Published in final edited form as:
J Immunol. 2009 December 15; 183(12): 7623–7629. doi:10.4049/jimmunol.0902425.

Inflammasome-Associated Nucleotide-Binding Domain, Leucine-Rich Repeat Proteins and Inflammatory Diseases[1]

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Abstract

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 nucleotide-binding domain (NBD), 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 zymosan 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

[1]This work was supported by National Institutes of Health Grant AI-067798 and a grant from the Radiation Countermeasures Center of Research Excellence (to J.P.-Y.T.).

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Disclosures
The authors have no financial conflict of interest.

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-1 β and pro-IL-18 into mature IL-1 β and IL-18, respectively. Although IL-1 β 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, 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 muramyldipeptide (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-β (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 adaptor 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; Nlrp1a, Nlrp1b, and Nlrp1c (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; Nlrp1a lacks the N-terminal pyrin domain, Nlrp1b lacks both the pyrin and NAD domains, and Nlrp1c 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 Nlrp1b 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-1 β 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-1 β 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-1 β 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 (intrinsic 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 (extrinsic 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-1 β secretion even in the absence of a stimulus in vitro (36, 52, 53). All CAPS are characterized by increased levels of IL-1 β 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.

Hoffman et al. sequenced a region within chromosome 1q44 that was previously known to contain mutations that lead to FCAS and MWS (57). This screening approach led to the discovery of four distinct mutations in exon 3 of a nine-exon gene that segregated with the disorder in three families with FCAS and one family with MWS. This gene is now referred to as NLRP3. NLRP3 is a cytoplasmic protein that is expressed in monocytes, macrophages, granulocytes, dendritic cells, nonkeratinized epithelial cells, osteoblasts, and uroepithelial cells (58, 59). NLRP3 is composed of three distinct domains: an N-terminal pyrin domain, a central NBD, and C-terminal LRRs. All 84 of the disease-associated mutations lie within exon 3, which encodes the central NBD of NLRP3 (60). The pyrin domain of NLRP3 is essential for homotypic interactions with the pyrin domain of other proteins. The NBD is thought to be involved in the oligomerization of NLRP3 to form the inflammasome complex. The LRR domain is suggested to mediate interaction with intracellular or extracellular PAMPs or DAMPs, albeit no evidence has been reported. Additional studies reported the role of NLRP3 in response to bacterial RNA, dsRNA, viral RNA, uric acid crystals, TLR ligands, bleomycin, and ATP using Nlrp3-deficient mice (12, 13, 18, 28). Recently, the Strober and Hoffman laboratories separately generated mice expressing mutations corresponding to human FCAS or MWS mutations (61, 62). Meng et al. generated a mouse expressing the R258W mutation corresponding to the human R260W substitution (62). Brydges et al. generated two lines of mice carrying the A350V and L351P mutations that correspond to the human A352V and L353P mutations downstream of a LoxP – flanked neomycin resistance cassette in reverse orientation (61). When these mice were crossed with Cre recombinase-expressing mice they would express the mutated NLRP3 protein in all tissues (CreZ), in myeloid cells only (CreL), or after exposure to tamoxifen (CreT). The mice generated by both studies developed severe cutaneous lesions associated with inflammatory cell infiltrates, recapitulating some of the urticaria-like skin lesions in MWS patients. Interestingly, both studies could recapitulate human disease by either expressing mutant NLRP3 only in myeloid cells (61) or by the generation of bone marrow chimeras with the mutant R258W protein in bone marrow cells (62).

Vitiligo

This is an autoimmune disease resulting from destruction of melanocytes causing patches of depigmented skin in patients. Vitiligo patients are at a higher risk for the development of other autoimmune diseases such as rheumatoid arthritis, diabetes, lupus, and thyroid disease. Fine scale association analyses of patients with vitiligo identified Nlrp1 variants that are 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-1 β release from murine macrophages in an NLRP3- and ASC-dependent manner (18). The importance of IL-1 β 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-1 β 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-1 β engagement to its receptor in this model.

Asbestosis 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-1 β release (22). Moreover, Nlrp3- deficient mice show decreased IL-1 β 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.

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-1 β 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-1 β pathway owing to its central role in several diseases (69). Modulation of IL-1 β function has been approached at three levels: firstly, the release of IL-1 β 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-1 β 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-1 β 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.

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 code 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-associate molecular pattern

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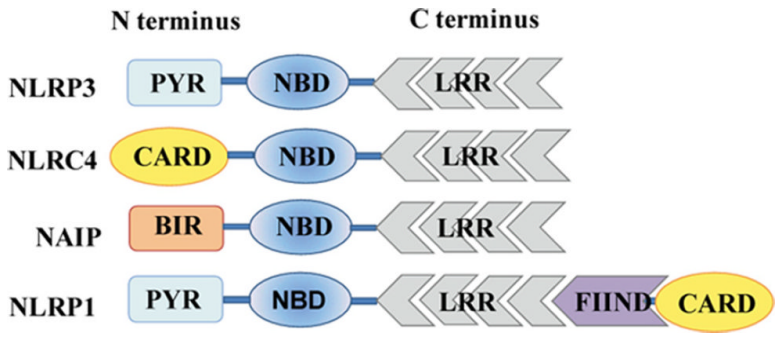

FIGURE 1.

Domain organization of inflammasome NLRs. NLR proteins have a conserved tripartite structure consisting of an N-terminal effector domain, a central NBD, and a variable number of C-terminal LRRs. Abbreviations not defined elsewhere: BIR, baculovirus inhibitor of apoptosis repeat; FIIND, function to find domain; PYR, pyrin domain.

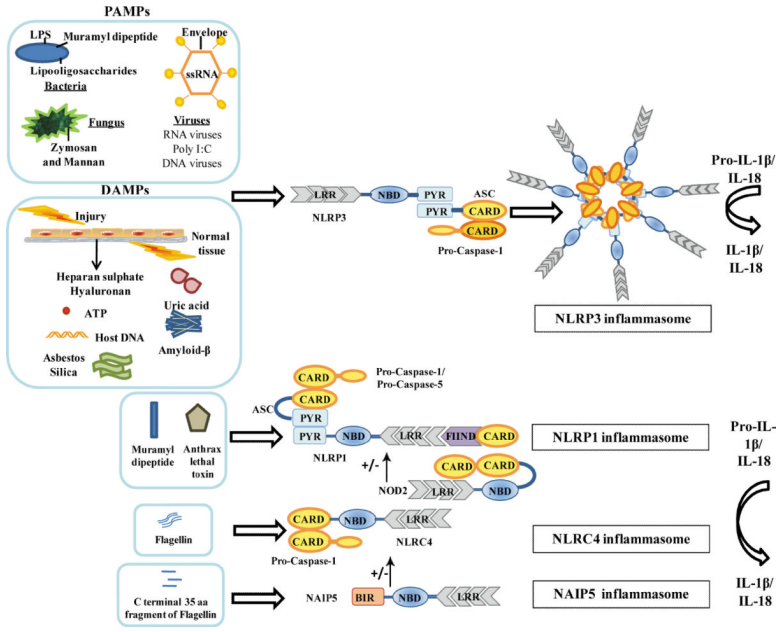

FIGURE 2.

NLR Inflammasomes. In response to PAMPs or DAMPs, the NLRs are activated to form multiprotein caspase-activating platforms referred to as inflammasomes. The NLRP1 inflammasome when activated by MDP or anthrax lethal toxin can recruit procaspase-1 via direct CARD-CARD interactions and cause its autocatalytic cleavage to mature caspase-1. The activated caspase-1 can then process IL-1 β and IL-18 from their inactive proproteins to mature active forms. The NLRP3 inflammasome is activated in response to several PAMPs and DAMPs, including but not restricted to nucleic acids (12–15), LPS (13), lipooligosaccharide (29),MDP(30), ATP(17), uric acid crystals (18), hyaluronan and heparan sulfate (31), amyloid- β (20), and asbestos and silica (21, 32). NLRP3forms a multiprotein inflammasome complex with the adaptor protein ASC and procaspase-1. Association of NLRP3 with ASC is required for recruitment of procaspase-1. The CARD domain of ASC is used to recruit procaspase-1 by CARD-CARD interactions, thus leading to the processing of procaspase-1 into active caspase-1. Caspase-1 is in turn critical for the processing and release of IL-1 β and IL-18. The NLRC4 inflammasome is a cytosolic sensor of flagellin and pathogens such as S. typhimurium, S. flexneri, and L. pneumophila (5–9, 11, 34). NLRC4 forms a homo-oligomeric inflammasome with caspase-1. The C-terminal, 35-aa fragment of flagellin is sensed by NAIP5 leading to a NAIP5-dependent cell death whereas full-length flagellin induces NAIP5-independent but NLRC4-dependent cell death and IL-1 β release (10). BIR, baculovirus inhibitor of apoptosis repeat; FIIND, function to find domain; PYR, pyrin domain.

Table I

Disease-associated mutations

NLR	Mutation(s) (Amino acid change)	Disease Association	Ref.
NLRP3	A439V, V198M, E627G, A352V	FCAS and MWS	57
	R260W, D303N, T348M, A439T, and G569R	FCAS and MWS	54
	F575S, Q306L, T436N, H358R, M662T, D303N, F309S	CINCA	58
	L264H, D303N, A374N, Y570C, F523L	CINCA	55
	L353P	FCAS	56
	T348M, E354D, L632N, R260L, R260P, D303N, D303G, F309S, T405P, T436I, Y570C	CINCA	51
NLRP1	L155H	Vitiligo	63
NLRP12	R284X, V635T	Guadeloupe variant periodic fever syndrome	66

Table II

Pharmacological inhibitors

Action	Target	Drug (Company)	Description	Ref.
Suppression of IL-1β production				
Caspase-1 inhibition	Caspase-1	Pranalcasan (Aventis/Vertex)	VX-740; VX-765	70
IL-1 β posttranslational processing	Unknown	CP424174, CP412245 (Pfizer)	Diarylsulphonyl urea	70
IL-1 β production inhibitor	Unknown	CJ14877, CJ14897 (Pfizer)	Pyridine-2-carboxylates	70
	Unknown	LL-Z1217a (Pfizer)	Terpenoid lactone	70
Suppression of IL-1 β release				
IL-1 β release inhibitors	Unknown	CP424174 (Pfizer)	Diarylsulphonyl urea	70
Neutralization of secreted IL-1 β	IL-1	Anakinra (Kineret, Amgen)	rhuIL-1Ra[a]	70
	IL-1	IL-1trap (Regeneron/Novartis)	Human IL-1R1:IgG1 protein	70
	IL-1	CDP-484 (Celltech)	PEGylated Ab	70
Inhibition of IL-1R signal transduction				
MyD88 inhibitors	MyD88	Hydrocinnamoyl-L-valyl pyrrolidine[15]	MyD88 mimic	70, 73
		ST2825 (Sigma-Tau)[b]	Peptidomimetic	74
IRAK-4 inhibitors	IRAK-4	Names unavailable[b]	Amides, imidazo [1,2-*a*] pyridine compounds	76-78

[a]Recombinant human IL-1Ra.

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