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NLRs join TLRs as innate sensors of pathogens

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Pathogen-recognition receptors (PRRs) are key components of immune systems and are involved in innate effector mechanisms and activation of adaptive immunity. Since their discovery in vertebrates, Toll-like receptors (TLRs) have become the focus of extensive research that has revealed their significance in the regulation of many facets of our immune system. What makes TLRs so central and fascinating is their ability to recognize microbes and directly initiate specific signal transduction cascades that alert the host defences. In this review, we discuss the function and biology of a new family of PRRs, the NACHT-LRRs (NLRs), which include both nucleotide-binding oligomerization domains (NODs) and NALPs [NACHT-, LRR- and pyrin domain (PYD)-containing proteins], and underline some intriguing similarities between NLRs and TLRs that emphasize the role of NLRs as a complementary system for host–microbe interactions.

Introduction

Innate immunity was thought formerly to be a non-specific immune response characterized by engulfment and digestion of microorganisms and foreign substances by macrophages and leukocytes. However, innate immunity acts with substantial specificity and is adept at discrimination between pathogens and self. Since the early nineties, Charles Janeway and his collaborators have proposed that the innate immune system can recognize key molecular signatures borne by pathogens, called pathogen-associated molecular patterns (PAMPs) [1]. These patterns represent molecules vital for microbial survival and are therefore unlikely to vary in their structures because any major changes would be disadvantageous. Such molecules include bacterial structural components, such as lipopolysaccharide (LPS) and peptidoglycans (PGNs), or viral RNA. Janeway predicted that host organisms develop a group of receptors that recognize these PAMPs, referred to as pathogen-recognition receptors (PRRs).

PAMPs are not the only trigger of innate immunity. Innate immunity can recognize abnormal self or danger signals, such as DNA, RNA or uric acid, which should not normally be present outside cells or at certain locations within the cell [2,3]. This proposed mechanism, also known as the 'danger model', is well illustrated in the case of the high mobility group box 1 (HMGB1) protein, a nuclear factor and secreted protein. In the cell nucleus, HMGB1 acts as an architectural chromatin-binding factor; however, outside the cell, it binds to RAGE (receptor for advanced glycation end-products), activates TLRs and is a potent mediator of inflammation [4,5]. Although the models of recognition of pathogen molecular patterns and the 'danger model' often appear to be opposed, they are not mutually exclusive because both systems might be necessary and might use the same collection of PRRs, converging on the same basic mechanism of activating inflammation and the adaptive immune system.

The activation of PRRs can lead to mobilization of soluble defence molecules, killing of the infected cells or tissues, acquisition of specialized functions by sentinel cells, induction of co-stimulatory molecules by antigen-presenting cells, and many other physiological responses. One of the modes of action of PRRs is the transcription activation [e.g. by the NF-κB or interferon-β (IFN-β) pathway], resulting in mobilization of the effectors of inflammation. Several of these PRRs have been identified; Toll-like receptors (TLRs) being the most studied [6]. Being transmembrane receptors, TLRs survey the extracellular fluids, including endosomal compartments, and have an important role in cross-presentation of particular pathogens to lymphocytes [7] (for more detailed information on TLRs, excellent reviews have been published recently [8,9]). Although it is well established that TLRs are key innate immune sensors, the recent discovery of novel PRR family members that are able to activate NF-κB or IFN-β suggests that some TLR-elicited responses might be redundant with similar signaling cascades triggered by other PRRs [10–12]. Interestingly, this hypothesis is supported by the unexpected observation that patients devoid of functional IRAK4, an important kinase for TLR signaling, show a relatively mild disease phenotype because they have a limited and transient susceptibility to infection by only a few pyogenic bacteria during childhood [13].

Here, we will review the function and biology of a family of recently identified intracellular PRRs, namely the NACHT [domain present in neuronal apoptosis inhibitory protein (NAIP), CIITA, HET-E and TP1]-LRRs (NLRs), whose emerging function is to detect intracellular pathogens or danger signals in general (Table 1). In

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Table 1. The human NLR family

NLRs	Name	Other names	Domain organization	Chromosomal localisation	Ligand	Proposed effector[b]	Genetic disease[c]
NALP	NALP1	DEFCAP; NAC; CARD7; CLR17.1	PYD-NACHT-NAD-LRR-FIIND-CARD	17p13	?	ASC/Caspase-1/5	
	NALP2	PYPAF2; NBS1; PAN1; CLR19.9	PYD-NACHT-NAD-LRR	19q13.42	?	ASC/Caspase-1	
	NALP3 or Cryopyrin	PYPAF1;CIAS1;-Cryopyrin; CLR1.1	PYD-NACHT-NAD-LRR	1q44	MDP	ASC/Caspase-1	FCU/MWS/ CINCA; NOMID
	NALP4	PYPAF4;PAN2; RNH2; CLR19.5	PYD-NACHT-NAD-LRR	19q13.43	?		
	NALP5	PYPAF8; MATER, PAN11; CLR19.8	PYD-NACHT-NAD-LRR	19q13.42	?		
	NALP6	PYPAF5; PAN3; CLR11.4	PYD-NACHT-NAD-LRR	11p15.5	?		
	NALP7	PYPAF3; NOD12; PAN7; CLR19.4	PYD-NACHT-NAD-LRR	19q13.42	?		
	NALP8	PAN4; NOD16; CLR19.2	PYD-NACHT-NAD-LRR	19q13.42	?		
	NALP9	NOD6; PAN12; CLR19.1	PYD-NACHT-NAD-LRR	19q13.42	?		
	NALP10	PAN5; NOD8; Pynod; CLR11.1	PYD-NACHT-NAD	11p15.4	?		
	NALP11	PYPAF6; NOD17; PAN10; CLR19.6	PYD-NACHT-NAD-LRR	19q13.42	?		
	NALP12	PYPAF7; Monarch1; RNO2; PAN6; CLR19.3	PYD-NACHT-NAD-LRR	19q13.42	?		
	NALP13	NOD14; PAN13; CLR19.7	PYD-NACHT-NAD-LRR	19q13.42	?		
	NALP14	NOD5; PAN8; CLR11.2	PYD-NACHT-NAD-LRR	11p15.4	?		
NOD	NOD1	CARD4; CLR7.1	CARD-NACHT-NAD-LRR	7p15-p14	Meso-DAP	RIP2	
	NOD2	CARD15; IBD1, PSORAS1; CLR16.3	CARD2x-NACHT-NAD-LRR	16q12	MDP	RIP2	Crohn's disease Blau syndrome
	NOD3	CLR16.2	(CARD)-NACHT-NAD-LRR[a]	16p13.3	?		
	NOD4	NOD27; CLR16.1	(CARD)-NACHT-NAD-LRR[a]	16q13	?		
	NOD5	NOD9; CLR11.3	X-NACHT-NAD-LRR	11q23.3	?		
CIITA	CIITA	MHC2TA, C2TA	(CARD)-NACHT-NAD-LRR	16p13	?	Transcriptional co-activators	BLSII
IPAF	IPAF	CARD12; CLAN; CLR2.1	CARD-NACHT-LRR	2p22-p21	?	Caspase-1	
NAIP	NAIP	BIRC1; CLR5.1	BIR3x-NACHT-LRR	5q13.1	?		Legionella disease, SMA

[a]NOD3 and NOD4 contain an atypical CARD domain.
[b]Effector proteins identified only through overexpression studies are excluded.
[c]Abbreviations: BLS, bare lymphocyte syndrome; CINCA, chronic infantile neurologic cutaneous articular syndrome; FCAS, familial cold autoinflammatory disease; FCU, familial cold urteceria; GC, germ cells, IC, immune cells; IPAF, ICE-protease activating factor; MWS, Muckle-Wells syndrome; NOMID, neonatal-onset multisystem inflammatory disease; SMA, spinal muscular atrophy.

particular, we focus on recent insights gained into the emerging roles of nucleotide-binding oligomerization domains (NODs) and NALP3 [NACHT-, LRR- and pyrin domain (PYD)-containing protein 3] in inflammation. For more general reviews on NLRs, we refer to two recently published articles [12,14].

Intracellular PRRs: the NLRs

Innate immunity is not only capable of detecting extracellular pathogens and danger through TLRs and other membrane-bound PRRs but can also sense their presence in the cytoplasm with great efficiency. For example, the presence of viral RNA is detected by RIG-I, which leads to the activation of classical immune defence systems, such as NF-κB and IFN-β [11]. The largest known family of intracellular PRRs, however, comprises the NACHT-LRRs (NLRs). Alternative names for this family include CATERPILLER, NOD and NOD-LRR [12,14–16].

NLRs have high structural and functional homology to R genes in plants, which detect the presence of pathogens and trigger the mitogen-activated protein kinase (MAPK) activation and eventually cell death (Box 1). Searches in the human genome database reveal a substantial number (at least 22) of R-gene homologues [12,15,17] (Table 1). Three structural domains characterize these intracellular proteins (Figure 1, Table 1). (i) At the C terminus is a leucine-rich repeat (LRR) domain that is also present in TLRs. The LRR domain is considered to be the ligand-sensing motif, able to recognize conserved microbial patterns or other ligands. For example, LRRs of TLR3 sense the presence of double-stranded RNA [18]. (ii) The intermediary NACHT (NBS; NOD) domain is essential for

Box 1. Plant R genes

Plants can activate an effective arsenal of inducible defense responses, such as cell death of infected cells [the hypersensitive response (HR)] or antibiotic production at the site of infection. These local responses can, in turn, trigger a long lasting systemic response [systemic acquired resistance (SAR)] that primes the plant for resistance against a broad spectrum of pathogens. Recognition of pathogens is mediated by disease resistance (R) genes, which encode cytosolic or membrane-bound proteins. The largest known class of R proteins includes those that contain a nucleotide-binding site, the NB-ARC domain, and a LRR domain [53] (Figures 1 and 2).

There are >149 predicted R proteins in *Arabidopsis* [54]. This highlights the importance of these innate immune recognition systems in plants, which are devoid of a true adaptive immunity. The NB-ARC domain of plants binds ATP and is thought to be involved in oligomerization, an important process for cell signaling. The crucial role of oligomerization is well established for the NB-ARC of the mammalian apoptotic protein Apaf-1 [21]. It is interesting to note that particular mutations in the NB-ARC can confer a gain of function and result in constitutively active R proteins [55,56]. R genes were essentially studied in 'gene for gene' relationships, in which an avirulence (*Avr*) gene of the pathogen is associated with a specific R gene; however, a more general role for R genes in more basic cellular processes cannot be excluded. In R proteins, the Avr recognition module is assumed to be the LRR that follows the oligomerization module. Although there is no clear evidence that this domain is involved directly in the recognition of pathogens, this assumption is probably based on the similarities with mammalian counterparts.

Only one direct interaction has been reported between the LRR of an NB-ARC–LRR resistance protein and the corresponding Avr protein [57]. The lack of detectable R–Avr interactions leads to the formulation of the 'guard hypothesis' [58]. This model, which can be compared to the 'danger model' in vertebrates, proposes that R genes activate resistance when they interact with another plant protein that is targeted and modified by pathogens during the process of infection. Resistance is triggered when the R protein detects an attempt to attack its guarded protein; this does not require direct interaction between the R and Avr proteins [59]. Compelling evidence for this model was recently reported for two *Arabidopsis* R proteins, RPM1 and RPS2. These sentinel genes guard the bacterial virulence target RIN4. When the *Avr* genes affect RIN4 by phosphorylation [60] or by degradation of RIN4 [61,62], R genes become activated, which leads to resistance [63,64].

the oligomerization and activation of the NLRs. NACHT domains are part of P-loop NTPases, which have predicted nucleotide triphosphatase activity [19] and are closely related to the oligomerization module found in AAA^+ ATPases [20]. A similar nucleotide-binding motif, called the nucleotide-binding ARC domain (NB-ARC), is found in the Apaf-1 (apoptotic protease-activating factor-1) protein, in which it is responsible for binding to dATP and cytochrome-*c*-dependent oligomerization, which results in activation of pro-caspase-9 [21]. Oligomerization of the NACHT domain is a prerequisite for transduction of the signal mediated by the third, (iii) N-terminal effector domain, which can be a pyrin domain (PYD), a caspase recruitment domain (CARD) or a baculovirus inhibitor of apoptosis protein repeat (BIR) domain.

The PYD and CARD of NALPs and NODs normally link the receptor to downstream adaptor and effector proteins, such as RIP-kinases or initiator caspases, through homotypic interactions connecting PYD–PYD or CARD–CARD, similar to the manner in which Toll–interleukin-1 (IL-1) receptor (TIR) domains link the TLRs to downstream TIR-containing adaptors. The function of the BIR domains in NAIP is currently unclear. The caspase-inhibitory cavity has been postulated to be similar to the BIRs present in X-inhibitor of apoptosis protein (IAP) [22]. However, BIR domains present in most other proteins show no obvious affinity for caspases, thus their function in NAIP remains to be determined. The activation domain (AD) of the class II transactivator (CIITA) acts as a co-activator of MHC II transcription [23].

Based on the phylogenetic history of the NACHT domain (which is the only domain present in all NLRs) and the particular type of effector domain, the 22 members of the NLR family can be further classified [15]. We can distinguish two large subfamilies, the CARD-containing NOD (NOD5 might not have a CARD) and the PYD-containing NALP groups. The remaining three NLR members are the CARD-containing CIITA, ICE-protease activating factor (IPAF) and NAIP with its three BIR domains (Figure 1, Table 1).

NLR activation is proposed to occur by a mechanism similar to the mechanism described for the apoptosome [24]. The NLR proteins are normally present in the cytoplasm in an inactive, autorepressed form (Figure 2). The LRRs fold intramolecularly back onto the NACHT domain, thereby inhibiting spontaneous oligomerization (and activation) of the NLR protein. On direct or indirect binding of a PAMP to the LRR, the molecule undergoes a conformational rearrangement, exposing the NACHT domain and thereby triggering oligomerization. In turn, NLRs expose the effector domains. Through a homotypic interaction, CARDs and PYDs recruit CARD- and PYD-containing effector molecules, bringing them into close proximity with each other and leading to their activation. Because NACHT domains are part of the larger AAA^+ domain family, which are known to form hexamers or heptamers, NLRs probably form oligomers of this size and consequently will assemble six or seven effector molecules. For example, the NB-ARC of APAF (another member of the AAA^+ family) assembles into heptamers, which recruits seven caspase-9 molecules [24].

Expression patterns of most NLRs have not been studied in detail. Expression of most NLRs is widespread; however, the expression of some NLRs seems to be restricted, for example, NALP3 is expressed mainly in immune cells, whereas NALP5 (Mater) is expressed in germ cells. The availability of antibodies that are able to specifically recognize the various NLRs is essential for obtaining more detailed information concerning the cells and tissues expressing the NLRs.

NLRs and inflammatory disorders

Polymorphisms or mutations in human or mouse NLRs are associated with susceptibility to inflammatory disorders, which further strengthens the idea that these molecules are important in inflammation and immunity. Loss-of-function mutations in CIITA are responsible for type II bare lymphocyte syndrome (BLS), a genetically inherited disorder characterized by MHC II deficiency [25]. Mutations in CIITA directly impair the ability of CIITA to act as a transcriptional activator, resulting in decreased MHC II expression. MHC II is fundamental for antigen presentation to T cells, a key event in the

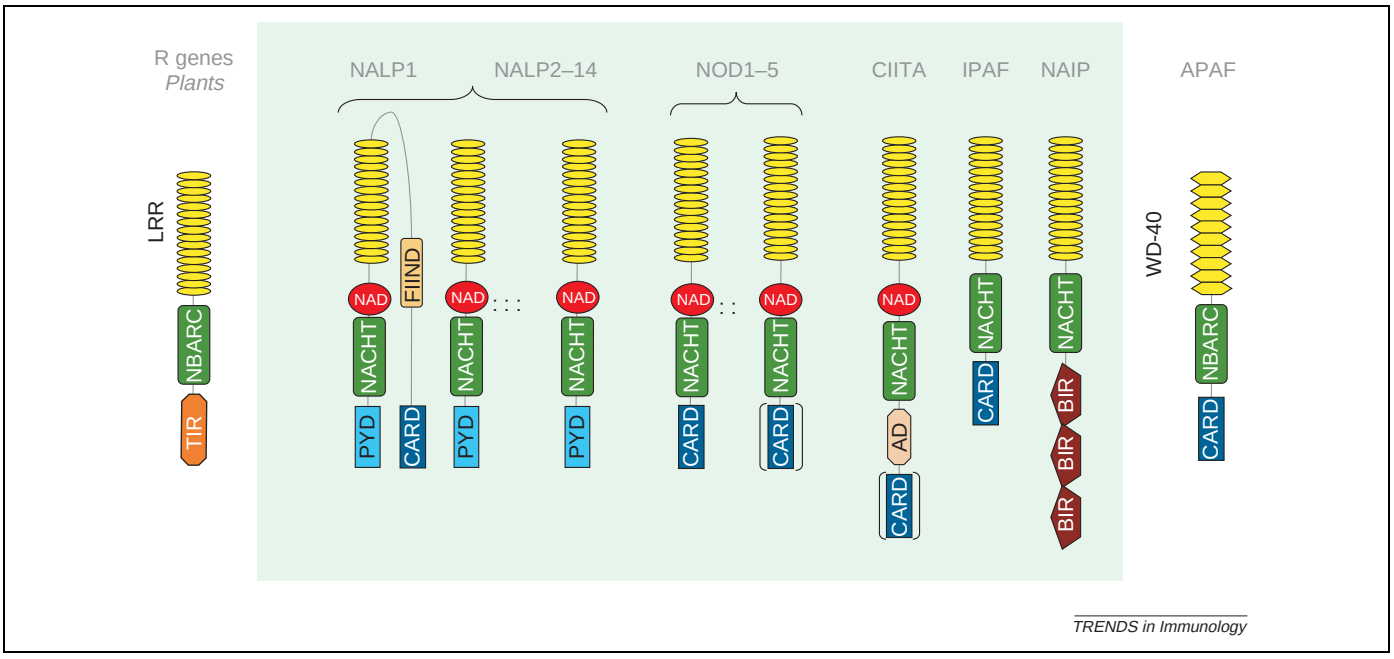

Figure 1. Domain organization of representative NLRs. For comparison, the structural organization of a plant R gene and Apaf are shown. NLRs are characterized by three distinct domains: the ligand-sensing LRRs (yellow), the NACHT domain (green), which is responsible for the capacity of NLRs to oligomerize, and the effector domain, which can be a PYD (blue), CARD (dark blue) or BIR domain (purple). Most NLRs also contain a NACHT-associated domain (NAD) (red) [65] C-terminal of the NACHT domain. NLRs are constituted by two large subfamilies: the 14 members of the PYD-containing NALP clan and the five members of the CARD-containing NODs. The two CARD-containing CIITA and IPAF and the three BIR-containing NAIP form the remaining NLR members (Table 1). Abbreviation: FIIND, function to find; NAD, NACHT-associated domain; WD-40, protein–protein interaction domain.

activation of adaptive immunity. As a result of this deficiency, patients suffering from BLS are extremely vulnerable to various viral, bacterial and fungal infections. In mammals, it has never been shown that CIITA can be activated directly by microbes or pathogens; however, based on the similarity of this molecule to other NLRs and TLRs that are known to induce co-stimulatory signals involving antigen presentation following microbial stimulation, it is tempting to speculate that this might be a plausible scenario. Such a mechanism might cooperate with the induction of co-stimulatory signals for antigen presentation by other NLRs or TLRs. In line with this hypothesis, some NLR-related R genes in plants have acquired transcriptional domains, which enable them to directly regulate transcription on infection [26].

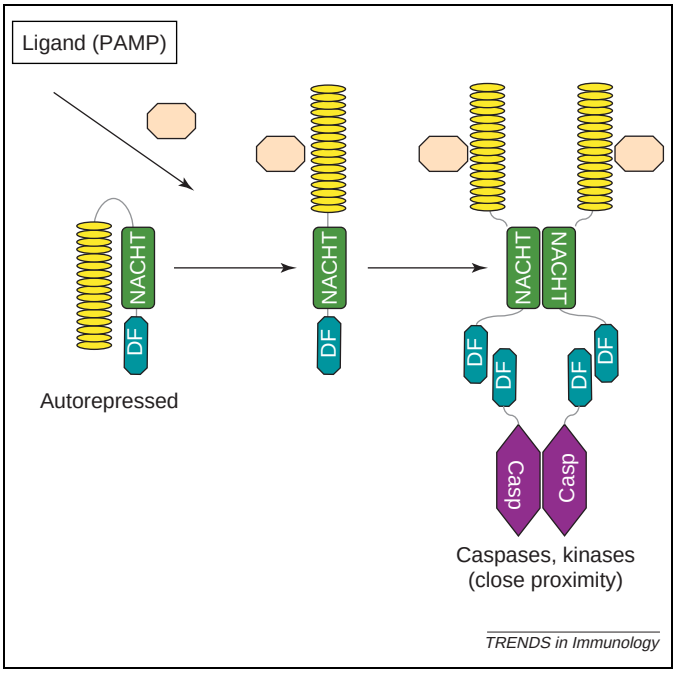

Figure 2. Proposed mechanism of NLR activation. On binding to a PAMP, the autorepressed NLR protein undergoes a conformational change, exposing the NACHT oligomerization domain. NLRs then probably form hexamers or heptamers (for the sake of simplicity, only two protomers are shown). In parallel, the death fold (DF)-containing effector domains, PYDs or CARDs, become accessible and are able to interact with downstream PYD- or CARD-containing effector molecules, such as caspases, kinases or adaptor proteins.

Genetic studies in mice revealed that the paralogue of NAIP, NAIP5, is involved in susceptibility to *Legionella pneumophila* in macrophages [27]. The molecular role of NAIP molecules in host–pathogen interactions is still unknown. In humans, NAIP was proposed to be an inhibitor of caspases and was linked to susceptibility to spinal muscular atrophy (SMN), a neurodegenerative disorder. However, SMN appears to be dependent on a mutation of *SMN1*, a neighbouring gene. In this context, NAIP deletion might contribute to the severity of the disease [28].

Mutations in *NOD2* are found in patients with Crohn's disease and Blau syndrome. Crohn's disease is a common condition associated with an enhanced mucosal permeability and inflammation of the small bowel. Both genetic and environmental factors are involved in this pathology. The disease-associated mutations, which affect mainly the LRR part of the NOD2 protein [29,30], lead to an apparent dysfunctional response to stimulation *in vitro* by a bacterial product in Crohn's disease patients [31–33], suggesting that the disease is caused by a lack of NOD2 responsiveness following bacterial insult [34]. In line with this, NOD2 appears to control secretion of the

antibacterial α-defensins [35]. This lack of function that underlies susceptibility is also supported by the homozygous pattern of the mutation that is frequent in this disease. In addition, it has been proposed that NOD2 negatively regulates TLR2-mediated Th1 responses [36], suggesting a possible crosstalk between TLRs and NLRs. Although peripheral blood mononuclear cells from individuals homozygous for the major disease-associated L1007fsinsC mutation in *NOD2* show a clear loss of function phenotype [32], macrophages from mice engineered to harbor the corresponding orthologous mutation exhibit elevated NF-κB activation and more efficient processing and secretion of IL-1β [37]. The function of NOD2 in the biology in Crohn's disease is apparently complex and will require further investigation.

Blau syndrome is a rare autosomal dominant genetic disorder not related directly to Crohn's disease, and which is characterized by granulomatous arthritis, uveitis and skin lesions. All the missense mutations in the *NOD2* gene associated with this disease [38,39] are localized in the NACHT domain of NOD2 but do not seem to affect crucially conserved residues in the NACHT domain. These mutations might therefore confer a gain of function to NOD2 rather than disrupting the folding of this domain. This concept is supported in overexpression studies [31,40] and is consistent with the heterozygosity pattern that is found in affected patients. Therefore, in this case, inflammation might be due to an aberrant activation of NOD2 in the absence of pathogenic stimuli.

Missense mutations in the NACHT domain of NALP3, also known as Cryopyrin or CIAS1, are involved in three autosomal dominant diseases, familial cold autoinflammatory syndrome (FCAS), Muckle Wells syndrome (MWS) and chronic infantile neurological cutaneous and articular syndrome (CINCA), which is also known as neonatal onset multisystemic inflammatory disease (NOMID) [20,41]. All three disorders are closely related autoinflammatory syndromes characterized by periodic fever, skin rashes, amyloidosis and, in the case of CINCA, the eventual development of neurological complications. The mutations in NALP3 are localized in the NACHT domain and resemble the NOD2 mutations found in the Blau syndrome. For example, the NALP3 R260W mutations correspond structurally to the R334W mutation in the NACHT of NOD2 found in Blau syndrome patients. Specifically, this amino acid change in the NACHT of NALP3 confers a gain of function to the protein, resulting in a constitutively active NALP3 in Muckle Wells patients [42].

Signals triggered by NLRs

Little is known on the signaling cascades and effector mechanisms initiated by most of the NLRs. Here, only the signals proposed to be triggered by NOD1, NOD2 and some NALPs are reviewed.

The NODs and RIP2 activation

NOD1 and NOD2 both contain N-terminal CARD domains, which link the NODs to the activation of the inflammatory kinase RIP2. Oligomerization of NODs induces physical proximity of RIP2 proteins and the IκB kinase (IKK) subunits, leading to NF-κB activation [12]. NOD2 activation also induces the ubiquitination of NEMO [43], a process involved in driving specific NF-κB responses [44]. NOD1- and NOD2-mediated NF-κB activation is deficient in RIP2 knockout mice, supporting the key role of this kinase in signaling by NODs. Surprisingly, RIP2-deficient macrophages display an impaired cytokine response to many TLR agonists, suggesting that RIP2 might be involved in both TLR and NOD signaling [45]. TLR and NODs might converge on similar signaling cascades, possibly involved in similar redundant functions.

NALPs and the inflammasome

Through genomic database searches, 14 NALPs were identified in the human genome [15]. Although only a few NALPs are well characterized, a common feature of NALPs (at least for NALP1, NALP2, NALP3, NALP6 and NALP12) is their ability to recruit the adaptor protein ASC through a homotypic PYD–PYD interaction, which in turn recruits caspase-1 through a CARD–CARD interaction [20]. The oligomerization of NALPs is believed to bring the inflammatory caspases into close proximity, leading to their activation within a complex known as the inflammasome [46] (Figure 3). Activation of caspase-1 is an essential step for the processing and maturation of the proinflammatory cytokines IL-1β and IL-18. IL-1β and IL-18 bind and activate the IL-1 receptor (IL-1R) and IL-18 receptor (IL-18R) complexes, respectively. These two receptors not only resemble TLRs but also share TLR intracellular signaling components. Both IL-1R and IL-18R, similar to TLRs, have a cytoplasmic TIR domain that, on activation, recruits TIR adaptors, such as MyD88, which is, in turn, responsible for activating NF-κB and other signaling cascades. Therefore, the activation of NALPs can be seen as an upstream step in the IL-1R and IL-18R signaling cascades, directly linking intracellular pathogen sensing to signaling pathways shared with TLRs (see later).

Peptidoglycans as activators of NOD1, NOD2 and NALP3

What are the signals that activate the NLRs? Early studies on the inflammasome revealed that NALP activation can be triggered *in vitro* by hypotonic stress. This 'stress'-induced activation process possibly results from the release or activation of a 'danger signal' or from the disruption of a guarding complex as proposed for R-gene activation [46]. Other studies using knockout mice showed that ATP-induced activation of caspase-1 is dependent on ASC, suggesting that NALPs are activated on P2X7 (ATP receptor) stimulation [47]. The mechanism and the ligand involved in these two types of activation, however, remain elusive. More information is available concerning the activation of NLRs by bacterial compounds because recent studies have identified bacterial peptidoglycans (PGNs) as activators of NODs and NALP3 [12,32,48,49]. PGN is a component of the bacterial cell wall and is composed of glycan chains of repeated N-acetylglycosamine (GlcNac) and N-acetylmuramic acid (MurNac), which are linked by short peptides. PGN can be degraded by bacterial hydrolases or, once phagocytosed, by host hydrolases, such as lysozyme (Figure 3). PGN degradation leads to the formation of muropetides that

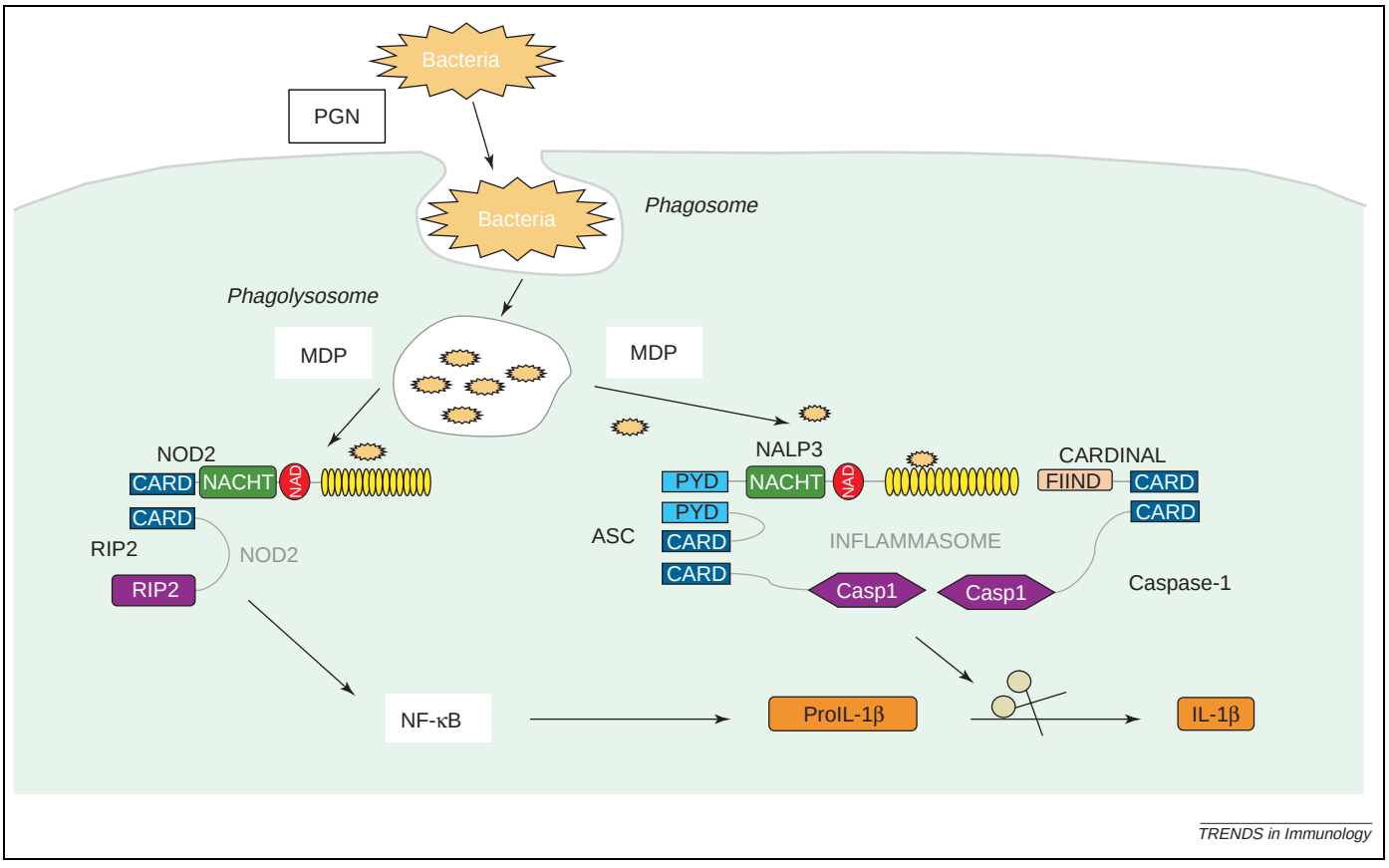

Figure 3. Model for the activation of the inflammasome by PGN. When bacteria are phagocytosed, PGN present in the bacterial wall is degraded to produce muropeptides, which are similar to MDP. Subsequent translocation of MDP into the cytoplasm (by an enigmatic mechanism) leads to the direct or indirect activation of NOD2, which results in NOD2–RIP2 complex formation. The resulting RIP2 activation leads to NF-κB activation and proIL-1β synthesis. Release of MDP also triggers NALP3 (Cryopyrin) activation, which results in the formation of the inflammasome complex (NALP3, cardinal, ASC and caspase-1). Caspase-1 activation then induces cleavage and maturation of IL-1β. Abbreviations: FIIND, function to find; NAD, NACHT-associated domain.

have strong immunomodulatory properties [50]. NOD1 recognizes specifically meso-diaminopimelic acid (meso-DAP), a PGN product mainly found in Gram-negative bacteria, and NALP3 and NOD2 sense muramyl dipeptide (MDP), a compound present in both Gram-positive and -negative bacteria (Figure 3). The mechanism involved in muropeptide recognition by NODs and NALPs has not been characterized to date and no direct interaction has been identified. It is therefore possible that the recognition is indirect and occurs by means of a linker protein. Originally, several reports indicated that TLR2 was the receptor involved in sensing PGNs. However, recent results demonstrate that TLR2 does not recognize PGN but rather minor contaminants, such as lipoproteins or lipoteichoic acids, present in most PGN preparations [51]. Moreover, NOD2 knockout mice show reduced activation of innate and adaptive immunity in the presence of MDP, suggesting a physiological role for this protein in the control of adaptive immunity [35]. Taken together, PGN products do not appear to influence TLRs but instead specifically activate intracellular NODs and some NALPs to regulate innate and adaptive immunity.

Similarity between NOD and NALP activation and the *Drosophila* Toll pathway

Another interesting parallel between TLRs and NLRs can be drawn if we compare the model proposed for PGN sensing by NODs and NALP3 with typical Toll receptor activation in *Drosophila* (Figure 4). The *Drosophila* Toll receptor and mammalian IL-1R do not recognize microbial patterns directly. Both receptors are activated by the endogenous cytokines, Spätzle and IL-1β, respectively, which both require processing and maturation for their activation [52]. Processing of Spätzle and IL-1β is mediated by specific proteases, which are activated by microbial compounds, such as PGN. The machinery involved in activation of Spätzle leads to the activation of serine protease cascades. In mammals, PGN activation of NOD2 might lead to the production of the IL-1β precursor and cooperate with PGN activation of the NALP3 inflammasome, thus resulting in the maturation of IL-1β. Such a system enables the indirect sensing of distant infections by means of circulating PGN recognition proteins (PGRPs) present in the haemolymph of the insect or by means of NLRs present in intracellular compartments. This results in the activation of signaling cascades that are evolutionary highly conserved between flies and mammals (Figure 4).

Conclusion

The recent characterization of NLRs has revealed interesting overlaps between the signaling pathways used by some NLRs and TLRs, suggesting redundancy and cooperation between these pathways. NLRs thus join

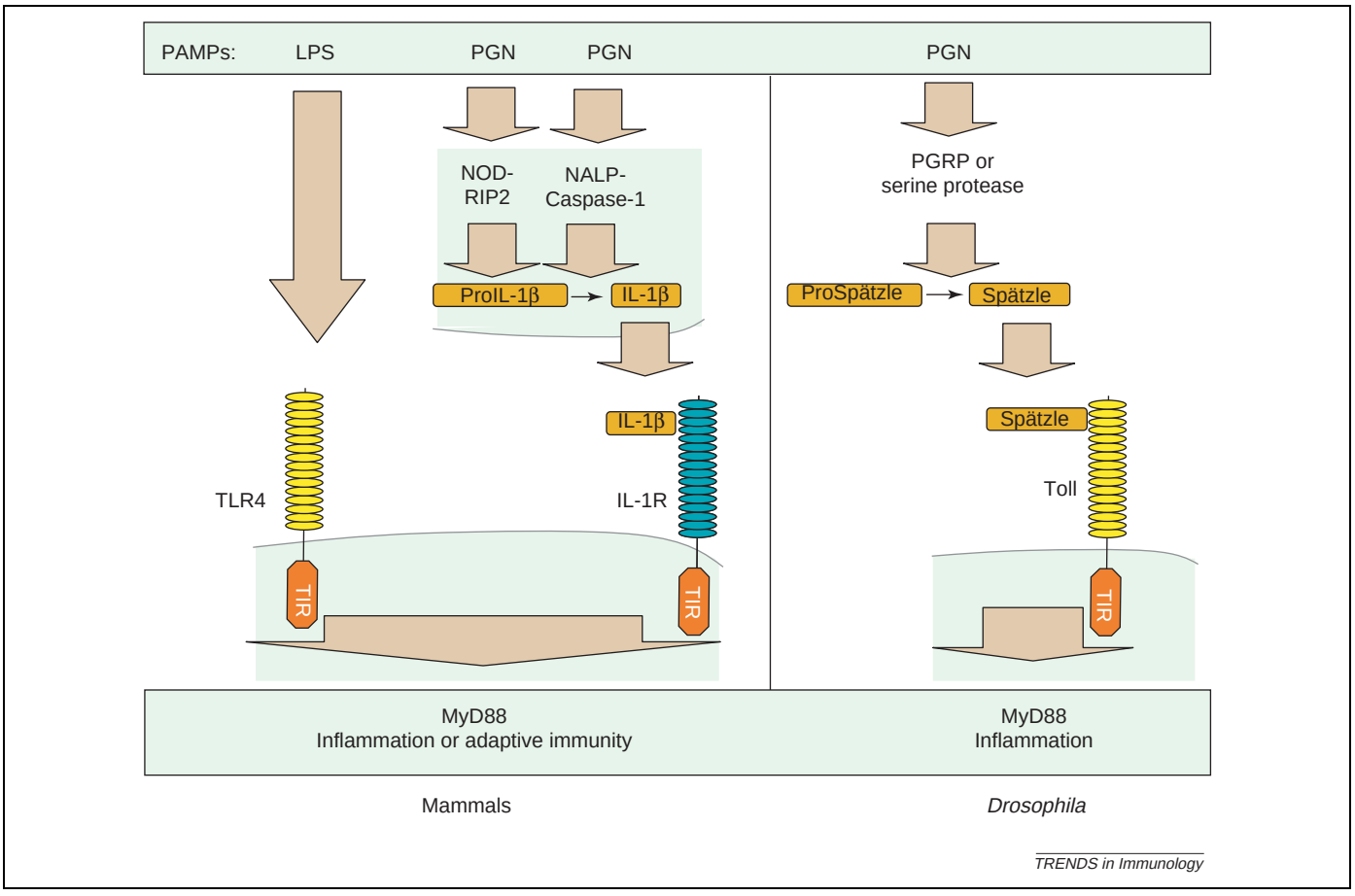

Figure 4. Model for PGN recognition by NODs and NALPs, and parallels with Toll in *Drosophila*. Recognition of PGN in mammals and in *Drosophila* leads to the activation of proteases that induce processing of cytokines involved in the activation of the TIR-containing receptors, IL-1R and Toll, respectively. In mammals, TLRs directly sense the PAMP, as illustrated for lipopolysaccharide (LPS) and TLR4 activation.

TLRs as crucial innate sensors of pathogens. Although some progress has been made in the characterization of some NLRs, this is an emerging field of research with a plethora of open questions. For instance, the function of many NLRs, such as NAIP or many NALPs, is not clearly known. Another important issue that remains unaddressed is the identification of additional ligands, their specificity and mechanisms of NLR activation. Finally, the investigation of the physiological function of those proteins involved in inflammation and immunity will shed more light on the respective roles of NLRs in human infections and inflammatory diseases.

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