs. Striking evidence for this comes from the recent discovery that some members of the NACHT (domain present in NAIP, CIITA, HET-E and TP1)-LRR (leucine-rich repeat) family (the NLR family) — which includes both nucleotide-binding oligomerization domain (NOD) proteins and NACHT-, LRR- and pyrin-domain-containing proteins (NALPs)[2] — function in a similar manner in that they also recognize microbial components, albeit in the cytosol rather than at the cell surface or in vesicles[2,3]. The similarity extends to the structure of receptors in the two systems in that both TLRs and NLRs rely on pathogen recognition by an LRR domain. However, the downstream signalling pathways that are involved in the TLR and NLR systems are markedly different. This, together with the microbial components sensed by NLRs probably being distinct from those sensed by TLRs, makes it probable that the NLRs will be found to function independently of the TLRs[2,3].

The NOD proteins NOD1 (which is encoded by the caspase-recruitment domain 4 gene, *CARD4*) and NOD2 (which is encoded by *CARD15*) recognize peptidoglycan (PGN), a component of bacterial cell walls, and are mainly expressed by two cell types that are exposed to this component under physiological conditions: antigen-presenting cells (APCs) and epithelial cells[3]. So, the study of NOD1 and NOD2 might allow us to improve our understanding of how mucosal cells respond to commensal organisms in the normal gastrointestinal tract. However, a more compelling interest in the function of these proteins arises from the recent finding that they have a role in the pathogenesis of human gastrointestinal disease: *CARD15* is a susceptibility gene for Crohn's disease[4,5]; and polymorphisms in *CARD4* are associated with inflammatory bowel disease[6] and asthma[7]. Moreover, *CARD4* is involved in host defence against *Helicobacter pylori* infection of the gastric mucosa, a chronic infection that can lead to peptic ulcers and gastric cancer[8]. In this Review, we focus on the signalling function of NOD1 and NOD2, with the aim of clarifying how such signalling contributes to inflammation and host defence. In doing so, we attempt to provide a framework for understanding how mutations in *CARD15* might lead to Crohn's disease.

## Structure of NOD1 and NOD2

As alluded to earlier, NOD1 and NOD2 are members of the phylogenetically conserved NLR protein family[2,3,9,10], which encompasses proteins that were previously identified as members of the CATERPILLER (CARD, transcription enhancer, R (purine)-binding, pyrin, lots of LRRs), NOD, NOD-LRR and NALP groups of proteins. In general, members of this family share a tripartite domain structure that consists of the following: a carboxy (C)-terminal LRR domain, which is involved in ligand recognition; a central NOD (also known as a NACHT domain), which facilitates self-oligomerization and has ATPase activity; and an amino (N)-terminal

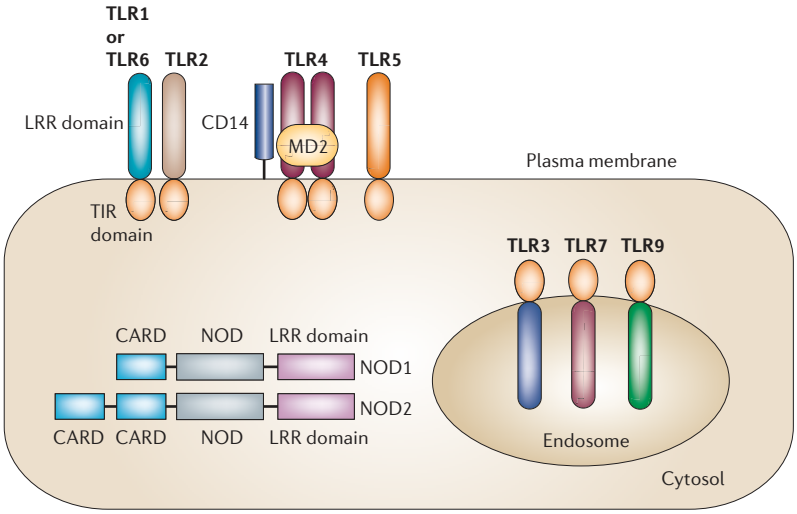


Figure 1 | **Structure and cellular location of TLRs and NOD1 and NOD2.** The NOD (nucleotide-binding oligomerization domain) proteins NOD1 and NOD2 are members of the NACHT (domain present in NAIP, CIITA, HET-E and TP1)-LRR (leucine-rich repeat) family (the NLR family). NOD1 and NOD2 are cytosolic molecules that are composed of three main domains: a carboxy-terminal LRR domain, which is involved in ligand recognition; a central NOD (also known as a NACHT domain), which facilitates self-oligomerization and has ATPase activity; and an amino-terminal domain composed of a caspase-recruitment domain(s) (CARD). By contrast, the Toll-like receptors (TLRs) are membrane-associated proteins that function as cell-surface or endosomal receptors. Both NLRs (in most cases) and TLRs use LRR domains to recognize microbial components, but these families use different domains for downstream signalling. TLRs use a Toll/interleukin-1 receptor (TIR) domain, whereas NLRs use either CARDs or pyrin domains. It should also be noted that TLR2 forms a heterodimer with either TLR1 or TLR6 in responding to its various ligands.

domain that is composed of protein–protein interaction cassettes, such as CARDs or pyrin domains. Although NLR-family members that contain CARDs interact with different downstream adaptor molecules than those that contain pyrin domains, they are functionally related in that both types of molecule activate nuclear factor-κB (NF-κB) and/or caspases; in addition, mutations in both CARD-containing and pyrin-domain-containing NLRs have been linked to inflammatory diseases[9,11].

For further details about the structure of NOD proteins and the function of their component domains, see previous reviews that focus on this topic[2,3]. Briefly, NOD1 consists of a C-terminal LRR, a central NOD and an N-terminal effector domain that contains a single CARD, whereas NOD2 has a C-terminal LRR, a central NOD and an N-terminal effector domain that contains two CARDs (FIG. 1). Although members of the NOD protein family have similar structures, this does not necessarily imply that they have similar functions. As discussed later, NOD1 and NOD2 have distinct activities.

## Expression of NOD1 and NOD2

Whereas TLRs are associated with the plasma membrane or, in some cases, with lysosomal and/or endosomal vesicles, both NOD1 and NOD2 are expressed mainly in the cytosol[3]. So, whereas TLRs function as cell-surface receptors, NOD1 and NOD2 do not have this property. However, there is recent evidence that, at least in epithelial cells, NOD2 contains molecular sequences that allow association with the plasma membrane[12]. This pattern of intracellular expression implies that the ligands for these molecules are not native microbial components *per se* but, instead, products that are derived from microbial components. Indeed, NOD1 and NOD2 have been shown to recognize peptides that are derived from the degradation of PGN, which is a component of bacterial cell walls[13–16].

Not surprisingly, NOD1 and NOD2 are mainly expressed by two cell types that are exposed to and/or deal with microorganisms that express PGN: APCs and epithelial cells. In both humans and mice, APCs such as macrophages and dendritic cells (DCs) express NOD1 and NOD2, whereas other haematopoietic cells (such as T cells and B cells) do not express these proteins[3,17,18]. Most (but not all) intestinal epithelial cell (IEC) lines and, more importantly, most primary epithelial cells express NOD1 (REFS 19–22). However, whereas most IECs express NOD2 at the mRNA level, expression at the protein level is low or undetectable[19–22], at least using the antibodies that are available at present. In addition, among primary epithelial-cell populations, NOD2 expression seems to be limited to Paneth cells, which are located at the base of the intestinal crypts[23].

The expression of both NOD1 and NOD2 is regulated by pro-inflammatory cytokines, but this occurs in a slightly different way for each. In the case of NOD1, constitutive (but variable) baseline expression by epithelial cell lines is upregulated by interferon-γ (IFNγ) acting through the transcription factor IFN-regulatory factor 1 (IRF1) at the *CARD4* promoter; however, NOD1 expression is not upregulated by tumour-necrosis factor (TNF)[21]. In the case of NOD2, baseline expression of protein by epithelial cells is low, and TNF induces upregulation of expression; furthermore, this positive effect is augmented by IFNγ[24]. NF-κB-binding sites in the *CARD15* promoter are involved in this response to TNF, implying that, when NOD2 activates NF-κB following activation by its ligand, NOD2 can upregulate itself. A similar regulatory system for expression of NOD1 and NOD2 has not yet been reported for APCs. Finally, consistent with the role of NOD proteins in innate immunity, type-I-IFN-mediated signalling might be involved in the expression of NOD1 and NOD2. This is indicated by the finding that upregulation of NOD1 and NOD2 expression by wild-type mice after challenge with *Listeria monocytogenes* was not seen in IFNβ-deficient mice[25].

## Signalling through NOD1 and NOD2

***Bacteria-derived ligands recognized by NOD1 and NOD2.*** Whereas initial studies identified lipopolysaccharide (LPS) as a NOD2 ligand[5], it is now well established that the NOD1 and NOD2 ligands are the PGN-derived peptides γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP)[14,15] and muramyl dipeptide (MDP)[13,16], respectively. Because PGN from both Gram-positive and Gram-negative bacteria contains MDP, NOD2 functions as a general sensor of most, if not all, bacteria. By contrast, because PGNs from Gram-positive bacteria do not

NOD
(nucleotide-binding oligomerization domain). The domain that defines NLRs (NACHT-LRR proteins). Its most characteristic feature is that it contains sequences that, on binding of ligand to the leucine-rich repeat (LRR) domain, facilitate self-oligomerization, and it also has ATPase activity. This oligomerization might be a key activation event that allows NLRs to bind to, and activate, downstream effector molecules through their caspase-recruitment domain or pyrin domain.

CARD or pyrin domain (caspase-recruitment domain or pyrin domain). The amino-terminal effector-binding domain of NLRs (NACHT-LRR proteins) usually consists of a CARD or a pyrin cassette for promoting molecular interactions. Proteins that contain a pyrin or CARD cassette in this region are also known as NALPs (NACHT-, LRR- and pyrin-domain-containing proteins) or nucleotide-binding oligomerization domain (NOD) proteins, respectively.

contain iE-DAP (except for PGNs derived from specific Gram-positive bacteria such as *Listeria* and *Bacillus* spp. and from many Gram-positive bacteria in the soil)[26], NOD1 mainly senses products from Gram-negative bacteria. Confirmation of these specificities has come from the finding that macrophages isolated from NOD1- or NOD2-deficient mice are completely unresponsive to their respective ligands[14,27].

The NOD-protein ligands need to reach the LRR domains of the respective NOD protein for activation of this protein to be initiated. However, information on how this is accomplished is sparse at present, especially in the case of APCs. One possibility that relates to phagocytic cells such as macrophages or DCs is that these cells generate the peptide ligands by ingesting whole bacteria and then digesting them in phagolysosomes[28,29]. In epithelial cells, a slightly different process might occur in that the apical peptide transporter PEPT1 seems to have a role in the delivery of MDP. This is indicated by the finding that MDP that is taken up by PEPT1 into colonic epithelial cells subsequently mediates the activation of NF-κB[30]. In addition, it has recently been shown that *H. pylori* can 'inject' PGN into cells through a type IV secretion system, which is encoded by a pathogenicity island[8]. This discovery indicates that PGN can enter cells by various mechanisms that involve bacteria–host interactions.

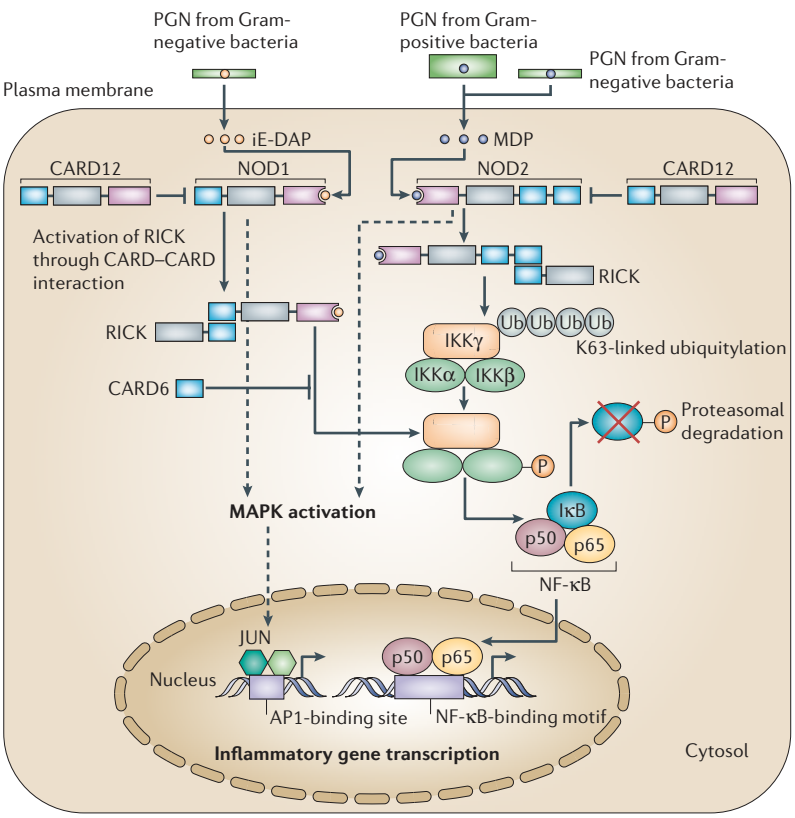


Figure 2 | **Signalling pathways of NOD1 and NOD2.** Recognition of muramyl dipeptide (MDP) and γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP) through leucine-rich repeat (LRR) domains activates the NOD (nucleotide-binding oligomerization domain) proteins NOD2 and NOD1, respectively, which then recruit receptor-interacting serine/threonine kinase (RICK) through caspase-recruitment domain (CARD)–CARD interactions. In the case of NOD2, activation of RICK leads to K63 (Lys63)-linked polyubiquitylation of IKKγ, the scaffold of the inhibitor of NF-κB (IκB)-kinase complex (the IKK complex), which also consists of IKKα and IKKβ. This is followed by the phosphorylation of IKKβ, as well as the phosphorylation of IκB and the release of nuclear factor-κB (NF-κB) for translocation to the nucleus. In the case of NOD1, ubiquitylation of IKKγ by RICK has not been studied, and the mechanism of NF-κB activation is not clear. CARD12 negatively regulates RICK-mediated NF-κB activation by both NOD1 and NOD2, whereas CARD6 negatively regulates only RICK-mediated NF-κB activation by NOD1 (for further details, see the main text). In addition to NF-κB activation, NOD1 and NOD2 signalling gives rise to the activation of mitogen-activated protein kinases (MAPKs) such as JUN amino-terminal kinase (JNK), extracellular-signal-regulated kinase (ERK) and p38MAPK by as-yet-unknown mechanisms (denoted by dashed arrows). AP1, activator protein 1; PGN, peptidoglycan.

Our understanding of this change comes, in part, from studies of activation of apoptotic-protease-activating factor 1 (APAF1), an NLR-family member that is involved in caspase activation and apoptosis[34,35]. Activation of APAF1 is initiated by the interaction of its WD40 domain with its ligand (cytochrome *c*), as well as by the binding of dATP or ATP to an ATP-binding cassette (ABC) or oligomerization cassette in the NOD. The molecule then undergoes self-oligomerization, which enables it to bind its downstream effector molecule, caspase-9, through a CARD–CARD interaction. The large molecular complex that is formed in this way, which is known as the apoptosome, then facilitates activation of the bound caspase-9, possibly by bringing caspase molecules into juxtaposition[34,35]. That this activation model applies to NLRs in general (and to NOD1 and NOD2 in particular) is indicated by the presence of structural similarities between NOD proteins and APAF1: the N-terminal region of the central NOD in NLRs contains both an ABC and an oligomerization module. At least in the case of NOD2, the introduction of mutations into the ABC region abolishes NOD2 signalling[33]. In addition, it has been shown that both NOD1 and NOD2 undergo self-oligomerization following the binding of PGN-derived ligand[32,33]. In one model of NOD-protein activation based on the APAF1–caspase-9 pathway, Inohara *et al.*[26] proposed that oligomerization of NOD1 or NOD2 also allows binding to a downstream effector molecule through a CARD–CARD interaction, in this case involving the serine/threonine kinase RICK (receptor-interacting serine/threonine kinase; also known as RIP2 or CARDIAK), and this, in turn, leads to cross-activation of RICK. However, further work is necessary to establish this possibility.

***Signalling pathways.*** One of the main outcomes of NOD1 and NOD2 activation by their respective ligands is the activation of NF-κB (FIG. 2). Whereas such activation was clearly evident in iE-DAP- or MDP-stimulated epithelial cells that had been transfected with constructs encoding wild-type NOD1 or NOD2, it was reduced in

cells that were transfected with constructs encoding a mutated NOD2 that had alterations in the LRR domain[13]. Consistent with this, after stimulation with MDP, translocation of NF-κB subunits to the nucleus is observed in human and mouse APCs that have intact NOD2 but not in APCs that are deficient in NOD2 or have a mutation in NOD2 (REFS 13,36).

The activation of NF-κB by NOD1 and NOD2 occurs exclusively through the downstream effector molecule RICK. This is shown by the finding that transfection of RICK-deficient fibroblasts with constructs encoding NOD1 or NOD2 results in severely defective NF-κB activation[37]. It should be noted, however, that RICK-deficient macrophages also have reduced cytokine responses following stimulation with LPS, lipoteichoic acid and PGN, indicating that TLR2 and TLR4 might also use RICK as a downstream effector molecule[37], although the existence of a TLR4–RICK pathway is controversial (discussed later).

RICK is a CARD-containing serine/threonine kinase that physically associates with the CARD(s) of NOD1 and NOD2 through CARD–CARD interactions[18,38]. As shown recently by Abbott *et al.*[39], following its activation by NOD2, RICK mediates K63-linked polyubiquitylation of inhibitor of NF-κB (IκB)-kinase-γ (IKKγ; also known as NEMO; the key member of the IKK complex) at a unique ubiquitylation site (the lysine residue at position 285). As shown previously, K63-linked polyubiquitylation is associated with activation of the NF-κB pathway[40], and in the case of the RICK–IKKγ interaction, it is, indeed, followed by phosphorylation of IKKβ and downstream activation of NF-κB, leading to the translocation of transcriptional components of NF-κB to the nucleus. So, in activating the IKK complex, RICK either activates an E3 ubiquitin ligase that promotes K63-linked polyubiquitylation or inhibits an enzyme (such as cylindromatosis protein, CYLD) that de-ubiquitylates proteins that are modified with K63-linked polyubiquitin, so RICK does not require its own kinase activity for this function.

Recently, it has been shown that activated NOD2 (but not NOD1) also interacts with the intracellular molecule GRIM19 (gene associated with retinoid-IFN-induced mortality 19) and that such an interaction might be required for optimal NF-κB activation. However, neither the structural basis of this interaction nor the mechanism of its relation to NF-κB activation is known[41].

Another outcome of NOD1 and NOD2 activation is the activation of the mitogen-activated protein kinase (MAPK) pathway. So, stimulation of wild-type macrophages, but not NOD2-deficient macrophages, with MDP leads to activation of p38MAPK and extracellular-signal-regulated kinase (ERK)[27,42]. In addition, activation of NOD1 by its ligand leads to the activation of JUN N-terminal kinase (JNK)[43]. The mechanism of such NF-κB-independent signalling is unknown at present. Finally, it has been shown in transfection and immunoprecipitation studies that NOD2 binds procaspase-1, and when cells are transfected with constructs encoding NOD2 and procaspase-1, NOD2 induces interleukin-1β (IL-1β) secretion[44]. Because caspase-1 is required for processing pro-IL-1β into mature IL-1β[45], NOD2 might bind procaspase-1 through a CARD–CARD interaction in the same way that it binds RICK and, in doing so, convert the procaspase into a caspase. However, whether NOD2 has this function under physiological conditions remains to be seen.

***Effects of heterologous NOD–NOD and/or CARD–CARD interactions.*** The ability of the NOD and CARD(s) of NOD1 and NOD2 to interact with other proteins that contain these domains introduces the possibility that the function of NOD1 and NOD2 is subject to regulation by other NLR-family members or other proteins that contain a CARD. One such interaction involves the NLR-family member CARD12 (also known as IPAF or CLAN), a protein with a domain structure that is identical to that of NOD1. In recent studies, it has been shown that, in cells that overexpress CARD12, CARD12 binds both NOD1 and NOD2 through NOD–NOD interactions[46]; in addition, such binding affects NOD-protein signalling in that the CARD12-overexpressing cells show inhibition of NOD1- and NOD2-mediated activation of NF-κB and production of IL-1β[44]. That CARD12 also has an effect on NOD-protein signalling under physiological conditions is indicated by the finding that CARD12 associates with NOD2 in macrophages that are exposed to PGN[44]. This finding, as well as more recent work[47], indicates that CARD12 is activated, through its LRR, by a component of PGN and then interacts with NOD2 that has been activated by MDP (or with NOD1 that has been activated by iE-DAP). To the extent that such interactions would necessarily interfere with the normal activation of NOD1 or NOD2, this would result in inhibition of NOD-protein signalling. However, this has not been shown to occur under physiological conditions, and CARD12 does have NOD1- and NOD2-independent function. This is shown by the finding that invasive bacteria lead to the activation of CARD12 and the induction of expression of IL-1β[48].

Regulation of NOD-protein signalling through a heterologous interaction is also shown by the interaction of NOD1 with CARD6, a protein that contains an N-terminal CARD. CARD6 has been shown to physically interact with NOD1 and RICK (but not with NOD2), and its overexpression leads to suppression of the NF-κB activation that is mediated by NOD1 or RICK[49]. So, CARD6 seems to affect NOD1 signalling by competing for RICK binding.

At present, NOD–NOD and CARD–CARD interactions affecting NOD1 and/or NOD2 signalling are represented by only the two examples that are are discussed above. It is possible, however, that more such interactions will be discovered in the future, and if so, NOD-protein signalling will increasingly be seen not as a highly discrete response but, instead, as part of a mosaic of responses by a network of interacting family members. This view is underscored by the recent discovery that cryopyrin (also known as NALP3 or CIAS1), an NLR-family member that is structurally similar to NOD2 except that it contains a pyrin domain in place of a CARD, is also activated by MDP[50]. In this case, however, the molecule activates procaspase-1 and therefore facilitates IL-1β secretion.

Type IV secretion system
A type of molecular syringe of Gram-negative bacteria. It enables the bacteria to deliver DNA (delivered by species such as *Agrobacterium* spp.) and protein effectors (delivered by species such as *Helicobacter pylori*) into eukaryotic cells.

Pathogenicity island
The genetic element, the 'island of evil', in the genome of an organism that is responsible for the capacity of the organism to cause disease (that is, its pathogenicity). The virulence of the organism is modulated by genes harboured on this island.

K63-linked polyubiquitylation
Ubiquitylation that involves polyubiquitin chains linked through the lysine residue at position 63 of ubiquitin.

### The interrelationship of NOD2 and TLRs

***NOD2 activity in APCs.*** Studies of NOD2 activity in APCs are complicated by the fact that intact PGN is postulated to signal through TLR2, and after uptake of PGN and release into the cytosol, a component of PGN (MDP) can signal through NOD2. So, in theory, it is possible that different molecular forms of the same microbial component can modify each other's function (FIG. 3). In a recent study in which interactions between TLRs and NOD2 were examined, such cross-regulation does seem to occur. In particular, it was found that NOD2-deficient splenic macrophages that were stimulated *in vitro* with a wide range of TLR ligands produced markedly more IL-12 than NOD2-sufficient macrophages, but this occurred only when they were stimulated with PGN and not with other TLR ligands[36]. By contrast, NOD2-deficient APCs produced normal amounts of TNF and IL-10 when they were stimulated with PGN, indicating that NOD2 does not affect all functions of TLR2 (REF. 36). These *in vitro* results were corroborated by *in vivo* studies in which it was shown that NOD2-deficient mice mounted far higher serum IL-12 responses to systemic challenge

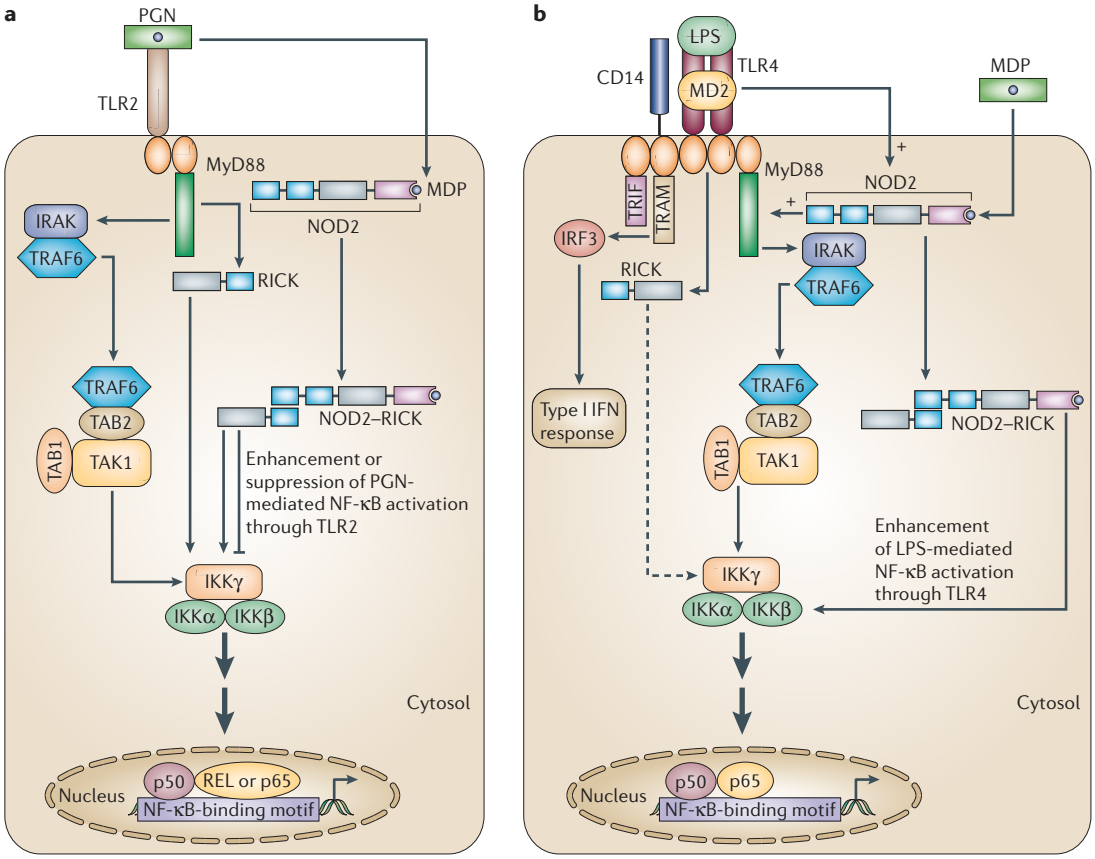


Figure 3 | **Models of the molecular interactions between TLR- and NOD2-signalling pathways in antigen-presenting cells. a** | Peptidoglycan (PGN) potentially activates both cell-surface Toll-like receptor 2 (TLR2) and cytosolic NOD2 (nucleotide-binding oligomerization-domain protein 2; through the generation of muramyl dipeptide, MDP). Stimulation of TLR2 triggers association with MyD88 (myeloid differentiation primary-response protein 88) and then recruitment of IRAK4 (interleukin-1 receptor (IL-1R)-associated kinase 4) and IRAK1. TRAF6 (tumour-necrosis-factor-receptor-associated factor 6) binds IRAK1 and then dissociates to form a complex with transforming-growth-factor-β-activated kinase 1 (TAK1), TAK1-binding protein 1 (TAB1) and TAB2. This complex is required for the ubiquitylation of TRAF6 and for the activation of the kinase activity of TAK1, which then leads to the phosphorylation of components of the IKK complex (the inhibitor of NF-κB (IκB)-kinase complex) and gives rise to downstream activation of nuclear factor-κB (NF-κB). TLR2 also triggers the activation of receptor-interacting serine/threonine kinase (RICK), which ubiquitylates the IKKγ subunit of the IKK complex (FIG. 2). Concomitant activation of NOD2 by MDP leads to the activation of RICK by NOD2 and the downmodulation of the TLR2-signalling pathway (for a discussion of the possible mechanism involved, see the main text). **b** | Lipopolysaccharide (LPS) activates the co-receptor complex of TLR4 and MD2 and initiates both MyD88-dependent and -independent signalling pathways. The MyD88-dependent pathway is similar to the TLR2 pathway described in **a**. By contrast, the MyD88-independent pathway involves TRIF (Toll/IL-1R-domain-containing adaptor protein inducing interferon-β, IFNβ) and TRAM (TRIF-related adaptor molecule), and induces IRF3 (IFN-regulatory factor 3) activation and type I IFN production. NOD2 might augment the MyD88-dependent pathway (and subsequent cytokine secretion), because activation of TLR4 increases the expression of NOD2, and NOD2 increases the expression of MyD88 (denoted by + symbols). In addition, NOD2 might increase IKKγ activation through an independent positive effect on RICK that, in the case of TLR4 signalling, is not negated by a dominant downmodulatory effect as is seen for TLR2 signalling. The dashed arrow denotes a pathway that has not been confirmed.

with PGN than did their wild-type counterparts. A final point is that stimulation of splenocyte populations from NOD2-deficient mice with PGN led to greatly increased IFNγ production by T cells, indicating that the increased IL-12 production by the splenocytes of these mice has the expected effect on downstream cytokine production.

A reasonable interpretation of these results is that PGN-mediated activation of TLR2 signalling is negatively regulated by MDP-mediated activation of NOD2 signalling and that, in the absence of NOD2, the negative regulation is released[36]. To test this hypothesis, APCs from NOD2-sufficient and NOD2-deficient mice were stimulated with PGN and increasing concentrations of exogenous MDP[36]. It was observed that this co-stimulation was associated with a dose-dependent inhibition of PGN-induced IL-12 production, but this occurred only in NOD2-sufficient APCs and not in NOD2-deficient APCs. A similar effect was noted for the much lower level of stimulation that was obtained by using the 'pure' TLR2 ligand tripalmitoyl-*S*-glyceryl cysteine ($Pam_3Cys$).

One clue to how NOD2 might negatively regulate PGN-induced IL-12 production is provided by its effect on nuclear translocation of the NF-κB subunit REL. Although the activation and nuclear translocation of the NF-κB subunits p50 (also known as NF-κB1) and p65 (also known as REL-A) is increased in the absence of NOD2, the activation and nuclear translocation of REL is increased to a far greater extent[36]. This specific ability of NOD2 deficiency to increase REL activation correlates with previous findings that both the p35 and the p40 subunits of IL-12 are particularly dependent on the activation of this NF-κB subunit[51,52]. These data allow a deeper question to be framed: how does NOD2 signalling negatively regulate activation of REL and thereby downregulate induction of IL-12 expression?

One speculative but appealing answer is that, in the absence of NOD2, PGN activates NF-κB through TLR2 in a RICK-dependent manner that leads to a different type of ubiquitylation, one that is more conducive to the activation of REL. This explanation derives some support from the finding that PGN-mediated signalling through TLR2 and MDP-mediated signalling through NOD2 both have RICK as a downstream effector molecule[37] and that, after NOD2 activation, RICK is at least partially sequestered from the PGN-mediated TLR2-signalling pathway[53]. Also consistent with this idea is that stimulation of cells with PGN induces a direct physical interaction between TLR2 and RICK[37]. A potential objection to this model is that NOD2 does not downregulate TLR4 signalling, which has also been suggested to involve RICK[37]. However, it is possible that the LPS preparations that were used in these experiments were contaminated with NOD2 ligand and that the RICK-mediated effects that were observed were a result of NOD2 signalling and not TLR4 signalling.

It should be noted that the observation that MDP downregulates PGN-mediated signalling through TLR2 has not been corroborated (and, in some cases, it has been contradicted) by other investigators, including those who have reported studies of mice with *Card15* abnormalities[42,54], so this model is still controversial. For example, in a study of macrophages from a different NOD2-deficient mouse from that previously discussed, responses to various TLR ligands were generally equivalent in wild-type and NOD2-deficient mice, and the addition of MDP to $Pam_3CSK_4$ ($Pam_3$Cys-Ser-Lys-Lys-Lys-Lys; a TLR2 ligand) resulted in an increase in IL-6 production in wild-type mice but not in NOD2-deficient mice[42]. In addition, these NOD2-deficient mice had a reduced total IgG and IgG1 response to antigen plus MDP, indicating that they have reduced cytokine production by T helper ($T_H$) cells[42]. Moreover, in a study of macrophages from mice carrying a knocked-in gene encoding a NOD2 molecule comparable to that containing the frameshift mutation that is observed in human Crohn's disease (discussed later), no differences between wild-type and mutated mice were seen after stimulation with various TLR ligands or TLR2 ligand (PGN) plus MDP, except in the case of stimulation with MDP alone, in which IL-1β production was increased. In addition, in this study, IL-12 responses were not increased in mutated mice either in the presence or absence of inflammation[54]. It is important to remember, however, that the observation that co-stimulation of macrophages with PGN (or $Pam_3Cys$) and MDP leads to decreased IL-12 (especially IL-12p70) responses[36] was carried out using wild-type mice. This finding, together with similar findings obtained using normal human monocyte-derived DCs stimulated with PGN and MDP (T.W. and W.S., unpublished observations), is therefore not subject to possible artefacts arising from the study of *Card15* knockout and knock-in mice or indeed patients with mutations in *CARD15* (the gene encoding NOD2). Similar studies of normal mice and humans have not yet been reported by other investigators, so additional studies will be necessary to resolve the existing discrepancies. (Studies of mice with *Card15* mutations and patients with *CARD15* mutations are discussed further later.)

Another concern that needs to be considered in relation to this view of the function of MDP-mediated signalling through NOD2 involves reports that question the role of PGN as a TLR2 ligand and therefore potentially undermine the two-signal hypothesis that is implied by the regulatory role of NOD2 (REF. 55). It should be noted, however, that the downregulating effect of MDP was not seen in the presence of an antibody that blocks TLR2 (T.W., A.K. and W.S., unpublished observations). So, whether PGN or a contaminant in PGN is the relevant TLR2 ligand, it is probable that MDP is regulating a TLR2 signal. In addition, we have accumulated data showing that the magnitude of the TLR2 responses that are obtained after stimulation with PGN cannot be achieved by any of the possible PGN contaminants at the concentration that is likely to be present (T.W., A.K. and W.S., unpublished observattions). Perhaps more importantly, a recent study of highly purified PGN showed that PGN is indeed a TLR2 ligand and loses this activity after digestion with muramidase, which affects the integrity of PGN but not its contaminants[56].

***Positive effects of NOD2 on TLR function.*** Another feature of NOD2 function in APCs is that, although it might downregulate IL-12 production that is induced through TLR2 activation, it somewhat paradoxically upregulates CXC-chemokine ligand 8 (CXCL8; also known as IL-8), TNF, IL-1β, IL-6 and IL-10 production through TLR2, TLR3, TLR4, TLR5, TLR7 and TLR9 in APCs[41,57–61]. Although the mechanism of these enhancing effects of NOD2 on TLRs is not clear, it might relate to changes in the expression of key components in the respective signal-transduction pathways. For example, LPS and MDP upregulate the expression of NOD2 and MyD88, respectively[17,62]. In addition, the enhancing effects might result from synergistic activation of NF-κB or MAPKs by the NOD-protein- and TLR-signalling pathways. Although this synergism does not seem to apply to PGN–TLR2-mediated induction of IL-12 expression, presumably because (as discussed earlier) NOD2 has a specific negative effect on PGN–TLR2-mediated signalling through NF-κB, it might apply to the expression of other genes mediated by PGN and TLR2. Whatever the mechanisms that are involved, these findings emphasize that NOD2 exerts a complex effect on TLR-induced cytokine responses: it might be suppressive in some cases, but it is additive or synergistic in others.

Given the similarities between NOD1 and NOD2 both in structure and in activating ligand, it is reasonable to assume that NOD1 could also exert negative effects on TLR-induced cytokine production. However, recent reports show that activation of NOD1 by iE-DAP increases TLR4-induced IL-6 production by mouse macrophages and TLR2-, TLR4-, TLR5-, TLR7- or TLR9-induced CXCL8, TNF, IL-1β and IL-10 production by human APCs[14,57,63,64]. So, at present, there is no evidence that NOD1 has a negative-regulatory role in TLR-induced responses.

***NOD1 and NOD2 function in epithelial cells.*** IECs are generally hyporesponsive to extracellular bacterial products. Accordingly, they mount weak responses to the TLR2 and TLR4 ligands[65]. With respect to TLR4 signalling, this can be attributed, in part, to low cell-surface expression of TLR4 and its co-receptor MD2, because several IEC lines show NF-κB activation in response to LPS when they are transfected with constructs encoding these receptors[66,67]. With respect to TLR2 signalling, IECs also do not express cell-surface TLR2 (although they do express intracellular TLR2); in addition, they seem to have increased expression of Toll-interacting protein (TOLLIP), an inhibitor of a TLR-signalling-pathway component that is essential for signalling through TLR2 and TLR4 (REFS 68,69). This unresponsiveness of IECs to stimulation of TLRs might, however, disappear under inflammatory conditions, given the recent finding that IECs in mice that lack the capacity to mount TLR-induced responses because of MyD88 deficiency are more vulnerable to apoptosis during dextran-sulphate sodium salt (DSS)-induced colitis. So, it is possible that, during inflammation, the capacity to respond to TLR signals is 'upgraded', and this leads to the secretion of cytoprotective factors, such as IL-6 and CXCL1 (also known as KC1 or GROα), that preserve IEC integrity[70–72]. The unresponsiveness of IECs to TLR signals, at least in the absence of inflammation, sets the stage for the function of NOD proteins as important sensors for the detection of bacteria invading the epithelium. This view is supported by the observation that Caco-2 cells expressing a dominant-negative form of NOD1 fail to activate NF-κB and, consequently, have deficient CXCL8 production on infection with entero-invasive *Escherichia coli*[22]. In addition, microinjection of IECs with supernatants from *Shigella flexneri* (which contain NOD1 ligand) activates translocation of the NF-κB subunit p65 to the nucleus in IECs from wild-type mice but not in IECs from NOD1-deficient mice[15]. These data, together with recent data showing that NOD1-deficient mice are susceptible to infection with *H. pylori*[8], indicate that NOD1 signalling is an important component of the innate immune response of the gut epithelium to infection by pathogenic bacteria, and more specifically, these data show the capacity of the epithelium to secrete chemokines under these circumstances.

In contrast to NOD1, NOD2 seems to have little function as a bacterial sensor in most IECs. A potentially important exception to this rule, however, is that NOD2 is expressed by Paneth cells, which are the only cells other than APCs that strongly express NOD2 at the protein level[20,23]. Furthermore, evidence has emerged that the production of α-defensins, which are antimicrobial peptides produced exclusively by Paneth cells in the gut, depends on the activation of NOD2 (REFS 20,23). So, it seems that, in a subset of IECs at the base of the epithelial crypts, activation of NOD2 is associated with an innate antibacterial response.

## NOD proteins and disease

The importance of NOD1 and NOD2 in innate immunity has been highlighted by the findings that mutations affecting the function of these proteins are associated with the occurrence of disease[4,5,73–75] and that these proteins are important host-defence factors[8,76] (TABLE 1). For further details about diseases that are associated with pyrin-domain-containing members of the NLR family, see reviews by Inohara *et al.*[3,26]

***Crohn's Disease and mutations of* CARD15.** Several years ago, genetic studies of large cohorts of patients with Crohn's disease showed that homozygous mutations in *CARD15* (which encodes NOD2) accounts for 10–15% of patients with this disease[4,5]. These mutations result in the following amino-acid sequence changes: Arg702Trp, Gly908Arg and Leu1007fsinsCys (where fsinsCys denotes a frameshift as a result of insertion of a cysteine residue). These are all localized to the LRR domain of NOD2 and therefore interfere with its ability to recognize ligand. So, cells that carry such mutant forms of NOD2 have a reduced capacity to induce NF-κB activation on stimulation with MDP[13,39].

Our current understanding of how these mutations give rise to susceptibility to Crohn's disease is best discussed in the context of the general immunological abnormalities that underlie Crohn's disease in all patients

Dextran-sulphate-sodium-salt-induced colitis (DSS-induced colitis). An animal model of colitis induced by administration of DSS in the drinking water. DSS changes epithelial-cell barrier function and creates a condition in which antigen-presenting cells or epithelial cells in the gut are exposed to mucosal microflora.

Table 1 | **Autoimmune and infectious diseases associated with NOD1 and NOD2**

| Disease | Mutations* | Comments | References |
|---|---|---|---|
| *NOD1* | | | |
| *Helicobacter pylori* infection | No mutation | Delivery of PGN to epithelial cells through type IV secretion system | 8 |
| *Chlamydophila pneumoniae* infection | No mutation | Activation of NF-κB in endothelial cells | 76 |
| Inflammatory bowel disease | Deletion polymorphism in LRR domain | Risk factor for inflammatory bowel disease | 6 |
| Asthma and high IgE levels | Insertion polymorphism in LRR domain | Risk factor for asthma | 7 |
| *NOD2* | | | |
| Crohn's disease | Arg702Trp, Gly908Arg, Leu1007fsinsCys | Defective NF-κB activation in response to MDP | 13,16 |
| Blau syndrome | Arg334Trp, Arg334Gln, Leu469Phe | Constitutive NF-κB activation | 73 |
| Early-onset sarcoidosis | Arg334Trp, His496Leu, Thr605Pro | Constitutive NF-κB activation | 74 |
| Graft-versus-host disease | Arg702Trp, Gly908Arg, Leu1007fsinsCys | Risk factor for graft-versus-host disease | 75 |

*Mutations indicated are substitutions at the indicated amino-acid residue or are frameshifts as a result of insertion of a cysteine residue (fsinsCys). LRR, leucine-rich repeat; MDP, muramyl dipeptide; NF-κB, nuclear factor-κB; NOD, nucleotide-binding oligomerization domain; PGN, peptidoglycan.

(BOX 1). Briefly, Crohn's disease is a consequence of a disturbance in the normal immunological unresponsiveness of the mucosal immune system to components of the mucosal microflora. The hyper-responsiveness to these components that ensues gives rise to the $T_H1$-cell-mediated inflammation that underlies all forms of the disease.

Within this conceptual framework, three main views of how *CARD15* mutations are associated with Crohn's disease are being considered at present. The first is based on the possibility[36] that NOD2 normally functions as a negative regulator of IL-12 production mediated by PGN through TLR2 (discussed earlier), and in the absence of this regulation, PGN elicits an excessive NF-κB-dependent IL-12 response by APCs that drives the inflammation of Crohn's disease (FIG. 4a).

In this model, it is assumed that DCs and macrophages residing in the tissues of the mucosal immune system are normally exposed to PGN that is derived from commensal bacteria in the intestinal microflora. This finds ample support in recent studies showing that germ-free mice that are mono-associated with commensal organisms contain remnants of these organisms in Peyer's patches and mesenteric lymph nodes in the absence of inflammation or other causes of loss of epithelial integrity. It is also supported by studies showing that bacterial remnants are present in the lamina propria of the terminal part of the small intestine of normal mice[77,78]. It is postulated that, in normal individuals, such bacterial penetration 'sets the stage' for an innate immune response to PGN, but this immune response would be reasonably weak, owing to NOD2 modulation, and would therefore be contained by mucosal regulatory T cells. By contrast, in individuals with a *CARD15* mutation, the innate immune response to PGN is much stronger, and the resultant IL-12 and/or IL-23 production is sufficient to create a milieu that could support $T_H1$-cell-induced colitis. However, it is probable that, for such inflammation to occur, T cells that are specific for mucosal antigens are also required. This is indicated by studies showing that NOD2-deficient mice to which antigen-specific T cells from T-cell-receptor-transgenic mice are adoptively transferred are susceptible to $T_H1$-cell-induced colitis that is elicited by intestinal bacteria expressing the antigen recognized by the transferred T cells (T.W., A.K. and W.S., unpublished observations). So, in this model of the association between mutated NOD2 and colitis, the mutation leads to a dysregulated innate immune response that is mediated by a PGN-driven TLR2 response, which then facilitates a strong adaptive $T_H1$-cell response driven by various specific antigens in the mucosal environment.

Whether this explanation fits the results of studies of cytokine responses of patients with mutations is not yet clear, because these studies have not focused on the key $T_H1$ cytokine, IL-12p70 (possibly because the types of cell studied make it difficult to do so). However, in general, such studies have shown that patients with *CARD15* mutations have reduced cytokine responses (TNF and IL-10) to PGN[79] or reduced cytokine responses (TNF, CXCL8, IL-1β and IL-10) to several TLR ligands in the presence of MDP[57–61]. This indicates that MDP-mediated signalling through NOD2 can augment, rather than inhibit, TLR responses in normal individuals. These data do not provide an adequate explanation for the increased cytokine production that is characteristic of Crohn's disease, unless one were to discount the role of innate immunity in disease pathogenesis. A recent study of monocyte-derived DCs from patients with *CARD15* mutations shows that production of IL-12p70 in response to PGN is higher in patients with *CARD15* mutations than in individuals without mutations in *CARD15*, thereby supporting the regulatory role of NOD2 as the basic cause of disease[80].

The second model for the role of *CARD15* mutations in Crohn's disease is based on the role of NOD2 in mucosal host defence. In this view, the abnormality is located in IECs in that epithelial cells expressing mutated NOD2 have defective activation of NF-κB

Adherent invasive *E. coli*
A type of *Escherichia coli* that has the ability to adhere to intestinal epithelial cells, to invade epithelial cells through a mechanism involving actin polymerization and microtubules, and to survive and replicate within macrophages.

on stimulation with MDP[13], as well as a reduced capacity to restrict proliferation of *Salmonella enterica* serovar Typhimurium in monolayer cultures[19]. This type of abnormality could be a consequence, in part, of impaired NOD2-dependent α-defensin production by Paneth cells and could therefore lead to colonization of the intestine with bacteria that cause Crohn's disease[20,23,81] (FIG. 4b). This possibility is supported by recent evidence showing the role of α-defensins in host defence in the intestine: human α-defensin-5 has been shown to have antimicrobial activity against *E. coli*, *L. monocytogenes*, *S. typhimurium* and *Candida albicans in vitro*[82], and human α-defensin-5-transgenic mice are resistant to oral challenge with *S. typhimurium*[83]. In addition, although almost all patients with Crohn's disease have reduced expression of two α-defensins in the ileal mucosa, human α-defensin-5 and human α-defensin-6, this reduction is more pronounced in patients with Crohn's disease who have *CARD15* mutations[84]. In addition, it has been reported that Paneth cells in NOD2-deficient mice have defective production of mRNA encoding α-defensins. Also, whereas NOD2-deficient and wild-type mice are equally susceptible to infection of the liver with *L. monocytogenes* on systemic challenge with this bacterium, NOD2-deficient mice show increased infection of the liver on oral challenge with this bacterium[42]. Finally, as alluded to earlier, NOD2 interacts with GRIM19, a molecule that has been shown to have a role in protecting epithelial cells from bacterial invasion *in vitro*. So, it is possible that *CARD15* mutations might weaken host defence at the IEC interface by affecting GRIM19 function[41].

The above data supporting the view that *CARD15* mutations lead to susceptibility to Crohn's disease as a consequence of impaired host defence and α-defensin production are counterbalanced by several objections. First, the cited published data that relate to a specific defect in α-defensin production in patients with *CARD15* mutations are based on measurements of mRNA encoding defensins, which might not reflect the amounts of secreted polypeptide[84]. Perhaps more importantly, neither mice in which the Paneth-cell population has been ablated nor mice that are deficient in matrilysin (also known as MMP7), which lack mature cryptidins (corresponding to human α-defensins), develop evidence of acute or chronic inflammation under pathogen-free conditions[85,86]. Second, with regard to the bacterial overgrowth that one might expect to find in inflammation as a result of defective defensin secretion, the data as a whole are difficult to interpret, and one cannot say whether the abnormalities that are found are causes or effects. So, although there is evidence that increased numbers of normal (non-pathogenic) bacteria adhere to the intestinal mucosa of patients with Crohn's disease, increases are also seen in patients with other forms of mucosal inflammation and even in patients with cancer[87,88]. In addition, in two recent studies, increased numbers of 'adherent invasive *E. coli*', an organism with poorly defined pathogenicity, were found in patients with Crohn's disease[88,89]. However, the significance of this finding was mitigated by the fact that, in one study, the organism was found in many control tissues and, in the other study, in an equal number of healthy tissues from patients[88,89]. Last, the conclusions drawn from studies of NOD2-deficient mice that were administered *L. monocytogenes*[42] are uncertain, because this organism did not cause mucosal infection in the NOD2-deficient mice studied. Despite these objections, the idea that *CARD15* mutations cause a host-defence defect remains credible and is not mutually exclusive with the idea that these mutations act through loss of NOD2 regulation, because the putative increase in exposure to microflora that is brought about by a lack of α-defensins could aggravate the proposed increase in PGN-induced TLR2 responses.

Recently, evidence of a third explanation for the association between *CARD15* mutations and Crohn's disease has been reported (FIG. 4c). This evidence is derived from the analysis of knock-in mice expressing NOD2 that contains the frameshift mutation that is associated with Crohn's disease (Leu1007fsinsCys)[54], which theoretically mimics the genetic defect in Crohn's disease. It was found that, on stimulation with MDP, macrophages from these mice had increased IL-1β production[54]. These data, together with the finding that the knock-in mice were more susceptible to DSS-induced colitis, indicated that the frameshift mutation that is associated with Crohn's

Box 1 | **The immunopathogenesis of Crohn's disease**

Genetically, mutations in the caspase-recruitment domain 15 gene (*CARD15*; which encodes nucleotide-binding oligomerization-domain protein 2, NOD2) or *DLG5* (*Drosophila melanogaster* discs large homologue 5 gene)[93] are associated with the development of Crohn's disease.

Several types of evidence point to Crohn's disease arising from an inappropriate hyper-responsiveness to commensal organisms in the normal mucosal microflora rather than from a response to a specific pathogenic organism[94]. Perhaps the most persuasive is the observation that, in numerous mouse models of mucosal inflammation, regardless of the underlying cause, inflammation does not develop under germ-free conditions but does develop under specific-pathogen-free conditions[95]. Because antigens that are associated with the normal mucosal microflora are present for the life of an individual and have access to the internal milieu, they can be considered to be equivalent to self-antigens. So, the reactivity of patients to antigens in the mucosal microflora in Crohn's disease can be thought of as a type of autoimmunity. Finally, in any given individual with Crohn's disease, the autoimmune-like response is likely to be directed against a group of antigens from microflora rather than against any one antigen, although some antigens might be more frequently involved than others[96]. Invariably, however, the inflammation is driven by an interleukin-12 (IL-12)–IL-23-producing T-helper-1-cell response that is mediated by nuclear factor-κB[94].

The hyper-responsiveness of Crohn's disease constitutes a break in oral tolerance: that is, a break in the normal immunological state of unresponsiveness to antigens in the microflora and to those derived from food. So, the inflammation in Crohn's disease could arise from either an abnormally strong effector-cell response that overcomes normal tolerance mechanisms or a defect in tolerance mechanisms that leads to inflammation driven by a normal effector-cell response. Oral tolerance is a complex phenomenon that is partially mediated by regulatory T cells[95,97]. This implies that abnormalities in the function of these cells might be the cause of disease in some patients. However, evidence for this is mainly derived from the study of mouse models of mucosal inflammation[95,97].

The recognition that mutations in the gene that encodes NOD2 might result in Crohn's disease has strengthened the idea (evident from the study of mouse models of mucosal inflammation) that innate immune factors also have a role in disease pathogenesis.

Blau syndrome
A rare, autosomal dominant disorder that is characterized by granulomatous arthritis, uveitis, skin rash and cranial neuropathy.

disease is a gain-of-function mutation that results in disease associated with increased IL-1β (and perhaps IL-6) production. However, this model cannot explain *in vitro* data showing that epithelial cells transfected with *CARD15* that contains Crohn's-disease-associated mutations have defective NF-κB activation in response to stimulation with MDP[13,16] and, more importantly, it also cannot explain why peripheral-blood mononuclear cells isolated from patients with Crohn's disease that have a frameshift mutation in *CARD15* show a marked defect in IL-1β production, rather than increased IL-1β production[61,90]. Finally, these knock-in mice did not have any abnormality in the production of $T_H1$ cytokines, which is an almost universal finding in Crohn's disease. So, although this model is of interest as an experimental model of mucosal inflammation, these discrepancies cast doubt on the idea that it explains the association between NOD2 defects and Crohn's disease.

***Blau syndrome and mutations of* CARD15.** Mutations of *CARD15* that occur in the NOD, rather than in the LRR domain, lead to two granulomatous diseases: Blau syndrome and early-onset sarcoidosis[73,74]. Because activation of NOD2 involves oligomerization initiated by the NOD[33], mutations in this domain might decrease the threshold for spontaneous oligomerization of NOD2. This might explain the finding that epithelial cells expressing mutated forms of NOD2 that are associated with Blau syndrome

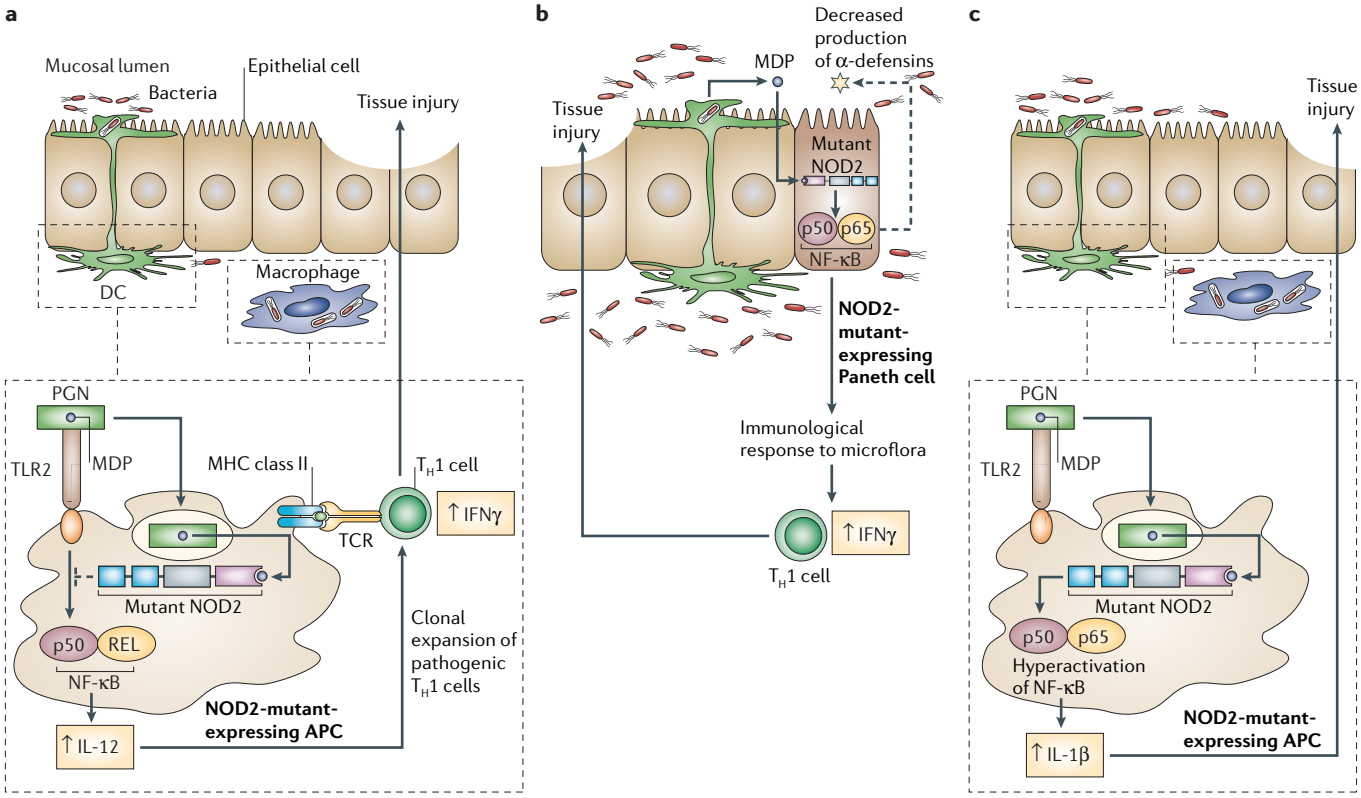


Figure 4 | **Possible mechanisms of Crohn's disease in patients with mutations in *CARD15*. a** | Peptidoglycan (PGN) derived from intestinal microflora signals to antigen-presenting cells (APCs) through Toll-like receptor 2 (TLR2) and (after digestion in dendritic cells (DCs) or macrophages to release muramyl dipeptide, MDP) through the NOD (nucleotide-binding oligomerization domain) protein NOD2, which is encoded by the caspase-recruitment domain 15 gene (*CARD15*). In mucosa in which there is normal expression of non-mutated NOD2, TLR2-induced activation of nuclear factor-κB (NF-κB) is negatively regulated by activation of NOD2; consequently, an excessive effector-cell response — that is, production of the T helper 1 ($T_H1$) cytokines interleukin-12 (IL-12) and interferon-γ (IFNγ) — that would support inflammation does not occur. By contrast, in mucosa that is deficient in *CARD15* (owing to the presence of mutations in *CARD15*), PGN-mediated NF-κB activation through TLR2 is not negatively regulated, and an effector-cell response that supports the $T_H1$-cell-mediated inflammation of Crohn's disease ensues. **b** | APCs residing in the lamina propria use DC dendrites that extend into the mucosal lumen to take up commensal bacteria and generate the NOD2 ligand, MDP. MDP that is generated by DCs diffuses into Paneth cells and activates NOD2, which then directly or indirectly induces secretion of antimicrobial peptides known as α-defensins. So, in mucosa in which NOD2 is not mutated, the commensal-bacteria population is downregulated, and no inflammation occurs. By contrast, in mucosa in which NOD2 is deficient, the lack of α-defensin production leads to a type of bacterial overgrowth that triggers the inflammatory response of Crohn's disease. This response necessarily involves inflammatory pathways other than those that depend on NOD2. **c** | In APCs with mutations in NOD2, MDP that is derived from PGN induces excessive IL-1β production rather than excessive IL-12 production. The result is a gain-of-function defect that mediates the inflammation of Crohn's disease. For a more extensive description of these mechanisms and an assessment of their validity, see the main text. TCR, T-cell receptor.

Early-onset sarcoidosis
A rare disease that usually manifests in children younger than 4 years of age. This disease is characterized by non-caseating epithelioid granulomas in the skin, joints and eyes. Unlike typical sarcoidosis seen in adults, pulmonary involvement is not observed.

and early-onset sarcoidosis show increased NF-κB activation, even in the absence of stimulation with MDP[74,91]. These are probably gain-of-function mutations that lead to excessive NOD2 signalling independent of MDP.

***Infectious disease and NOD-protein function.*** Evidence has recently been presented showing that infection with *H. pylori* leads to the activation of NOD1 in gastric epithelial cells and to the induction of host-defence factors, such as chemokines, through an as-yet-undefined signalling pathway[8]. Consistent with this, NOD1-deficient mice develop higher bacterial loads after infection with *H. pylori* than do wild-type mice. The delivery of PGN into the cells occurs by a type IV secretion system that is mediated by the *H. pylori* Cag pathogenicity island[8]. These findings indicate a general mechanism by which epithelial cells sense, and then react to, the presence of non-invasive organisms. In addition, they indicate that abnormalities in NOD1 function might lead to the development of chronic infection with *H. pylori* and to the subsequent development of peptic-ulcer disease and gastric carcinoma[92].

## Concluding remarks

The discovery that the NLR family of proteins constitutes a microbial-recognition system that is parallel to the TLR microbial-recognition system opens up a challenging new area of study of the innate immune response. As discussed here, it is evident that this system is largely independent of the TLR system and, as such, might positively or negatively modulate TLR responses. The full extent of such modulation, however, awaits a better definition of the range of bacterial components that can stimulate NLR responses. The study of this system has already yielded insights into the pathogenesis of human disease, and we can expect many such additional insights in the future.


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### Competing interests statement
The authors declare no competing financial interests.

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