ACTA ABP BIOCHIMICA POLONICA

Vol. 60, No 3/2013
351–360
on-line at: www.actabp.pl

Review

NOD1 and NOD2 receptors: integral members of the innate and adaptive immunity system

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NOD-like proteins (NLR) are a specialized group of intracellular receptors, which constitute an essential component of the host innate immune system. They were discovered more than a decade ago, but research on this particular class of microbial detectors is still ongoing to allow for a better understanding of the mechanisms, recognition of microorganisms, transmission of signals, and carrying out the activation of inflammatory signaling pathways. In this review, we discuss the construction of NOD1 and NOD2 receptors, their functions, and significance in the pathogenesis of inflammatory diseases in humans.

Key words: NOD1, NOD2, CARD, NF-κB

Received: 26 April, 2013; revised: 28 June, 2013; accepted: 23 July, 2013; available on-line: 31 July, 2013

INTRODUCTION

Response to impulses from the environment is possible through the presence of the PRR (pattern recognition receptors), which selectively recognize specific conserved structures known as pathogen associated molecular patterns (PAMPs) (Takeuchi *et al.,* 2010), as well as DAMPs (damage-associated molecular pattern molecules) that are released by stressed cells undergoing necrosis and can initiate and perpetuate immune response in the noninfectious inflammatory response (Foell *et al.*, 2007). These receptors constitute an integral component of the non-specific and specific immune system. PRR can be associated with the cell membrane, the endosome membrane, or they can be found in the cytoplasm. PRR include Toll-like receptors (TLR), lectin receptors C-type (CLR), RIG-I-like receptors (RLR), and NOD-like receptors (NLR). All of these play important roles in the response to microbial infection (Akira *et al.,* 2006; Takeuchi *et al.,* 2010; Kvarnhammar *et al.,* 2012). The structure of NLR proteins is unique and contains several domains, which are highly conserved (Inohara *et al.,* 2005; Martinon *et al.,* 2005). Their common feature is the presence of a centrally located, conservative nucleotide-binding oligomerization domain (NBD), responsible for oligomerization of the receptor, the C-terminal domain of leucine rich repeats (LRR), and the N-terminal effector domain (Fig. 1). The NLR receptor family contains 23 members, which are divided into five subfamilies, mainly due to the type of the N-terminal effector domain (Tig *et al.,* 2008).

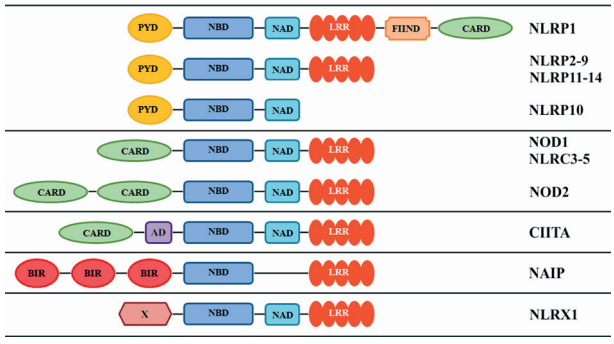

Figure 1. The Nod-like receptor subfamilies (Inohara *et al.*, 2002; Benko *et al.*, 2008; Carneiro *et al.*, 2008)

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Abbreviations: A20, ubiquitin modifying enzyme; AAMP, angio-associated migratory cell protein; Abl, Abelson tyrosine kinase; AP1, activator protein 1; ATG16L1, autophagy related 16-like 1; ATP, adnosine triphosphate; BS, Blau syndrome; CagA, cytotoxin-associated protein A; CARD, caspase-recruting domain; CARDIAK, CARD-containing ice-associated kinase; CARDIF, CARD adaptor inducting IFNB; CD, Crohn disease; CENTβ1, centaurin β1; CLR, lectin receptors C-type; D-Ala, D-alanine; DAMP, damage-associated molecular pattern molecules; D-Glu, D-glutamic acid; DUOX2, Dual oxidase 2 protein; EOS, early-onset sarcoidosis; GlcNAc, N-acetylglucosamine; GRIM19, gene associated with retinoic-interferon-induced mortality 19; GTPase Rac1, ras-related C3 botulinum toxin substrate 1 GTPase; IBD, inflammatory bowel disease; iEDAP, γ-D-glutamyl-meso-diaminopimelic acid; IFN, interferon; IKK, IκB kinase; IL, interleukin; IPAF, ice protease-activating factor; IPS-1, interferon-β promoter stimulator 1; IRF, interferon regulatory factor; ISRE, IFN-stimulated response elements; IκBα, inhibitor of κB; JNKBP1, c-Jun N-terminal kinase-binding protein; L-Ala, L-alanine; L-Lys, L-lysine; LRR, leucine rich repeats; MAPK, mitogen-activated kinases; MAVS, mitochondrial antiviral-signaling; MDA5, melanoma differentiation-associated gene 5; mDAP, meso-diaminopimelic acid; MDP, muramyl dipeptide; MHC II, major histocompatibility complex class II; MIP, macrophage inflammatory protein; MurNAc, N-acetylmuramic acid; MyD88, myeloid differentation primary response protein 88; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate oxidase; NBD, nucleotide-binding oligomerization domain; NEMO, NF-κB essential modulator; NF-κB, nuclear transctiption factor kappaB; NLR, NOD-like receptors; NLRC, NLR family CARD-containing; NMR, nuclear magnetic resonance spectroscopy; NOD, nucleotide-binding oligomerization domain-containing proteins; PAI, pathogenicity island; PAMP, pathogen associated molecular pattern; PG, peptidoglycan; PRR, pattern recognition receptors; RICK, RIP-like interacting clarp kinase; RIG-I, retinoic acid inducible gene I; RIP2, receptor-interacting protein 2; RLR, RIG-I-like receptors; RSV, respiratory syncytial virus; SFKs, src-family protein tyrosine kinases; siRNA, small interfering RNA; SPI, *Sallmonella* pathogenicity island; ssRNA, single-stranded RNA; T3SS, TTSS, type III secretion system; T4SS,TFSS, type IV secretion system; TAK1, transforming growth factor β-activated kinase1; TBK1, TANK binding kinase 1; TIRAP, TIR domain-containing adapter protein; TLR, toll-like receptor; TM, transmembrane domain; TNF-α, tumor necrosis factor alpha; TRAF, tumor necrosis factor receptor-associated factor; TRIF, TIR-domain-containing adapter-inducing interferon-β; TRIM27, T-cell receptor molecule interacting 27; Ub, ubiquitin; UC, ulcerative colitis; VISA, virus-induced signaling adapter.

NOD SUBFAMILY

The NOD (nucleotide-binding oligomerization domain-containing proteins) subfamily includes five proteins: NOD1, NOD2, NLRC3, NLRC4, NLRC5. NOD1, and NOD2 (gene products *CARD4* and *CARD15*, respectively). These are large cytoplasmic proteins, which are best known proteins in this group. The NOD1 receptor consists of 953 amino acids, its molecular weight is 97 kDa, while the molecular weight of NOD2 is 110 kD and it contains 1040 amino acids (Inohara *et al.,* 1999; McDonald *et al.,* 2005; Kufer *et al.,* 2008). The NOD1 N-terminal effector domain contains a CARD (caspase-recruting domain) domain, while NOD2 contains two CARD domains. CARD domain interacts with caspases involved in apoptosis and inflammation, including caspase 1. It was also revealed that the CARD-mediated caspase-independent interaction (Akira *et al.,* 2006; Chen *et al.,* 2009) is responsible for binding of signaling proteins and intracellular signal transduction. The central NBD domain is responsible for ATP-dependent regulation by oligomerization of the receptor, while the C-terminal domain for detection of ligands (microbial components) (Benko *et al.,* 2008; Magalhaes *et al.,* 2011). The NOD subfamily also contains the NLRC4 receptor, known as IPAF. It interacts with procaspase-1 through the CARD domain, and could be involved in the activation of caspase-1 in response to pro-inflammatory and apoptotic stimuli. In spite of similar construction to NOD1 and NOD2, this protein is not involved in the activation of the transcription nuclear factor kappaB (NF-$\varkappa$B) (Poyet *et al.,* 2001). This group also contains the NOD3 receptor, which inhibits the TLR-dependent activation of the NF-$\varkappa$B transcription factor (Schneider *et al.,* 2012).

Tissue localization of human NOD1 and NOD2 receptors

NOD1 receptor is common in many tissues, however the presence of NOD2 is confined only to leukocytes and epithelial cells (Ogura *et al.,* 2001; Uehara *et al.,* 2007; Correa *et al.,* 2012). The mature cells of peripheral blood demonstrated the highest level of NOD2 expression in monocytes, granulocytes, and dendritic cells (Gutierrez *et al.,* 2002), as well as low NOD2 expression in T lymphocytes (Girardin *et al.,* 2003). NOD1 and NOD2 expression was also demonstrated in peripheral B-type blood cells, while in tonsillar B cells only the expression of NOD1 was discovered (O'Neill *et al.,* 2011).

NOD1 and NOD2 ligands

NOD1 and NOD2 were shown to recognize fragments of the bacterial cell wall component called peptidoglycan (PG). PG is a glycol heteropolymer composed of a sugar chain consisting of glycan chains of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). These molecules are linked with β-1,4-glycosidic bonds. An integral part of PG are protein components, which contain unique amino acids such as the L-alanine (L-Ala), D-glutamic acid (D-Glu), meso-diaminopimelic acid (mDAP), L-lysine (L-Lys), and D-alanine (D-Ala) (Karaś *et al.*, 2006; Carneiro *et al.*, 2008; Bugla-Płoskońska *et al.*, 2008). An important differentiation feature is an amino acid located at the third position of the peptide chain. In Gram-positive bacteria it is the L-lysine (Lys-PG), while in the majority of Gram-negative bacteria it is the meso-diaminopimelic acid (DAP-PG) (Szilvia *et al.,* 2008). The NOD1 receptors recognize the PG moieties containing γ-D-glutamyl-meso-diaminopimelic acid (iEDAP), M-TetraDAP (MurNAc-L-Ala-D-Glu-mDAP-D-Ala), and M-TriDAP (MurNAc-L-Ala-D-Glu-mDAP), particularly present in Gram-negative bacteria and some Gram-positive bacteria, such as *Listeria monocytogenes* and *Bacillus spp.* A minimal structure recognized by NOD1 is dipeptide iEDAP (Girardin *et al.*, 2003; Chamaillard *et al.*, 2003; Carneiro *et al.*, 2008; Correa *et al.*, 2012). The NOD2 receptor detects and binds directly to muramyl dipeptide (MDP) (which consists of MurNAc, L-alanine, and D-glutamic acid) and Muramyl-TriLys (Szilvia *et al.*, 2008). These structures are present both in Gram-positive and Gram-negative bacteria (Fig. 2).

On the basis of the cytosolic localization, NOD1 and NOD2 receptors may respond primarily to: a/ bacteria, which evade being detected by TLR, b/ bacterial components delivered to the cytosol by the secretion systems or by created pores, c/ bacterial products, which are delivered by phagocytosis or pinocytosis. NOD1 and NOD2 receptors appear to recognize both invasive and non-invasive forms of the bacteria. In the latter case, stimulation of intracellular reconnaissance system of the NOD receptors is carried out by a functional bacterial system type III secretion (T3SS or TTSS) and IV (T4SS or TFSS) functioning in Gram-negative bacteria. Proteins involved in these processes are encoded in the genome of bacteria in regions called pathogenicity sites, which for T3SS are referred to as SPI-2, and cag PAI for the T4SS type (Wong *et al.,* 2004). The T3SS type allows to "inject" bacterial proteins directly into the cytoplasm of the host cell by passing the periplasmic space of external environment (Galan *et al.,* 2006). On the other hand, the T4SS type is a primary mechanism whereby bacteria secrete and absorb molecules of DNA or proteins. Its precise mechanism of action remains unknown. It has been shown that *Helicobacter pylori* uses T4SS to insert CagA proteins and peptidoglycan degradation products directly into intestinal epithelial cells from the bacterial cytosol (Hatakeyama *et al.,*

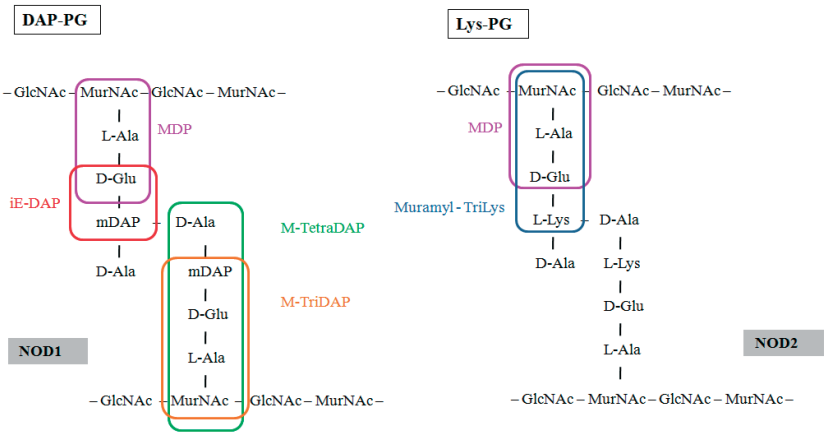

Figure 2. The structure of peptydoglycan (PG) motifs recognized by NOD1 and NOD2 (Benko *et al.*, 2008)

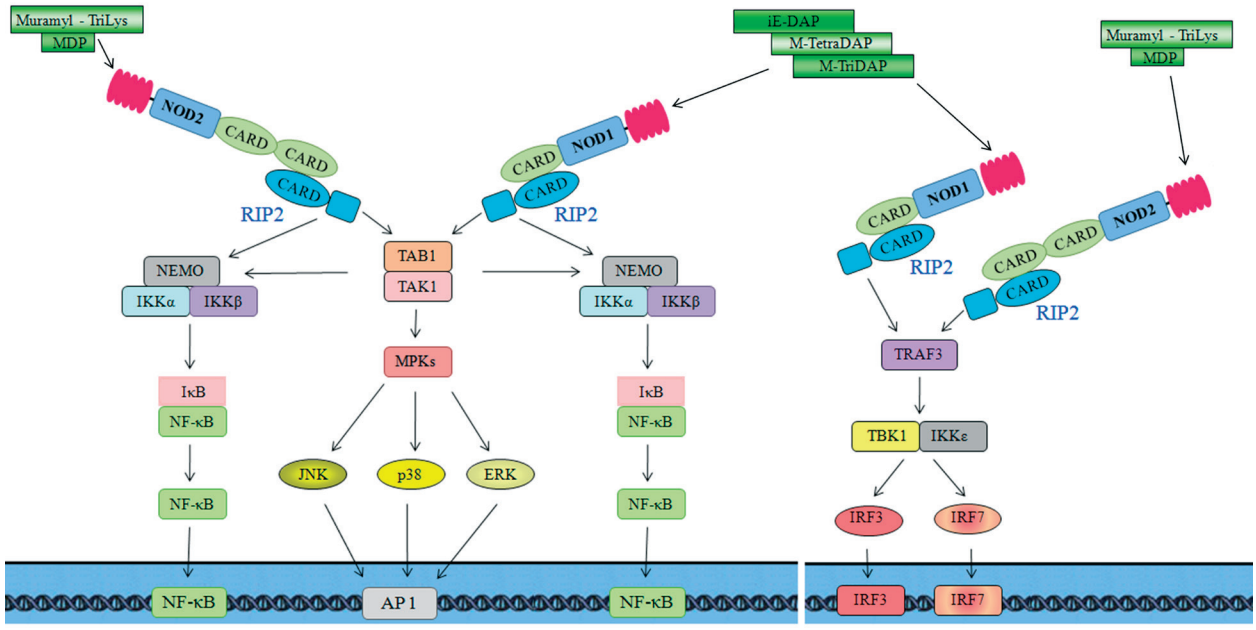

Figure 3. NOD1 and NOD2 signaling pathway (compiled by HA, MO)

2005; Kusters *et al.,* 2006). The CagA (cytotoxin-associated protein A) is a large (120-145 kDa) and highly immunogenic protein, encoded by the *cagA* gene, located within the pathogenicity site *cag PAI*. After an entry into host cells CagA is phosphorylated by two types of interacting cellular kinases: SFKs kinase (src-family protein tyrosine kinases) and Abl kinase (abelson tyrosine kinase). The CagA phosphorylation level determines the way of interaction with a variety of proteins in eukaryotic cell and induction of signaling pathways. Unchanged pathogenicity sites *cag* are required for proper NOD1signaling. *H. pylori* strains that do not have *cag* sites have reduced activation level of NF-κB (Viala *et al.,* 2004). Research of Boughan *et al.* (2006) and Viala *et al.* (2004) have proven the role of NOD1 in identifying these bacteria. They have revealed that NOD1-deficient mice exhibit an increased susceptibility to *H. pylori* infection, which was associated with disorders in production of an antimicrobial peptide: β-defensin. *In vitro* studies have shown that physical contact between host cells and living bacteria is not necessary. The NOD1 agonists can get into the cell via endocytosis (Girardin *et al.,* 2003) and phagocytosis (dead bacteria) (Opitz *et al.,* 2004). Bacterial secretion systems allow to create pores through pathogens, what enables them to leave the phagosome after phagocytosis and to transport bacterial products. For instance, listeriolysin O — a toxin creating pores, is an important virulence factor of *Listeria monocytogenes*. Lack of listeriolysin O completely excludes pathogenicity. It allows to deliver PG into the host cells and enables its recognition by NOD1 and NOD2 (Opitz *et al.,* 2006; Lenz *et al.,* 2006).

Signal transmission through NOD1 and NOD2

The main function of PRR includes the activation of inflammatory signaling pathways. In some aspects, NLR signaling is very similar to the extracellular receptor TLR stimulation. After the TLR membrane recognizes relevant PAMPs, the adapter proteins such as MyD88 (myeloid differentation primary response protein 88), TIRAP (TIR domain-containing adapter protein), and TRIF (TIR-domain-containing adapter-inducing interferon-β) become involved, and they lead to activate signaling pathways of mitogen-activated kinases (MAPK) and nuclear transcription factor (NF-κB), resulting in the induction of proinflammatory mediators and agents such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and IL-1β (Kawai & Akira 2006). Similarly, stimulation of intracellular NOD1 and NOD2 activates signaling pathways, whose main targets are: NF-κB, MAPK, and caspase-1. As a consequence of this activation, the corresponding genes transcription and inflammatory mediators production starts. Proper activation of the signaling pathway of the NOD1 and NOD2 receptor is tightly regulated by post-translational modifications such as phosphorylation and ubiquitination (Jiang *et al.,*2011; Tigno-Aranjuez *et al.,* 2012). Recognition of the agonists by NOD1 and NOD2 initiate their own oligomerization and exposure of the CARD domain for recruitment and activation of the RIP2 (Receptor-interacting protein 2) adapter protein, also known as RICK (RIP-like interacting clarp kinase) or CARDIAK (CARD-containing ice –associated kinase) (Inohara *et al.,* 2005; Nembrini *et al.,* 2009). The RIP protein family consists of seven members characterized by the presence of serine-threonine properties domain. The C-terminal RIP2 contains the CARD domain, similar to the CARD domain of the NOD1 and NOD2 receptor. Transmission of signals from receptors to RIP2 is possible through CARD-CARD interactions. The RIP2 protein is a strategic moment for the proper functioning of the signaling pathway (Park *et al.,* 2007). Active RIP2 kinase can directly lead to ubiquitination of NEMO (NF-κB essential modulator) and thus the activation of the IKK complex (IκB kinase). Active IKK complex phosphorylates the NF-κB inhibitor **(**IκBα- inhibitor of kappaB). Phosphorylated IκBα is degraded, and release free NF-κB occurs followed by its translocation into the nucleus. In the nucleus, NF-κB inducts the expression of over 200 genes including genes encoding proinflammatory cytokines, growth factors, factors responsible for stimulation of immune cells, and others (Burns *et al.,* 2004; Rahighi *et al.,* 2009; Jiang *et al.,* 2011). RIP2 also activates TAK1 kinase (transforming growth factor

β-activated kinase1), which can affect the IKK complex, and activate one of the three main families of MAP kinases: p38, JNK, and ERK. As a result of the pathway activation involving MAPK, a release of the AP1 transcription factor responsible for control of cell proliferation, cell differentiation, and apoptosis occurs (Karin *et al.,* 1995; Kobayashi *et al.,* 2005). NOD1 and NOD2 also activate other signaling pathways of not completely understood mechanisms, although the adapter proteins active in these pathways are well documented in TLR3, TLR7, TLR8, and TLR9 signaling pathways. Upon binding the bacterial ligand, NOD1 or NOD2 induce own-oligomerization and recruitment of RIP2, which binds TRAF3 (tumor necrosis factor receptor-associated factor 3). This protein activates the TBK1 kinase (TANK binding kinase 1) and IKKε (kinase inhibitor of nuclear factor kappaB), which phosphorylates and activates regulatory factors IRF3 and IRF7 (interferon regulatory factor 3, 7). Activated IRF3 and IRF7 form homodimers and/or heterodimers, they pass into the nucleus, where they bind with the molecules of ISRE (IFN-stimulated response elements) inducing the IFN gene expression of type I (Takeuchi & Akira 2009; McCartney *et al.,* 2009; Correa *et al.,* 2012). Type I interferons activate dendritic cells and NK cells. They are also responsible for carrying out the effector functions of B and T lymphocytes thus linking the innate and adaptive immune responses. The type I IFN releases subsequent mediators, which directly interact with viruses and inhibit their proliferation (Theofilopoulos *et al.,* 2005) (Fig. 3).

Antiviral RIP2-independent pathway

For nearly 10 years, NOD2 have been connected with the functioning of RIP2 in mediating the induction of NF-κB and pro-inflammatory responses induced by PG. Recent studies have shown that NOD2 has a wider biological activity including antiviral response through alternative way. It was demonstrated *in vitro* that after stimulation of NOD2 with viral ssRNA (single-stranded RNA) an activation of IRF3 occurs. This observation was confirmed *in vivo* in patients infected with respiratory syncytial virus (RSV) (Sabbah *et al.,* 2009). In mouse models, Sabbah *et al.* (2009) revealed that NOD2 (but not NOD1) binds with a virus ssRNA, then this complex translocates towards the mitochondria and activates the mitochondrial antiviral signaling protein MAVS (mitochondrial antiviral-signaling), also known as VISA (virus-induced signaling adapter), IPS-1 (interferon-β promoter stimulator 1), or CARDIF (CARD adaptor inducting IFNB). Complex MAVS–NOD2 is created through NBD and LRR domains. The NOD2 CARD domain is not essential for the interaction with MAVS. Two important functional domains, CARD and TM, are distinguished in the MAVS protein structure. The C-terminal transmembrane domain (TM) links with the outer mitochondrial membrane, while the N-terminal domain CARD interacts with the CARD of cytoplasmic RNA helicases that recognize viral infections such as RIG-I (retinoic acid inducible gene I) and MDA5 (melanoma differentiation-associated gene 5) (Yoneyama *et al.,* 2004; Kawai *et al.,* 2005; Fritz *et al.,* 2006). As a consequence of this interaction, MAVS binds to IKKα/β kinases (activating NF-κB). The interaction of TBK1 and IKKε results in the activation of IRF3. Coordinated activation of signaling pathways of NF-κB and IRF3 leads to the creation of a multiprotein reinforcement complex that accelerates the expression of interferon-β (IFN-β) and hence the antiviral resistance (Sun *et al.,* 2006; Qi *et al.,*

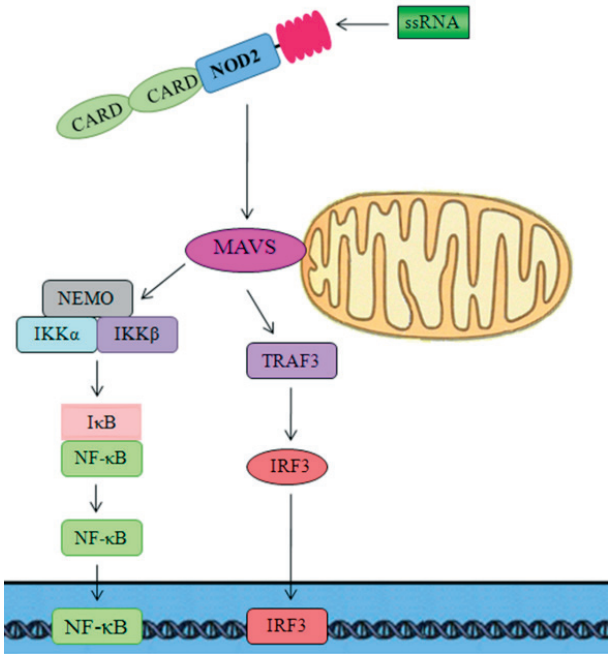

Figure 4. ssRNA virus-induced activation of NOD2 (compiled by HA, MO)

2007; Lecat *et al.,* 2010; Ting *et al.,* 2010). It has been shown that MAVS also connects directly to TRAF3 but its exact role in this pathway and the IKK complex activation is not well studied (Correa *et al.,* 2012). However, many viruses have developed strategies that interfere and derange these innate signaling events thus inhibiting the IFN-β production. In this mechanism, an activated serine protease cleaves the TM domain from the MAVS molecule *via* proteolytic degradation, thereby disconnecting the MAVS protein from the mitochondria and causing its dysfunction (Correa *et al.,* 2012) (Fig. 4).

Other functions of NOD1 and NOD2 in the processes of pathogens elimination

The NOD1 and NOD2 receptors are involved in the process of autophagy (autophagocytosis), what has been proven in plants and animals including mammals. (Travassos *et al.,* 2010). This process involving NOD1 and NOD2 is based on their cooperation with the ATG16L1 (autophagy related 16-like 1) protein, a component of the protein complex acting as a regulator of this mechanism (Homer *et al.,* 2010). This mechanism is independent from the adapter protein RIP2 and transcription factor NF-κB (Travassos *et al.,* 2010). In ATG16L deficient cells, both creation of autophagosomes and degradation of long-lived proteins are seriously impaired (Saitoh *et al.,* 2008). The NOD and ATG16L receptors are essential for induction of autophagy in intestinal epithelial cells after pathogen stimulation (Ravikumar *et al.,* 2004). During the bacterial infection both associated proteins move toward the cell membrane and participate in the formation of autophagosome follicles around the bacteria (Wickner, 2002). Autophagosome and lysosome fusion leads to degradation of bacteria followed by the presentation of bacterial antigenic determinants to T cells, in the context of the MHC class II antigens. The role of NOD2 in the induction of autophagy is confirmed in studies revealing that NOD2 mutation prevents the induction of autophagy (Shaw *et al.,* 2011).

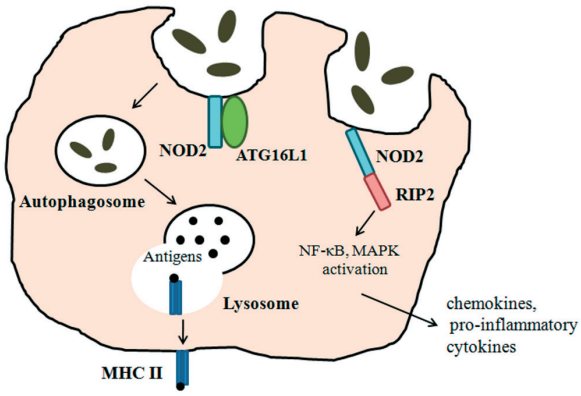

Figure 5. Autofagocytosis activation mediated by NOD2 (Shaw *et al.*, 2011) — modified

The most recent reports indicate that the control of signal transduction pathways through NOD1 and NOD2 is also held *via* CARD and ubiquitin (Ub) (Ver Heul *et al.,* 2013).

A small hydrophobic surface of CARD that binds Ub was identified by nuclear magnetic resonance spectroscopy (NMR) and structure-guided mutagenesis. *In vivo* studies with the CARD domain mutated in Ub binding site have demonstrated the inability of Ub connection, while retaining the ability to bind RIP2 and ATG16L. Such mutation leads to a significant hyperactivity of the NOD1 and NOD2 receptors after ligand-stimulation. These data suggest that Ub-binding provides a negative-feedback loop of the NOD-dependent activation of RIP2.

NOD1 and NOD2 are also involved in the mechanism of reactive oxygen species formation (ROS) - an essential part of the antimicrobial response (Tan *et al.,* 2012). ROS directly activates the DUOX2 protein (dual oxidase 2 protein). DUOX2 is a protein subunit, one of the isoforms of the NADPH oxidase (reduced form of nicotinamide adenine dinucleotide phosphate oxidase), a key enzyme involved in the first line of defense against pathogens. Hydrogen peroxide, which has antibacterial properties, is released in phagocytes via the DUOX2 protein during an infection (Lipinski *et al.,* 2009; Kersse *et al.,* 2011). A higher expression level of DOUX2 was proven in inflamed cells when compared to the normal tissue (Ha *et al.,* 2005; Lipinski *et al.,* 2009).

Biological response to NOD1 and NOD2 signaling

The consequence of NF-κB and MAPK activation mediated by NOD1 and NOD2 is triggering of transcription and production of inflammatory mediators. Inflammatory cytokines IL-1β, IL-6, TNFα, IL-8, IL-18, IL-12p40, IL-12p70 (Carneiro *et al.,* 2008; Kersse *et al.,* 2011), oxide nitrogen (Tötemeyer *et al.,* 2006; Carneiro *et al.,* 2008), and cell adhesion molecules (Carneiro *et al.,* 2008) are produced in the dendritic cells, lymphocytes, monocytes and macrophages. The epithelial cells, through the activation of the NOD1 and NOD2 receptors, produce pro-inflammatory mediators such as TNF, IL-6, IL-8, MIP2 (macrophage inflammatory protein 2), and antimicrobial peptides (e.g. β-defensin2) (Carneiro *et al.,* 2008; Kersse *et al.,* 2011), what significantly expedites fighting pathogens. NOD1 stimulation in epithelial and mesothelial cells induces chemokine production and recruitment of immune effector cells, including neutrophils *in vivo* (Park *et al.,* 2007). *In vitro* studies have revealed that overexpression of NOD1 and NOD2 may induce apoptosis (Ogura *et al.,* 2001). These pathways are not completely elucidated, although it has been shown that in the case of NOD1 the process involves caspase-8 and caspase-9 and requires the RIP2 protein (Windheim *et al.,* 2007).

Moreover, obtained results suggest that NOD1 and NOD2 stimulation induces an increased expression of a major histocompatibility complex class II (MHC II) proteins on the surface of antigen presenting cells (macrophages, dendritic cells, B lymphocytes) (Homer *et al.,* 2010; Travaassos *et al.,* 2010; Shaw *et al.,* 2011), what indicates that these receptors are essential in coordinating the adaptive immune response (Fritz *et al.,* 2007).

Another factor indicating the role of NOD2 in this type of response is an observation that NOD2 agonist (muramyl dipeptide) may function as an effective adjuvant for antigen-specific T-cell response and antibody production by B lymphocytes (Sugimoto *et al.,* 1978).

NOD1 and NOD2 signaling regulation

It has been shown that NOD1 and NOD2 interact with different intracellular molecules that may positively or negatively regulate their signaling pathway (Table 1). The most significant inhibitory effect on NOD2 is caused by the Erbin protein. This protein is combined with intact CARD and LRR NOD2 domains inhibiting the receptor's function. In case of the NOD2 LRR domain's damage, the function of Erbin is not observed (McDonald *et al.,* 2005). Yamamoto-Furusho *et al.* (2006), using siRNA against CENTβ1 (centaurin β1), confirmed the theory that CENTβ1 overexpression inhibits the activation of NOD2-dependent NF-κB, which places them in a group of the NOD1 and NOD2 inhibitory proteins. Another inhibitory protein is the AAMP (angio-associated migratory cell protein), because of the involvement of this protein in angiogenesis. AAMP combines with the CARD domain of the NOD2 receptor preventing the formation of a NOD2–RIP2 complex (Bielig *et al.,* 2009). GTPase Rac1 (ras-related C3 botulinum toxin substrate 1 GTPase) negatively affects the NOD2-dependent release of IL-8 (Eitel *et al.,* 2008). Another negative regulator of NOD2 was described by Zurek *et al.* (2012) RIG-I (retinoic acid-inducible gene 1). This cytoplasmic receptor protein, which recognizes viruses through its CARD domain, can bind to different regions of the NOD2, inhibiting the release of NF-κB (Morosky *et al.,* 2011). Another identified inhibitor is the JNKBP1 (c-Jun N-terminal kinase-binding protein) cytoskeleton protein, characterized by the presence of the WD-40 domain, via which it combines with the NOD2 receptor following the MDP activation. JNKBP1-NOD2 complex interferes with the oligomerization of NOD2, prevents the activation of NF-κB, secretion of IL-8, and antibacterial response (Lecat *et al.,* 2012). The recently identified inhibitor TRIM27 (T-cell receptor molecule interacting 27), which interacts with NOD2 via the NBD domain, inhibits the pro-inflammatory response. It was noted that the increased expression of TRIM27 occurs in patients with Crohn's disease (Zurek *et al.,* 2012). A negative impact on the mechanisms of innate immunity also appears in the TRAF4 protein (TNF receptor-associated factor 4), as a sole representative of the TRAF's family. By binding to NOD2, it inhibits the activation of NF-κB (Marinis *et al.,* 2012).

The proteins, whose mechanism of action is poorly understood, stimulating the function of NOD2 have also been identified (Table 1). It was stated that GRIM19

Table 1. Proteins involved in the regulation of the NOD1 and NOD2 receptors (compiled by HA, MO)

Molecule	NOD1		NOD2		Reference
	Positive	Negative	Positive	Negative	
Erbin				+	McDonald *et al.* (2005)
CENTβ1		+		+	Yamamoto-Furusho *et al.* (2006)
Rac1GTPase				+	Eitel *et al.* (2008)
RIG-1				+	Morosky *et al.* (2011)
JNKBP1				+	Lecat *et al.* (2012)
AAMP				+	Bielig *et al.* (2009)
TRIM27				+	Zurek *et al.* (2012)
TRAF4				+	Marinis *et al.* (2012)
GRIM19			+		Barnich *et al.* (2005)
CARD9			+		Hsu *et al.* (2007)
Vimentin			+		Stevens *et al.* (2012)

(Barnich *et al.,* 2005), CARD9 (Hsu *et al.,* 2007), and Vimentin (Stevens *et al.,* 2012) are required for the activation of NF-κB through NOD2.

MUTATIONS IN THE NOD1 AND NOD2 GENES AS A CAUSE OF DISEASES IN HUMANS

The physiological role of various NLR is to defend the host against bacterial infections. More often however, the role of these receptors is attributed to maintaining organ homeostasis. This is substantiated by the fact that many non-inflammatory disease processes originate in NLR defective signaling, caused by mutations within its genes**.** Dysregulation of NLR function has been described in a variety of disorders, including chronic inflammations, autoimmunity and cancer predisposition (Carneiro *et al.,* 2008; Davis *et al.,* 2011). It was revealed that disruption of the NOD1 and NOD2 function caused by its gene mutations may contribute to the pathogenesis of inflammatory bowel diseases (Boughan *et al.,* 2006; Uehara *et al.,* 2007; Zilbauer *et al.,* 2007). Still little is known about the mechanism of regulation in different diseases. So far, mutations and polymorphisms within the NOD1 and NOD2 receptors have been identified, however, the importance of the level of ubiquitination and phosphorylation remains to be established. It has only been proven that mutations in the NOD2 receptor and A20 (ubiquitin modifying enzyme) associated with Crohn's disease can affect the condition of ubiquitination. An increased level of RIP2 ubiquitination was observed in the A20 null cells. After activating NOD2, NEMO also undergoes ubiquitination and thus activates IKK. It is interesting that in Crohn's disease, where mutations of NOD2 are ascertained, NEMO ubiquitination and NF-κB signaling is inhibited, which further emphasizes the significance of proper regulation of ubiquitination in NOD2 signaling pathways (Wertz & Dixit, 2010, Chen *et al.,* 2009).

Inflammatory bowel disease and Leśniowski-Crohn disease

IBD includes two different inflammatory conditions such as ulcerative colitis (UC) and Leśniowski-Crohn disease, often called Crohn's disease only (CD). Despite similar clinical symptoms, UC and CD are distinguished by clinical and histological features. UC is a chronic inflammation confined to the rectum or colon mucous membrane, whereas CD most commonly affects the distal part of the terminal ileum and the beginning of the colon. Inappropriate immune responses and impaired epithelial barrier functions appear to be engaged in the pathogenesis of CD. Performed studies indicate that both environmental and genetic factors contribute to its etiology. Among families affected by CD, eight loci (*IBD1-IBD8*) were identified, which were suspected to cause the disease (Brant & Shugart, 2004). Through genetic and functional studies, the risk region of *IBD1* has been identified and proven to be the *CARD15/NOD2* gene (Ogura *et al.,* 2001; Hugot *et al.,* 2001; Fernandez *et al.,* 2004). A considerable number of polymorphisms within the *CARD15/NOD2* gene has been revealed but as so far three of them appear to be significantly associated with susceptibility to the disease. These are known as R702W (Arg702Trp), G908R (Gly908Arg), and L1007fsinsC polymorphisms. Arg-702Trp and Gly908Arg mutations are single amino acid changes within the LRR domain, whereas the L1007fsinsC mutation is caused by a deletion causing reading frame shift leading to the loss of 33 amino acids (Lesage *et al.,* 2002). In case of homozygotes the risk of developing CD presents a 40-fold increase, whereas in case of heterozygotes only a 2–4-fold increase.

In homozygotes, the disease has proceeded asymptomatically for 10–15 years, which implies that the development of the disease is also affected by other genetic factors and environmental influences apart from *CARD15* gene mutations (Eckmann *et al.,* 2005; Carneiro *et al.,* 2008). There are reports indicating that type of mutation determines the location of CD and disease behavior. Therefore, L1007fs homozygotes develop the gastroduodenal involvement and also have increased risk for early-onset of CD (Mardini *et al.,* 2005). CD-associated mutations result in loss of phenotype functions. Inohara *et al.* (2003) showed that disease-associated mutations lead to diminished NF-κB activation upon MDP stimulation, which is consistent with the finding that mutations occur near or within the LRR NOD2 domain (Ogura *et al.,* 2001; Hugot *et al.,* 2001). Furthermore, it was found that monocytes isolated from CD patients harboring the L1007fsinsC mutation produce less of pro-inflammatory cytokines such as TNF-α, IL-6, IL-8, as well as anti-inflammatory cytokine IL-10 (Netea *et al.,* 2004; Van Heel *et al.,* 2005). An impaired production of IL-1β was observed in MDP-induced monocytes from a patient with NOD2 mutation (Inohara *et al.,* 2003; Van Heel *et al.,* 2005). In addition, CD patients with mutant NOD2 have reduced expression of two α-defensins: α-defensin-5 and α-defensin-6 in the ileal mucosa (Wehkamp *et al.,* 2004).

Due to similarity between the NOD1 and NOD2 structure and signaling, appropriate tests were performed to determine whether NOD1 is associated with susceptibility to inflammatory bowel disease (IBD). The results of different research groups are contradictory. The research of Molnar *et al.* (2007) and McGovern *et al.* (2007) confirmed that relationship. However, there are reports that deny such connection (Van Limbergen *et al.,* 2007). However, each of these studies concerned different populations, so it shall not be excluded that the incidence of CD depends not only on NOD1 polymorphism, but also on the population characteristics.

In turn, the studies of Travassos *et al.* (2010) prove the existence of functional connection between NOD1, NOD2, ATG16L1, the autophagy induction and Crohn's disease.

Blau Syndrome

Blau syndrome (BS) is a rare, inflammatory disorder inherited in an autosomal dominant pattern associated with the defect in the *CARD15* gene. Blau syndrome is characterized by early-onset granulomatous dermatitis causing persistent rash and skin granulomas, granulomatous arthritis (inflammation of the synovium), and uveitis (Blau, 1985). The NOD2 gene mutations specifically in the NBD domain such as 334Q, R334W, and L469F are known to cause Blau syndrome (Miceli-Richard *et al.,* 2001). NOD2 activation requires oligomerization initiated by the NBD domain, so mutations within this domain may disturb this process. These mutations also lead to the enhancement of functioning and constitutive activation of NF-κB, even in the absence of MDP stimulation (Chamaillard *et al.,* 2003; Tanabe *et al.,* 2004). These observations gave the origin to the hypothesis that Blau syndrome is an autoimmune disease. It has not been studied yet whether mutations in the *CARD15* gene have an impact on the course of Blau syndrome, and whether there is a correlation between the mutation and disease phenotype (Sfriso *et al.,* 2012).

Sarcoidosis

Sarcoidosis is an autoimmune disorder of unknown etiology characterized as a systemic granulomatous disease. Sarcoid granulomas are most frequently located in the lungs and the lymph nodes. This disease may affect any organ though. However, studies indicate an increased susceptibility to this disease in patients with a genetic variant of NOD1, which has a significantly reduced expression and causes impaired activation of NF-κB in response to both iEDAP and *Propionibacterium acnes* infection (Tanabe *et al.,* 2006). No correlation between NOD2 mutations and adult sarcoidosis was observed (Ho *et al.,* 2005).

NOD2 mutations have been shown by Kanazawa *et al.* (2005) to predispose to early-onset sarcoidosis (EOS). EOS differs from adult sarcoidosis aside from young age at onset, its symptoms are similar to those observed in Blau syndrome. EOS is characterized by a classic symptom triad consisting of skin, eye, and joint lesions, with pulmonary involvement on rare occasions (Cancrini *et al.,* 1998).

In a small study group of 10 Japanese patients diagnosed with EOS, nine had heterozygous missense mutations in the NBD domain of CARD15. In total, six different variants have been identified, five of which were identified as the new forms. Only one variant was identical to that reported in Blau syndrome. Regardless of the NOD2 variants associated with EOS, a basic activity increase of NF-κB was shown as compared with the wild type. These results correspond to those in Blau syndrome (Kanazawa *et al.,* 2005).

Allergic diseases

Allergies are associated with increased reactivity to antigens, IgE overproduction, and therefore are inflammatory in nature. Bacterial infection and recognition of intracellular, microbiological, products by NOD1 may constitute a cause of many cases of allergies.

It has been revealed that certain genetic NOD1 variants are associated with an increased risk of developing asthma and dermatitis atopia (Hysi *et al.,* 2005). The NOD1 polymorphism may modify the protective effect of early detection of allergens (Eder *et al.,* 2006).

The NOD1 and NOD2 receptors in the process of carcinogenesis

Reports concerning the importance of NLR in neoplasia or possible cancer prevention are very limited and mostly refer to *in vitro* experiments. However, a tumor-suppressor function of NOD1 has been suggested in breast cancer xenograft models by da Silva Correia *et al.* (2006). Results of TLR signaling studies suggest a potential connection between NLR, and neoplasia should be considered (Rakoff-Nahoum *et al.,* 2007; Fukata *et al.,* 2007).

The search for a relationship between the NOD1 and NOD2 receptors activation and tumor development has begun, particularly in the gastrointestinal tract,which is rich in microorganisms. That aspect was taken into inconsideration because of the increased incidence of colorectal cancer in CD patients with 3020InsC, R702W, and G908R polymorphisms within the struc-

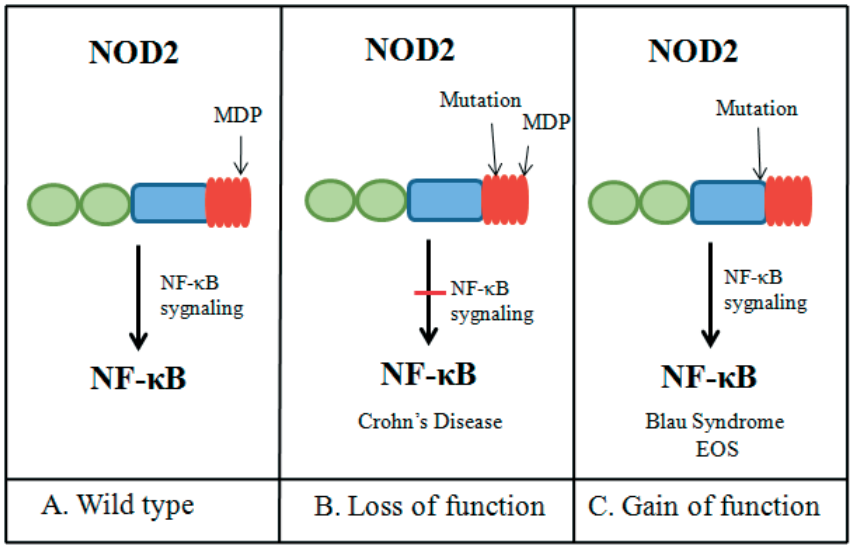

Figure 6. Diseases caused by changes within the NOD2 receptor (Jeanette et al., 2008) — modified

ture of NOD2. Kurzawski and colleagues have reported as the first, a relationship between the NOD2 3020insC variant and colorectal carcinoma in Polish population (Kurzawski *et al.,* 2004; Papaconstantinou *et al.,* 2005).

Polymorphisms in human NOD1 and NOD2 genes correlated with increased cancer risk. An increased risk of gastric cancer has been described in *Helicobacter pylori* carriers who also showed 802C-T polymorphism in NOD2, however the studies conducted on larger population have not confirmed this dependency (Hnatyszyn *et al.,* 2010). An increased incidence of colon and rectum tumors was demonstrated in mouse NOD1-/- models, suggesting the importance of the NOD1 receptor in the pathogenesis of cancer (Chen *et al.,* 2008). However, spontaneous growth of tumors has not been observed in NOD1-deficient mice.

CONCLUSIONS

Identification of the NLR, as well as initial characterization and understanding of their functions has enabled a wider outlook on pathogen detection and eradication mechanisms as well as host immune system responses. The involvement of NLRs in the pathogenesis of several genetic diseases indicates that these proteins play an important role in the regulation of immune and inflammatory responses. Multicenter studies have enabled researchers to obtain the evidence that the NOD1 and NOD2 receptors, like other members of the NLR family, recognize conserved microbial molecules and trigger signal transduction pathways leading to the activation of kinases, caspases, and transcription factors. Regulation of gene expression under the control of NF-*κ*B, AP1, and IRF leads in turn to stimulation of the genes responsible for the production of pro-inflammatory cytokines, chemokines, and interferons. It has been proven that NOD1 and NOD2 are involved in the process of autophagy, in the mechanism of creating the reactive oxygen species, and also in the activation of the antiviral response after NOD2 is activated by the viral ssRNA. An interaction between NLR and TLR has been demonstrated as a response against pathogens. The complete operating range of cellular mechanisms, which are responsible for regulating the activity of NOD1 and NOD2, has not been established yet. Many questions remain unanswered, including the understanding of the processes via which mutations in the NLR could be associated with the vulnerability not only to inflammation, but also cancer. This knowledge would allow for a rational therapy of diseases in general.

Acknowledgemets

This study was supported by grants from the Medical University of Lublin: DS 221/13.

The paper was developed using the equipment purchased within "The equipment of innovative laboratories doing research on new medicines used in the therapy of civilization and neoplastic diseases" Project of the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

REFERENCES

Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* **124**: 783–801.

Barnich N, Hisamatsu T, Aguirre JE, Xavier R, Reinecker HC, Podolsky DK (2005) GRIM-19 interacts with nucleotide oligomerization domain 2 and serves as downstream effector of antibacterial function in intestinal epithelial cells. *J Biol Chem* **280**: 19021–19026.

Benko S, Philpott DJ, Girardin SE (2008) The microbial and danger signals that activate Nod-like receptors. *Cyokine* **43**: 368–373.

Bielig H, Zurek B, Kutsch A, Menning M, Philpott DJ, Sansonetti PJ, Kufer TA (2009) A function for AAMP in Nod2-mediated NF-kappaB activation. *Mol Immunol* **46**: 2647–2654.

Blau EB (1985) Familial granulomatous arthritis, iritis, and rash. *J Pediatr* **107**: 689–693.

Boughan PK, Argent RH, Body-Malapel M, Park JH, Ewings KE, Bowie AG, Ong SJ, Cook SJ, Sorensen OE, Manzo BA, Inohara N, Klein NJ, Nuñez G, Atherton JC, Bajaj-Elliott M (2006) Nucleotide-binding oligomerization domain-1 and epidermal growth factor receptor: critical regulators of β-defensins during Helicobacter pylori infection. *J Biol Chem* **281**: 11637–11648.

Brant SR, Shugart YY (2004) Inflammatory bowel disease gene hunting by linkage analysis: rationale, methodology, and present status of the field. *Inflamm Bowel Dis* **10**: 300–311.

Bugla- Płoskońska G (2008) Organizacja struktur zewnętrznych bakterii Gram-ujemnych a proces aktywacji układu immunologicznego. *Post Mikrobiol* **47**: 191–197.

Burns KA, Martinon F (2004) Inflammatory diseases: is ubiquitinated NEMO at the hub? *Curr Biol* **14**: 1040–1042.

Cancrini C, Angelini F, Colavita M, Cortis E, Chini L, Mammone F, Rossi P, De Sanctis R (1998) Erythema nodosum: a presenting sign of early onset sarcoidosis. *Clin Exp Rheumatol* 16: 337–339.

Carneiro LA, Magalhaes JG, Tattoli I, Philpott DJ, Travassos LH (2008) Nod-like proteins in inflammation and disease. *J Pathol* **214**: 136–148.

Chamaillard M, Hashimoto M, Horie Y, Masumoto J, Qiu S, Saab L, Ogura Y, Kawasaki A, Fukase K, Kusumoto S, Valvano MA, Foster SJ, Mak TW, Nuñez G, Inohara N (2003) An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nat Immunol* **4**: 702–707.

Chamaillard M, Philpott D, Girardin SE, Zouali H, Lesage S, Chareyre F, Bui TH, Giovannini M, Zaehringer U, Penard-Lacronique V, Sansonetti PJ, Hugot JP, Thomas G (2003) Gene-environment interaction modulated by allelic heterogeneity in inflammatory diseases. *Proc Natl Acad Sci USA* **100**: 3455–3460.

Chen GY, Shaw MH, Kim YG, Nuñez G (2009) Nod-like receptors: role in innate immunity and inflammatory disease. *Annu Rev Pathol* **4**: 365–398.

Chen GY, Shaw MH, Redondo G, Nuñez G (2008) The innate immune receptor Nod1 protects the intestine from inflammation-induced tumorigenesis. *Cancer Res* **68**: 10060–10067.

Chen ZJ, Sun LJ (2009) Nonproteolytic of ubiquitin in cell signaling. *Mol Cell* **33**: 275–286.

Correa RG, Milutinovic S, Reed JC (2012) Roles of NOD1 (NLRC1) and NOD2 (NLRC2) in innate immunity and inflammatory diseases. *Biosci Rep* **32**: 597–608.

da Silva Correia J, Miranda Y, Austin-Brown N, Hsu J, Mathison J, Xiang R, Zhou H, Li Q, Han J, Ulevitch RJ (2006) Nod1-dependent control of tumor growth. *Proc Natl Acad Sci USA* **103**: 1840–1845.

Davis BK, Wen H, Ting, JP (2011) The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu Rev Immunol* **29**: 707–735.

Eckmann L, Karin M (2005) NOD2 and Crohn's Disease: Loss or Gain of Function? *Immunity* **22**: 661–667.

Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrländer C, Nowak D, Holst O, Martinez FD (2006) Association between exposure to farming, allergies and genetic variation in CARD4/NOD1. *Allergy* **61**: 1117–1124.

Eitel J, Krüll M, Hocke AC, N'Guessan PD, Zahlten J, Schmeck B, Slevogt H, Hippenstiel S, Suttorp N, Opitz B (2008) Beta-PIX and Rac1 GTPase mediate traffi cking and negative regulation of NOD2. *J Immunol* **181**: 2664–2671.

Fernandez L, Mendoza JL, Martinez A, Urcelay E, Fernandez-Arquero M, Garcia-Paredes J, Peña AS, Diaz-Rubio M, de la Concha EG (2004) IBD1 and IBD3 determine location of Crohn's disease in the Spanish population. *Inflamm Bowel Dis* **10**: 715–722.

Foell D, Wittkowski H, Roth J (2007) Mechanisms of disease: a 'DAMP' view of inflammatory arthritis. *Nat Clin Pract Rheumatol* 3: 382–390.

Fritz JH, Ferrero RL, Philpott DJ, Girardin SE (2006) Nod-like proteins in immunity, inflammation and disease. *Nat Immunol* **7**: 1250–1257.

Fritz JH, Le Bourhis L, Sellge G, Magalhaes JG, Fsihi H, Kufer TA, Collins C, Viala J, Ferrero RL, Girardin SE, Philpott DJ (2007) Nod1-mediated innate immune recognition of peptidoglycan contributes to the onset of adaptive immunity. *Immunity* **26**: 445–459.

Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, Hsu D, Xu R, Harpaz N, Dannenberg AJ, Subbaramaiah K, Cooper HS, Itzkowitz SH, Abreu MT (2007) Toll-like receptor–4 promotes the development of colitis-associated colorectal tumors. *Gastroenterology* **133**: 1869–1881.

Galan JE, Wolf-Watz H (2006) Protein delivery into eukaryotic cells by type III secretion machines. *Nature* **444**: 567–573.

Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem* **278**: 8869–8872.

Girardin SE, Boneca IG, Carneiro LA, Antignac A, Jéhanno M, Viala J, Tedin K, Taha MK, Labigne A, Zähringer U, Coyle AJ, DiStefano PS, Bertin J, Sansonetti PJ, Philpott DJ (2003) Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. *Science* **300**: 1584–1587.

Gutierrez O, Pipaon C, Inohara N, Fontalba A, Ogura Y, Prosper F, Nuñez G, Fernandez-Luna JL (2002) Induction of Nod2 in myelomonocytic and intestinal epithelial cells *via* nuclear factor-kappa B activation. *J Biol Chem* **277**: 41701–41705.

Ha EM, Oh CT, Bae YS, Lee WJ (2005) A direct role for dual oxidase in drosophila gut immunity. *Science* **310**: 847–850.

Hatakeyama M, Higashi H (2005) Helicobacter pylori CagA: a new paradigm for bacterial carcinogenesis. *Cancer Sci* **96**: 835–843.

Hnatyszyn A, Szalata M, Stanczyk J, Cichy W, Slomski R (2010) Association of c.802C>T polymorphism of NOD2/CARD15 gene with the chronic gastritis and predisposition to cancer in H. pylori infected patients. *Exp Mol Pathol* **88**: 388–393.

Ho LP, Merlin F, Gaber K, Davies RJ, McMichael AJ, Hugot JP (2005) CARD 15 gene mutations in sarcoidosis. *Thorax* **60**: 354–355.

Homer CR, Richmond AL, Rebert NA, Achkar JP, McDonald C (2010) ATG16L1 and NOD2 interact in an autophagy-dependent antibacterial pathway implicated in Crohn's disease pathogenesis. *Gastroenterology* **139**: 1630–1641.

Hsu YM, Zhang Y, You Y, Wang D, Li H, Duramad O, Qin XF, Dong C, Lin X (2007) The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. *Nat Immunol* **8**: 198–205.

Hugot JP, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R,Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G (2001) Association of NOD2 leucinerich repeat variants with susceptibility to Crohn's disease. *Nature* **411**: 599–603.

Hysi P, Kabesch M, Moffatt MF, Schedel M, Carr D, Zhang Y, Boardman B, von Mutius E, Weiland SK, Leupold W, Fritzsch C, Klopp N, Musk AW, James A, Nunez G, Inohara N, Cookson WO **(**2005) NOD1 variation, immunoglobulin E and asthma. *Hum Mol Genet* **14**: 935–941.

Inohara N, Chamaillard C, McDonald C, Nuñez G (2005) NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. *Annu Rev Biochem* **74**: 355–383.

Inohara N, Koseki T, del Peso L, Hu Y, Yee C. Chen S, Carrio R, Merino J, Liu D, Ni J, Nuñez G (1999) Nod1, an Apaf-1-like activator of caspase-9 and nuclear factor-kB. *J Biol Chem* **274**: 14560–14567.

Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, Fukase K, Inamura S, Kusumoto S, Hashimoto M, Foster SJ, Moran AP, Fernandez-Luna JL, Nuñez G (2003) Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* **278**: 5509–5512.

Jiang X, Chen ZJ (2011) The role of ubiquitylation in immune defence and pathogen evasion. *Nat Rev Immunol* **12**: 35–48.

Kanazawa N, Okafuji I, Kambe N, Nishikomori R, Nakata-Hizume M, Nagai S, Fuji A, Yuasa T, Manki A, Sakurai Y, Nakajima M, Kobayashi H, FujiwaraI,Tsutsumi H, Utani A, Nishigori C, Heike T, Nakahata T, Miyachi Y (2005) Early-onsetsarcoidosis and *CARD15* mutations with constitutivenuclearfactor-kappaBactivation: commongeneticetiology with Blau syndrome. *Blood* **105**: 1195–1197.

Karaś M, Drożański W, Russa R (2006) Peptydoglikan gram-ujemnych bakterii pałeczkowatych. *Post Mikrobiol* **45**: 27–38.

Karin M (1995) The Regulation of AP-1 Activity by Mitogen-activated Protein Kinases. *J Biol Chem* **270**: 16483–16486.

Kawai T, Akira S (2006) TLR signaling. *Cell Death Differ* **13**: 816–825.

Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi, S Akira (2005) SIPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction *Nature Immunology* **6**: 981–988

Kersse K, Bertrand MJ, Lamkanfi M, Vandenabeele P (2011) NOD-like receptors and the innate immune system: Coping with danger, damage and death. *Cytokine Growth Factor Rev* **22**: 257–276.

Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nuñez G, Flavell RA (2005) Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* **307**: 731–734.

Kufer TA, Kremmer E, Adam AC, Philpott DJ, Sansonetti PJ (2008) The pattern-recognition molecule Nod1 is localized at the plasma membrane at sites of bacterial interaction. *Cell Microbiol* **10**: 477–486.

Kurzawski G, Suchy J, Kladny J, Grabowska E, Mierzejewski M, Jakubowska A, Debniak T, Cybulski C, Kowalska E, Szych Z, Domagala W, Scott RJ, Lubinski J (2004) The NOD2 3020insC mutation and the risk of colorectal cancer. *Cancer Res* **64**: 1604–1606.

Kusters JG, van Vliet AH, Kuipers EJ (2006) Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev* **19**: 449–490.

Kvarnhammar AM, Cardell LO (2012) Pattern-recognition receptors in human eosinophils. *Immunology* **136**: 11–20.

Lecat A, Piette J, Legrand-Poels S (2010) The protein Nod2: An innate receptor more complex than previously assumed. *Biochem Pharmacol* **80**: 2021–2031.

Lecat A, Di Valentin E, Somja J, Jourdan S, Fillet M, Kufer TA, Habraken Y, Sadzot C, Louis E, Delvenne P, Piette J, Legrand-Poels S (2012). The c-Jun N-terminal kinase (JNK)-binding protein (JNK-BP1) acts as a negative regulator of NOD2 protein signaling by inhibiting its oligomerization process. *J Biol Chem* **287**: 29213–29226.

Lenz LL, Mohammadi S, Geissler A, Portnoy DA (2003) SecA2-dependent secretion of autolytic enzymes promotes Listeria monocytogenes pathogenesis. *Proc Natl Acad Sci USA* **100**: 12432–12437.

Lesage S, Zouali H, Cézard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP (2002) CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* **70**: 845–857.

Lipinski S, Till A, Sina C, Arlt A, Grasberger H, Schreiber S, Rosenstiel P (2009) DUOX2-derived reactive oxygen species are effectors of NOD2-mediated antibacterial responses. *Cell Sci* **122**: 3522–3530.

Magalhaes JG, Sorbara MT, Girardin SE, Philpott DJ (2011) What is new with Nods? *Curr Opin Immunol* **23**: 29–34.

Mardini HE, Gregory KJ, Nasser M, Selby L, Arsenescu R Winter TA, de Villiers WJ (2005) Gastroduodenal Crohn's disease is associated with NOD2/CARD15 gene polymorphism, particulary L1007P homozygosity. *Dig Dis Sci* **50**: 2316–2322.

Marinis JM, Hutti JE, Homer CR, Cobb BA, Cantley LC, McDonald C, Abbott DW (2012) IκB kinase α phosphorylation of TRAF4 downregulates innate immune signaling. *Mol Cell Biol* **32**: 2479–2489.

Martinon F, Tschopp J (2005) NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* **26**: 447–454.

McCartney SA, Colonna M (2009) Viral sensors: diversity in pathogen recognition. *Immunol Rev* **227**: 87–94.

McDonald C, Chen FF, Ollendorff V, Ogura Y, Marchetto S, Lécine P, Borg JP, Nuñez G (2005) Erbin in the regulation of Nod2-dependent NF-kappaB signaling. *J Biol Chem* **280**: 40301–40309.

McDonald C, Chen FF, Ollendorff V, Ogura Y, Marchetto S, Lécine P, Borg JP, Nuñez G (2005) A role for Erbin in the regulation of Nod2-dependent NF-kappaB signaling. *J Biol Chem* **280**: 40301–40309.

McGovern DP, Hysi P, Ahmad T, van Heel DA, Moffatt MF, Carey A, Cookson WO, Jewell DP (2005) Association between a complex insertion/deletion polymorphism in NOD1 (CARD4) and susceptibility to inflammatory bowel disease *Hum Mol Genet* **14**: 1245–1250.

Miceli-Richard C, Lesage S, Rybojad M, Prieur AM, Manouvrier-Hanu S, Häfner R, Chamaillard M, Zouali H, Thomas G, Hugot JP (2001) CARD15 mutations in Blau syndrome. *Nat Genet* **29**: 19–20.

Molnar T, Hofner P, Nagy F, Lakatos PL, Fischer S, Lakatos L, Kovacs A, Altorjay I, Papp M, Palatka K, Demeter P, Tulassay Z, Nyari T, Miheller P, Papp J, Mandi Y, Lonovics J (2007) NOD1 gene E266K polymorphism is associated with disease susceptibility but not with disease phenotype or NOD2/CARD15 in Hungarian patients with Crohn's disease. *Dig Liver Dis* **39**: 1064–1070.

Morosky SA, Zhu J, Mukherjee A, Sarkar SN, Coyne CB (2011) Retinoic acid-induced gene-I (RIG-I) associates with nucleotide-binding oligomerization domain-2 (NOD2) to negatively regulate inflammatory signaling. *J Biol Chem* **286**: 28574–28583.

Nembrini C, Kisielow J, Shamshiev AT, Tortola L, Coyle AJ, Kopf M, Marsland BJ (2009) The Kinase activity of Rip2 determines its stability and consequently Nod1- and Nod2-mediated immune responses. *J Biol Chem* **284**: 19183–19188.

Netea MG, Kullberg BJ, de Jong DJ, Franke B, Sprong T, Naber TH, Drenth JP, Van der Meer JW (2004) NOD2 mediates anti-inflammatory signals induced by TLR2 ligands: implications for Crohn's disease. *Eur J Immunol* **34**: 2052–2059.

O'Neill L (2011) Editorial: Synergism between NOD-like receptors and Toll-like receptors in human B lymphocytes. *J Leukoc Biol* **89**: 173–175.

Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, KirschnerBS,Hanauer SB, Nuñez G, Cho JH (2001) A frameshiftmutation in NOD2 associated with susceptibility to Crohn'sdisease. *Nature* **411**: 603–606.

Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nuñez G (2001) Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-κB. *J Biol Chem* **276**: 4812–4818.

Opitz B, Püschel A, Beermann W, Hocke AC, Förster S, Schmeck B, van Laak V, Chakraborty T, Suttorp N, Hippenstiel S (2006) Listeria monocytogenes activated p38 MAPK and induced IL-8 secretion in a nucleotide-binding oligomerization domain 1–dependent manner in endothelial cells. *J Immunol* **176**: 484–490.

Opitz B, Püschel A, Schmeck B, Hocke AC, Rosseau S, Hammerschmidt S, Schumann RR, Suttorp N, Hippenstiel S (2004) Nucleotide-binding oligomerization domain proteins are innate immune

receptors for internalized *Streptococcus pneumoniae*. *J Biol Chem* **279**: 36426–36432.
Papaconstantinou I, Theodoropoulos G, Gazouli M, Panoussopoulos D, Mantzaris GJ, Felekouras E, Bramis J (2005) Association between mutations in the CARD15/NOD2 gene and colorectal cancer in a Greek population. *Int J Cancer* **114**: 433–435.
Park JH, Gi Kim Y, McDonald C, Thirumala-Devi Kanneganti TG, Hasegawa M Body-Malapel M, Inohara N, NuñezG (2007) Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract, RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs. *J Immunol* **178**: 2380–2386.
Park JH, Kim YG, Shaw M, Kanneganti TD, Fujimoto Y, Fukase K, Inohara N, Nuñez G (2007) Nod1/RICK and TLR signaling regulate chemokine and antimicrobial innate immune responses in mesothelial cells. *J Immunol* **179**: 514–521.
Poyet JL, Srinivasula SM, Tnani M, Razmara M, Fernandes-Alnemri T, Alnemri ES (2001) Identification of Ipaf, a human caspase-1-activating protein related to Apaf-1. *J Biol Chem* **276**: 28309–28313.
Qi, B, Huang Y, Rowe D Halliday G (2007) VISA — a pass to innate immunity. *Int J Biochem Cell Biol* **39**: 287–291.
Rahighi S, Ikeda F, Kawasaki M, Akutsu M, Suzuki N, Kato R, Kensche T, Uejima T, Bloor S, Komander D, Randow F, Wakatsuki S, Dikic I (2009) Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. *Cell* **136**: 1098–1109.
Rakoff-Nahoum S, Medzhitov R (2007) Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science* **317**: 124–127.
Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ, Rubinsztein DC (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet* **36**: 585–595.
Sabbah A, Chang TH, Harnack R, Frohlich V, Tominaga K, Dube PH, Xiang Y, Bose S (2009) Activation of innate immune antiviral responses by Nod2. *Nat Immunol* **10**: 1073–1780.
Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M, Tanaka K, Kawai T, Tsujimura T, Takeuchi O, Yoshimori T, Akira S (2008) Loss of the autophagy protein Atg16L1 enhancesendotoxin-induced IL-1beta production. *Nature* **456**: 264–268.
Schneider M, Zimmermann AG, Roberts RA, Zhang L, Swanson KV, Wen H, Davis BK, Allen IC, Holl EK, Ye Z, Rahman AH, Conti BJ, Eitas TK, Koller BH, Ting JP (2012) The innate immune sensor NLRC3 attenuates Toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-κB. *Nat Immunol* **13**: 823–831.
Sfriso P, Caso F, Tognon S, Galozzi P, GavaA,Punzi L (2012) Blau syndrome, clinical and genetic aspects. *Autoimmun Rev* **12**: 44–51.
Shaw MH, Kamada N, Warner N, Kim YG, Nuñez G (2011) The ever-expanding function of NOD2: autophagy, viral recognition, and T cell activation. *Trends Immunol* **32**: 73–79.
Stevens C, Henderson P, Nimmo ER, Soares DC, Dogan B, Simpson K. W (2013). The intermediate filament protein, vimentin, is a regulator of NOD2 activity. *Gut* **62**: 695–707.
Sugimoto M, Germain RN, Chedid L, Benacerraf B (1978) Enhancement of carrier-specific helper T cell function by the synthetic adjuvant, N-acetyl muramyl-l-alanyl-d-isoglutamine (MDP). *J Immunol* **120**: 980–982.
Sun Q, Sun L, Liu HH, Chen X, Seth RB, Forman J, Chen ZJ (2006) The specific and essential role of MAVS in antiviral innate immune responses. *Immunity* **24**: 633–642.
Takeuchi O, Akira S (2009) Innate immunity to virus infection. *Immunol Rev* **227**: 75–86.
Takeuchi O, Akira S (2010) Pattern recognition receptors and inflammation. *Cell* **140**: 805–820.
Tan X, Li WW, Guo J, Zhou JY (2012) Down-regulation of NOD1 in neutrophils of periparturient dairy cows .*Vet Immunol Immunopathol* **150**: 133–139.
Tanabe T, Ishige I, Suzuki Y, Aita Y, Furukawa A, Ishige Y, Uchida K, Suzuki T, Takemura T, Ikushima S, Oritsu M, Yokoyama T, Fujimoto Y,Fukase K, Inohara N, Nuñez G, Eishi Y (2006) Sarcoidosis and NOD1 variation with impaired recognition of intracellular *Propionibacterium acnes*. *Biochim Biophys Acta* **1762**: 794–801.
Tanabe T, Chamaillard M, Ogura Y, Zhu L, Qiu S, Masumoto J, Ghosh P, Moran A, Predergast MM, Tromp G, Williams CJ, Inohara N, Nuñez G (2004) Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. *EMBO J* **23**: 1587–1597.
Theofilopoulos AN, Baccala R, Beutle B, Kono DH (2005) Type I interferons (a/b) in immunity and autoimmunity. *Annu Rev Immunol* **23**: 307–336.
Tigno-Aranjuez JT, Abbott DW (2012) Ubiquitination and phosphorylation in the regulation of NOD2 signaling andNOD2-mediated disease. *Biochim Biophys Acta* **1823**: 2022–2028.
Ting JP, Duncan JA, Lei Y (2010) How the noninflammasome NLRs function in the innate immune system. *Science* **327**: 286–290.
Ting JP, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK, Flavell RA, Girardin SE, Godzik A, Harton JA, Hoffman HM, Hugot JP, Inohara N, Mackenzie A,Maltais LJ, Nuñez G, Ogura Y, Otten LA, Philpott D, Reed JC, Reith W, Schreiber S, Steimle V, Ward PA (2008) The NLR gene family: a standard nomenclature. *Immunity* **28**: 285–287.
Tötemeyer S, Sheppard M, Lloyd A, Roper D, Dowson C, Underhill D, Murray P, Maskell D, Bryant C (2006) IFN-γ enhances production of nitric oxide from macrophages via a mechanism that depends on nucleotide oligomerization domain-2. *J Immunol* **176**: 4804–4810.
Travassos LH, Carneiro LAM, Ramjeet M, Hussey S, Kim Y-Gi, Magalhães J G, Yuan L, Soares F, Chea E, Le Bourhis L, Boneca IG, Allaoui A, Jones NL, Nuñez G, Girardin SE, Philpott DJ (2010) Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat Immunol* **11**: 55–62.
Uehara A, Fujimoto Y, Fukase K, Takada H (2007) Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce antimicrobial peptides, but not proinflammatory cytokines. *Mol Immunol* **44**: 3100–3111.
van Heel DA, Ghosh S, Butler M, Hunt KA, Lundberg AM, Ahmad T, McGovern DP, Onnie C, Negoro K, Goldthorpe S, Foxwell BM, Mathew CG, Forbes A, Jewell DP, Playford RJ (2005) Muramyl dipeptide and toll-like receptor sensitivity in NOD2-associated Crohn's disease. *Lancet* **365**: 1794–1796.
Van Limbergen J, Nimmo ER, Russell RK, Drummond HE, Smith L, Anderson NH, Davies G, Arnott ID, Wilson DC, Satsangi J (2007) Investigation of NOD1/CARD4 variation in inflammatory bowel disease using a haplotype-tagging strategy. *Hum Mol Genet* **16**: 2175–2186.
Van Limbergen J, Russell RK, Nimmo ER, Törkvist L, Lees CW, Drummond HE, Smith L, Anderson NH, Gillett PM, McGrogan P, Hassan K, Weaver LT, Bisset WM, Mahdi G, Arnott ID, Sjöqvist U, Lördal M, Farrington SM, Dunlop MG, Wilson DC, Satsangi J (2007) Contribution of the NOD1/CARD4 insertion/deletion polymorphism +32656 to inflammatory bowel disease in Northern Europe. *Inflamm Bowel Dis* **13**: 882–889.
Ver Heul AM, Fowler CA, Ramaswamy S, Piper RC (2013) Ubiquitin regulates caspase recruitment domain-mediated signaling by nucleotide-binding oligomerization domain-containing proteins NOD1 and NOD2. *J Biol Chem* **288**: 6890–6902.
Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Mémet S, Huerre MR, Coyle AJ, DiStefano PS, Sansonetti PJ, Labigne A, BertinJ,Philpott DJ, Ferrero RL (2004) Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. *Nat Immunol* **5**: 1166–1174.
Wehkamp J, Harder J, Weichenthal M, Schwab M, Schäffeler E, Schlee M, Herrlinger KR, Stallmach A, Noack F, Fritz P, Schröder JM, Bevins CL, FellermannK, Stange EF (2004) NOD2(CARD15) mutations in Crohn's disease are associated with diminished mucosal α-defensin expression. *Gut* **53**: 1658–1664.
Wertz IE, Dixit VM (2010) Signaling to NF-κB: Regulation by Ubiquitination Cold Spring *Harb Perspect Biol* 2: a003350
Wickner W (2002) Yeast vacuoles and membrane fusion pathways. *EMBO J* **21**: 1241–1247.
Wilmanski JM, Petnicki-Ocwieja T, Kobayashi KS (2008) NLR proteins: integral members of innate immunity and mediators of inflammatory diseases *J Leukoc Biol* **83**: 13–30.
Windheim M, Lang C, Peggie M, Plater LA, Cohen P (2007) Molecular mechanisms involved in the regulation of cytokine production by muramyl dipeptide. *Biochem J* **404**: 179–190.
Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS (2004) Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* **291**: 187–194.
Yamamoto-Furusho JK, Barnich N, Xavier R, Hisamatsu T, Podolsky DK (2006) Centaurin beta1 down-regulates nucleotide-binding oligomerization domains 1- and 2-dependent NF-kappaB activation. *J Biol Chem* **281**: 36060–36070.
Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T (2004) The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* **5**: 730–737.
Zilbauer M, Dorrell N, Elmi A, Lindley KJ, Schüller S, Jones HE, Klein NJ, Nuñez G, Wren BW, Bajaj-Elliott M (2007) A major role for intestinal epithelial nucleotide oligomerization domain 1 (NOD1) in eliciting host bactericidal immune responses to *Campylobacter jejuni*. *Cell Microbiol* **9**: 2404–2416.
Zurek B, Schoultz I, Neerincx A, Napolitano LM, Birkner K, Bennek E, Sellge G, Lerm M, Meroni G, Söderholm JD, Kufer TA (2012) TRIM27 negatively regulates NOD2 by ubiquitination and proteasomal degradation. *PLoS One* **7**: e41255.