Cellular Microbiology (2008) **10**(1), 1–8
doi:10.1111/j.1462-5822.2007.01059.x
First published online 18 October 2007

Microreview

Intracellular NOD-like receptors in innate immunity, infection and disease

Luigi Franchi, Jong-Hwan Park, Michael H. Shaw, Noemi Marina-Garcia, Grace Chen, Yun-Gi Kim and Gabriel Núñez*
Department of Pathology and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI 48109, USA.

Summary

The innate immune system comprises several classes of pattern-recognition receptors, including Toll-like receptors (TLRs) and nucleotide binding and oligomerization domain-like receptors (NLRs). TLRs recognize microbes on the cell surface and in endosomes, whereas NLRs sense microbial molecules in the cytosol. In this review, we focus on the role of NLRs in host defence against bacterial pathogens. Nod1 and Nod2 sense the cytosolic presence of molecules containing meso-diaminopimelic acid and muramyl dipeptide respectively, and drive the activation of mitogen-activated protein kinase and NF-κB. In contrast, Ipaf, Nalp1b and Cryopyrin/Nalp3 promote the assembly of inflammasomes that are required for the activation of caspase-1. Mutation in several NLR members, including NOD2 and Cryopyrin, is associated with the development of inflammatory disorders. Further understanding of NLRs should provide new insights into the mechanisms of host defence and the pathogenesis of inflammatory diseases.

Introduction

Upon encountering pathogenic microorganisms, the immunocompetent host activates two distinct effector mechanisms, the innate and the adaptive immune defences, to ensure effective elimination of the invading microbe. Unlike adaptive immune responses, the innate immune system relies on phagocytic and non-haematopietic cells to sense the presence of pathogens. The initial recognition of microbes is mediated by a set of germline-encoded pattern-recognition receptors (PRRs) that sense highly conserved microbial motifs, so-called pathogen-associated molecular patterns (PAMPs) (Kawai and Akira, 2006). PRRs can be found in the extracellular space, integrated in cellular membranes or in the cytosol. Among the membrane-bound PRRs, the best-known PRRs are the Toll-like receptors (TLRs) that sense a wide array of microbial ligands at the cell surface or within endosomes (Kawai and Akira, 2006). Cytoplasmic PRRs include the caspase-recruiting domain (CARD) helicases, such as retinoic acid-inducible protein I and melanoma-differentiation-associated protein 5, which are involved in antiviral responses (Kawai and Akira, 2006), and the nucleotide binding oligomerization domain (NOD)-like receptor (NLR) family that recognize primarily microbial molecules of bacterial origin (Inohara *et al*., 2005). In humans, the NLR family is composed of 23 cytosolic proteins characterized by the presence of a conserved NOD domain and leucine-rich repeats (LRRs) (Inohara *et al*., 2005). The general domain structure of the NLR family members includes an amino-terminal effector region that consists of a protein–protein interaction domain such as the CARD, Pyrin or BIR domain, a centrally located NOD domain, and carboxyl-terminal LRRs that are involved in microbial sensing (Inohara *et al*., 2005). Some members of the NLR family, namely Nod1 and Nod2, mediate activation of NF-κB and mitogen-activated protein kinases (MAPKs) in response to peptidoglycan-related molecules (McDonald *et al*., 2005). A different set of NLRs, including Nalp1, Cryopyrin/Nalp3 and Ipaf, are involved in the activation of the protease caspase-1 (Franchi *et al*., 2006a). Ipaf is activated by bacterial flagellin (Franchi *et al*., 2006b; Miao *et al*., 2006); mouse Nalp1b by lethal toxin produced by *Bacillus anthracis* (Boyden and Dietrich, 2006); Cryopyrin is activated in response to a variety of microbial molecules (Kanneganti *et al*., 2006; 2007; Mariathasan *et al*., 2006; Sutterwala *et al*., 2006) as well as endogenous ligands, such as uric acid crystals (Martinon *et al*., 2006). While certain microbial molecules, such as meso-diaminopimelic acid (iE-DAP) and muramyl dipeptide (MDP) (McDonald *et al*., 2005), are exclusively recognized by NLRs, other PAMPs are also sensed by TLR

Received 1 August, 2007; revised 6 September, 2007; accepted 13 September, 2007. *For correspondence. E-mail bclx@umich.edu; Tel. (+1) 734 764 8514; Fax (+1) 734 647 9654.

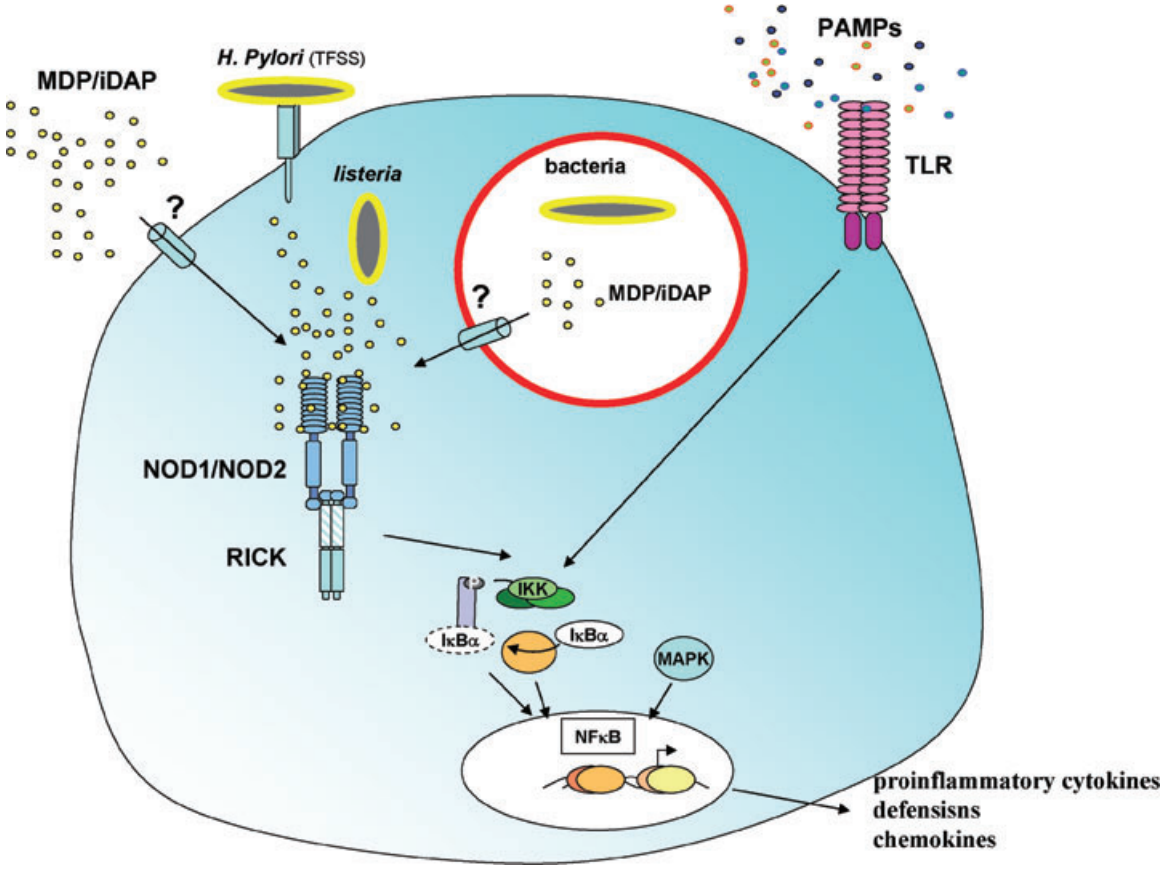

Fig. 1. Model for activation of Nod1 and Nod2 signalling pathways. The NLR proteins NOD1 and NOD2 sense intracellular iE-DAP and MDP respectively, leading to recruitment of the adaptor proteins RICK. Extracellular PAMPs are recognized by TLRs, which signal through MyD88, IRAK proteins and TRAF members independently of NOD1/NOD2. For clarity, the TLR pathway has been simplified. The subsequent activation of NF-κB and MAPKs results in the transcriptional upregulation of pro-inflammatory and host defence genes.

family members. Flagellin, for example, is recognized by both Ipaf (Franchi *et al*., 2006b; Miao *et al*., 2006) and TLR5 (Hayashi *et al*., 2001), although the amino acid residues of flagellin that are recognized by these two PRRs appear to be different (Franchi *et al*., 2007a).

Nod1 and Nod2

Early studies revealed that Nod1 and Nod2 induce NF-κB activation when overexpressed in mammalian cells and enhance the response to specific microbial products independently of TLRs (Inohara *et al*., 2001). Subsequent studies revealed that Nod1 recognizes peptidoglycan-related molecules containing iE-DAP, which is found in many Gram-negative and certain Gram-positive bacteria, including the genus *Listeria* and *Bacillus* (Chamaillard *et al*. 2003; Girardin *et al*., 2003a). In contrast, Nod2 is activated by MDP, a peptidoglycan motif which is present in all Gram-positive and Gram-negative bacteria (Girardin *et al*., 2003b; Inohara *et al*., 2003). Upon ligand recognition, Nod1 and Nod2 undergo conformational changes, resulting in self-oligomerization via the NOD domain and recruitment of RICK (RIP2), a serine threonine kinase that is required for Nod1- and Nod2-mediated NF-κB and MAPK activation (Inohara *et al*., 2000; Girardin *et al*., 2001; Park *et al*., 2007a,b) (Fig. 1). Whereas Nod1 is ubiquitously expressed in various cell types, Nod2 is expressed at higher levels in phagocytic cells and Paneth cells of the small intestine (Inohara *et al*., 2005). Administration of Nod1 ligands to cells and mice induce chemokine production and recruitment of neutrophils *in vivo* (Masumoto *et al*., 2006). Furthermore, Nod1 stimulation contributes to adaptive immune responses, although the mechanism involved remains unclear (Fritz *et al*., 2007). *In vitro* studies have demonstrated that many bacteria express Nod1-stimulatory activity, which is highest in *Bacillus* species (Hasegawa *et al*., 2007). Moreover, infection of host cells by several pathogenic bacteria, including *Shigella flexneri* (Girardin *et al*., 2001), enteroinvasive *Escherichia coli* (Kim *et al*., 2004), *Listeria monocytogenes* (Park *et al*., 2007b) and *Campylobacter jejuni* (Zilbauer *et al*., 2007), results in Nod1-dependent NF-κB activation. However, the role of Nod1 during *in vivo* infection with the exception of *Helicobacter pylori* remains

poorly understood (Viala *et al*., 2004; Boughan *et al*., 2006).

Similar to Nod1, Nod2 has been implicated in the detection of several pathogenic bacteria and induction of innate immune responses to *Streptococcus pneumoniae* (Opitz *et al*., 2004), *Mycobacterium tuberculosis* (Ferwerda *et al*., 2005), *Staphylococcus aureus* (Kapetanovic *et al*. 2007) and *L. monocytogenes* (Kobayashi *et al*., 2005; Herskovits *et al*., 2007). The mechanism involved in the entry of MDP into the host cytosol is still unknown, although in epithelial cells, an active transport mechanism for MDP through the peptide transporter hPepT1 has been proposed (Ismair *et al*., 2006). MDP might also be generated from phagocytosed bacteria and recognized via Nod2 in the cytosol (Herskovits *et al*., 2007). Stimulation of TLRs and Nod1 or Nod2 by their respective agonists results in synergistic production of pro-inflammatory cytokines (Fritz *et al*., 2005; Kobayashi *et al*., 2005; Tada *et al*., 2005). This type of cooperative signalling may increase the sensitivity of bacterial detection and reduce the threshold for Nod1/Nod2 and TLR activation.

Nod2 is associated with inflammatory disease

Genetic variation in Nod2 is associated with susceptibility to several inflammatory diseases. Crohn's disease (CD), a chronic inflammatory disorder of the intestinal wall, is associated with three common mutations (R702W, G908R and L1007insC) involving amino acid residues near or within the LRRs of Nod2 (Hugot *et al*., 2001; Ogura *et al*., 2001). Functional studies have revealed that the human CD-associated Nod2 variants exhibit reduced or loss of activity when compared with the wild-type protein (Inohara *et al*., 2003). Intriguingly, mouse macrophages, but not human monocytes, expressing the disease-associated L1007InsC NOD2 variant exhibit increased IL-1β levels when stimulated with MDP (van Heel *et al*., 2005; Maeda *et al*., 2005). Although the mechanism by which Nod2 mutations increase the susceptibility to CD remains poorly understood, impaired sensing of bacteria may trigger an abnormal inflammatory response to unclear bacteria. Alternatively, reduced expression of α-defensins in Paneth cells or dysregulated TLR2 signalling have been proposed (Watanabe *et al*., 2004; Kobayashi *et al*., 2005). Clearly, further studies are needed to understand the link between Nod2 mutations and the development of CD. In addition, several missense mutations involving amino acid residues in the NOD domain of Nod2 cause two autosomal dominant disorders characterized by granulomatous inflammation in multiple organ tissues, called Blau syndrome (BS) and early-onset sarcoidosis (EOS) (Miceli-Richard *et al*., 2001; Kanazawa *et al*., 2005). In contrast to CD, the Nod2 mutations associated with BS and EOS represent gain-of-function mutations (Tanabe *et al*., 2004), which is consistent with the dominant mode of inheritance of these diseases.

The inflammasome: a molecular machinery for caspase-1 activation

Caspase-1 is the prototypical inflammatory caspase and mediates the proteolytic maturation of the cytokines IL-1β and IL-18 (Lamkanfi *et al*., 2007a). Upon detection of specific microbial motifs, some NLRs switch conformation and assemble a molecular platform, the inflammasome, which is responsible for the processing and activation of pro-caspase-1 into the enzymatically active heterodimer composed of a 10 kDa and a 20 kDa chain (Fig. 2). Ipaf senses cytosolic flagellin (Amer *et al*., 2006; Franchi *et al*., 2006b; Miao *et al*., 2006), while the detection of microbial molecules by Cryopyrin depends on membrane pore formation induced by several toxins, including maitotoxin and nigericin, which are thought to aid the translocation of microbial products into the host cytosol, where they can be detected by NLRs (Mariathasan *et al*., 2006; Sutterwala *et al*., 2006; Kanneganti *et al*., 2007). The delivery of microbial products into the cytosol is also promoted by endogenous molecules, such as ATP, which activate the P2X7 receptor and the opening of a large pore mediated by the hemichannel pannexin-1 (Kanneganti *et al*., 2007). The bipartite adaptor protein ASC has been implicated in the activity of the NALP1-3 and Ipaf-containing inflammasomes by linking the interaction between NLR proteins and inflammatory caspases (Tschopp *et al*., 2003; Franchi *et al*., 2006a). ASC plays a central role in the assembly of the inflammasomes and the activation of caspase-1 in response to a broad range of PAMPs and intracellular pathogens (Tschopp *et al*., 2003; Franchi *et al*., 2006a). Although production of pro-IL-1β is induced through TLR stimulation, activation of caspase-1 via NLRs is independent of TLR signalling (Kanneganti *et al*., 2007). The dissociation between pro-IL-1β production and caspase-1 activation via TLRs and NLRs may serve to tailor the quality of the inflammatory response against invasive microbes and to safeguard against IL-1β overproduction.

Dysregulated inflammasome activation can result in the development of inflammatory disorders. For example, point mutations in Cryopyrin are the cause of familial cold autoinflammatory syndrome, Muckle–Wells syndrome, and neonatal-onset multisystem inflammatory disease. Functional studies revealed that the Cryopyrin mutants exhibit enhanced activity to induce IL-1β secretion (Dowds *et al*., 2004) and mononuclear cells from patients with autoinflammatory syndromes spontaneously secrete IL-1β (Agostini *et al*., 2004). These observations suggest

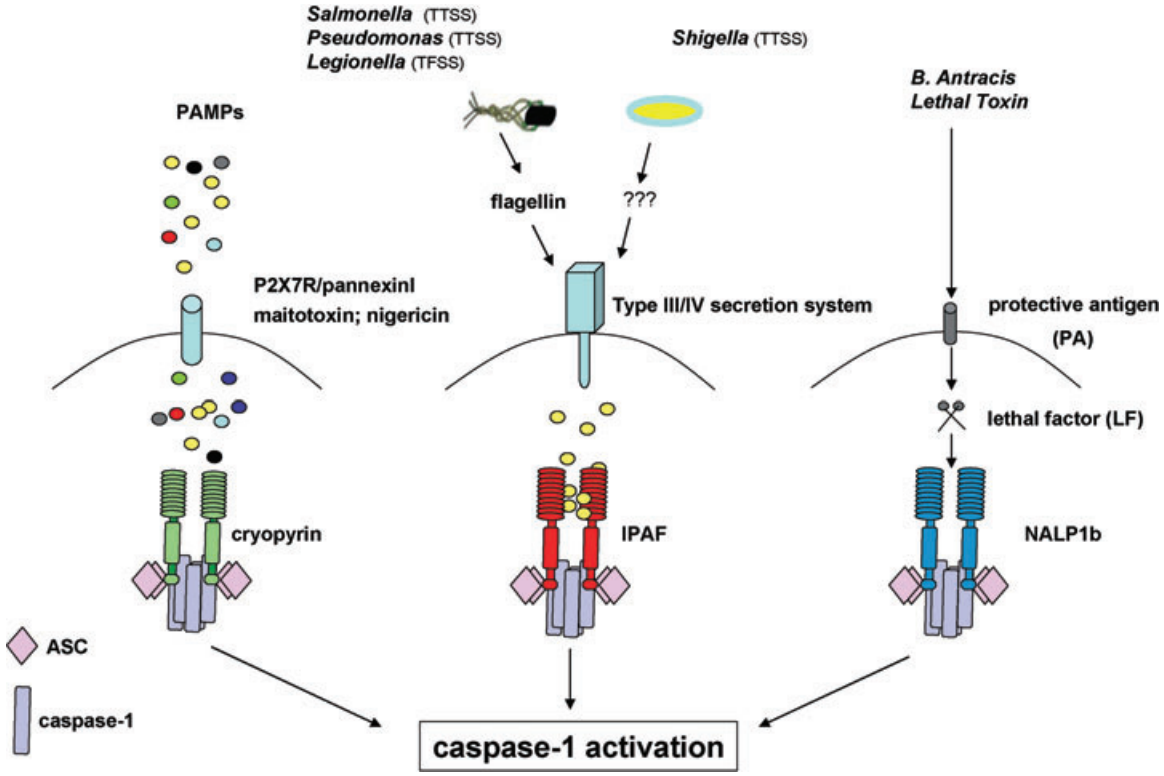

Fig. 2. Model for NLR-mediated caspase-1 activation. Bacteria and bacterial products enter the cytosol via pore-forming toxins, type III or IV secretion systems, or ATP-mediated activation of the pannexin-1 pore. Activation of NLR proteins by cytosolic PAMPs results in the formation of caspase-1-activating inflammasomes independently of TLRs. The inflammasome adaptor ASC is required for recruitment of caspase-1. *Salmonella* and *Legionella* flagellin are sensed by Ipaf, whereas mouse Nalp1b recognizes anthrax lethal toxin. Cryopyrin/Nalp3 mediates caspase-1 activation in response to a wide variety of microbial components and the endogenous danger signal uric acid. Active caspase-1 processes the IL-1β precursor into the mature cytokine and mediates its secretion by a poorly understood mechanism.

that the disease-associated mutations confer a state of constitutive activation to Cryopyrin, leading to increased caspase-1 activity. Importantly, treatment with IL-1 receptor antagonist is effective in controlling the disease activity in patients with these autoinflammatory syndromes, indicating a critical role for IL-1β in pathogenesis of these diseases (Hoffman *et al*., 2004).

Role of NLRs in bacterial infection

Nucleotide binding oligomerization domain-like receptors represent an immune surveillance system that detect the presence of microbial molecules inside the cell. In the following sections, we have selected certain pathogenic bacteria to illustrate the regulation of host immune responses through Nod1 and Nod2 as well as the inflammasome.

Salmonella: caspase-1 mediates inflammation and cell death

Salmonella species cause human diseases that range from self-limiting gastroenteritis to systemic infection. The virulence of *Salmonella* is mainly due to genes within the pathogenicity islands SPI-1 and SPI-2 that encode for type III secretion systems (TTSS). While SPI-1 is crucial for enteric colonization, SPI-2 is important in the systemic phase of the infection (Hueffer and Galan, 2004). Recent studies have revealed that caspase-1 activation in response to *Salmonella* is mediated by Ipaf and the adaptor ASC (Mariathasan *et al*., 2004) through the detection of bacterial flagellin (Franchi *et al*., 2006b; Miao *et al*., 2006). The sensing of flagellin requires the expression of the TTSS encoded by SPI-1 and is independent of TLR5 (Franchi *et al*., 2006b; Miao *et al*., 2006). The role of TTSS in the delivery of flagellin for Ipaf recognition, however, remains unclear. One possibility is that flagellin leaks through a pore formed by the TTSS. Alternatively, flagellin may be produced by the small number of bacteria that, through the action of the TTSS, escape the vacuolar compartment. Once activated, Ipaf induces the activation of caspase-1, which, in turn, mediates the maturation of IL-1β and IL-18 and the induction of cell death. Notably, while the adaptor ASC is required for the activation of caspase-1 and production of IL-1β, it is dispensable for the induction of macroph-

age cell death (Mariathasan *et al*., 2004). The dissociation between Ipaf/caspase-1 and ASC for the induction of cell death may be explained by the observation that ASC exerts a prosurvival effect through the activation of NF-κB (Masumoto *et al*., 2003). In agreement with the hypothesis that Ipaf activation induces a host response that confers protection to mice infected with *Salmonella*, caspase-1 deficiency is associated with increased susceptibility to the oral-gastric infection with *Salmonella* (Lara-Tejero *et al*., 2006). It is also interesting to note that during *Salmonella* infection, the inflammatory response mediated by TLR5 has a detrimental role for the host (Uematsu *et al*., 2006).

Shigella: caspase-1 mediates inhibition of autophagy

Shigella are highly adapted human pathogens that cause bacillary dysentery. The intestinal epithelial barrier represents the first line of defence against *Shigella.* Experiments in human intestinal cell lines showed that sensing of *Shigella* is largely mediated by Nod1, which is required for the activation of JNK and the secretion of IL-8 (Girardin *et al*., 2001). Macrophages are another component in the host defence against *Shigella*. Upon infection of macrophages, *Shigella* can escape from within the membrane vacuoles and enter the cytosol. This event is dependent on IpaB and triggers the activation of caspase-1, which, in turn, is responsible for the induction of pyroptosis, a form of cell death, and the production of the pro-inflammatory cytokines IL-1β and IL-18 (Hilbi *et al*., 1998). Recent studies have identified Ipaf as the critical NLR responsible for caspase-1 activation in *Shigella*-infected macrophages (Suzuki *et al*., 2007). *Shigella* do not express flagellin and, accordingly, the activation of caspase-1 in *Shigella*-infected macrophages is flagellin-independent. Thus, Ipaf mediates both flagellin-dependent and independent caspase-1 activation in response to pathogenic bacteria.

Autophagy, an intracellular degradation system employed for the turnover of cytoplasmic constituents, is another host response mechanism that is induced by the presence of cytoplasmic bacteria (Levine, 2005). In epithelial cells infected with *Shigella*, autophagy is triggered by the recognition of the bacterial effector protein VirG by ATG5, which is involved in autophagy (Ogawa *et al*., 2005). The bacterium avoids the autophagic response via IcsB (Ogawa *et al*., 2005). In macrophages, however, the induction of autophagy occurs independently of VirG and is inhibited by Ipaf and caspase-1. Notably, the negative regulation of autophagy mediated by the inflammasome is stimulus-dependent, as Ipaf and caspase-1 do not regulate autophagy induced by serum starvation (Suzuki *et al*., 2007). Thus, NLR proteins not only play a role in the induction of the inflammatory response, but also appear to regulate other host defence mechanisms, such as the autophagic response.

Legionella: caspase-1 mediates the maturation of the phagolysosome

Legionella pneumophila is a Gram-negative intracellular facultative pathogen that is responsible for Legionnaires' disease. In human macrophages, *Legionella* manipulates the endosome–lysosome pathway, avoiding the fusion of late endosomes with lysosomes, a feature that contributes to the creation of a vacuolar replicative niche known as the *Legionella*-containing vacuole (LCV). In contrast, macrophages from most inbred mouse strains restrict *Legionella* replication by promoting the fusion of the LCV with lysosomes (Fortier *et al*., 2005). The latter is mediated by the recognition of bacterial flagellin, delivered to the cytosol via a type IV secretion system (Amer *et al*., 2006; Molofsky *et al*., 2006; Ren *et al*., 2006; Zamboni *et al*., 2006). Consistently, flagellin-deficient *Legionella* multiply inside macrophages from mouse strains that are normally restrictive to *Legionella* replication (Amer *et al*., 2006; Molofsky *et al*., 2006; Ren *et al*., 2006; Zamboni *et al*., 2006). The fusion of the LCV with lysosomes is regulated by two different NLRs, Ipaf and Naip5. Ipaf senses the presence of flagellin inside the host cell and promotes phagolysosome fusion through the activation of caspase-1 (Amer *et al*., 2006). Accordingly, macrophages lacking Ipaf or caspase-1 exhibit impaired LCV maturation and *Legionella* degradation, which allows replication of the bacterium (Amer *et al*., 2006). Consistently, mice deficient in Ipaf show increased bacterial burden after pulmonary infection (Amer *et al*., 2006). In contrast to Ipaf, the role of Naip5 in the regulation of *Legionella* replication is less clear. Initial studies suggested that Naip5 controls replication of the bacterium via flagellin- and caspase-1-mediated cell death (Zamboni *et al*., 2006). However, recent experiments indicate that Naip5 acts independently of flagellin and caspase-1 to regulate the replication of *Legionella* in macrophages by controlling phagolysosome formation (Lamkanfi *et al*., 2007b). At present, it is unclear how Naip5 senses *Legionella* and NLRs control LCV maturation.

Listeria: a role for NLRs in intestinal inflammation

Immunocompromised individuals are particularly vulnerable to infection with *Listeria* and can develop septicemia and meningitis. To clarify the role of Nod1 in host immune responses to *Listeria* infection, *in vitro* studies have been performed in several cell types, such as endothelial cells, mesothelial cells and macrophages (Opitz *et al*., 2006; Boneca *et al*., 2007; Park *et al*., 2007b). Opitz *et al.* (2006) revealed that only invasive *Listeria* can induce activation of p38 MAPK and IL-8 secretion in endothelial cells via Nod1.

Similarly, secretion of the neutrophil chemoattractant factor KC induced by *Listeria* infection was reduced in Nod1- and RICK-deficient mesothelial cells (Park *et al*., 2007a). In macrophages, there is evidence for a redundant role for TLRs and Nod1/Nod2 in cytokine production induced by *Listeria* (Park *et al*., 2007a). Consistently, RICK-deficient mice are more susceptible to *Listeria* infection delivered intravenously (Hsu *et al*., 2007). In contrast, mice lacking Nod2 exhibit impaired *Listeria* clearance only when infected orogastrically (Kobayashi *et al*., 2005), suggesting a critical role for Nod2 in the intestinal tract. Although the mechanism responsible for this phenotype is unclear, mRNA levels of several α-defensins, including defensin-related cryptidin 4 (Defcr4), which has potent antimicrobial activity, was reduced in the terminal ileum from Nod2-deficient mice (Kobayashi *et al*., 2005).

There is clear evidence that cytosolic invasion by Listeria is required for IL-1β/IL-18 production as well as caspase-1 activation (Mariathasan *et al*., 2006; Ozoren *et al*., 2006; Franchi *et al*., 2007b). Furthermore, caspase-1 activation induced by *Listeria* is TLR-independent, but requires the adaptor ASC (Ozoren *et al*., 2006). However, the specific NLR involved in the regulation of caspase-1 activation in response to *Listeria* remains controversial. Cryopyrin was necessary for IL-1β and IL-18 production as well as caspase-1 activation in macrophages treated with heat-killed *Listeria* in the presence of ATP (Kanneganti *et al*., 2007) or in the presence of the pore-forming protein Streptolysin O (Kanneganti *et al*., 2007). However, while some studies suggested a critical role for Cryopyrin in caspase-1 activation induced by *Listeria* infection (Mariathasan *et al*., 2006), other studies did not support such a role (Franchi *et al*., 2007b).

Concluding remarks

There is now conclusive evidence that several members of the NLR family play important roles in innate immune responses to pathogenic bacteria. These include the activation of caspase-1 and NF-κB in response to several intracellular bacteria. However, the mechanisms involved in microbial recognition, including the delivery of PAMPS to the cytosol, and the interplay between TLRs and NLRs, require further elucidation. This will require additional studies with mutant mice deficient in NLR genes and better characterization of the players involved in NLR signalling pathways that are activated during the host immune response.

References

Agostini, L., Martinon, F., Burns, K., McDermott, M.F., Hawkins, P.N., and Tschopp, J. (2004) NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity* **20:** 319–325.

Amer, A., Franchi, L., Kanneganti, T.D., Body-Malapel, M., Ozoren, N., Brady, G., *et al.* (2006) Regulation of *Legionella* phagosome maturation and infection through flagellin and host IPAF. *J Biol Chem* **281:** 35217–35223.

Boneca, I.G., Dussurget, O., Cabanes, D., Nahori, M.A., Sousa, S., Lecuit, M., *et al.* (2007) A critical role for peptidoglycan N-deacetylation in *Listeria* evasion from the host innate immune system. *Proc Natl Acad Sci USA* **104:** 997–1002.

Boughan, P.K., Argent, R.H., Body-Malapel, M., Park, J.H., Ewings, K.E., Bowie, A.G., *et al.* (2006) Nucleotide-binding oligomerization domain-1 and epidermal growth factor receptor: critical regulators of beta-defensins during *Helicobacter pylori* infection. *J Biol Chem* **281:** 11637–11648.

Boyden, E.D., and Dietrich, W.F. (2006) Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat Genet* **38:** 240–244.

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.

Dowds, T.A., Masumoto, J., Zhu, L., Inohara, N., and Núñez, G. (2004) Cryopyrin-induced interleukin 1{beta} secretion in monocytic cells: enhanced activity of disease-associated mutants and requirement for ASC. *J Biol Chem* **279:** 21924–21928.

Ferwerda, G., Girardin, S.E., Kullberg, B.J., Le Bourhis, L., de Jong, D.J., Langenberg, D.M., *et al.* (2005) NOD2 and toll-like receptors are nonredundant recognition systems of *Mycobacterium tuberculosis*. *PloS Pathog* **1:** 279–285.

Fortier, A., Diez, E., and Gros, P. (2005) Naip5/Birc1e and susceptibility to *Legionella pneumophila*. *Trends Microbiol* **13:** 328–335.

Franchi, L., McDonald, C., Kanneganti, T.D., Amer, A., and Núñez, G. (2006a) Nucleotide-binding oligomerization domain-like receptors: intracellular pattern recognition molecules for pathogen detection and host defense. *J Immunol* **177:** 3507–3513.

Franchi, L., Amer, A., Body-Malapel, M., Kanneganti, T.D., Ozoren, N., Jagirdar, R., *et al.* (2006b) Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat Immunol* **7:** 576–582.

Franchi, L., Stoolman, J., Kanneganti, T.D., Verma, A., Ramphal, R., and Núñez, G. (2007a) Critical role for Ipaf in *Pseudomonas aeruginosa*-Induced caspase-1 activation. *Eur J Immunol* (in press).

Franchi, L., Kanneganti, T.D., Dubyak, G.R., and Núñez, G. (2007b) Differential requirement of P2X7 receptor and intracellular K+ for Caspase-1 activation induced by intracellular and extracellular bacteria. *J Biol Chem* **282:** 18810–18818.

Fritz, J.H., Girardin, S.E., 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.

Fritz, J.H., Le Bourhis, L., Sellge, G., Magalhaes, J.G., Fsihi,

H., Kufer, T.A., *et al.* (2007) Nod1-mediated innate immune recognition of peptidoglycan contributes to the onset of adaptive immunity. *Immunity* **26:** 445–459.

Girardin, S.E., Tournebize, R., Mavris, M., Page, A.L., Li, X., Stark, G.R., *et al.* (2001) CARD4/Nod1 mediates NF-kappaB and JNK activation by invasive *Shigella flexneri*. *EMBO Report* **2:** 736–742.

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

Girardin, S.E., Boneca, I.G., Viala, J., Chamaillard, M., Labigne, A., Thomas, G., *et al.* (2003b) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem* **278:** 8869–8872.

Hasegawa, M., Kawasaki, A., Yang, K., Fujimoto, Y., Masumoto, J., Breukink, E., *et al.* (2007) A role of lipophilic peptidoglycan-related molecules in induction of Nod1-mediated immune responses. *J Biol Chem* **282:** 11757–11764.

Hayashi, F., Smith, K.D., Ozinsky, A., Hawn, T.R., Yi, E.C., Goodlett, D.R., *et al.* (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* **410:** 1099–1103.

van Heel, D.A., Ghosh, S., Butler, M., Hunt, K.A., Lundberg, A.M., Ahmad, T., *et al.* (2005) Muramyl dipeptide and toll-like receptor sensitivity in NOD2-associated Crohn's disease. *Lancet* **365:** 1794–1796.

Herskovits, A.A., Auerbuch, V., and Portnoy, D.A. (2007) Bacterial ligands generated in a phagosome are targets of the cytosolic innate immune system. *PLoS Pathog* **3:** e51.

Hilbi, H., Moss, J.E., Hersh, D., Chen, Y., Arondel, J., Banerjee, S., *et al.* (1998) Shigella-induced apoptosis is dependent on caspase-1 which binds to IpaB. *J Biol Chem* **273:** 32895–32900.

Hoffman, H.M., Rosengren, S., Boyle, D.L., Cho, J.Y., Nayar, J., Mueller, J.L., *et al.* (2004) Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. *Lancet* **364:** 1779–1785.

Hsu, Y.M., Zhang, Y., You, Y., Wang, D., Li, H., Duramad, O., *et al.* (2007) The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. *Nat Immunol* **8:** 198–205.

Hueffer, K., and Galan, J.E. (2004) Salmonella-induced macrophage death: multiple mechanisms, different outcomes. *Cell Microbiol* **6:** 1019–1025.

Hugot, J.P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J.P., Belaiche, J., *et al.* (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **411:** 599–603.

Inohara, N., Koseki, T., Lin, J., del Peso, L., Lucas, P.C., Chen, F.F., *et al.* (2000) An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways. *J Biol Chem* **275:** 27823–27831.

Inohara, N., Ogura, Y., Chen, F.F., Muto, A., and Núñez, G. (2001) Human Nod1 confers responsiveness to bacterial lipopolysaccharides. *J Biol Chem* **276:** 2551–2554.

Inohara, N., Ogura, Y., Fontalba, A., Gutierrez, O., Pons, F., Crespo, J., *et al.* (2003) Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* **278:** 5509–5512.

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

Ismair, M.G., Vavricka, S.R., Kullak-Ublick, G.A., Fried, M., Mengin-Lecreulx, D., and Girardin, S.E. (2006) hPepT1 selectively transports muramyl dipeptide but not Nod1-activating muramyl peptides. *Can J Physiol Pharmacol* **84:** 1313–1319.

Kanazawa, N., Okafuji, I., Kambe, N., Nishikomori, R., Nakata-Hizume, M., Nagai, S., *et al.* (2005) Early-onset sarcoidosis and CARD15 mutations with constitutive nuclear factor-kappaB activation: common genetic etiology with Blau syndrome. *Blood* **105:** 1195–1197.

Kanneganti, T.D., Body-Malapel, M., Amer, A., Park, J.H., Whitfield, J., Franchi, L., *et al.* (2006) Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J Biol Chem* **281:** 36560–36568.

Kanneganti, T.D., Lamkanfi, M., Kim, Y.G., Chen, G., Park, J.H., 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.

Kapetanovic, R., Nahori, M.A., Balloy, V., Fitting, C., Philpott, D.J., Cavaillon, J.M., and Adib-Conquy, M. (2007) Contribution of phagocytosis and intracellular sensing for cytokine production by *Staphylococcus aureus*-activated macrophages. *Infect Immun* **75:** 830–837.

Kawai, T., and Akira, S. (2006) Innate immune recognition of viral infection. *Nat Immunol* **7:** 131–137.

Kim, J.G., Lee, S.J., and Kagnoff, M.F. (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.

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

Lamkanfi, M., Kanneganti, T.D., Franchi, L., and Núñez, G. (2007a) Caspase-1 inflammasomes in infection and inflammation. *J Leukoc Biol* **82:** 220–225.

Lamkanfi, M., Amer, A., Kanneganti, T.D., Munoz-Planillo, R., Chen, G., Vandenabeele, P., *et al.* (2007b) The Nod-like receptor family member Naip5/Birc1e restricts *Legionella pneumophila* growth independently of caspase-1 activation. *J Immunol* **178:** 8022–8027.

Lara-Tejero, M., Sutterwala, F.S., Ogura, Y., Grant, E.P., Bertin, J., Coyle, A.J., *et al.* (2006) Role of the caspase-1 inflammasome in *Salmonella typhimurium* pathogenesis. *J Exp Med* **203:** 1407–1412.

Levine, B. (2005) Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. *Cell* **120:** 159–162.

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

Maeda, S., Hsu, L.C., Liu, H., Bankston, L.A., Iimura, M., Kagnoff, M.F., *et al.* (2005) Nod2 mutation in Crohn's

disease potentiates NF-kappaB activity and IL-1beta processing. *Science* **307:** 734–738.
Mariathasan, S., Newton, K., Monack, D.M., Vucic, D., French, D.M., Lee, W.P., *et al.* (2004) Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* **430:** 213–218.
Mariathasan, S., Weiss, D.S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., *et al.* (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* **440:** 228–232.
Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* **440:** 237–241.
Masumoto, J., Dowds, T.A., Schaner, P., Chen, F.F., Ogura, Y., Li, M., *et al.* (2003) ASC is an activating adaptor for NF-kappa B and caspase-8-dependent apoptosis. *Biochem Biophys Res Commun* **303:** 69–73.
Masumoto, J., Yang, K., Varambally, S., Hasegawa, M., Tomlins, S.A., 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.
Miao, E.A., Alpuche-Aranda, C.M., Dors, M., Clark, A.E., Bader, M.W., Miller, S.I., and Aderem, A. (2006) Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via Ipaf. *Nat Immunol* **7:** 569–575.
Miceli-Richard, C., Lesage, S., Rybojad, M., Prieur, A.M., Manouvrier-Hanu, S., Hafner, R., *et al.* (2001) CARD15 mutations in Blau syndrome. *Nat Genet* **29:** 19–20.
Molofsky, A.B., Byrne, B.G., Whitfield, N.N., Madigan, C.A., Fuse, E.T., Tateda, K., and Swanson, M.S. (2006) Cytosolic recognition of flagellin by mouse macrophages restricts *Legionella pneumophila* infection. *J Exp Med* **203:** 1093–1104.
Ogawa, M., Yoshimori, T., Suzuki, T., Sagara, H., Mizushima, N., and Sasakawa, C. (2005) Escape of intracellular *Shigella* from autophagy. *Science* **307:** 727–731.
Ogura, Y., Bonen, D.K., Inohara, N., Nicolae, D.L., Chen, F.F., Ramos, R., *et al.* (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* **411:** 603–606.
Opitz, B., Puschel, A., Schmeck, B., Hocke, A.C., 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., Puschel, A., Beermann, W., Hocke, A.C., 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.
Ozoren, N., Masumoto, J., Franchi, L., Kanneganti, T.D., Body-Malapel, M., Erturk, I., *et al.* (2006) Distinct roles of TLR2 and the adaptor ASC in IL-1beta/IL-18 secretion in response to *Listeria monocytogenes*. *J Immunol* **176:** 4337–4342.
Park, J.H., Kim, Y.G., McDonald, C., Kanneganti, T.D., Hasegawa, M., Body-Malapel, M., *et al.* (2007a) RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs. *J Immunol* **178:** 2380–2386.
Park, J.H., Kim, Y.G., Shaw, M., Kanneganti, T.D., Fujimoto, Y., Fukase, K., *et al.* (2007b) Nod1/RICK and TLR signaling regulate chemokine and antimicrobial innate immune responses in mesothelial cells. *J Immunol* **179:** 514–521.
Ren, T., Zamboni, D.S., Roy, C.R., Dietrich, W.F., and Vance, R.E. (2006) Flagellin-deficient *Legionella* mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog* **2:** e18.
Sutterwala, F.S., Ogura, Y., Szczepanik, M., Lara-Tejero, M., Lichtenberger, G.S., Grant, E.P., *et al.* (2006) Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity* **24:** 317–327.
Suzuki, T., Franchi, L., Toma, C., Ashida, H., Ogawa, M., Yoshikawa, Y., *et al.* (2007) Differential regulation of caspase-1 activation, pyroptosis and autophagy via Ipaf and ASC in *Shigella*-infected macrophages. *PloS Pathog* **3:** e111.
Tada, H., Aiba, S., Shibata, K., Ohteki, T., and 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.
Tanabe, T., Chamaillard, M., Ogura, Y., Zhu, L., Qiu, S., Masumoto, J., *et al.* (2004) Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. *EMBO J* **23:** 1587–1597.
Tschopp, J., Martinon, F., and Burns, K. (2003) NALPs: a novel protein family involved in inflammation. *Nat Rev Mol Cell Biol* **4:** 95–104.
Uematsu, S., Jang, M.H., Chevrier, N., Guo, Z., Kumagai, Y., Yamamoto, M., *et al.* (2006) Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal CD11c+ lamina propria cells. *Nat Immunol* **7:** 868–874.
Viala, J., Chaput, C., Boneca, I.G., Cardona, A., Girardin, S.E., Moran, A.P., *et al.* (2004) Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island. *Nat Immunol* **5:** 1166–1174.
Watanabe, T., Kitani, A., Murray, P.J., and Strober, W. (2004) NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. *Nat Immunol* **5:** 800–808.
Zamboni, D.S., Kobayashi, K.S., Kohlsdorf, T., Ogura, Y., Long, E.M., Vance, R.E., *et al.* (2006) The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of *Legionella pneumophila* infection. *Nat Immunol* **7:** 318–325.
Zilbauer, M., Dorrell, N., Elmi, A., Lindley, K.J., Schuller, S., Jones, H.E., *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.

