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REVIEW

Therapeutic targeting of NOD1 receptors

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The nucleotide-binding oligomerization domain 1 (NOD1) protein is an intracellular receptor for breakdown products of peptidoglycan (PGN), an essential bacterial cell wall component. NOD1 responds to γ-D-glutamyl-meso-diaminopimelic acid, which is an epitope unique to PGN structures from all Gram-negative bacteria and certain Gram-positive bacteria. Upon ligand recognition, NOD1 undergoes conformational changes and self-oligomerization mediated by the nucleotide-binding NACHT domains, followed by the recruitment and activation of the serine threonine kinase receptor-interacting protein 2 leading to the activation of NF-κB and MAPK pathways and induction of inflammatory genes. Much of our knowledge is derived from seminal studies using mice deficient in NOD1 and confirming an essential role for NOD1 in the host immune response against gastrointestinal and respiratory pathogens. In addition, recent studies have revealed a role for intracellular NOD1 receptors in the regulation of vascular inflammation and metabolism. This review will discuss our current understanding of intracellular NOD1 receptors in host immunity and chronic inflammatory disorders with a focus on cardiovascular diseases. Although therapeutic advances may have to wait until the complex interplay with pathogens, danger signals, other pattern recognition receptors and overlapping metabolic pathways is further unravelled, the steadily growing body of knowledge suggest that NOD1 antagonism might represent attractive candidate to reduce excessive inflammation associated to intestinal, cardiovascular and metabolic diseases.

Abbreviations

BIRs, baculovirus inhibitor repeats; CARD, caspase recruitment domain; DAMPs, damage-associated molecular patterns; ECs, endothelial cells; MDP, muramyl dipeptide; meso-DAP, γ-D-glutamyl-meso-diaminopimelic acid; NOD, nucleotide oligomerization domain; PAMPs, pathogen-associated molecular patterns; PRRs, pattern recognition receptors; PYD, pyrin domain; RIP2, receptor-interacting protein 2; TLRs, Toll-like receptors

The innate immune system has been regarded as the first line of defence against invading pathogens. There has recently been a dramatic increase in our knowledge of early pathogen recognition. A number of germ-line receptors termed pattern recognition receptors (PRRs) have been identified. These PRRs specifically bind preserved molecular signatures, so-called pathogen-associated molecular patterns (PAMPs), present on invading bacteria, fungi and viruses. Four major subfamilies of PRRs have been identified to date: Toll-like receptors (TLRs), which are transmembrane proteins located at the cell surface or in endosomes; nucleotide oligomerization domain (NOD)-like receptors (NLRs), which are located in the cytoplasm; RIG-like receptors, which are also located intracellularly and are primarily involved in antiviral responses; C-type lectin receptors, which are characterized by the presence of a carbohydrate-binding domain and include the mannose receptor and surfactant proteins A and D.

Since the initial studies of Hoffmann and colleagues in *Drosophila* (Lemaitre *et al.*, 1997), our appreciation of the complex roles of PRRS signalling in host immunity has increased dramatically. It is now evident that PRRs can also recognize damage-associated molecular patterns (DAMPs),

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which comprised non-infectious material that can cause tissue damage (i.e. asbestos or silica), and endogenous molecules, which are released during cellular injury (including high-mobility group box 1, heat shock proteins, purine metabolites or extracellular matrix fragments; Chen and Nunez, 2010). Furthermore, we are increasingly aware that, in addition to immune cells, structural cells of the CVS, including endothelial cells (ECs) and vascular smooth muscle cells, sense pathogens and danger signals directly clearly contributing to the development of cardiovascular diseases including septic shock and atherosclerosis (Jimenez *et al.*, 2005; Cartwright *et al.*, 2007a,b; Mitchell *et al.*, 2007).

In this review, we will discuss our current understanding of intracellular NOD1 receptors in host immunity and chronic inflammatory disorders with a focus on cardiovascular diseases. Examples of agonists and antagonists will be provided to illustrate the potential utility of drugs targeting innate immunity.

The NLR family

The NLRs represent a relatively new addition to the family of PRRs. These are specialized intracellular proteins that act as cytosolic pathogen sensors. Like the TLRs, the NLRs are evolutionally conserved proteins. Homologues to the NLRs can be found in plant biology where they play a crucial role in host defence against bacteria and fungi (R genes). There are 23 identified NLRs in man and over 34 identified genes in mice. The structure of NLRs is composed of three domains. A variable N-terminal domain comprising of a caspase recruitment domain (CARD), pyrin domain (PYD), acidic domain or baculovirus inhibitor repeats (BIRs), a centrally located NOD, and C-terminal leucine-rich repeats that allow pathogen sensing. The NLRs are subdivided into groups according to the variable N-terminal domain, that is, four groups for CARD, PYD, BIR and acidic domains and an extra group for N-terminal domains bearing no known homology (Ting *et al.*, 2008).

The best studied NLRs are NOD1 and NOD2, which recognize breakdown products of peptidoglycan (PGN), an essential bacterial cell wall component. NOD1 responds to γ-D-glutamyl-meso-diaminopimelic acid (meso-DAP), which is an epitope unique to PGN structures from all Gram-negative bacteria and certain Gram-positive bacteria (Chamaillard *et al.*, 2003; Girardin *et al.*, 2003). In contrast, NOD2 is activated by muramyl dipeptide (MDP), a PGN motif present in Gram-negative and Gram-positive bacteria (McDonald *et al.*, 2005). Evidence for NOD1/2 stimuli that are not related to pathogens are scarce. To date, the best characterized of the NLRs that responds to DAMPs is the NLRP3 inflammasome, which is responsible for the activation of caspase 1 and 8 and cleavage of pro-IL1b and IL-18 on their biologically active forms (McGettrick and O'Neill, 2013). However, recent reports suggest that NOD1 receptors may also be activated by potential DAMPs and they will be discussed below.

NOD1 is ubiquitous, whereas NOD2 expression pattern seems to be more restricted to immune cells (Bertin *et al.*, 1999; Ogura *et al.*, 2001; Shaw *et al.*, 2008). Interestingly, the expression pattern of the NOD receptors alone does not predict function, as experiments in our own group and others have shown. For example, administration of a specific NOD1 agonist to rats induced specific effects in vascular tissues despite the ubiquitous expression of the receptor (Cartwright *et al.*, 2007b; Nishio *et al.*, 2011). The relative importance of NOD signalling in structural versus haematopoietic cells remains to be elucidated although the lack of profound immunosuppression in NOD 1/2 knockout mice may suggest a lesser role in 'professional' immune cells (Stroo *et al.*, 2012).

The earliest research that can be linked to NOD signalling involved studies on immunoactive acrylopeptides similar in structure to bacterial PGN. FK156 was the first such peptide extracted from fermentation broths of *Streptomyces violaceus* and other *Streptomyces* species (Gotoh *et al.*, 1982). Screening of synthetic derivatives identified FK565 as a peptide with antibacterial properties (Yokota *et al.*, 1983) and strong immune priming and anti-tumour effects (Inamura *et al.*, 1985). Both FK156 and FK565 are indeed DAP-containing tetrapeptide muropeptides but it was only later that NOD1 was identified as the receptor via which these DAP-containing peptides signal. With the discovery of meso-DAP as the structure recognized by NOD1, investigators have studied the effects of related proteins on professional immune cells and other cell types and have started to elucidate the signalling pathways activated by NOD1 receptors (Strober *et al.*, 2006; Shaw *et al.*, 2008; Correa *et al.*, 2012). Upon ligand recognition, NOD1 and NOD2 undergo conformational changes and self-oligomerization mediated by their centrally located NACHT (Koonin and Aravind, 2000), followed by the recruitment and activation of the serine threonine kinase receptor-interacting protein 2 (RIP2; Figure 1). The exact role of the kinase activity of RIP2 is not fully understood but *in vitro* and *in vivo* studies with RIP2 null mutations show an inability to activate NF-κB in response to NOD stimulation (Kobayashi *et al.*, 2002; Park *et al.*, 2007). Activated RIP2 binds and activates NF-κB essential modulator (NEMO). The subsequent degradation of the NF-κB inhibitor α releases NF-κB and allows it to translocate to the nucleus to initiate transcription of target genes. Recent studies have demonstrated that K63-linked regulatory ubiquitination of RICK is essential for the recruitment of TGF-β-activated kinase, a kinase required for the activation of NF-κB and MAPK pathways (Kumar *et al.*, 2009) and induction of inflammatory genes such as IL-6, CXCL8 or macrophage inflammatory protein-2 (MIP-2) (Chamaillard *et al.*, 2003; Buchholz and Stephens, 2008).

In vivo, the infected organism is exposed to multiple PAMPs and the resulting immune response is likely to depend on a complex interplay between differentially activated PRRs. This raises interesting questions as to how the respective PRRs may interact in biological systems when co-stimulated by their relevant PAMPs. Perhaps the best example of cooperation between TLR and NLR signalling is that of NLRP3-mediated inflammasome activation to release the cytokine IL-1β where initial activation of TLR and/or NOD2 signalling produce pro-IL-1β, but release of the mature cytokine requires a second signal directly activating the NLRP3 inflammasome (Kanneganti *et al.*, 2007). NOD1/2 signalling itself has not been demonstrated via inflam-

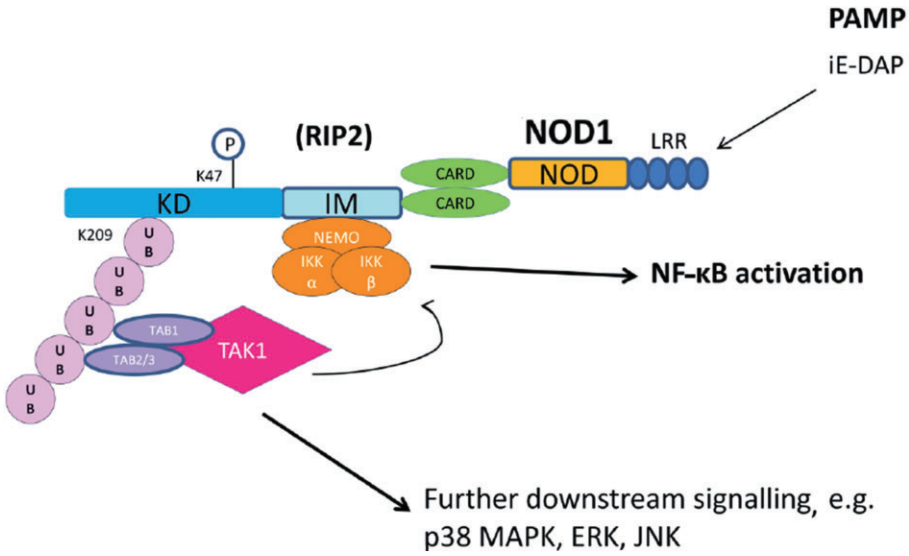

Figure 1

NOD1 signalling via RIP2. Recognition of meso-DAP (iE-DAP) through LRR domains activates the NOD1 protein, which then associates with RIP2 via CARD–CARD interactions. Subsequent K63-linked polyubiquitination at K209 in RIP2 allows for recruitment of the TAK1/TAK1-binding protein (TAB1, 2, 3) complex, which leads to interaction with the NF-κB inhibitor NEMO and release of NF-κB to initiate gene transcription. TAK1 is also required for the activation of the MAPK pathways. K47 and K209, lysine residues at RIP2; KD, kinase domain; LRR, leucine-rich repeat; TAK1, TGF-β-activated kinase. Adapted from Hasegawa *et al.*, 2008.

masomes but there are well-characterized examples of TLR and NOD interaction. For example, NOD1 and NOD2 agonists significantly enhance TLR-induced cytokine secretion in monocytes (Yang *et al.*, 2001; van Heel *et al.*, 2005; Uehara *et al.*, 2005) or dendritic cells (Tada *et al.*, 2005). Similarly, signalling via the viral PRR, TLR3, has been demonstrated to enhance the lethality of subsequent bacterial infection in mice in a NOD1/2- and RIP2-dependent fashion (Kim *et al.*, 2011), therefore providing a potential mechanism by which prior activation of certain PRRs may influence subsequent infections. In addition, experiments in wild-type and NOD1/2 knockout macrophages illustrated the potential crosstalk between NOD receptors (Kim *et al.*, 2008). Wild-type macrophages pre-stimulated with a combination of LPS (TLR4 agonist) and MDP (NOD2 agonist) were rendered hyporesponsive to subsequent challenge with pathogenic bacteria and this bacterial tolerance was impaired in NOD2 null macrophages and abolished in NOD1/NOD2 double knockout macrophages. Interestingly, the authors also showed cross-tolerization between NOD1 and NOD2 ligands in wild-type macrophages, whereas responses to NOD1 ligands were intensified in NOD2 null cells pre-stimulated with LPS and MDP. As described above, the signalling pathways downstream of NOD1 and NOD2 converge on the signalling kinase RIP2 thus representing an interesting modulator of synergistic or inhibitory interaction between both receptors. Hopefully as mechanisms of interaction between TLR and NLR signalling pathways become clear, the functional relevance of multiple PRR activation will emerge. This of course is potentially of great importance if we seek to therapeutically target NOD receptors with pharmacological inhibitors in the future.

NOD1 and host defence

Role of NOD1 in the regulation of pulmonary innate immunity

A number of studies have demonstrated a role for NOD1 in pulmonary innate immune responses. A critical role for NOD1 in the host responses during lung infections with *Chlamydia pneumonia* (Opitz *et al.*, 2005), *Pseudomonas aeruginosa* (Travassos *et al.*, 2005) and other intracellular bacteria such as *Listeria monocytogenes* (*Opitz et al., 2006*) or *Shigella flexneri* (Girardin *et al.*, 2001) has been described. For instance, the respiratory pathogen *C. pneumonia* activates human ECs via NOD1 (Opitz *et al.*, 2005). Accordingly, NOD1- and RIP2-knockout mice infected with *C. pneumonia* showed delayed bacterial clearance and delayed neutrophil recruitment to the lungs with evidence for impaired NO and cytokine production. At later phase of infection, however, knockout mice developed more severe and chronic lung inflammation, which led to increased mortality (Shimada *et al.*, 2009). In contrast, NOD2 is probably the main NLR involved in *Mycobacterium tuberculosis* infection (Ferwerda *et al.*, 2005), whereas both receptors mediate host responses during *Streptococcus pneumoniae* (Opitz *et al.*, 2004) and *Legionella pneumophila* (Berrington *et al.*, 2010) infections. However, the study by Berrington *et al.* reveals that activation of these receptors apparently modulates the *in vivo* pulmonary response to *L. pneumophila* differently. Thus, the authors found decreased levels of proinflammatory cytokines, impaired neutrophil recruitment to the alveolar space and impaired bacterial clearance in NOD1-deficient mice as compared to wild-type mice. In contrast, increased lung

neutrophils and proinflammatory cytokines were seen in the NOD2-deficient mice. Furthermore, the lungs of both NOD1- and NOD2-deficient mice had significantly increased proinflammatory cytokine levels at later stages of infection (Berrington *et al.*, 2010). However, recent evidence suggests a more complex role of NOD1 in the regulation of pulmonary innate immunity. Thus, a recent study has identified NOD1 as the strategy employed by *Klebsiella pneumoniae* to counteract host defence inflammatory responses (Regueiro *et al.*, 2010). Likewise, the respiratory syncytial virus inhibits NOD1 gene expression in macrophages (Senft *et al.*, 2009) thereby representing an interesting mechanism potentially underlying viral-induced secondary bacterial infection. In contrast, Kim and colleagues have shown that murine norovirus infection promotes bacterial superinfection and lethality via up-regulation of NOD1 and NOD2 pathways (Kim *et al.*, 2011), which could suggest a clear beneficial role of NOD1/2 inhibition. Finally, NOD1 has also been implicated in non-infectious lung inflammatory disease. Thus, oxidant stress induced by either cigarette smoke extract or hydrogen peroxide primed human monocytes for stimulation with TLR and NOD1/2 agonists (Paul-Clark *et al.*, 2008). In addition, Hysi and colleagues reported association of an insertion-deletion polymorphism in NOD1 (ND(1)+32656) with IgE variation and with a greater than fivefold increased risk of childhood asthma (Hysi *et al.*, 2005). The same mutation was also implicated in the development of inflammatory bowel disease (McGovern *et al.*, 2005).

Role of NOD1 in the regulation of intestinal innate immunity

The intestinal lumen is exposed to a large concentration of commensal bacteria that express a wide array of PAMPs but the organism has developed mechanisms to avoid innate recognition and harmful inflammatory responses at intestinal sites, including low expression levels of functional TLRs (4, 5 and 6, 17) and expression of molecules that inhibit TLR signalling (Abreu *et al.*, 2005). How the host intestinal epithelium detects invasive bacteria while avoiding recognition of symbiotic bacteria is not well understood. However, increasing evidence suggest that NOD1 may be a relevant player in the recognition of invasive bacteria by intestinal epithelial (Girardin *et al.*, 2001; Chamaillard *et al.*, 2004; Kim *et al.*, 2004).

NOD1 is expressed in macrophages and epithelial cells, including those lining the intestinal mucosa and its expression is induced by IFN-γ in intestinal epithelial cells (Hisamatsu *et al.*, 2003). Oral PGN activates intestinal mast cells and induces diarrhoea in mice in a dose-dependent manner, which is inhibited by blockade of NOD1 signalling, providing potential therapeutic significance (Feng *et al.*, 2007). In addition, intestinal epithelial cell lines have shown increased sensitivity to NOD1 ligands as compared to macrophages, dendritic cells or splenocytes (Masumoto *et al.*, 2006). Thus, NOD1 has been associated during the last 10 years to an effective host response against a wide range of bacterial pathogens that infect the gastrointestinal tract including enteroinvasive *Escherichia coli* (Kim *et al.*, 2004; Chaouche-Drider *et al.*, 2009), *Helycobacter pylori* (Viala *et al.*, 2004), *Listeria monocytogenes* (O'Connell *et al.*, 2005), *S. flexneri* (Girardin *et al.*, 2001; Fukazawa *et al.*, 2008), *Campylobacter jejuni* (Zilbauer *et al.*, 2007), *Vibrio cholera* (Chatterjee and Chaudhuri, 2013), *Clostridium difficile* (Hasegawa *et al.*, 2011), *Helicobacter pylori* (van Heel *et al.*, 2005; Eckmann, 2006) and *Salmonella* colitis (Hasegawa *et al.*, 2011). Moreover, dietary fatty acids are also known to modulate immune responses and intestinal epithelial cells, as major absorptive sites, are exposed to a relatively higher concentration of fatty acids during each meal. Although the molecular mechanisms by which fatty acids cause inflammation and insulin resistance are not fully understood, it has been demonstrated that NOD1 can be activated by saturated fatty acids in human intestinal epithelial HCT116 cells (Zhao *et al.*, 2007). All these studies suggest that NOD1 can modulate inflammation and mediate efficient clearance of bacteria from the mucosal tissue during both infectious and non-infectious intestinal pathologies. Indeed, both single NOD1 and double NOD1/NOD2 knockout mice display increased intestinal permeability and increased susceptibility to colitis (Stroo *et al.*, 2012) and inflammation-induced colon tumorigenesis (Chen *et al.*, 2008). Thus, preliminary data suggest that carriage of the NOD1 G796A mutation increases the susceptibility of gastric epithelial cells for intestinal metaplasia and atrophy when infected by certain *H. pylori* strains (Kara *et al.*, 2010). The diminished epithelial chemokine production observed in NOD1 knockout mice is thought to compromise cell recruitment and thereby host defence against the bacteria. More recently, a role for NOD1 in the development of gut immunity has also been identified, particularly in the development of isolated lymphoid follicles (Bouskra *et al.*, 2008). During the last few years, a broader role for the microbiota as a major modulator of systemic immunity has been proposed. In this regard, recognition of PGN from the microbiota by NOD1 has been shown to enhance systemic innate immunity (Clarke *et al.*, 2010) suggesting also a role for NOD1 in priming systemic innate defences. Finally, an interesting study recently published suggests that the gut microbiota can selectively activate mucosal endothelial and mesenchymal cells to promote specific angiogenic responses in a TLR- and NLR-dependent fashion. This innate immunity-mediated response may expand the mucosal microvascular network, foster immune cell recruitment and contribute to chronic intestinal inflammation (Schirbel *et al.*, 2012).

In summary, evidence accumulating during recent years identify NOD1 as an important receptor involved in innate host defence against gastrointestinal pathogens and in the regulation of inflammatory responses, suggesting that further insights into their physiological functions may yield new pharmacological strategies for treating intestinal inflammatory conditions (Hruz and Eckmann, 2008; Chen and Nunez, 2009).

Role of NOD1 in cancer

In 2002, it was estimated that 18% of cancers worldwide were attributable to infectious agents (Parkin, 2006), particularly gastric (*H. pylori*), liver (hepatitis B and C viruses) and cervix (human papillomavirus) cancers.

NOD1 receptors may have an impact in cancer risk because, in addition to *H. pylori*, other potentially carcinogenic infectious agents are sensed by NOD1 receptors such as *C. pneumonia* (a possible risk factor for lung cancer) or enteropathogenic *E. coli* and *S. flexneri* (associated to colorectal

cancer). However, whether activation of NOD1 receptors may have a beneficial effect by protecting the host against these invading microorganisms or may indeed promote carcinogenesis is far from being elucidated. As discussed earlier, NOD1 knockout mice seem to display increased susceptibility to inflammation-induced colon tumorigenesis (Chen *et al.*, 2008). However, two independent studies have found increased levels of NOD1 receptors in gastric tumour tissues, when compared with paired non-tumour samples (Allison *et al.*, 2013), whereas no differences were found between normal and tumour prostatic tissues (Kang *et al.*, 2012). Similarly, studies looking for association of NOD1/CARD4 gene polymorphism and cancer risk have yielded contradictory results as recently reviewed (Kutikhin, 2011). Thus, preliminary data suggested that carriage of the NOD1 G796A mutation increases the susceptibility of gastric epithelial cells for intestinal metaplasia and atrophy when infected by certain *H. pylori* strains (Kara *et al.*, 2010), but these findings have not been corroborated in later studies (Kupcinskas *et al.*, 2011).

NOD1 and cardiovascular diseases

The vasculature plays a prominent role in the inflammatory response through vasodilatation and the trafficking of leucocytes to sites of infection. However, excessive vascular inflammation may result in deleterious effects on the host exemplified by septic shock, acute lung injury and in a more chronic setting, atherosclerosis. While specialized immune cells such as the macrophage have predominantly been used to study PRR responses, it is clear that blood vessels themselves can sense pathogens and danger signals clearly contributing to the development of cardiovascular diseases including septic shock and atherosclerosis (Jimenez *et al.*, 2005; Cartwright *et al.*, 2007a,b; Mitchell *et al.*, 2007).

Role of NOD1 in septic shock

Sepsis represents a systemic inflammatory response to infection. Severe sepsis is associated with multi-organ dysfunction and may progress to septic shock with hypotension, vascular dysfunction and a high mortality rate of up to 50% (Martin *et al.*, 2009). Management is exclusively supportive and the lack of approved pharmacological therapy reflects major deficiencies in our understanding of the pathogenesis of sepsis/septic shock. A large number of treatments have failed to improve survival, including glucocorticosteroids (Patel and Balk, 2011) or activated protein C (Marti-Carvajal *et al.*, 2008). Indeed, despite initial promising clinical outcome data, activated protein C has now been withdrawn from clinical practice because of poor efficacy (Mitka, 2011).

A number of studies support the notion that PGN is a contributing factor in the development of sepsis. Thus, PGN has been shown to induce the release of inflammatory cytokines *in vitro* (Myhre *et al.*, 2006) and to cause organ injury, organ inflammation and systemic inflammation alone (Wang *et al.*, 2004) or in synergy with lipoteichoic acid or LPS *in vivo* (De Kimpe *et al.*, 1995; Wray *et al.*, 2001). Most notably, some investigators noted that the biological response to PGN was enhanced by sonication (Rosenthal and Dziarski, 1994; Wang *et al.*, 2004). Similarly, *in vitro* studies have confirmed that although NOD1 agonists seem to be relatively weak inducers of inflammatory responses in some cells, NOD1 activation may synergize with TLR signalling to orchestrate immune responses. Thus, NOD1 stimulation has been shown to induce cytokine release, production of antibacterial peptides and to promote autophagia in dendritic cells, macrophages, monocytes (Chamaillard *et al.*, 2003; Fritz *et al.*, 2005; Tada *et al.*, 2005; Uehara *et al.*, 2005), ECs (Moreno *et al.*, 2010; Gatheral *et al.*, 2012) or epithelial cells (Kim *et al.*, 2004; Uehara *et al.*, 2007) *in vitro*. *In vivo*, administration of the NOD1 agonist KF1B induced chemokine production and neutrophil recruitment (Masumoto *et al.*, 2006), whereas the NOD1 agonist FK565 reproduced the cardiovascular and organ failure associated with septic shock (Cartwright *et al.*, 2007b). Intriguingly, despite the profound induction of vascular shock seen with FK565, there was little impact on neutrophil ingress into the lung compared to LPS suggesting a predominant vascular effect of the NOD1 ligand. Indeed, NOD1 agonists are sensed directly by rodent and human vascular cells in culture (i.e. endothelial and smooth muscle cells; Moreno *et al.*, 2010; Gatheral *et al.*, 2012) and by whole blood vessels *in vitro* (Cartwright *et al.*, 2007b; Nishio *et al.*, 2011). Furthermore, NOD1/2 double knockout are protected against sepsis-induced acute renal disease (Stroo *et al.*, 2012) and renal ischaemia reperfusion (I/R) injury (Shigeoka *et al.*, 2010), thereby suggesting that endogenous damage signals released (i.e. DAMPs) during I/R injury are also sensed by NOD1/2. Thus, increasing evidence suggest that therapeutic targeting of NOD1 might be useful for the treatment of vascular complications derived from both infectious- and non-infectious-related multiorgan failure.

Role of NOD1 in atherosclerosis

In addition to sepsis and shock, pathogen-sensing pathways are also now implicated in more chronic forms of cardiovascular disease including atherosclerosis. Atherosclerosis is a multifactorial disease of large- and medium-sized arteries caused by deposition of fatty materials and fibrous elements within the intima of the vessel wall over years. Considerable evidence supports the early involvement of the monocyte/macrophage, the most prominent cellular component of the innate immune response, during atherogenesis. Symptoms become apparent acutely in the late stages of the disease, when plaque formation, vascular remodelling and luminal obstruction results in diminished oxygen supply to target organs.

As a chronic inflammatory condition, atherosclerosis is associated with the presence of conventional cardiovascular risk factors such as hypercholesterolaemia, hypertension, diabetes, smoking and genetic factors. However, the incidence of atherosclerosis is not fully explained by these risk factors (Katz and Shannon, 2006) and infectious agents have been suggested to contribute to the development of atherosclerosis. Indeed, various infectious agents have been identified in atheroma including *C. pneumoniae*, *Cytomegalovirus*, *Herpes simplex* virus, Epstein–Barr virus, HIV, *H. pylori* and hepatitis B and C (Libby *et al.*, 1996; Epstein *et al.*, 1999; Saikku, 2000; Espinola-Klein *et al.*, 2002; Laberge *et al.*, 2005). Due to the number of seroepidemiological and animal studies linking especially *C. pneumoniae* infection to the development of chronic vascular lesions and coronary heart disease, it was

anticipated that Chlamydia infections might be a treatable risk factor for coronary heart disease. However the failure of antibiotic trials (Andraws *et al.*, 2005) and the experimental data reporting that atherogenesis is not altered in mice held in germ-free environment (Wright *et al.*, 2000) has prompted many researchers to criticize the 'infection hypothesis' of atherosclerosis. However, despite these criticisms, a number of recent reports support the notion that innate immune receptors might be involved in this inflammatory process. Thus, the atherogenic potential of PRRs has gained increasing attention during the last few years. Activation of the innate immune systems by PAMPs and DAMPs (such as cholesterol crystals, saturated fatty acids or oxidized low density lipoproteins) represents an attractive candidate as potential atherogenic stimuli as they are well-established promoters of the three key processes of atherosclerosis (EC activation, monocyte adhesion and foam cell formation) and retain their properties independently of bacterial viability (Frantz *et al.*, 2007; Erridge, 2008). Thus, several PAMPs including PGN, LPS and bacterial DNA have been identified as constituents of human atheroma (Erridge, 2008). Specifically, PGN has been found in macrophage-rich atherosclerotic plaques associated with an inflammatory unstable phenotype (Laman *et al.*, 2002) and a clinical study found lower systemic IgM-specific antibody levels against PG in atherosclerotic patients compared to control patients (Nijhuis *et al.*, 2004). More recently, this group has extended these findings by demonstrating an increased expression in human atherosclerotic lesions of the intracellular receptors for PGN breakdown products, NOD1 and NOD2. In this study, NOD1 expression was associated with the presence of smooth muscle cells and macrophages, whereas NOD2 was found in macrophages within inflammatory areas and in ECs (Nijhuis, 2006).

Further supporting a role for NOD1, Opitz and colleagues reported that NOD1 receptors are essential for an intracellularly triggered prolonged and profound activation of ECs by intracellular Chlamydia (Opitz *et al.*, 2005). In addition, chronic administration of selective NOD1 ligands induces coronary arteritis and valvulitis in mice (Nishio *et al.*, 2011). However, this site-specific vascular inflammation was apparently not related to NOD1 expression levels but appeared to be due to a site-specific production of chemokine/cytokine by respective vascular structures. Whether these vascular-targeted effects can be explained by differences in the expression levels of certain molecules involved in NOD1 signalling pathway remains to be determined.

Finally, early evidence suggest a role for NOD1 receptors in myocardial diseases. Fernandez-Velasco *et al.* (2012) have recently confirmed that NOD1 receptors are not just expressed and functional in immune or vascular cells, but are also readily detectable in both cardiac myocytes and cardiac fibroblasts. Activation of NOD1 receptors induced apoptotic pathways in isolated adult murine cardiomyocytes and promoted activation of pro-fibrotic mediators (such as TGF-β pathway) in cardiac fibroblasts. Furthermore, the specific agonist C12-ieDAP induced cardiac dysfunction characterized by a decreased ejection fraction and increased cardiac fibrosis and cardiomyocyte apoptosis (Fernandez-Velasco *et al.*, 2012). Although a previous report (El Mokhtari *et al.*, 2006) failed to identify significant associations between coronary heart disease and the functional CARD4 mutations ND1 +32656, rs2075822 and rs2907748 (linked to inflammatory bowel disease and asthma), these new findings suggesting a contribution of NOD1 to some cardiac diseases with a proinflammatory background such as heart failure warrants further investigation.

NOD1 and metabolic diseases

Insulin resistance is a major defect underlying the development of type 2 diabetes and is a central component of the metabolic syndrome, a constellation of abnormalities including obesity, hypertension, glucose intolerance and dyslipidemia. Insulin resistance results from a complex interplay between nutrient overload, systemic fatty acids excess, inflammation of the adipose tissue, endoplasmic reticulum and oxidative stress and hypoxia of the adipose tissue (Hotamisligil, 2006). Because NOD1 can be found in glucose-metabolizing tissues, including muscle cells (Moreno *et al.*, 2010), liver and adipose (Dharancy *et al.*, 2009), there is a potential that NOD1 in these cells may act as a sensor for intracellular fats and lead to impaired insulin sensitivity. Accordingly, stimulation of NOD1 with synthetic ligands (FK565 or Tri-DAP) has been shown to induce proinflammatory cytokine secretion and impaired insulin-stimulated glucose uptake directly in adipocytes and hepatocytes *in vitro* (Schertzer *et al.*, 2011; Zhao *et al.*, 2011). Furthermore, in the key paper published by Schertzer *et al.* (2011), the authors compared responses in both wild-type control mice and NOD1/2 double gene knockout mice, which had been fed a high-fat diet (HFD) and subsequently exposed to NOD ligands. These authors showed that NOD1/2 double knockout mice were protected from HFD-induced inflammation, lipid accumulation and peripheral insulin intolerance. Furthermore, NOD1 ligands induced peripheral and hepatic insulin resistance within 6 h in wild-type (with NOD2 having a much smaller effect) but not genetically deficient mice. Although NOD1 ligands elicited only minor changes in circulating proinflammatory mediators, they were shown to cause inflammation in adipose tissue and insulin resistance in muscle and hepatic tissues. In this regard, the study by Zhao *et al.* previously discussed showing that saturated fatty acids are able to activate NOD1 receptors in human intestinal epithelial cells (Zhao *et al.*, 2007) might provide an insight on the molecular mechanisms by which HFD may cause inflammation and insulin resistance. However, in contrast to the direct actions previously reported by NOD1 ligands on adipocytes and hepatocytes, skeletal muscle cells in culture only became insulin resistant when exposed to NOD2 ligand (Tamrakar *et al.*, 2010). These findings point to important differences in NOD protein activation between muscle and adipose cells.

Similarly, an independent study revealed that early onset of HFD-induced hyperglycaemia was characterized by an increased bacterial translocation from intestine towards mesenteric adipose tissue and blood, which was dependent on NOD1, CD14 and Myd88, but not NOD2 (Amar *et al.*, 2011).

Further supporting a role for NOD genes in dietary fat-induced inflammatory responses and insulin resistance, a recent study has linked the Glu266Lys polymorphism in the NOD1 gene between dietary saturated fatty acid intake and

insulin sensitivity, despite a lack of correlation with other classical biomarkers of the metabolic syndrome (including waist circumference, triglycerides, high density lipoproteins, glucose, systolic and diastolic blood pressure; Cuda *et al.*, 2012). In summary, recent evidence illustrate the complex relationship between systems of immune defence and metabolic regulation but may also offer exciting new therapeutic avenues in type 2 diabetes.

Concluding remarks

The discovery of PRRs as the sentinels of the innate immune system and regulators of adaptive immunity has presented the opportunity to develop new therapies to combat inflammatory and infectious disease (Paul-Clark *et al.*, 2012). Indeed, research in this field is attractive for drug development and has already led to the development of specific therapies such as Imiquimod for the treatment of basal cell carcinoma.

Although NLR are still a relatively new addition to this research field, it is appealing to postulate based on the recent advances within this research field that modulation of specific NOD1 responses could be beneficial for the treatment of acute infections and relevant inflammatory diseases. Because one of the main functions of NLRs is to help to fight against microbial infection, the first potential application of these receptors includes the treatment of acute infections. Prior to the discovery of Toll-like or NLRs, pharmacologists have exploited the modulation of these receptors. Indeed, the vaccine adjuvant complete Freund's adjuvant developed as early as 1937 is indeed a mixed cocktail of TLR, NOD1 and NOD2 agonists. FK565 represents the first synthetic NOD1 agonist employed in research with potent immune boosting and anti-tumour effects. Following the discovery of ie-DAP as the minimal structure able to stimulate NOD1, additional commercial NOD1 agonists have been developed with improved cell membrane permeability and increased potency (i.e. C12-ie-DAP).

The increasing evidence associating NOD1 signalling with a number of conditions associated to chronic inflammation reviewed herein is unravelling the antagonism of this intracellular receptor as an attractive candidate to help reducing excessive inflammation during infection and chronic inflammatory diseases. Current pharmacological strategies pursuing NOD1 blockade include direct targeting of NOD1 proteins by small-molecule drugs, such as Noditinib-1 (Correa *et al.*, 2011; Khan *et al.*, 2011) or GSK'217 (Gatheral *et al.*, 2012), and specific signalling pathway inhibitors, particularly RIP2 inhibitors such as the recently described inhibitor GSK'214 (Gatheral *et al.*, 2012). It is however important to consider the extensive crosstalk between NOD receptors and other PRRs as exemplified by synergistic interaction with TLRs as discussed earlier. Thus, therapeutic manipulation of NOD1 receptor signalling might have unforeseen consequences on alternative inflammatory pathways. Moreover, as new endogenous DAMPs activating NOD1 receptors are unveiled, further studies will be needed to understand how they may be modulated for the benefit of the host.

In conclusion, although this is a new research field and years of experimental and clinical studies lie ahead, the results discussed in this review suggest that the most promising and clear use for NOD1 agonists is as adjuvants for the treatment of microbial infections that do not respond well to antibiotics. Although therapeutic advances may have to wait until the complex interplay with pathogens, danger signals, other PRR and overlapping metabolic pathways is further unravelled, the steadily growing body of knowledge suggest that NOD1 antagonists might represent attractive candidates to reduce excessive inflammation associated to intestinal, cardiovascular and metabolic diseases (Figure 2).

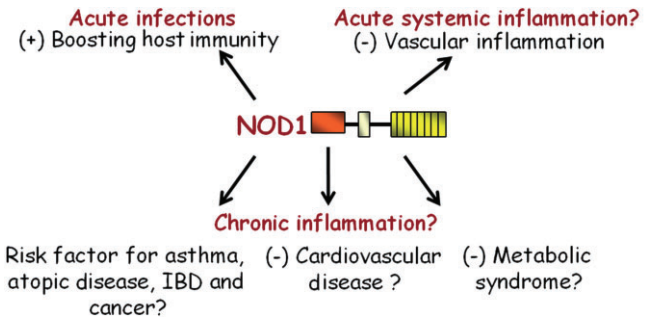

Figure 2
Therapeutic potential of NOD1 modulation. The most promising and clear use of NOD1 agonists is as adjuvants for the treatment of microbial infections not responding well to antibiotics, particularly for gastrointestinal and respiratory infections. In addition, the growing body of research suggest that NOD1 modulation may open a new lead for the development of more selective anti-inflammatory drug targeting when attempting to reduce collateral damage from the septic immune response. Finally, NOD1 activation has been linked to a number of chronic inflammatory diseases suggesting that NOD1 antagonists represent attractive candidates to reduce excessive inflammation associated to intestinal, cardiovascular and metabolic diseases. IBD, inflammatory bowel disease.

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Conflict of interest

The authors have read the journal's policy and have the following conflicts: Timothy Gatheral has received honoraria from GlaxoSmithKline for participation in educational meetings regarding the management of asthma and COPD. This does not alter the authors' adherence to all the *British Journal of Pharmacology* policies on sharing data and materials.

References

Abreu MT, Fukata M, Arditi M (2005). TLR signaling in the gut in health and disease. J Immunol 174: 4453–4460.

Allison CC, Ferrand J, McLeod L, Hassan M, Kaparakis-Liaskos M, Grubman A *et al*. (2013). Nucleotide oligomerization domain 1 enhances IFN-gamma signaling in gastric epithelial cells during *Helicobacter pylori* infection and exacerbates disease severity. J Immunol 190: 3706–3715.

Amar J, Chabo C, Waget A, Klopp P, Vachoux C, Bermudez-Humaran LG *et al*. (2011). Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. EMBO Mol Med 3: 559–572.

Andraws R, Berger JS, Brown DL (2005). Effects of antibiotic therapy on outcomes of patients with coronary artery disease: a meta-analysis of randomized controlled trials. JAMA 293: 2641–2647.

Berrington WR, Iyer R, Wells RD, Smith KD, Skerrett SJ, Hawn TR (2010). NOD1 and NOD2 regulation of pulmonary innate immunity to *Legionella pneumophila*. Eur J Immunol 40: 3519–3527.

Bertin J, Nir WJ, Fischer CM, Tayber OV, Errada PR, Grant JR *et al*. (1999). Human CARD4 protein is a novel CED-4/Apaf-1 cell death family member that activates NF-kappaB. J Biol Chem 274: 12955–12958.

Bouskra D, Brezillon C, Berard M, Werts C, Varona R, Boneca IG *et al*. (2008). Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. Nature 456: 507–510.

Buchholz KR, Stephens RS (2008). The cytosolic pattern recognition receptor NOD1 induces inflammatory interleukin-8 during *Chlamydia trachomatis* infection. Infect Immun 76: 3150–3155.

Cartwright N, McMaster SK, Sorrentino R, Paul-Clark M, Sriskandan S, Ryffel B *et al*. (2007a). Elucidation of Toll-like receptor and adapter protein signaling in vascular dysfunction induced by gram-positive *Staphylococcus aureus* or gram-negative *Escherichia coli*. Shock 27: 40–47.

Cartwright N, Murch O, McMaster SK, Paul-Clark MJ, van Heel DA, Ryffel B *et al*. (2007b). Selective NOD1 agonists cause shock and organ injury/dysfunction in vivo. Am J Respir Crit Care Med 175: 595–603.

Chamaillard M, Hashimoto M, Horie Y, Masumoto J, Qiu S, Saab L *et al*. (2003). An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat Immunol 4: 702–707.

Chamaillard M, Inohara N, Nunez G (2004). Battling enteroinvasive bacteria: NOD1 comes to the rescue. Trends Microbiol 12: 529–532.

Chaouche-Drider N, Kaparakis M, Karrar A, Fernandez MI, Carneiro LA, Viala J *et al*. (2009). A commensal *Helicobacter* sp. of the rodent intestinal flora activates TLR2 and NOD1 responses in epithelial cells. PLoS ONE 4: e5396.

Chatterjee D, Chaudhuri K (2013). *Vibrio cholerae* O395 outer membrane vesicles modulate intestinal epithelial cells in a NOD1 protein-dependent manner and induce dendritic cell-mediated Th2/Th17 cell responses. J Biol Chem 288: 4299–4309.

Chen GY, Nunez G (2009). Gut immunity: a NOD to the commensals. Curr Biol 19: R171–R174.

Chen GY, Nunez G (2010). Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol 10: 826–837.

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

Clarke TB, Davis KM, Lysenko ES, Zhou AY, Yu Y, Weiser JN (2010). Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. Nat Med 16: 228–231.

Correa RG, Khan PM, Askari N, Zhai D, Gerlic M, Brown B *et al*. (2011). Discovery and characterization of 2-aminobenzimidazole derivatives as selective NOD1 inhibitors. Chem Biol 18: 825–832.

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.

Cuda C, Badawi A, Karmali M, El-Sohemy A (2012). Effects of polymorphisms in nucleotide-binding oligomerization domains 1 and 2 on biomarkers of the metabolic syndrome and type II diabetes. Genes Nutr 7: 427–435.

De Kimpe SJ, Kengatharan M, Thiemermann C, Vane JR (1995). The cell wall components peptidoglycan and lipoteichoic acid from *Staphylococcus aureus* act in synergy to cause shock and multiple organ failure. Proc Natl Acad Sci U S A 92: 10359–10363.

Dharancy S, Body-Malapel M, Louvet A, Berrebi D, Gantier E, Gosset P *et al*. (2009). Neutrophil migration during liver injury is under nucleotide-binding oligomerization domain 1 control. Gastroenterology 138: 1546–1556, 1556 e1541–1545.

Eckmann L (2006). Sensor molecules in intestinal innate immunity against bacterial infections. Curr Opin Gastroenterol 22: 95–101.

El Mokhtari NE, Ott SJ, Nebel A, Simon R, Schreiber S (2006). A functional variant in the CARD4 gene and risk of premature coronary heart disease. Int J Immunogenet 33: 307–311.

Epstein SE, Zhou YF, Zhu J (1999). Potential role of cytomegalovirus in the pathogenesis of restenosis and atherosclerosis. Am Heart J 138 (5 Pt 2): S476–S478.

Erridge C (2008). The roles of pathogen-associated molecular patterns in atherosclerosis. Trends Cardiovasc Med 18: 52–56.

Espinola-Klein C, Rupprecht HJ, Blankenberg S, Bickel C, Peth S, Kopp H *et al*. (2002). [Manifestations of atherosclerosis in various vascular regions. Similarities and differences regarding epidemiology, etiology and prognosis]. Med Klin (Munich) 97: 221–228.

Feng BS, He SH, Zheng PY, Wu L, Yang PC (2007). Mast cells play a crucial role in *Staphylococcus aureus* peptidoglycan-induced diarrhea. Am J Pathol 171: 537–547.

Fernandez-Velasco M, Prieto P, Terron V, Benito G, Flores JM, Delgado C *et al*. (2012). NOD1 activation induces cardiac dysfunction and modulates cardiac fibrosis and cardiomyocyte apoptosis. PLoS ONE 7: e45260.

Ferwerda G, Girardin SE, Kullberg BJ, Le Bourhis L, de Jong DJ, Langenberg DM *et al*. (2005). NOD2 and Toll-like receptors are nonredundant recognition systems of *Mycobacterium tuberculosis*. PLoS Pathog 1: 279–285.

Frantz S, Ertl G, Bauersachs J (2007). Mechanisms of disease: Toll-like receptors in cardiovascular disease. Nat Clin Pract Cardiovasc Med 4: 444–454.

Fritz JH, Girardin SE, Fitting C, Werts C, Mengin-Lecreulx D, Caroff M *et al*. (2005). Synergistic stimulation of human monocytes and dendritic cells by Toll-like receptor 4 and NOD1- and NOD2-activating agonists. Eur J Immunol 35: 2459–2470.

Fukazawa A, Alonso C, Kurachi K, Gupta S, Lesser CF, McCormick BA *et al*. (2008). GEF-H1 mediated control of NOD1 dependent NF-kappaB activation by *Shigella* effectors. PLoS Pathog 4: e1000228.

Gatheral T, Reed DM, Moreno L, Gough PJ, Votta BJ, Sehon CA *et al*. (2012). A key role for the endothelium in NOD1 mediated vascular inflammation: comparison to TLR4 responses. PLoS ONE 7: e42386.

Girardin SE, Tournebize R, Mavris M, Page AL, Li X, Stark GR *et al.* (2001). CARD4/Nod1 mediates NF-kappaB and JNK activation by invasive *Shigella flexneri*. EMBO Rep 2: 736–742.

Girardin SE, Boneca IG, Carneiro LA, Antignac A, Jehanno M, Viala J *et al.* (2003). Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science 300: 1584–1587.

Gotoh T, Nakahara K, Iwami M, Aoki H, Imanaka H (1982). Studies on a new immunoactive peptide, FK-156. I. Taxonomy of the producing strains. J Antibiot (Tokyo) 35: 1280–1285.

Hasegawa M, Fujimoto Y, Lucas PC, Nakano H, Fukase K, Nunez G *et al.* (2008). A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-kappaB activation. EMBO J 27: 373–383.

Hasegawa M, Yamazaki T, Kamada N, Tawaratsumida K, Kim YG, Nunez G *et al.* (2011). Nucleotide-binding oligomerization domain 1 mediates recognition of *Clostridium difficile* and induces neutrophil recruitment and protection against the pathogen. J Immunol 186: 4872–4880.

van Heel DA, Ghosh S, Butler M, Hunt K, Foxwell BM, Mengin-Lecreulx D *et al.* (2005). Synergistic enhancement of Toll-like receptor responses by NOD1 activation. Eur J Immunol 35: 2471–2476.

Hisamatsu T, Suzuki M, Podolsky DK (2003). Interferon-gamma augments CARD4/NOD1 gene and protein expression through interferon regulatory factor-1 in intestinal epithelial cells. J Biol Chem 278: 32962–32968.

Hotamisligil GS (2006). Inflammation and metabolic disorders. Nature 444: 860–867.

Hruz P, Eckmann L (2008). Caspase recruitment domain-containing sensors and adaptors in intestinal innate immunity. Curr Opin Gastroenterol 24: 108–114.

Hysi P, Kabesch M, Moffatt MF, Schedel M, Carr D, Zhang Y *et al.* (2005). NOD1 variation, immunoglobulin E and asthma. Hum Mol Genet 14: 935–941.

Inamura N, Nakahara K, Kino T, Gotoh T, Kawamura I, Aoki H *et al.* (1985). Activation of tumoricidal properties in macrophages and inhibition of experimentally-induced murine metastases by a new synthetic acyltripeptide, FK-565. J Biol Response Mod 4: 408–417.

Jimenez R, Belcher E, Sriskandan S, Lucas R, McMaster S, Vojnovic I *et al.* (2005). Role of Toll-like receptors 2 and 4 in the induction of cyclooxygenase-2 in vascular smooth muscle. Proc Natl Acad Sci U S A 102: 4637–4642.

Kang MJ, Heo SK, Song EJ, Kim DJ, Han SY, Han JH *et al.* (2012). Activation of Nod1 and Nod2 induces innate immune responses of prostate epithelial cells. Prostate 72: 1351–1358.

Kanneganti TD, Lamkanfi M, Kim YG, Chen G, Park JH, Franchi L *et al.* (2007). Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. Immunity 26: 433–443.

Kara B, Akkiz H, Doran F, Bayram S, Erken E, Gumurdullu Y *et al.* (2010). The significance of E266K polymorphism in the NOD1 gene on *Helicobacter pylori* infection: an effective force on pathogenesis? Clin Exp Med 10: 107–112.

Katz JT, Shannon RP (2006). Bacteria and coronary atheroma: more fingerprints but no smoking gun. Circulation 113: 920–922.

Khan PM, Correa RG, Divlianska DB, Peddibhotla S, Sessions EH, Magnuson G *et al.* (2011). Identification of inhibitors of NOD1-induced nuclear factor-kappaB activation. ACS Med Chem Lett 2: 780–785.

Kim JG, Lee SJ, Kagnoff MF (2004). Nod1 is an essential signal transducer in intestinal epithelial cells infected with bacteria that avoid recognition by Toll-like receptors. Infect Immun 72: 1487–1495.

Kim YG, Park JH, Daignault S, Fukase K, Nunez G (2008). Cross-tolerization between Nod1 and Nod2 signaling results in reduced refractoriness to bacterial infection in Nod2-deficient macrophages. J Immunol 181: 4340–4346.

Kim YG, Park JH, Reimer T, Baker DP, Kawai T, Kumar H *et al.* (2011). Viral infection augments Nod1/2 signaling to potentiate lethality associated with secondary bacterial infections. Cell Host Microbe 9: 496–507.

Kobayashi K, Inohara N, Hernandez LD, Galan JE, Nunez G, Janeway CA *et al.* (2002). RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. Nature 416: 194–199.

Koonin EV, Aravind L (2000). The NACHT family – a new group of predicted NTPases implicated in apoptosis and MHC transcription activation. Trends Biochem Sci 25: 223–224.

Kumar H, Kawai T, Akira S (2009). Toll-like receptors and innate immunity. Biochem Biophys Res Commun 388: 621–625.

Kupcinskas J, Wex T, Bornschein J, Selgrad M, Leja M, Juozaityte E *et al.* (2011). Lack of association between gene polymorphisms of angiotensin converting enzyme, Nod-like receptor 1, Toll-like receptor 4, FAS/FASL and the presence of *Helicobacter pylori*-induced premalignant gastric lesions and gastric cancer in Caucasians. BMC Med Genet 12: 112.

Kutikhin AG (2011). Role of NOD1/CARD4 and NOD2/CARD15 gene polymorphisms in cancer etiology. Hum Immunol 72: 955–968.

Laberge MA, Moore KJ, Freeman MW (2005). Atherosclerosis and innate immune signaling. Ann Med 37: 130–140.

Laman JD, Schoneveld AH, Moll FL, van Meurs M, Pasterkamp G (2002). Significance of peptidoglycan, a proinflammatory bacterial antigen in atherosclerotic arteries and its association with vulnerable plaques. Am J Cardiol 90: 119–123.

Lemaitre B, Reichhart JM, Hoffmann JA (1997). Drosophila host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. Proc Natl Acad Sci U S A 94: 14614–14619.

Libby P, Geng YJ, Aikawa M, Schoenbeck U, Mach F, Clinton SK *et al.* (1996). Macrophages and atherosclerotic plaque stability. Curr Opin Lipidol 7: 330–335.

McDonald C, Inohara N, Nunez G (2005). Peptidoglycan signaling in innate immunity and inflammatory disease. J Biol Chem 280: 20177–20180.

McGettrick AF, O'Neill LA (2013). How metabolism generates signals during innate immunity and inflammation. J Biol Chem R113.486464.

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

Marti-Carvajal A, Salanti G, Cardona AF (2008). Human recombinant activated protein C for severe sepsis. Cochrane Database Syst Rev (1): CD004388.

Martin G, Brunkhorst FM, Janes JM, Reinhart K, Sundin DP, Garnett K *et al.* (2009). The international PROGRESS registry of

patients with severe sepsis: drotrecogin alfa (activated) use and patient outcomes. Crit Care 13: R103.

Masumoto J, Yang K, Varambally S, Hasegawa M, Tomlins SA, Qiu S *et al*. (2006). Nod1 acts as an intracellular receptor to stimulate chemokine production and neutrophil recruitment in vivo. J Exp Med 203: 203–213.

Mitchell JA, Ryffel B, Quesniaux VF, Cartwright N, Paul-Clark M (2007). Role of pattern-recognition receptors in cardiovascular health and disease. Biochem Soc Trans 35 (Pt 6): 1449–1452.

Mitka M (2011). Drug for severe sepsis is withdrawn from market, fails to reduce mortality. JAMA 306: 2439–2440.

Moreno L, McMaster SK, Gatheral T, Bailey LK, Harrington LS, Cartwright N *et al*. (2010). Nucleotide oligomerization domain 1 is a dominant pathway for NOS2 induction in vascular smooth muscle cells: comparison with Toll-like receptor 4 responses in macrophages. Br J Pharmacol 160: 1997–2007.

Myhre AE, Aasen AO, Thiemermann C, Wang JE (2006). Peptidoglycan – an endotoxin in its own right? Shock 25: 227–235.

Nijhuis MM (2006). Peptidoglycan in atherosclerotic plaque formation and vulnerability. Available at: http://igitur-archive.library.uu.nl/dissertations/2006-0620-200920 (accessed 30/6/2013).

Nijhuis MM, van der Graaf Y, Melief MJ, Schoneveld AH, de Kleijn DP, Laman JD *et al*. (2004). IgM antibody level against proinflammatory bacterial peptidoglycan is inversely correlated with extent of atherosclerotic disease. Atherosclerosis 173: 245–251.

Nishio H, Kanno S, Onoyama S, Ikeda K, Tanaka T, Kusuhara K *et al*. (2011). Nod1 ligands induce site-specific vascular inflammation. Arterioscler Thromb Vasc Biol 31: 1093–1099.

O'Connell RM, Vaidya SA, Perry AK, Saha SK, Dempsey PW, Cheng G (2005). Immune activation of type I IFNs by Listeria monocytogenes occurs independently of TLR4, TLR2, and receptor interacting protein 2 but involves TNFR-associated NF kappa B kinase-binding kinase 1. J Immunol 174: 1602–1607.

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

Opitz B, Puschel A, Schmeck B, Hocke AC, Rosseau S, Hammerschmidt S *et al*. (2004). Nucleotide-binding oligomerization domain proteins are innate immune receptors for internalized *Streptococcus pneumoniae*. J Biol Chem 279: 36426–36432.

Opitz B, Forster S, Hocke AC, Maass M, Schmeck B, Hippenstiel S *et al*. (2005). Nod1-mediated endothelial cell activation by *Chlamydophila pneumoniae*. Circ Res 96: 319–326.

Opitz B, Puschel A, Beermann W, Hocke AC, Forster S, Schmeck B *et al*. (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.

Park JH, Kim YG, McDonald C, Kanneganti TD, Hasegawa M, Body-Malapel M *et al*. (2007). RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs. J Immunol 178: 2380–2386.

Parkin DM (2006). The global health burden of infection-associated cancers in the year 2002. Int J Cancer 118: 3030–3044.

Patel GP, Balk RA (2011). Systemic steroids in severe sepsis and septic shock. Am J Respir Crit Care Med 185: 133–139.

Paul-Clark MJ, Sorrentino R, Bailey LK, Sriskandan S, Mitchell JA (2008). Gram-positive and Gram-negative bacteria synergize with oxidants to release CXCL8 from innate immune cells. Mol Med 14: 238–246.

Paul-Clark MJ, George PM, Gatheral T, Parzych K, Wright WR, Crawford D *et al*. (2012). Pharmacology and therapeutic potential of pattern recognition receptors. Pharmacol Ther 135: 200–215.

Regueiro V, Moranta D, Frank CG, Larrarte E, Margareto J, March C *et al*. (2010). *Klebsiella pneumoniae* subverts the activation of inflammatory responses in a NOD1-dependent manner. Cell Microbiol 13: 135–153.

Rosenthal RS, Dziarski R (1994). Isolation of peptidoglycan and soluble peptidoglycan fragments. Methods Enzymol 235: 253–285.

Saikku P (2000). *Chlamydia pneumoniae* in atherosclerosis. J Intern Med 247: 391–396.

Schertzer JD, Tamrakar AK, Magalhaes JG, Pereira S, Bilan PJ, Fullerton MD *et al*. (2011). NOD1 activators link innate immunity to insulin resistance. Diabetes 60: 2206–2215.

Schirbel A, Kessler S, Rieder F, West G, Rebert N, Asosingh K *et al*. (2012). Pro-angiogenic activity of TLRs and NLRs: a novel link between gut microbiota and intestinal angiogenesis. Gastroenterology 144: 613–623 e619.

Senft AP, Taylor RH, Lei W, Campbell SA, Tipper JL, Martinez MJ *et al*. (2009). Respiratory syncytial virus impairs macrophage IFN-alpha/beta- and IFN-gamma-stimulated transcription by distinct mechanisms. Am J Respir Cell Mol Biol 42: 404–414.

Shaw MH, Reimer T, Kim YG, Nunez G (2008). NOD-like receptors (NLRs): bona fide intracellular microbial sensors. Curr Opin Immunol 20: 377–382.

Shigeoka AA, Kambo A, Mathison JC, King AJ, Hall WF, Correia J *et al*. (2010). Nod1 and nod2 are expressed in human and murine renal tubular epithelial cells and participate in renal ischemia reperfusion injury. J Immunol 184: 2297–2304.

Shimada K, Chen S, Dempsey PW, Sorrentino R, Alsabeh R, Slepenkin AV *et al*. (2009). The NOD/RIP2 pathway is essential for host defenses against *Chlamydophila pneumoniae* lung infection. PLoS Pathog 5: e1000379.

Strober W, Murray PJ, Kitani A, Watanabe T (2006). Signalling pathways and molecular interactions of NOD1 and NOD2. Nat Rev Immunol 6: 9–20.

Stroo I, Butter LM, Claessen N, Teske GJ, Rubino SJ, Girardin SE *et al*. (2012). Phenotyping of Nod1/2 double deficient mice and characterization of Nod1/2 in systemic inflammation and associated renal disease. Biol Open 1: 1239–1247.

Tada H, Aiba S, Shibata K, Ohteki T, Takada H (2005). Synergistic effect of Nod1 and Nod2 agonists with Toll-like receptor agonists on human dendritic cells to generate interleukin-12 and T helper type 1 cells. Infect Immun 73: 7967–7976.

Tamrakar AK, Schertzer JD, Chiu TT, Foley KP, Bilan PJ, Philpott DJ *et al*. (2010). NOD2 activation induces muscle cell-autonomous innate immune responses and insulin resistance. Endocrinology 151: 5624–5637.

Ting JPY, Lovering RC, Alnemri ES, Bertin J, Boss JM, Davis BK *et al*. (2008). The NLR gene family: a standard nomenclature. Immunity 28: 285–287.

Travassos LH, Carneiro LA, Girardin SE, Boneca IG, Lemos R, Bozza MT *et al*. (2005). Nod1 participates in the innate immune response to *Pseudomonas aeruginosa*. J Biol Chem 280: 36714–36718.

Uehara A, Yang S, Fujimoto Y, Fukase K, Kusumoto S, Shibata K *et al*. (2005). Muramyldipeptide and diaminopimelic acid-containing desmuramylpeptides in combination with chemically synthesized Toll-like receptor agonists synergistically induced production of interleukin-8 in a NOD2- and

NOD1-dependent manner, respectively, in human monocytic cells in culture. Cell Microbiol 7: 53–61.

Uehara A, Fujimoto Y, Fukase K, Takada H (2007). Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce anti-microbial peptides, but not proinflammatory cytokines. Mol Immunol 44: 3100–3111.

Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP *et al*. (2004). Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. Nat Immunol 5: 1166–1174.

Wang JE, Dahle MK, Yndestad A, Bauer I, McDonald MC, Aukrust P *et al*. (2004). Peptidoglycan of *Staphylococcus aureus* causes inflammation and organ injury in the rat. Crit Care Med 32: 546–552.

Wray GM, Foster SJ, Hinds CJ, Thiemermann C (2001). A cell wall component from pathogenic and non-pathogenic gram-positive bacteria (peptidoglycan) synergises with endotoxin to cause the release of tumour necrosis factor-alpha, nitric oxide production, shock, and multiple organ injury/dysfunction in the rat. Shock 15: 135–142.

Wright SD, Burton C, Hernandez M, Hassing H, Montenegro J, Mundt S *et al*. (2000). Infectious agents are not necessary for murine atherogenesis. J Exp Med 191: 1437–1442.

Yang S, Tamai R, Akashi S, Takeuchi O, Akira S, Sugawara S *et al*. (2001). Synergistic effect of muramyldipeptide with lipopolysaccharide or lipoteichoic acid to induce inflammatory cytokines in human monocytic cells in culture. Infect Immun 69: 2045–2053.

Yokota Y, Mine Y, Wakai Y, Watanabe Y, Nishida M, Goto S *et al*. (1983). Immunoactive peptides, FK-156 and FK-565. II. Restoration of host resistance to microbial infection in immunosuppressed mice. J Antibiot (Tokyo) 36: 1051–1058.

Zhao L, Kwon MJ, Huang S, Lee JY, Fukase K, Inohara N *et al*. (2007). Differential modulation of Nods signaling pathways by fatty acids in human colonic epithelial HCT116 cells. J Biol Chem 282: 11618–11628.

Zhao L, Hu P, Zhou Y, Purohit J, Hwang D (2011). NOD1 activation induces proinflammatory gene expression and insulin resistance in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 301: E587–E598.

Zilbauer M, Dorrell N, Elmi A, Lindley KJ, Schuller S, Jones HE *et al*. (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.