Immunity
Review

Regulation of the Antimicrobial Response by NLR Proteins

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Nucleotide-binding, oligomerization domain (NOD)-like receptor (NLR) proteins are a family of innate immune receptors that play a pivotal role in microbial sensing, leading to the initiation of antimicrobial immune responses. Dysregulation of the function of multiple NLR family members has been linked, both in mice and humans, to a propensity for infection and autoinflammatory disease. Despite our increased understanding of NLR function and interactions, many aspects related to mechanisms of sensing, downstream signaling, and in vivo functions remain elusive. In this review, we focus on key members of the NLR family, describing their activation by diverse microbes, downstream effector functions, and interactions with each other and with other innate sensor protein families. Also discussed is the role of microbial sensing by NLR receptors leading to activation of the adaptive immune arm that collaborates in the antimicrobial defense.

Introduction

Nucleotide-binding, oligomerization domain-like receptor (NLR) proteins are a family of proteins with diverse functions in the immune system, characterized by a shared domain architecture that includes a nucleotide-binding domain (NBD) and a leucine-rich repeat (LRR) domain. The latter, which is shared with other innate immune proteins such as the Toll-like receptor (TLR) family, is thought to play a role in recognition and autoregulation of pathogen- and danger-associated molecular patterns (PAMPs and DAMPs, respectively). The NBD can bind nucleotides and is possibly involved in the induction of conformational changes and self-oligomerization that are necessary for NLR function. On the basis of the presence of additional domains, NLRs were grouped into subfamilies (Table 1) (Ting et al., 2008). Typical domains present in NLRs are the caspase activation and recruitment domains (CARDs) and the pyrin domains (PYDs). These domains are involved in homeotypic protein interactions and allow the recruitment of downstream effector molecules.

In this review, we will highlight the function of several members of the NLR family that are involved in the regulation of the antimicrobial immune response and focus on concepts of pathogen recognition as well as their interplay with other innate immune receptors.

NOD1 and NOD2

Two widely studied members of the NLRC (NOD-like receptor containing a CARD domain) family are NOD1 and NOD2. The discovery that mutations in NOD2 are strongly associated with Crohn's disease, an autoinflammatory disorder that is thought to be driven by aberrant immune response against intestinal microbes (reviewed in Cho, 2008), highlighted the importance of NLRs in the regulation of antimicrobial responses. NOD1 and NOD2 are cytosolic receptors that recognize distinct building blocks of peptidoglycan (PGN), a polymer consisting of glycan chains crosslinked to each other via short peptides (Fritz et al., 2006). In Gram-positive bacteria, it is the major building block of the cell wall. Although it is present only in smaller amounts in Gram-negative bacteria, its general abundance and its highly conserved structure make PGN a prime target for recognition by pattern recognition receptors (PRRs). NOD2 recognizes a minimal motif of muramyl dipeptide (MDP) that is found in all PGNs. In contrast, NOD1 recognizes muropeptides (iE-DAPs) that are found in the PGN of Gram-negative and only some Gram-positive bacteria (Figure 1). Because PGN structures are actively remodeled during bacterial cell growth and division, the constant release of NOD1 and NOD2 ligands from bacteria allows the innate immune system to survey its surrounding for the presence of bacteria. Interestingly, recent studies have identified additional agonists for NOD2, N-glycolyl muramyl dipeptide from mycobacteria (Coulombe et al., 2009) and viral ssRNA (Sabbah et al., 2009) (discussed below) demonstrating that NOD1 and NOD2 initiate innate responses upon recognition of a larger variety of pathogen-derived molecules. The mechanisms by which these agonists cross the host's cell membrane to stimulate NOD1 and NOD2 remain not fully understood. This is important given that only a minority of bacteria replicates in the cytoplasm, where they can be directly sensed, yet bacteria localized in phagosomes and outside the cell can effectively activate both NOD1 and NOD2. Several transport systems, including pannexin, PepT1, and PepT2, as well as endocytosis, have been demonstrated to enable hydrophilic muramyl peptides to cross to the cytoplasm (Lee et al., 2009; Marina-García et al., 2009; Vavricka et al., 2004).

Signaling downstream of NOD1 and NOD2 was thought to mainly result in the activation of NF-κB signaling (Fritz et al., 2006; Kanneganti et al., 2007) (Figure 1). Indeed, recognition of PGN ligands by NOD1 and NOD2 leads to a conformational change that activates receptor-interacting serine-threonine kinase 2 (RIP2) via cellular inhibitors of apoptosis 1 and 2 (cIAP1 and 2) (Bertrand et al., 2009), subsequently leading to ubiquitination of NF-κB essential modulator (NEMO) and the activation of the proinflammatory NF-κB pathway. In parallel,

Table 1. The NLR Family

Subfamily	Human	Mouse	N Terminus	Other Names
NLRA				
	CIITA		CARD	NLRA;MHCIITA;C2TA
		CIIta	CARD	*Nlra; MHCIITA;C2ta*
NLRB				
	NAIP		BIR	BIRC1;CLR5.1
		Naip1-7	BIR	*Birc1a-g*
NLRC				
	NOD1		CARD	NLRC1;CARD4;CLR7.1
		Nod1	CARD	*Nlrc1;Card4*
	NOD2		CARD	NLRC2;CARD15;CD;BLAU;IBD1;PSORAS1; CLR16.3
		Nod2	CARD	*Nlrc2;Card15*
	NLRC3		CARD[a]	NOD3;CLR16.2
		Nlrc3	CARD[a]	*Clr16.2*
	NLRC4		CARD	IPAF;CARD12;CLAN;CLR2.1
		Nlrc4	CARD	*Ipaf;Card12;CLAN*
	NLRC5		CARD[a]	NOD27;NOD4;CLR16.1
		Nlrc5	CARD[a]	
NLRP				
	NLRP1		PYD	NALP1;CARD7;NAC;DEFCAP;CLR17.1
		Nlrp1a-c	PYD	*Nalp1a-c*
	NLRP2		PYD	NALP2;PYPAF2;NBS1;PAN1;CLR19.9
		Nlrp2	PYD	*Nalp2;Pypaf2;Nbs1;Pan1*
	NLRP3		PYD	NALP3;Cryopyrin;CIAS1;PYPAF1;CLR1.1
		Nlrp3	PYD	*Nalp3;Cryopyrin;Cias1;Pypaf1;Mmig1*
	NLRP4		PYD	NALP4;PYPAF4;PAN2;RNH2;CLR19.5
		Nlrp4a	PYD	*Nalp4a;Nalp-eta;Nalp0D*
		Nlrp4b	PYD	*Nalp4b;Nalp-gamma;Nalp9E*
		Nlrp4c	PYD	*Nalp4c;Nalp-alpha;RNH2*
		Nlrp4d	PYD	*Nalp4d;Nalp-beta*
		Nlrp4e	PYD	*Nalp4e;Nalp-epsilon*
		Nlrp4f	PYD	*Nalp4f;Nalp-kappa;Nalp9F*
		Nlrp4g	PYD	*Nalp4g*
	NLRP5		PYD	NALP5;PYPAF8;MATER;PAN11;CLR19.8
		Nlrp5	PYD	*Mater;Op1*
	NLRP6		PYD	NALP6;PYPAF5;PAN3;CLR11.4
		Nlrp6	PYD	*Nalp6*
	NLRP7		PYD	NALP7;PYPAF3;NOD12;PAN7;CLR19.4
	NLRP8		PYD	NALP8;PAN4;NOD16;CLR19.2
	NLRP9		PYD	NALP9;NOD6;PAN12;CLR19.1
		Nlrp9a	PYD	*Nalp9a;Nalp-theta*
		Nlrp9b	PYD	*Nalp9b;Nalp-delta*
		Nlrp9c	PYD	*Nalp9c;Nalp-zeta*
	NLRP10		PYD	NALP10;PAN5;NOD8;PYNOD;CLR11.1
		Nlrp10	PYD	Nalp10;Pynod
	NLRP11		PYD	NALP11;PYPAF6;NOD17;PAN10;CLR19.6
			PYD	
	NLRP12		PYD	NALP12;PYPAF7;Monarch1;RNOS;PAN6;CLR19.3
		Nlrp12	PYD	*Nalp12*
	NLRP13		PYD	NALP13;NOD14;PAN13;CLR19.7
			PYD	

Table 1. *Continued*

Subfamily	Human	Mouse	N Terminus	Other Names
	NLRP14		PYD	NALP14;NOD5;PAN8;CLR11.2
		Nlrp14	PYD	*Nalp14;Nalp-iota;GC-LRR*
NLRX1				
	NLRX1		CARD[a]	NOD9;CLR11.3
		Nlrx1	CARD[a]	

This table is adapted from Kanneganti et al. (2006) and Bryant and Fitzgerald (2009).
[a] Currently disputed as to whether it contains a CARD, PYD, or another N terminus binding domain.

MDP recognition can also lead to the activation of the mitogen-activated protein kinase (MAPK) pathway via RIP2, which contributes to cytokine production. Importantly, numerous studies have highlighted the interaction with other signaling pathways that stimulate NF-κB, including TLRs (Lee and Kim, 2007). Depending on the cell type, NOD agonists modulate the magnitude of TLR ligand-induced cytokine production. In dendritic cells, NOD1 and NOD2 agonists can synergize with TLR ligands leading to enhanced production of proinflammatory cytokines (Fritz et al., 2005). In contrast, in splenocytes MDP treatment leads to a hyporesponsive state and decreased TLR ligand-induced NF-κB signaling (Watanabe et al., 2004), indicating that the interaction between the different PRR pathways is strongly dependent on the cellular context as well as the inflammatory setting.

Both NOD1 and NOD2 are highly expressed in antigen-presenting cells (APCs) such as monocytes, macrophages, and dendritic cells (Fritz et al., 2006; Kanneganti et al., 2007). Several recent studies identified NOD2 expression also in other hematopoietic lineages (Petterson et al., 2011; Shaw et al., 2009). In addition, NOD1 is expressed in many epithelial cell subsets, whereas NOD2 seems to be more restricted to specialized cell types such as Paneth cells in the small intestine. NOD2 expression is potently induced by TLR ligands including LPS and by inflammatory mediators such as TNF-α, IFN-γ, and IL-17 in other nonhematopoietic tissues. Furthermore, a recent study demonstrated that NOD2 expression in the intestine is regulated by signals from the microbiota, given that germfree mice had lower NOD2 expression that was reversible upon monocolonization with commensal bacteria (Petnicki-Ocwieja et al., 2009).

Role of NOD1 in the Antimicrobial Response

A role for NOD1 as a modulator of the in vivo antimicrobial response was described first in infection with the pathogen *Helicobacter pylori*, in which *Nod1*$^{-/-}$ mice featured enhanced susceptibility to infection with this pathogen (Viala et al., 2004). This phenotype correlated with an impaired acute inflammatory response, probably due to decreased production of chemokines by gastric epithelial cells as well as to impaired innate immune cell recruitment. Subsequent studies established roles for NOD1 in the induction of cytokines, antimicrobial peptides, and type I interferons during *H. pylori* infection (Watanabe et al., 2010a). In the later study, Strober and colleagues found an unexpected signaling pathway in epithelial cells leading to the induction of IFN-β via RIP2 and TNF-receptor-associated factor 3 (TRAF3) (Watanabe et al., 2010b). NOD2-mediated immune control of the infection further required members of the canonical type I interferon cascade consisting of IFN-regulatory factor 7 (IRF7), and the IFN-stimulated gene factor 3 (ISGF3) complex. Similar to studies with TLR-deficient mice, *Nod1*$^{-/-}$ mice also had reduced T helper 1 (Th1) cell responses upon *H. pylori* infection, suggesting that the two pathways may cooperate in the induction of adaptive immune responses.

NOD1 has also been involved in the recognition of other pathogens including *Clostridium difficile* (Hasegawa et al., 2011), *Legionella pneumophila* (Berrington et al., 2010a; Frutuoso et al., 2010), *Listeria monocytogenes* (Boneca et al., 2007), *Staphylococcus aureus* (Travassos et al., 2004), and *Pseudomonas aeruginosa* (Travassos et al., 2005). In many of these studies, either an impaired recruitment or function of neutrophils was noted during the inflammatory process. An interesting finding was reported, in which impaired neutrophil function in *Nod1*$^{-/-}$ mice was observed already in the steady state that could have contributed to the results of the other studies (Clarke et al., 2010). It was hypothesized that PGNs derived from commensal bacteria circulating in the serum prime neutrophils at distant sites through NOD1 signaling. Accordingly, broad-spectrum antibiotic-treatment that leads to suppression of certain commensal microflora communities resulted in similarly diminished neutrophil-mediated antimicrobial responses, whereas injection of NOD1 ligands was able to restore the normal response.

NOD1 was also demonstrated to provide protection against infection with the intracellular parasite *Trypanosome cruzi*, the etiological agent of Chagas disease (Silva et al., 2010). *Nod1*$^{-/-}$ mice featured worsened disease, normal production of cytokines, and levels of parasitemia similar to those of *Myd88*$^{-/-}$ mice, which, in turn, featured a near complete lack of cytokine induction. The exacerbated disease in *Nod1*$^{-/-}$ mice was suggested to be the result of impaired ability of macrophages to kill intracellular parasites. These results indicate that, in some infections, TLRs and NOD1 may orchestrate different aspects of the immune response upon infection. Additional in vivo studies will be needed to understand the role of NOD1 during systemic immune responses, which may lead to new therapeutic approaches.

Role of NOD2 in the Antimicrobial Response

In vitro studies have implicated NOD2 in a number of bacterial infectious models including *Listeria monocytogenes* (Kobayashi et al., 2005), *Staphylococcus aureus* (Deshmukh et al., 2009), *Chlamydophila pneumoniae* (Shimada et al., 2009), *Streptococcus pneumoniae* (Opitz et al., 2004), and *Mycobacterium tuberculosis* (Divangahi et al., 2008). Interestingly, NOD2 was found to be required for immune control of *L. monocytogenes*

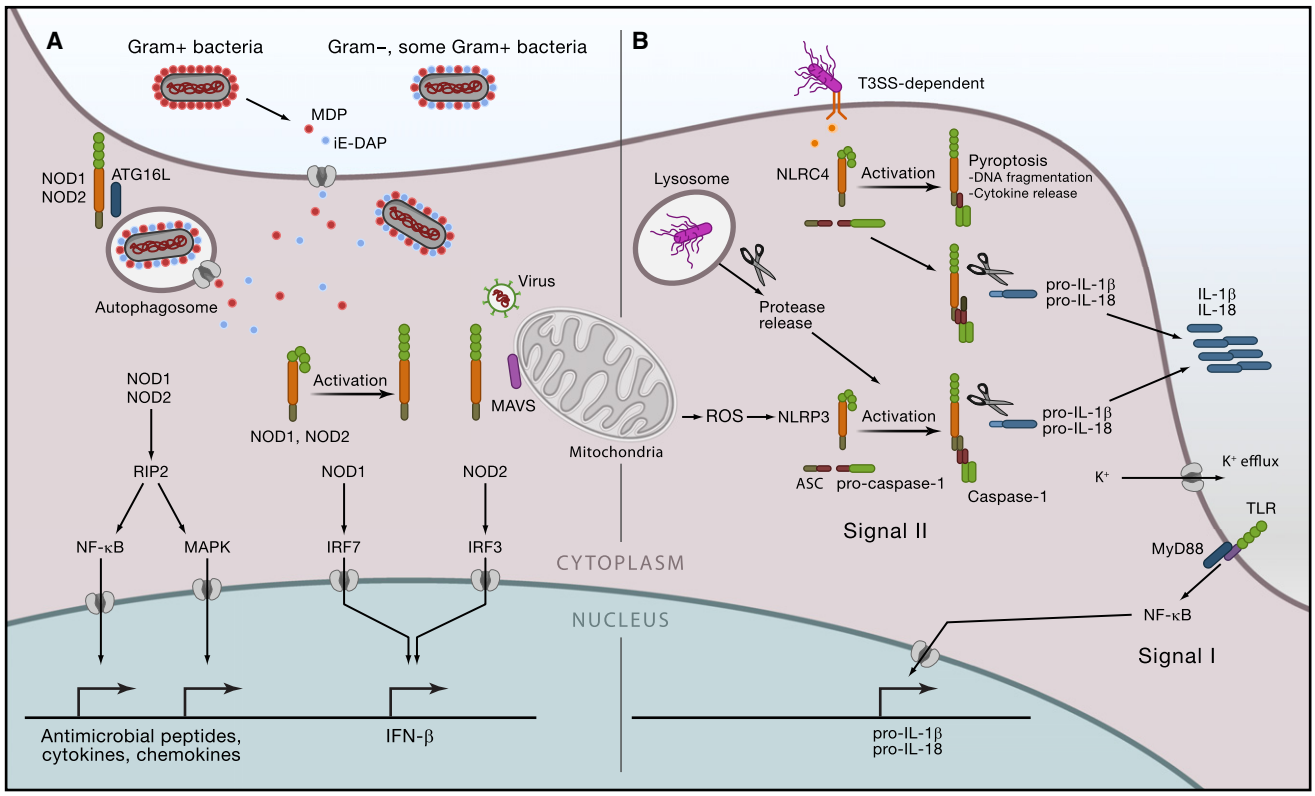

Figure 1. Microbial Activation of NLRs

(A) Activation of NOD1 and NOD2 results, depending on the recognized ligand, in transcription of genes encoding chemokines, cytokines, antimicrobial peptides, and type I interferons. MDPs and iE-DAPs are derived from extracellular, intracytosolic, or intravesicular bacteria, whose recognition can stimulate activation of the NF-kB and MAPK pathways. NOD1-mediated recognition of *H. pylori* and NOD2-mediated recognition of ssRNA stimulate type I interferon transcription via IRF7 and IRF3, respectively. NOD1 and NOD2 recruit ATG16L to sites of bacterial phagocytosis to initiate autophagy.

(B) Activation of NLRC4 and NLRP3 results in assembly of inflammasomes that activate caspase-1. This activation requires two signals. Signal I induces transcription of pro-IL-1β and pro-IL-18; signal II is provided by PAMPs and DAMPs. In the case of NLRC4, recognition of cytoplasmatic flagellin leads to pyropotosis, a specialized form of cell death, via an ASC-independent mechanism. NLRC4 also induces processing of pro-IL-1β and pro-IL-18 via ASC-dependent mechanisms. NLRP3 senses either directly microbial molecules or indirectly signals associated with cellular perturbations such as increased ROS production, release of lysosomal proteases into the cytoplasm upon "frustrated" phagocytosis, and potassium efflux. Assembly of the NLRP3 inflammasome leads to processing of pro-IL-1β and pro-IL-18 by caspase-1.

after oral, but not intravenous and intraperitoneal infection, indicating that NOD2 might have nonredundant functions in intestinal antimicrobial responses, whereas other PRRs such as TLRs could be sufficient for the host antimicrobial response when infected via different routes. A recent study highlighted collaboration between NOD1 and NOD2 in the *Salmonella typhimurium* colitis model (Geddes et al., 2010). Although mice deficient in either NOD1 or NOD2 had normal susceptibility for infection, mice deficient in both NOD1 and NOD2 featured decreased inflammation but increased bacterial colonization of the intestine. Notably, NOD1 and NOD2 expression in both hematopoietic and nonhematopoietic cells contributed to resistance against infection. Although this was in an apparent conflict with a previous study demonstrating that *Ripk2*$^{-/-}$ mice have comparable disease to wild-type (WT) controls (Bruno et al., 2009), the authors found that RIP2 (encoded by *Ripk2*) was only involved in *S. typhimurium*-induced colitis with bacteria expressing preferentially a particular type III secretion system (*Salmonella* pathogenicity island [SPI]-2). This suggests that pathogen-sensing requirements are not only distinct for different microbes, but may differ among subtypes within the same species.

The traditional view that NOD2 only recognizes MDP was recently challenged as *Nod2*$^{-/-}$ cells were found to be impaired in the expression of type I interferons upon stimulation with viral single-stranded RNA (ssRNA)(Sabbah et al., 2009). Interestingly, this pathway is independent of RIP2 and does not require the CARD domain, but is rather dependent on the mitochondrial antiviral signaling protein (MAVS) and interferon regulatory factor 3 (IRF3) (Figure 1). Strikingly, *Nod2*$^{-/-}$ mice have an enhanced susceptibility to infection with respiratory syncytial virus (RSV) and decreased production of IFN-β after influenza virus infection (Figure 2). The authors were able to demonstrate direct binding of NOD2 to viral ssRNA, but not host mRNA, by PCR methods. Therefore, NOD2 has the ability to recognize a larger variety of structural elements than previously appreciated.

Induction of Autophagy by NOD1 and NOD2

Autophagy is a lysosomal degradation pathway that was originally shown to be important during development and in metabolic states of stress such as starvation, but was subsequently demonstrated to be central in antimicrobial immunity (Münz, 2009). Recently, it has been reported that NOD1 and NOD2 are

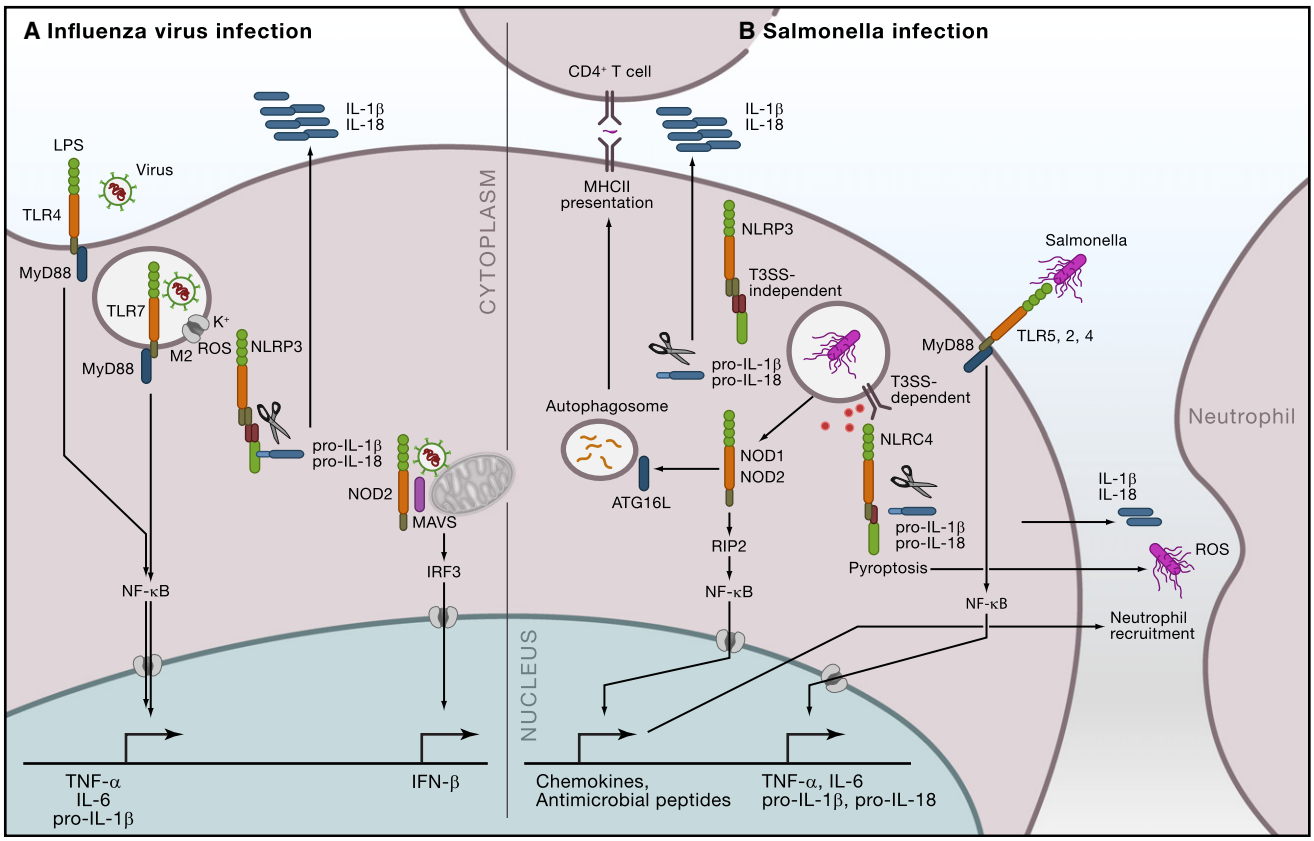

Figure 2. Crosstalk between NLRs and Other Innate Immune Pathways

(A) Influenza virus infection. Extracellular PRRs (TLR4) recognizes microbial products, whereas influenza virus is detected by TLR7. This leads to transcriptional activation of genes encoding proinflammatory cytokines (pro-IL-1β, pro-IL-18, TNF-α, and IL-6) in an NF-kB-dependent manner (signal I). Influenza virus M2 ionic channel mediates the activation of the NLRP3 inflammasome that leads to pro-IL-1β and pro-IL-18 processing and secretion (signal II). NOD2 recognizes influenza virus ssRNA stimulating type I interferon transcription via IRF3.

(B) *Salmonella* infection. *Salmonella* is recognized by extracellular PRRs (TLR2, TLR4, and TLR5) resulting in transcriptional activation of genes encoding pro-inflammatory cytokines (pro-IL-1β, pro-IL-18, TNF-α, and IL-6) in an NF-kB-dependent manner (signal I). Intracellular *Salmonella* activate NLRP3 and NLRC4 inflammasomes, which leads to pro-IL-1β, pro-IL-18 processing into their biological active forms. NLRC4 responds to flagellin injected by the T3SS, whereas NLRP3 is activated through an undefined T3SS-independent signal (signal II). NLRP3 inflammasome assembly is completely dependent on ASC, whereas NLRC4 may assemble an inflammasome without ASC. NLRC4 inflammasome activation also results in pyroptosis and release of bacteria into the extracellular space. Activation of NOD1 and NOD2 by *Salmonella* leads to chemokine secretion and neutrophil recruitment to the site of infection. NOD1 and NOD2 also target ATG16L to sites of bacterial phagocytosis to initiate autophagy.

able to regulate autophagy, as stimulation with NOD1 and NOD2 agonists leads to the NOD-dependent recruitment of ATG16L to the plasma membrane at sites of *Salmonella typhimurium* and *Shigella flexneri* intrusion (Figure 2) (Cooney et al., 2010; Travassos et al., 2010). This recruitment resulted in efficient degradation of bacteria in autophagosomes and subsequent processing of bacterial antigens for presentation on MHC class II molecules. In these studies, conflicting results were reported on RIP2 dependency of this pathway, which could potentially be attributed, at least partially, to the different model systems (human versus mouse) and cell types (dendritic cells versus fibroblasts). Further studies are needed to determine the details of this pathway. Mutations in NOD2 that are associated with Crohn's disease resulted in its impaired abilities to recruit ATG16L to the plasma membrane, induce autophagy, and promote antigen presentation. Importantly, this finding was able to link mutations that have been associated with Crohn's disease, i.e., NOD2 as well as ATG16L mutations, into a single pathway (Cho, 2008). It will be of extraordinary interest to understand the molecular details of this pathway, which may lead to aberrant bacterial processing and defective presentation of antigens to $CD4^+$ T cells, resulting in an inflammatory cascade characteristic of Crohn's disease.

Regulation of the Antimicrobial Adaptive Immune Response by NOD1 and NOD2

Beyond NOD1 and NOD2's roles in modulation of innate immunity, they also direct adaptive immune responses. Such effects were already demonstrated in early studies identifying MDP as the minimal adjuvant components of complete Freund's adjuvant (Strominger, 2007). Indeed, NOD1 and NOD2 were shown to be directly involved in the priming of adaptive immunity when cognate ligands were used as adjuvant (Kobayashi et al., 2005; Werts et al., 2007). Notably, nonhematopoietic cells required NOD1, indicting an important role for this molecule in epithelial and stromal cells upstream of APCs (Werts et al.,

2007). In addition to NOD2's role in the regulation of the adaptive immune response via APCs and the microenvironment, it was found to directly act in T cells as part of a modulatory network that controls the antimicrobial response. There is an enhanced susceptibility of *Nod2*$^{-/-}$ mice to *Toxoplasma gondii* infection, which was attributed to a diminished T cell IFN-γ production (Shaw et al., 2009). This cell-intrinsic effect was suggested to involve a defect in signaling downstream of the costimulatory molecule CD28. Studies in other T cell-dependent models, including graft-versus-host disease (GVHD) (Penack et al., 2009) and an in vivo Th2 cell model (Magalhaes et al., 2008), did not report NOD2-dependent intrinsic defects in T cell function, suggesting that NOD2 functions in a nonredundant manner in T cells under specific conditions, possibly depending on the level of CD28 requirement.

Inflammasome Forming NLR Proteins

In contrast to the NOD proteins, several other members of the NLR protein family (see Table 1) may form multiprotein complexes, named "inflammasomes." This results in activation of inflammatory caspases, cysteine proteases that are synthesized as inactive zymogens. Upon activation, caspases trigger cellular programs that lead to inflammation or cell death. Caspase-1 is the most prominent member of the proinflammatory group of caspases that also includes caspase-4, caspase-5, caspase-11, and caspase-12. Activation of pro-caspase-1 is essential for the processing of pro-IL-1β and pro-IL-18 and for the secretion of their mature active forms. Caspase-1's catalytic activity is tightly regulated by the inflammasomes, in a signal-dependent manner. Inflammasomes require two signals to accomplish their biological function. Signal I initiates transcriptional activation of inflammasome components and is often provided through TLR and NF-κB signaling, whereas signal II is required to initiate inflammasome assembly.

Known inflammasomes are composed of one of several NLR and PYHIN proteins, including NLRP1, NLRP3, NLRC4, and AIM2 that function as sensors of endogenous or exogenous PAMPs or DAMPs (Sutterwala et al., 2007b). After sensing the relevant signal, inflammasomes are assembled through homophilic CARD-CARD and PYD-PYD interactions between NLRs, apoptosis-associated speck-like protein containing a CARD (ASC), and pro-caspase-1 (Agostini et al., 2004; Martinon et al., 2002). Inflammasome components and the activation of downstream pathways depend on the nature of the stimuli, the sensor protein and, to some extent, on the presence of absence of ASC. Although there is conclusive evidence that NLRP1, NLRP3, and NLRC4 regulate proinflammatory responses through caspase-1 activation, domain structure conservation through the NLRP family suggests that other uncharacterized members might also regulate inflammasome assembly.

NLRC4

NLRC4 contains an N-terminal CARD, a central NOD domain, and a C-terminal LRR. NLRC4 is expressed mainly in lymphoid tissues, more specifically in myeloid cells, but also in the gastrointestinal tract (Hu et al., 2010). Activation of the NLRC4 inflammasome leads to caspase-1 activation, release of IL-1β, and a rapid form of cell death called pyroptosis (Sutterwala and Flavell, 2009) (Figure 1). Because NLRC4 contains a CARD domain, it can interact directly with pro-caspase-1; therefore, the role of ASC in NLRC4-mediated responses has remained elusive. Infection of ASC-deficient macrophages with NLRC4-activating bacteria results in defective caspase-1 activation and IL-1β secretion (Mariathasan et al., 2004; Sutterwala et al., 2007a; Suzuki et al., 2007), yet normal pyroptosis, suggesting that pathways downstream NLRC4 are independently regulated through the presence of ASC. In concordance, Broz and colleagues demonstrated that NLRC4-Inflammasomes containing ASC form a single large "focus" in which Caspase-1 undergoes autoproteolysis and processes IL-1β /IL-18. In contrast, NLRC4-ASC-independent inflammasomes activate Caspase-1 without autoproteolysis and do not form any large structures in the cytosol. Moreover, Caspase-1 mutants that were unable to undergo autoproteolysis promoted rapid cell death and processed IL-1β /IL-18 inefficiently, which suggests that NLRC4 forms spatially and functionally distinct inflammasomes complexes in response to bacterial pathogens (Broz et al., 2010b). Interestingly, NLRC4 and ASC probably do not interact directly as NLRC4 does not contain a PYD; therefore, it is possible that additional PYD-containing proteins are required for NLRC4-ASC-dependent processing of IL-1β in response to pathogens.

Bacterial Activation of the NLRC4 Inflammasome

The NLRC4 inflammasome activators are mainly Gram-negative bacteria that contain bacterial type III (T3SS) or type IV (T4SS) secretion systems. These include *Salmonella* (Mariathasan et al., 2004), *Legionella* (Zamboni et al., 2006), *Shigella* (Suzuki et al., 2007), *Pseudomonas* (Sutterwala et al., 2007a), and *Yersinia* (Brodsky et al., 2010). The microbial molecule flagellin is required to induce NLRC4-mediated caspase-1 activation during *Legionella*, *Salmonella*, and low burden infection of *Pseudomonas* (Franchi et al., 2006; Franchi et al., 2007; Miao et al., 2006; Miao et al., 2008; Molofsky et al., 2006). *L. pneumophila* and *S. typhimurium* mutant strains lacking flagellin or expressing point mutations in its gene are defective in their ability to induce caspase-1 activation (Franchi et al., 2006; Miao et al., 2006). Moreover, delivery of flagellin molecules to the cytosol through transfection or retroviral transduction results in caspase-1 activation in an NLRC4-dependent manner (Franchi et al., 2006; Lightfield et al., 2008; Miao et al., 2006). In contrast, extracellular flagellin is unable to induce the activation of the NLRC4 inflammasome, suggesting that additional factors are required to enable flagellin transport into the cytosol (Franchi et al., 2006; Miao et al., 2006). Because NLRC4 activation is dependent on functional bacterial secretion systems (T3SS, T4SS) (Franchi et al., 2006; Sutterwala et al., 2007a; Suzuki et al., 2007; Zamboni et al., 2006), it has been proposed that the T3SS can serve as a route for flagellin monomers to gain entry into the cytosol, leading to caspase-1 activation (Sun et al., 2007). *L. pneumophila* is unique in that the activation of the NLRC4 inflammasome by the C-terminal portion of its flagellin requires the presence of a second NLRP, NAIP5 (Coers et al., 2007; Zamboni et al., 2006). NLRC4 and NAIP5 had been suggested to physically interact to regulate caspase-1 activation; however, the exact role of NAIP5 in this process remains uncertain.

The NLRC4 inflammasome can also be activated in a flagellin-independent manner. The nonflagellated bacterium *S. flexneri*

and the *P. aeruginosa* mutant PAKΔ*fliC (flagellin-deficient)* have been shown to activate capase-1 through NLRC4 (Sutterwala et al., 2007a; Suzuki et al., 2007). These observations suggest that additional microbial molecules can trigger assembly of the NLRC4 inflammasome. Indeed, Miao and colleagues recently demonstrated that NLRC4 detects the rod protein of the T3SS of multiple Gram-negative bacteria through a sequence motif that is also found in flagellin (Miao et al., 2010b).

NLRC4 Interaction with Other Proinflammatory Signaling Pathways

NLRC4 plays a critical role during pulmonary and peritoneal *P. aeruginosa* infection (Franchi et al., 2007; Sutterwala et al., 2007a). However, NLRC4 contribution during other bacterial infections is less clearly defined, suggesting that it may have redundant or additive roles during host immune responses. *S. typhimurium*-infected NLRC4-deficient macrophages feature marked defects in caspase-1 activation and pyroptosis (Mariathasan et al., 2004). In contrast, in vivo studies suggested that the absence of caspase-1 but not of NLRC4 results in enhanced susceptibility to *S. typhimurium* infection (Lara-Tejero et al., 2006). The discrepancy between the in vitro and in vivo results suggests that additional pathways contribute to *Salmonella*-induced caspase-1 activation. In fact, although NLRC4 is activated by *Salmonella* flagellin, NLRP3 responds to another, undefined, *Salmonella*-T3SS-independent signal (Figure 2) (Broz et al., 2010a). Accordingly, mice lacking both NLRs were more susceptible to *S. typhimurium* infection (Broz et al., 2010a). Similarly, *Yersinia*-T3SS triggers ASC-dependent caspase-1 activation through the NLRP3 and NLRC4 inflammasomes (Brodsky et al., 2010). Interestingly, NLRP3 was not essential for the in vivo bacterial clearance in this study (Brodsky et al., 2010).

In vivo restriction of *L. pneumophila* is dependent on complex interactions between the MyD88, NOD1 and NOD2, and NLRC4 pathways. *Rip2*-deficient mice infected with a flagellin-deficient *L. pneumophila* (*ΔflaA*) were able to clear the bacteria, indicating that MyD88 signaling, independently of RIP2 and NLRC4, is able to protect the host against *L. pneumophila* infection (Archer et al., 2010). Concurrently, WT and *ΔflaA L. pneumophila* grew to comparable levels in the lung of *Myd88*$^{-/-}$ mice, which suggests that replication of *L. pneumophila* was not restricted severely by the NLRC4 pathway. However, *Myd88*$^{-/-}$ mice survived a high-dose challenge of WT *L. pneumophila* but succumbed to equal doses of *ΔflaA* strain; therefore, NLRC4 provides a level of host protection under high bacterial burden that is independent of MyD88 (Archer et al., 2010). In this context, signaling through RIP2 and MyD88 cooperate to attract neutrophils to the site of *L .pneumophila* infection; this can be of particular importance in light of recent evidence indicating that NLRC4-dependent, ASC-independent pyroptosis results in bacterial release from macrophages, exposing bacteria to uptake and killing by neutrophils (Miao et al., 2010a).

Finally, a crosstalk between NLRC4 and TLR5 (flagellin-extracellular receptor) has been recently identified. TLR5 has a protective role in *S. typhimurium* and *P. aeruginosa* infection in mice (Hawn et al., 2003; Morris et al., 2009). Likewise, humans carrying a dominant-negative allele of *TLR5* are more susceptible to *L. pneumophila* infection, suggesting that TLR5 and NLRC4 have nonredundant roles in these infections (Hawn et al., 2003). In contrast, flagellin and OVA immunization elicits a normal humoral immune response in single *Nlrc4*$^{-/-}$ or *Tlr5*$^{-/-}$ mice but not in *Nlrc4*$^{-/-}$*Tlr5*$^{-/-}$ mice (Vijay-Kumar et al., 2010).

NLRP3

NLRP3, like most other members of the NLRP subfamily (with the exception of NLRP10), consists of a carboxy-terminal LRR domain, a central NOD domain, and an amino-terminal PYD, mainly interacting with ASC (Schroder and Tschopp, 2010). NLRP3 is mainly expressed in multiple cells of the hematopoietic system, of the lymphocytic and myelogenic lineages, and in other cell types such as skin keratinocytes, transitional epithelium of the urinary tract, and osteoblasts. Noninfectious triggers for NLRP3 inflammasome formation include crystal activators such as monosodium urate (MSU), calcium pyrophosphate dihydrate (CPPD), asbestos, silica, and alum, protein aggregates such as fibrillar β-amyloid, haptens such as trinitrophenylchloride, and ultraviolet irradiation. A detailed description of these DAMPs is provided elsewhere (Schroder and Tschopp, 2010).

In general, inflammasome activation is believed to involve two steps, the first involving transcription of the "building blocks," e.g., pro- IL-1β, pro-IL-18, and pro-caspase-1, as well as expression of NLRP3 itself in the case of NLRP3 inflammasome activation. The second step, through poorly understood interactions of activators with the relevant NLRP, leads to inflammasome assembly that in turn drives cleavage and release of active IL-1β and IL-18. This process is best studied in the NLRP3 inflammasome. The first priming event of NLRP3 inflammasome activation involves intricate interaction with other PRR such as TLR and NOD2, resulting in NF-kB-driven transcriptional activation. TLR-induced priming can be performed through both the MyD88 and TRIF pathways, given that only deficiency of both adaptor proteins results in abrogated priming (Bauernfeind et al., 2009). Interestingly, cytokines including TNF-α, IL-1α, and IL-1β have been suggested to be capable to provide this priming signal for NLRP3 assembly, providing an alternative mechanism for inflammasome activation in "sterile inflammation" in the absence of microbial interactions (Franchi et al., 2009a). In vivo, some of the NLRP3 activators can induce inflammasome formation in the absence of a priming signal. Whether such signal is provided constitutively in the in vivo setting remains to be determined.

The precise mechanism whereby NLRP3 senses the presence of its activators remains elusive. Although physical interaction of microbial activators with NLRP3 may occur in some cases (Marina-García et al., 2008), a more general mechanism is thought to involve a cellular mediator(s) that interacts with the structurally diverse DAMPs and PAMPs to provide signals for NLRP3 inflammasome formation. Similar indirect innate triggering systems have been described in plants, in which pathogen-induced alterations in endogenous proteins are sensed by NLR-like R proteins that, in turn, trigger canonical resistance mechanisms against these pathogens (Mackey et al., 2002).

Three nonexclusive models for indirect NLRP3 activation have been proposed and were reviewed in (Schroder and Tschopp, 2010) (Figure 1). The first involves ATP-induced activation of the nonselective cation channel $P2X_7$, which in turn activates the larger pore-forming Pannexin-1, leading to potassium efflux and potentially cytoplasmic migration of PAMPs and DAMPs,

resulting in NLRP3 inflammasome activation. Nonetheless, cells devoid of Pannexin-1 activity retain normal potassium efflux despite failure to assemble the NLRP3 inflammasome. The second model suggests that NLRP3 inflammasome formation is triggered through production of ROS, e.g., following "frustrated" endocytosis, with NADPH oxidase suggested to be central in this process. However, although chemical inhibition of ROS synthesis or knockdown of NADPH oxidase subunits result in diminished inflammasome formation, monocytes derived from NADPH-oxidase-deficient patients feature normal IL-1β production (Meissner et al., 2010a). Recently, an alternative source for ROS was suggested to be functionally impaired mitochondria. Inhibition of key steps in the respiratory chain or abrogation of defective mitochondria clearance by autophagy or mitophagy resulted in enhanced mitochondrial production of ROS, leading to NLRP3 inflammasome activation (Zhou et al., 2011). The third mechanism involves lysosomal destabilization after phagocytosis, leading to cytoplasmic leakage of inflammasome activators such as cathepsin B. Interestingly, the pore-forming toxin nigericin, which triggers the NLRP3 inflammasome, promotes potassium efflux but may also promote lysosomal leakage. Nonetheless, cathepsin B-deficient mice feature normal NLRP3 inflammasome assembly, which could indicate that other lysosomal proteases are involved in proteolytic activation of the NLRP3 inflammasome. In summary, it remains unclear at this point whether the different proposed models are mutually exclusive or whether these pathways can interact at different levels regulating this important multiprotein complex.

Microbial Activators of the NLRP3 Inflammasome

Mounting evidence implicates the NLRP3 inflammasome as an important molecular pathway mediating the response against a myriad of pathogenic microbial infections. Although the precise mechanisms of microbial sensing remain elusive, it is becoming clear that many of the pathogens induce the formation of the NLRP3 inflammasome through conserved pathways. At some instances, activation of the NLRP3 inflammasome results in the potentiation of the host antimicrobial response, while in others it initiates pathogen-derived immune evasive mechanisms.

Bacterial Activators of the NLRP3 Inflammasome

Multiple bacterial pathogens were shown to activate the NLRP3 inflammasome. One common bacterial-mediated NLRP3 inflammasome-inducing mechanism involves secretion of pore-forming toxins that promote potassium efflux and resultant NLRP3 inflammasome formation. One such example is the Gram-positive rod *L. monocytogenes* in which NLRP3-dependent inflammasome activation depends on the pore-forming toxin listeriolysin (Mariathasan et al., 2006). *Nlrp3*$^{-/-}$ mice have an impaired immune response manifesting as delayed clearance of *L. monocytogenes*. Interestingly, clearance is unaltered during secondary infection of caspase-1-deficient mice, suggesting that other host-derived anti microbial mechanisms bypass the need for NLRP3 inflammasome sensing upon repeated infection (Tsuji et al., 2004). Likewise, secretion of Pneumolysin by *Streptococcus pneumoniae* or Streptolysin O by *Streptococcus pyogenes* induces NLRP3 inflammasome activation through both potassium efflux and lysosomal leakage induction, whereas *Nlrp3*$^{-/-}$ mice display impaired Pneumococcal clearance (Harder et al., 2009; McNeela et al., 2010). *Staphylococcus aureus* NLRP3 activation is mediated by secretion of one of several toxins (Craven et al., 2009; Mariathasan et al., 2006). Of these, α-hemolysin acts independent of TLR signaling but may require cosignaling by bacterial-released lipoproteins (Muñoz-Planillo et al., 2009). Interestingly, differences in responsiveness to α-hemolysin activation of the NLRP3 inflammasome were found between human and mouse cells, hinting toward species and cell-specific differences in the inflammasome-related host response (Craven et al., 2009).

Another mechanism of NLRP3 inflammasome activation is employed by Mycobacteria. Infection of mice with *Mycobacterium marinum* requires a functioning Esx-1 (type VII) secretion system that induced IL-1β secretion through formation of a NLRP3 inflammasome. Both *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice had attenuated disease compared to WT mice but an equal bacterial burden, suggesting that IL-1β may have a detrimental effect for the host in mycobacterium infection (Carlsson et al., 2010). Similarly in *Mycobacterium tuberculosis*, the Esx-1 secretion system was found to contribute to bacterial virulence through the activity of its substrate ESAT-6 that induced macrophage membrane disruption, enabling cytoplasmic translocation of PAMPs that, in turn, induced NLRP3 inflammasome activation (Mishra et al., 2010).

Finally, intracellular bacteria may activate the NLRP3 inflammasome directly, through cytoplasmic release of bacterial-derived molecules (enabling signal I) and concomitant activation of host-derived inflammasome activation pathways. One such example is the intracellular pathogen *Chlamydia trachomatis*, whose growth was enhanced in a cervical epithelial cell line, by the activation of the NLRP3 inflammasome in a type III secretion system-dependent manner through induction of potassium efflux and ROS production. Inflammasome-mediated pathogen growth in this study was mediated by unknown IL-1β-independent mechanisms. Nevertheless, the in vivo correlate of this observation remains to be determined (Abdul-Sater et al., 2009).

A NLRP3 and ASC-mediated host response to bacterial infections that does not require caspase-1 was recently discovered. It involves induction of necrosis of bacterially infected myeloid cells and was coined pyronecrosis to distinguish it from pyroptosis, which requires caspase-1. Examples of pathogens suggested to employ this mechanism include *S. aureus*, *S. flexneri*, *K. pneumonia*, *Porphyromonas gingivalis*, and *Neisseria gonorrhoeae* (Craven et al., 2009; Duncan et al., 2009; Huang et al., 2009; Willingham et al., 2009; Willingham et al., 2007). In these infections, monocyte pyronecrosis was shown to be induced by cytoplasmic leakage of cathepsin-B and to result in the release of the proinflammatory factor HMGB1 from necrotic cells. In vivo, *Nlrp3*$^{-/-}$ mice infected with *K. pneumonia* were characterized by attenuated lung inflammation, but diminished survival, suggesting a role for pyronecrosis and the ensuing inflammation in host protection against this infection.

Viral Activators of the NLRP3 Inflammasome

NLRP3 has been shown to be activated by viral ssRNA and dsRNA and to play a major modulatory role in several viral infections like influenza and sendai viruses (Allen et al., 2009; Kanneganti et al., 2006). Influenza virus is a potent inducer of IL-1β secretion by macrophages and dendritic cells (Allen et al.,

2009; Ichinohe et al., 2009; Ichinohe et al., 2010; Thomas et al., 2009). In *Nlrp3*$^{-/-}$ mice inoculated with influenza A, survival during viral infection was shown to be impaired. It is unclear whether this effect stems from defective clearance of the virus, delayed pulmonary epithelial repair, or a combination of the two. During influenza infection, transcriptional activation of the inflammasome components (signal I) was found to depend on viral RNA recognized through TLR7, whereas inflammasome activation (signal II) was mediated by the M2 ionic channel, through pH neutralization of the trans golgi network (TGN) that results in potassium efflux and ROS production (Figure 2) (Ichinohe et al., 2010). Importantly, the exact role of the NLRP3 inflammasome in modulation of antiviral adaptive immune response remains controversial. A recent study suggests that defined communities within the intestinal microbiota are required for NLRP3 function during influenza infection. Bacterial signals provide a constitutive "signal I" necessary for baseline expression of NLRP3 inflammasome components, enabling DC migration and resultant adaptive immune activation upon sensing of influenza infection by the NLRP3 inflammasome. Impaired baseline expression of inflammasome components as a consequence of oral antibiotic treatment was overcome by intestinal or pulmonary administration of TLR agonists, possibly providing the missing signal I (Figure 2) (Ichinohe et al., 2011). Detailed review of inflammasome regulation of influenza infection is given elsewhere (Pang and Iwasaki, 2011). Other viruses, such as the DNA viruses vaccinia (Delaloye et al., 2009), adenovirus (Barlan et al., 2011), and varicella zoster (Nour et al., 2011) and the RNA viruses encephalomyocarditis virus and vesicular stomatitis virus (Rajan et al., 2011) have been demonstrated to activate the NLRP3 inflammasome, yet in vivo infection of caspase-1-deficient mice with the later two viruses did not result in impaired survival. The physiological importance of these observations remains to be determined.

Fungal Activators of the NLRP3 Inflammasome

Several studies have recently shown that the NLRP3 inflammasome is essential for systemic antifungal immune response against *Candida albicans* and *Saccharomyces cerevisiae* (Gross et al., 2009; Hise et al., 2009; Kumar et al., 2009). In one study (Hise et al., 2009), the Candida PAMPs β-glucan and zymosan, which bind Dectin-1 and TLR2 respectively, were shown to induce pro-IL-1β transcriptional activation (signal I), whereas a yet uncharacterized *Candida*-derived molecule induced assembly of the NLRP3 inflammasome (signal II). Interestingly, inactivated immature hyphal forms were also able to induce this assembly. In another study (Gross et al., 2009), *C. albicans* was shown to specifically activate the NLRP3 inflammasome by signaling through Syk, which functions downstream of several PRR including Dectin-1. Syk was found to regulate both production of pro-IL-1β (signal I) and inflammasome formation (signal II), by inducing potassium efflux and production of ROS. *Nlrp3*$^{-/-}$ mice featured an impaired containment of *C. albicans*, manifested as widespread fungal dissemination leading to enhanced mortality. Interestingly, fungal β-glucan-induced humoral response was also found to be dependent upon NLRP3 (Kumar et al., 2009). Coupling Syk to inflammasome signaling may link the NLRP3 inflammasome pathway to other Syk-dependent pathways such as those downstream of C-type Lectin Receptors that also function as PRR (Kumar et al., 2009; Robinson et al., 2006). In vitro, *Aspergillus fumigatus* is similarly capable of activating the NLRP3 inflammasome, although it remains unknown whether NLRP3 is also required in vivo to limit infection (Saïd-Sadier et al., 2010).

Parasitic Activators of the NLRP3 Inflammasome

The role of NLRP3 in parasitic infections is just beginning to be revealed. The plasmodium-secreted crystal hemozoin was recently shown to induce IL-1β secretion through activation of the NLRP3 inflammasome via Syk and Lyn kinase-dependent mechanisms. In vivo, malaria severity was found to be attenuated in *Nlrp3*$^{-/-}$ mice (Dostert et al., 2009; Shio et al., 2009). Similarly in another study, *Nlrp3*$^{-/-}$ mice featured delayed onset of cerebral malaria, yet this phenotype was not observed in mice deficient of caspase-1, ASC, and IL-1 receptor, suggesting that this NLRP3-induced effect was not mediated by formation of an inflammasome (Reimer et al., 2010). Even less is known about possible inflammasome-related roles in helminthic infection. Schistosomal egg antigens activate the NLRP3 inflammasome (Ritter et al., 2010). This effect was dependent on the PRR dectin-2, as well as on potassium efflux, ROS production, and Syk activity. In vivo, Schistosoma-infected *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice featured an aberrant adaptive immune response, manifesting reduced IL-17A, IFN-γ, IL-5, and IL-10 levels, elevated IL-13 levels, and reduced hepatic granuloma formation.

NLRP1

Although the NLRP3 inflammasome has been the main focus of many research groups, the NLRP1 inflammasome was the first to be described (Martinon et al., 2002). NLRP1 is expressed in multiple cell types including granulocytes, monocytes, dendritic cells, T and B cells, neurons, and testes. In humans, a single gene encodes NLRP1 that contains a PYD, a NBD, a LRR, a FIIND, and a C-terminal CARD. In mice, three highly polymorphic paralogues (*Nlrp1a*, *Nlrp1b*, and *Nlrp1c*) have been described (Franchi et al., 2009b). Unlike human NLRP1, murine NLRP1 lacks functional PYD and FIIND domains and is predicted to not be able to interact with ASC. Indeed, NLRP1b has been shown to activate caspase-1 in an ASC-independent manner (Hsu et al., 2008).

Genetic studies identified the *Nlrp1b* gene as the key determinant of susceptibility to *Bacillus anthracis* lethal toxin (LeTx) in mice (Boyden and Dietrich, 2006). Subsequently, it was revealed that LeTx-induced macrophage cell death is dependent on caspase-1 activation by the *Nlrp1b*-sensitive allele (Boyden and Dietrich, 2006; Hsu et al., 2008). However, the mechanism of LeTx activation of the NLRP1 inflammasome remains to be determined. As is the case for the NLRP3 inflammasome (Marina-García et al., 2008), NLRP1 can also be activated by MDP. MDP was suggested to induce conformational changes in NLRP1, which enable its oligomerization, thus creating a platform for caspase-1 activation (Faustin et al., 2007). In addition, recent studies indicate that NLRP1 and NOD2 interaction is required for proper responses to LeTx and MDP in vivo. NOD2 promotes pro-IL-1β (signal I) transcription in an NF-kB-dependent manner and it regulates caspase-1 activation by MDP through direct interaction with NLRP1 (Hsu et al., 2008). Further studies are required to determine the physiological relevance of

NOD2-NLRP1 interactions and the individual contribution of the NLRP1 and NLRP3 inflammasomes during host responses to MDP.

The importance of the NLRP1 inflammasome during host responses is highlighted by the existence of host and microbial inhibitors to its assembly and function. The antiapoptotic proteins Bcl-2 and Bcl-X(L) bind and suppress NLRP1, reducing caspase-1 activation and IL-1β production (Bruey et al., 2007). $CD4^+$ effector and memory T cells suppress NLRP1 and NLRP3-inflammasome activation through TNF family ligands in a cell contact-dependent manner (Guarda et al., 2009). Similarly, type I interferons inhibit Nlrp1b and NLRP3 inflammasome-dependent caspase-1 activation through a yet to be determined STAT-1-mediated mechanism (Guarda et al., 2011). Finally, Kaposi sarcoma herpes virus Orf63 is a viral homolog of human NLRP1 that interacts with NLRP1, NLRP3, and NOD2. Orf63 blocks NLRP1-dependent innate immune responses and is required for reactivation and generation of progeny virus (Gregory et al., 2011).

Other NLR Proteins in Antimicrobial Responses

Multiple other NLR proteins have been identified in humans and mice, yet with a few exceptions their functions, including the host antimicrobial response, are currently unknown. The role of CIITA in regulating MHC class II expression has been reviewed elsewhere (Wright and Ting, 2006). NLRP2, NLRP6, and NLRP12 have been proposed to activate caspase-1 through inflammasome assembly and to negatively regulate proinflammatory signaling pathways (Bruey et al., 2004; Grenier et al., 2002; Wang et al., 2002). Interestingly, NLRP2 mediates the induction of human beta defensins (HBD) by *Fusubacterium nucleatum* in gingival epithelial cells, suggesting that it may be involved in regulation of microbial communities in the oral cavity (Ji et al., 2009). NLRP12 has been suggested to possess anti-inflammatory regulatory properties, potentially through modulation of IRAK1 phosphorylation, and during infection its levels drop sharply, enhancing TLR signaling (Williams et al., 2005). Interestingly, *Nlrp12*$^{-/-}$ mice were shown to exhibit attenuated inflammatory responses in two models of contact hypersensitivity because of reduced dendritic cell migration (Arthur et al., 2010). This mechanism could also potentially affect the antimicrobial host response.

NLRC5 is a highly conserved member of the same subfamily as the NOD proteins. It contains a large LRR region, a central NOD domain, and a C-terminal CARD-like domain (Schroder and Tschopp, 2010). The role of NLRC5 as a modulator of the inflammatory immune response remains unclear and controversial. Earlier studies showed that NLRC5 inhibits NF-κB and type I interferon pathways through interaction with IKKα and β and RIG-I and MDA5, respectively (Cui et al., 2010). Accordingly, knockdown of NLRC5 resulted in enhanced antiviral immunity (Cui et al., 2010). Benko et al. (2010) also demonstrated that NLRC5 limits activation of proinflammatory pathways via transcriptional repression. In contrast to these studies, two groups reported that NLRC5 potentiates antiviral responses (Kuenzel et al., 2010; Neerincx et al., 2010); moreover, macrophages from *Nlrc5*$^{-/-}$ mice stimulated ex vivo-induced normal levels of IFN-β, IL-6, and TNF-α after treatment with RNA viruses, DNA viruses, and bacteria (Kumar et al., 2011). These results indicate that NLRC5 may be dispensable for cytokine induction during viral infections under physiological conditions. Finally, two independent studies demonstrated that NLRC5 regulates processing of IL-1β upon overexpression or in response to NLRP3 inflammasome-activating molecular patterns (Kumar et al., 2011) and that NLRC5 physically interacts with NLRP3 (Davis et al., 2011). These results invoke the possibility that NLRC5 might cooperate with NLRP3 to activate the NLRP3 inflammasome. Interestingly, NLRC5 has been shown to be a transcriptional regulator of MHC class I genes, supporting the observation that NLRC5 is most similar to CIITA (Meissner et al., 2010b).

NLRX1 is a member of the NLR family that contains an N terminus CARD-related "X" domain. NLRX1 is targeted to the mitochondria matrix where it has been shown to interact with MAVS and modulate antiviral responses (Arnoult et al., 2009; Moore et al., 2008). Moreover, NLRX1 can also potentiate proinflammatory signaling pathways by inducing reactive oxygen species (ROS) in HeLa cells (Tattoli et al., 2008), which in turn impacts the growth of the intracellular bacteria *Chlamydia trachomatis* (Abdul-Sater et al., 2010). Analysis of mice deficient for NLRX1 is required to elucidate the role of this NLR in the regulation of host anti-microbial immune responses.

Finally, emerging evidence suggests that non-NLR innate receptors may cooperate with NLR proteins and with other inflammasome adaptors in microbial sensing. One such example is the non-NLR protein AIM2, which was recently shown to form an ASC-dependent inflammasome and to recognize dsDNA and was also shown to play a central role in cytosolic sensing of bacteria and DNA viruses, including *Fransicella tularensis*, vaccinia virus, mouse cytomegalovirus (MCMV), and to a certain extent *L. monocytogenes* (Fernandes-Alnemri et al., 2010; Jones et al., 2010; Rathinam et al., 2010). Another example is the viral nucleic acid recognition receptor RIG-I, which was recently shown to bind ASC and activate caspase-1 in response to viral infection, resulting in IL-1β production, a process that is independent of NLRP3 (Poeck et al., 2010). Altogether, these data suggest that cytosolic pathogen sensing may involve complex interactions, in which the NLR family members and inflammasome adaptors may actively interact with sensors and adaptors of other pathways, depending on the infectious context.

NLR Mutations in Human Infectious Disease

In recent years, multiple members of the NLR family have been associated with human disease (reviewed in Hoffman and Brydges, 2011). Examples include monogenic auto-inflammatory disorders such as Muckle–Wells syndrome (MWS), familial cold auto-inflammatory syndrome (FCAS), and chronic infantile neurological cutaneous and articular syndrome (CINCA), which are associated with NLRP3 gain-of-function mutations, whereas Blau syndrome is associated with NOD2 gain-of-function mutations (Borzutzky et al., 2010). The most well established NLR link to a multifactorial autoinflammatory disorder is the association between mutations in NOD2 and Crohn's disease, which occur in 30% of patients of European ancestry as compared to ≤1% in unaffected individuals (Cho, 2008). A number of aberrations have been found in patient immune cells including impaired cytokine secretion, reduced Paneth cell antimicrobial peptide production, diminished IL-10 secretion after microbial stimulation,

and defective regulation of autophagy. Lately, a systematic SNP analysis revealed that mutations within the NLRP3 locus are also strongly associated with risk factors for the development of Crohn's disease (Villani et al., 2009). Sporadic reports on successful IL-1 receptor antagonist (Anakinra) treatment in patients with intractable gout (Abdul-Sater et al., 2009) and pseudogout (suggested in mice to be regulated by the NLRP3 inflammasome) point to the possibility that human gout, and possibly other crystal deposition diseases, are also NLRP3 regulated (McGonagle et al., 2007). Similarly, mutations in NLRP1 are associated with some cases of vitiligo and Addison's disease (Jin et al., 2007; Zurawek et al., 2010).

Less studied are the possible associations between NLR mutations and the propensity for infection. Mutations in the NOD2 gene were recently suggested to be associated with an increased risk for the development of spontaneous bacterial peritonitis (SBP) in cirrhotic patients with ascites, possibly linking NOD2's effects on the intestinal barrier function to enhanced bacterial translocation in susceptible patient populations (Appenrodt et al., 2010; Nischalke et al., 2011). A recent genome-wide association analysis revealed that single-nucleotide polymorphisms (SNPs) related to several genes in the NOD2 signaling pathway were significantly associated with leprosy. This raised the possibility that Crohn's disease and multibacillary leprosy, both of which involve granuloma formation possibly driven by aberrant Th1 responses, share underlying NOD2 pathway aberrations (Berrington et al., 2010b; Zhang et al., 2009).

Future studies in human patients may unravel roles of these and other NLR proteins, through the mechanisms discussed in this review in other multifactorial diseases, including infectious diseases. Studying their mechanisms of action will be of significant clinical importance and may enable the development of novel therapeutic interventions for these disorders.

Concluding Remarks

NLRs are central mediators of microbial sensing. In most cases, NLR-mediated pathogen recognition is employed by the host as part of the antimicrobial immune response. In a minority of cases, this sensing is manipulated by pathogens as part of immune-evasive mechanisms. However, many important open questions remain. These include how microbial signals are sensed by the different NLRs, how different NLRs interact with each other and with other host signaling pathways, how NLR binding and activation leads to initiation of the anti-microbial response, how NLRs modulate the adaptive anti-microbial immune response, and what is the clinical significance of aberrations in NLR signaling with respect to host vulnerability to infection. Deciphering these mechanisms will undoubtedly enable further understanding of the innate immune response against pathogens.

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