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Brief Reviews

Inflammasome-Associated Nucleotide-Binding Domain, Leucine-Rich Repeat Proteins and Inflammatory Diseases

Sushmita Jha*† and Jenny P.-Y. Ting2†‡

The nucleotide-binding domain, leucine-rich repeat (NLR) proteins are a recently discovered family of intracellular pathogen and danger signal sensors. NLRs have emerged as important contributors to innate immunity in animals. The physiological impact of these genes is increasingly evident, underscored by the genetic association of variant family members with an array of inflammatory diseases. The association of mutations in NLR genes with autoinflammatory diseases indicates an important function of these genes in inflammation in vivo. This review summarizes the role of the inflammasome NLR proteins in innate immunity and inflammatory diseases and explores the possible utility of some of these NLRs as pharmacological targets. The Journal of Immunology. 2009, 183: 7623–7629.

The nucleotide-binding domain (NBD),3 leucine-rich repeat (LRR) (NLR) gene family is an evolutionarily conserved family of genes, important for immune function in animals (1–3). There are >20 NLR genes in humans. The NLR gene family members were discovered by their structural similarity to the MHC class II gene master regulator CIITA and other NBD-LRR-containing proteins (4). NLR genes encode cytoplasmic proteins with a tripartite domain structure that is conserved with a subclass of plant disease resistance genes (3). The tripartite structure of NLRs consists of a variable N-terminal effector domain, a central NBD, and a variable number of C-terminal LRRs. Fig. 1 provides schematics of the domain structures of the NLR proteins described in this review. The NLRs are responsible for rapid sensing of pathogen-associated molecular patterns (PAMPs) such as the bacterial cell wall components LPS, lipoproteins, and flagellin (5–11), bacterial and viral nucleic acids (12–15), and the fungal cell wall components zymo- san and mannan (16). In addition, NLRs also sense damage-associated molecular patterns (DAMPs) such as ATP (17), uric acid (18, 19), amyloid-β (20), asbestos (21, 22), silica (21), hyaluronan, and heparan sulfate (23). However a major unresolved issue in the field is how an NLR acts as a sensor, because direct evidence of NLR proteins binding to a specific pathogen- or non-pathogen-derived ligand is lacking. Regardless of the mechanism, the sensing of PAMPs and DAMPs by NLR proteins can result in the assembly of a caspase-1 activating multiprotein complex referred to as the “inflammasome” (2). This is similar to the cytoplasmic multiprotein complexes assembled for the activation of caspase-9 and caspase-8 referred to as the apoptosome (containing Apaf-1) (24) and the death-inducing signaling complex (Fas/CD95-DISC) (25), respectively. The protein components of the caspase-activating platforms are present as inactive monomers that oligomerize on exposure to the activating PAMP or DAMP signal. Inflammasome formation results in the cleavage of caspase-1 from its inactive proprotein form to its active mature form. This active caspase-1 then processes the cleavage of pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18, respectively. Although IL-1β and IL-18 are the most widely studied targets of caspase-1, two recent studies have identified >70 new targets of caspase-1 ranging from chaperones, cytoskeletal and translation machinery, and glycolysis and immune proteins (26, 27). There are several studies and related reviews analyzing the role of the NLR gene family in infectious diseases, but this review focuses on the role of the inflammasome NLRs in inflammatory diseases.

NLR gene family and NLR inflammasomes

The well known inflammasomes, the NLRP1, NLRP3, NLRC4, and NAIP5 inflammasome complexes, and their key component proteins will be discussed in brief in this section. Fig. 2 depicts the triggering PAMPs and DAMPs and the key component proteins of the four inflammasomes discussed below.

The NLRP3 inflammasome. The NLR family, pyrin domain-containing (NLRP) 3 (NLRP3; also called Cryopyrin, NALP3),

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3 Abbreviations used in this paper: NBD, nucleotide-binding domain; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; CAPS, cryopyrin-associated periodic syndrome; CARD, caspase activating and recruitment domain; CINCA, chronic infantile neurological, cutaneous and articular syndrome; DAMP, damage-associated molecular pattern; FCAS, familial cold autoinflammatory syndrome; LRR, leucine-rich repeat; NAD, NBD-associated domain; MDP, muramyl dipeptide; MWS, Muckle-Well syndrome; MSU, monosodium urate; NAIP, NLR apoptosis inhibitory protein; NLR, nucleotide-binding domain, leucine-rich repeat; NLRC, NLR family, CARD-containing (inflammasome); NLRP, NLR family, pyrin domain-containing (inflammasome); NOD, nucleotide oligomerization domain; NOMID, neonatal onset multisystem inflammatory disease; PAMP, pathogen-associated molecular pattern.
PYPAf1, CIAS1, and CLR1.1) inflammasome is activated by the presence of pathogen products such as nucleic acids (12–15), LPS (12, 13, 18, 28), lipooligosaccharide (29), and muramyl dipeptide (MDP) (30); certain toxins such as nigericin (Streptomyces hygroscopicus) and maitotoxin (marine dinoflagellates) (17); cellular danger signals such as ATP (17), uric acid crystals (18), hyaluronan and heparan sulfate (31), and amyloid-B (20), environmental danger signals such as asbestos and silica (21, 32); and alum and other particulate adjuvants (32, 33). NLRP3 forms a multiprotein complex, referred to as the NLRP3 inflammasome, with the adapter protein apoptosis-associated speck-like protein containing a caspase activating and recruitment domain (ASC) (28) and procaspase-1. Association of NLRP3 with ASC is required for recruitment of procaspase-1 (34). The caspase activating and recruitment domain (CARD) of ASC is used to recruit procaspase-1 by CARD-CARD interactions, thus leading to the processing of procaspase-1 into active caspase-1 (35).

The NLRP1 inflammasome. The human NLRP1 (also called CARD7, DEFCAP, and CLR17.1) inflammasome was the first caspase-1-activating inflammasome to be identified (36). There is only one Nlrp1 gene in humans in contrast to three paralogues in mice; Nlrd1a, Nlrd1b, and Nlrd1c (37). The NLRP1 protein in humans consists of an N-terminal pyrin domain central, an NBD, an NBD-associated domain (NAD), LRR and function to find domains (FIIND), and a C-terminal CARD domain. The mouse counterparts vary in structure from the human protein; Nlrd1a lacks the N-terminal pyrin domain, Nlrd1b lacks both the pyrin and NAD domains, and Nlrd1c lacks all but the NBD and LRR domains. Initial studies on NLRP1 using cell extracts suggested that the NLRP1 inflammasome in humans consisted of NLRP1, caspase-1, caspase-5 (not present in mice), and ASC (38, 39). Even though the presence of ASC is not required for processing of caspase-1 by the NLRP1 inflammasome, it does augment this function (40). The mouse Nlrd1b inflammasome is activated in response to Bacillus anthracis (41) and specifically to the lethal toxin. Faustin et al. used a cell-free system with recombinant NLRP1 inflammasome components to show inflammasome assembly and caspase-1 activation in response to the peptidoglycan component MDP (40). Hsu et al. showed that MDP stimulation of macrophages leads to association of NLRP1 with nucleotide oligomerization domain (NOD) 2 (41). Gel filtration experiments revealed a complex consisting of NLRP1, NOD2, and caspase-1. Moreover, Bacillus anthracis infection also induces NOD2- and caspase-1-dependent IL-1β secretion. These results suggest the existence of a NLRP1- and NOD2-containing inflammasome and the potential for MDP to activate both NLRP1 and NOD2. However there is no data to show that MDP binds to either NLRP1 or NOD2; thus, how MDP activates this pathway is unclear.

The NLRC4 inflammasome. NLR family, CARD-containing (NLRC) 4 (NLRC4; also called IPAF, NOD27, and CLR16.1) is a cytosolic sensor of flagellin, flagellated pathogens such as Salmonella typhimurium (6, 7, 34) and Legionella pneumophila (5), and nonflagellated pathogens such as Shigella flexneri (9), and Pseudomonas aeruginosa (11). NLRC4 forms a homo-oligomeric inflammasome with caspase-1 (34). Initial characterization of NLRC4 in human tissues and cell lines demonstrated its direct association with the CARD domain of procaspase-1 through CARD-CARD interactions (42, 43). This interaction can cause autocatalytic processing of procaspase-1 into caspase-1 (43). A constitutively active NLRC4 could cause autocatalytic processing of procaspase-1 leading to caspase-1-dependent apoptosis in transfected cells (43). In macrophages, caspase-1 activation and IL-1β release by cytoplasmic flagellin requires NLRC4 (6, 7, 34). NLRC4 can interact directly with procaspase-1 through CARD-CARD interaction; however, direct interaction of ASC with NLRC4 has not been demonstrated. Nonetheless, ASC-deficient macrophages show defective caspase-1 activation and IL-1β release in response to Salmonella, Shigella, and Pseudomonas infection, indicating that the function of NLRC4 is ASC dependent (9, 11, 34).

The NAIP5 inflammasome. The NLR apoptosis-inhibitory protein (NAIP) 5 (also called BIRC1 and NLRB1) is also a cytosolic sensor of flagellin. Although the human genome has one Naip5 gene, there are seven paralogues of NAIP, Naip1-7, in mice (44). Based on coimmunoprecipitation studies using overexpressed Myc-tagged NAIP and hemagglutinin-tagged NLRC4 in HEK293 cells, these two proteins can coassociate, suggesting that they can be part of the same caspase-1-activating inflammasome (45). Recently, Lightfield et al. reported a novel role for NAIP5 in inflammasome activation in response to the C terminus of flagellin and L. pneumophila infection (10). Interestingly, whereas transduction of macrophages with a C-terminal 35-aa fragment of flagellin led to NAIP5-dependent cell death, full-length flagellin induced NAIP5-independent but NLRC4-dependent cell death and IL-1β release, suggesting a separation of duty for NAIP5 and NLRC4. Moreover, because NLRC4 can sense some nonflagellated bacteria (9, 11), this might point to a mechanism for differential sensing of bacteria via the regulation of inflammasome components. However, NAIP5 has no caspase domain and needs NLRC4 to activate procaspase 1. Thus, NAIP5 appears to possess NLRC4-dependent and -independent functions.

Inflammasome NLRs and inflammatory disease

NLR-related inflammatory diseases can be classified into three categories based on disease resulting from the following: 1) mutation of core components of the inflammasome complexes (intrinsically inflammasomopathies); 2) aberrant activation of the inflammasome complex (acquired or complex inflammasomopathies); and 3) mutation of accessory or regulatory proteins upstream or downstream of the inflammasome complex (extrinsically inflammasomopathies) such as pyrin or the proline serine threonine phosphatase interaction protein PSTPIP1 (46). The first two will be discussed in this review,
but readers can refer to excellent reviews on the last group of proteins because they do not directly involve NLR proteins (46, 47). Table I provides a list of the disease-associated mutations discussed in this section.

**Intrinsic inflammasomopathies**

**Cryopyrin-associated periodic syndromes.** Autosomal dominant mutations in NLRP3 in humans leads to three autoinflammatory syndromes collectively referred to as cryopyrin-associated periodic syndromes (CAPS; also called cryopyrinopathies) (48–51). Gain-of-function mutations of NLRP3 cause a lowered activation threshold that leads to IL-1β secretion even in the absence of a stimulus in vitro (36, 52, 53). All CAPS are characterized by increased levels of IL-1β in the absence of infection. CAPS consist of a spectrum of diseases ranging from the mild, such as familial cold autoinflammatory syndrome (FCAS), to the intermediate, such as Muckle-Wells syndrome (MWS), to the severe, such as chronic infantile neurological, cutaneous and articular (CINCA) syndrome, also known as neonatal-onset multisystem inflammatory disease (NOMID). All three syndromes present with fever, urticaria-like rash, and varying degrees of arthropathy and neurological manifestations (4, 54–56). FCAS consists of the mildest symptoms, including cold-induced urticaria and mild arthralgia. MWS is characterized by spontaneous urticaria (not cold-induced), sensorineural hearing loss, arthralgia, and in some cases renal amyloidosis. CINCA is the most severe, with spontaneous urticaria, deforming arthropathy, sensorineural hearing loss, and chronic aseptic meningitis.
Vitiligo, an autoimmune disease associated with the development of vitiligo alone (63). The emergence of other autoimmune diseases such as rheumatoid arthritis, diabetes, lupus, and thyroid disease in vitiligo patients has been associated with the development of vitiligo alone (63). The mechanism by which NLRP1 leads to skin hypopigmentation in vitiligo remains unknown.

**Complex or acquired inflammasomopathies**

**Gout/pseudogout.** Gout and pseudogout are rheumatic diseases caused by deposition of monosodium urate (MSU) and calcium pyrophosphate dihydrate crystals respectively, in joints and periarticular tissues. This deposition can lead to acute or chronic inflammation of the joints. MSU and calcium pyrophosphate dihydrate crystals increase caspase-1 activation and IL-1β release from murine macrophages in an NLRP3- and ASC-dependent manner (18). The importance of IL-1β in gout studied in mice was further supported by the resistance of mice deficient in the IL-1 and TLR signaling adaptor protein MyD88 to MSU-induced inflammation (64). Although TLR-deficient mice still showed inflammation, the IL-1β receptor-deficient mice did not, thus indicating a specific role for IL-1 signaling in the pathology. Bone marrow reconstitution experiments established that IL-1R expression in nonhematopoietic and hematopoietic cells is required for the initiation of inflammation upon MSU stimulation, indicating IL-1β engagement to its receptor in this model.

**Asbestososis and silicosis.** Prolonged inhalation of asbestos and silica leads to two environmentally induced forms of pulmonary fibrosis referred to as asbestosis and silicosis respectively. Alveolar macrophages from individuals with prolonged exposure to asbestos exhibit enhanced IL-1β release (22). Moreover, Nlrp3-deficient mice show decreased IL-1β release in response to asbestos and silica (21, 32), indicating a role for NLRP3 in the immune response to asbestos and silica. Silica crystals, once phagocytosed, can cause lysosomal damage leading to release of the lysosomal protease, cathepsin B, which can activate the NLRP3 inflammasome. Inhibition of phagosomal acidification or cathepsin B impairs NLRP3 inflammasome activation (32). In the bleomycin-induced lung injury model of fibrosis, the NLRP3 inflammasome is triggered by local uric acid release in response to DNA damage and degradation after bleomycin injury, suggesting that uric acid may be one of the triggering DAMPs in lung fibrosis and disease (65).

**Guadeloupe variant periodic fever syndrome (FCAS2).** This syndrome was first reported in two families in Guadeloupe and thus named the Guadeloupe variant periodic fever syndrome (66). Based on the similarities in symptoms to FCAS, this syndrome is also referred to as FCAS2. Individuals with this syndrome present with cold-induced heterogeneous symptoms including fever, arthralgia, myalgia, sensorineural hearing loss, aphthous ulcers, and lymphadenopathy.

### Table I. Disease-associated mutations

<table>
<thead>
<tr>
<th>NLR</th>
<th>Mutation(s) (Amino acid change)</th>
<th>Disease Association</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLRP3</td>
<td>A439V, V198M, E627G, A352V</td>
<td>FCAS and MWS</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>R260W, D303N, T348M, A439T, and G569R</td>
<td>FCAS and MWS</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>F575S, Q306L, T436N, H358R, M662T, D303N, F309S</td>
<td>CINCA</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>L264H, D303N, A374N, Y570C, F523L</td>
<td>CINCA</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>L353P</td>
<td>FCAS</td>
<td>56</td>
</tr>
<tr>
<td>NLRP1</td>
<td>L155H</td>
<td>Vitiligo</td>
<td>63</td>
</tr>
<tr>
<td>NLRP2</td>
<td>R284X, V635T</td>
<td>Guadeloupe variant periodic fever syndrome</td>
<td>66</td>
</tr>
</tbody>
</table>
Table II.  Pharmacological inhibitors

<table>
<thead>
<tr>
<th>Action</th>
<th>Target</th>
<th>Drug (Company)</th>
<th>Description</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppression of IL-1β production</td>
<td>Caspase-1</td>
<td>Pranalcasan (Aventis/Vertex)</td>
<td>VX-740; VX-765</td>
<td>70</td>
</tr>
<tr>
<td>IL-1β posttranslational processing</td>
<td>Unknown</td>
<td>CP424174, CP412245 (Pfizer)</td>
<td>Diaryl sulphonlamide</td>
<td>70</td>
</tr>
<tr>
<td>IL-1β production inhibitor</td>
<td>Unknown</td>
<td>CJ14877, CJ14897 (Pfizer)</td>
<td>Pyridine-2-carboxylates</td>
<td>70</td>
</tr>
<tr>
<td>Unknown</td>
<td>LL-Z1217a (Pfizer)</td>
<td></td>
<td>Terpenoid lactone</td>
<td>70</td>
</tr>
<tr>
<td>Suppression of IL-1β release</td>
<td>IL-1β release inhibitors</td>
<td>CP424174 (Pfizer)</td>
<td>Diaryl sulphonlamide</td>
<td>70</td>
</tr>
<tr>
<td>Neutralization of secreted IL-1β</td>
<td>IL-1</td>
<td>Anakintra (Kineret, Amgen)</td>
<td>rhuIL-1 Ra*</td>
<td>70</td>
</tr>
<tr>
<td>IL-1</td>
<td>IL-1trap (Regeneron/Novartis)</td>
<td></td>
<td>Human IL-1RI:1gG1 protein</td>
<td>70</td>
</tr>
<tr>
<td>IL-1</td>
<td>CDP-484 (Celltech)</td>
<td></td>
<td>PEGylated Ab</td>
<td>70</td>
</tr>
<tr>
<td>Inhibition of IL-1R signal transduction</td>
<td>MyD88 inhibitors</td>
<td>Hydrocinnamolyl-l-valyl pyrrolidine</td>
<td>MyD88 mimic</td>
<td>70, 73</td>
</tr>
<tr>
<td>IRAK-4 inhibitors</td>
<td>IRAK-4</td>
<td>Names unavailablea</td>
<td>Amides, imidazo[1,2-a]pyridine compounds</td>
<td>76–78</td>
</tr>
</tbody>
</table>

*a* Recombinant human IL-1Ra.

*b* May also inhibit IL-18R and TLR signal transduction.

Genetic studies in patients with the Guadeloupe variant periodic fever syndrome revealed two missense mutations, one nonsense mutation, and one deletion mutation in the *Nlrp12* gene. The nonsense mutation caused a truncation within the NBD of the protein whereas the splice mutation caused a deletion of the C-terminal LRRs. NLRP12 was recognized as one of the few NLR proteins that can suppress NF-κB signaling (67, 68). Both of the missense mutations in NLRP12 caused a reduction in the suppression of NF-κB signaling by NLRP12, whereas the NBD mutation caused a more significant impact on normal NLRP12-induced NF-κB signaling as compared with the LRR mutation.

**NLRs as potential pharmacological targets**

Activation of the various inflammasome complexes discussed in this review leads to activation of caspase-1 and production of the proinflammatory cytokines IL-1β and IL-18. Although specific drugs that interfere with inflammasome components are under development, there have been several clinical studies exploring the modification of the IL-1β pathway owing to its central role in several diseases (69). Modulation of IL-1β function has been approached at three levels: firstly, the release of IL-1β can be blocked by the inhibition of upstream pathways (70, 71); secondly, the released cytokine can be neutralized or its receptor blocked to prevent downstream signaling (70, 72); and finally, the signaling mechanisms in the target cells can be blocked by disrupting further downstream signaling pathways (73–78). A detailed list of the available drugs targeting the above-mentioned steps of regulation for the IL-1β pathway along with their mechanisms of action is provided in Table II. There are some caveats in the use of some of the inhibitors because they can inhibit not only the IL-1β but also the IL-18 pathways. A better understanding of the underlying mechanism for each disease would provide more accurate targets. Target specificity would enable a more accurate control of pathology. CAPS symptoms remain the gold standard, as they can be reversed by treatments with the IL-1R antagonist Kineret. Although some of these drugs are efficacious in relieving symptoms (72, 74–78), several others are in clinical trials or remain to be tested in humans, awaiting further studies of their mode of action (70, 71).

**Conclusions**

The association of the NLRs with several immunological diseases suggests a role for these proteins in both innate and adaptive immunity. Recent studies are beginning to unfold the role of this family in immune regulation and dysregulation; however, a plethora of questions remain unanswered. Firstly, how is the diversity of PAMPs and DAMPs sensed and differentiated from self-molecules? Secondly, how does such a wide range of symptoms in CAPS arise from mutations that are relatively clustered in the NBD of NLRP3? Thirdly, is there a cross-talk between the different inflammasome pathways and do they compensate for each other? Finally, what are the DAMPs and PAMPs that might activate the inflammasome pathways in complex immune diseases such as type II diabetes, multiple sclerosis, and atherosclerosis? Considering the vibrant research in this field, significant progress is likely to resolve several of these issues.

**Disclosures**

The authors have no financial conflict of interest.

**References**


