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Nlrp3: an immune sensor of cellular stress and infection

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Abstract

Innate immune cells rely on pathogen recognition receptors such as the nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR) family to mount an appropriate immune response against microbial threats. The NLR protein Nlrp3 senses microbial ligands, endogenous danger signals and crystalline substances in the cytosol to trigger the assembly of a large caspase-1-activating protein complex termed the Nlrp3 inflammasome. Autoproteolytic maturation of caspase-1 zymogens in the Nlrp3 inflammasome leads to maturation and extracellular release of the pro-inflammatory cytokines interleukin (IL)-1 β and IL-18. Gain-of-function mutations in the NOD domain of Nlrp3 are associated with auto-inflammatory disorders characterized by skin rashes and prolonged episodes of fever. In addition, decreased Nlrp3 expression was recently linked with susceptibility to Crohn's disease in humans. In this review, we discuss recent developments on the role of the Nlrp3 inflammasome in innate immunity, its activation mechanisms and the auto-inflammatory disorders associated with deregulation of Nlrp3 inflammasome activity.

Keywords

NLR; Nlrp3; inflammasome; caspase-1; interleukin-1

Introduction

The innate immune system recognizes infections and cellular damage through pattern recognition receptors (PRRs) (Kumar et al., 2009). Recognition occurs through the detection of evolutionary conserved microbial ligands that are critical for microbial function such as flagellin and components of the bacterial cell wall or viral envelope (Kumar et al., 2009). Endogenous danger signals recognized by PRRs usually represent post-translational modifications of host proteins. The detection in an atypical location of molecules normally sequestered in a defined space represents a third way for signalling cellular dysfunction. Several classes of PRRs can be distinguished, including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (RLRs) and nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs) (Kawai and Akira, 2006). TLRs and CLRs detect

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microbes on the cell surface and in endosomes, whereas RLRs and NLRs sense microbial components in intracellular compartments (Kanneganti et al., 2007b).

The discovery of NLRs as cytosolic PRRs suggested that microbes evading extracellular surveillance encounter a second line of defense in the host cytosol. Bioinformatics studies revealed the existence of 22 human NLR genes and recent gene duplications gave rise to 34 mouse NLRs (Kanneganti et al., 2007b). The NLR family member Nlrp3 (also known as Nalp3, Cryopyrin, CIAS1, PYPAF1 and CLR1.1) was originally identified as the gene mutated in patients suffering from the autosomal-dominant periodic fever syndromes familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological cutaneous and articular syndrome (CINCA) (Hoffman et al., 2001, Feldmann et al., 2002). Our knowledge on the immune roles, signalling pathways and disease-associated mutations of Nlrp3 has dramatically increased in recent years and will be discussed below.

Structure

Nlrp3 is a protein of 1016 amino acids transcribed from the gene *CIAS1*, which is located on human chromosome 1q44 and consists of 9 coding exons (Figure 1). The architecture of Nlrp3 resembles that of a subset of plant disease-resistance (R) genes involved in the hypersensitive response against virulent plant pathogens (Lamkanfi and Dixit, 2009). Nlrp3 shares the presence of a centrally located NOD motif (also referred to as NBD or NACHT domain) with all other NLR family members (Kanneganti et al., 2007b). This NOD motif is flanked at the N-terminus by a pyrin domain to allow homotypic interactions with the bipartite adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) in the Nlrp3 inflammasome (see further). At the C-terminus, Nlrp3 contains an array of 12 leucine-rich repeat (LRR) motifs believed to be involved in modulating Nlrp3 activity and in sensing microbial ligands and endogenous alarmins (Lamkanfi and Dixit, 2009).

Nlrp3 expression and activation

Nlrp3 expression is detected mainly in the cytosol of granulocytes, monocytes, dendritic cells, T and B cells, epithelial cells and osteoblasts (McCall et al., 2008, Kummer et al., 2007), suggesting an important role in the primary defense mechanisms of the body against microbial threats. Thus, most studies characterizing the role of Nlrp3 signaling in immunity have been conducted in cells of the immune system.

Given that many molecules without obvious homology can induce Nlrp3 activation, this process is widely believed to involve the generation/activation of a secondary messenger. However, the precise nature of this factor and the way it leads to Nlrp3 activation are still debated. Nevertheless, several mutually non-exclusive mechanisms have been suggested (Figure 2) including K⁺ efflux, the generation of reactive oxygen species and lysosomal destabilization (Lamkanfi and Dixit, 2009).

Biological functions

Nlrp3 was initially shown to assemble a large (700 kDa) multiprotein complex coined the “inflammasome” that was sufficient to trigger activation of the cysteine protease caspase-1 under certain *in vitro* conditions (Martinon et al., 2004). Once activated, caspase-1 processes the precursor forms of the pro-inflammatory cytokines interleukin (IL)-1 β and IL-18 to generate biologically active IL-1 β and IL-18 (Kanneganti et al., 2007b, Lamkanfi and Dixit, 2009). The bipartite adaptor protein ASC plays a central role in the interaction between Nlrp3 and caspase-1 in the inflammasome (Figure 2). As a consequence, caspase-1 activation and the secretion of IL-1 β and IL-18 are abolished in ASC-deficient macrophages (Mariathasan et

al., 2004). ASC has a specific role in caspase-1 activation because secretion of the cytokines TNF- α and IL-6 is not affected by ASC deficiency.

Studies in mice with a gene-targeted deletion in *cias1* demonstrated that Nlrp3-dependent caspase-1 activation is stimulus-dependent under physiological conditions (Kanneganti et al., 2006, Mariathasan et al., 2006, Sutterwala et al., 2006). The Nlrp3 inflammasome is responsible for caspase-1 activation in macrophages and dendritic cells infected with *Staphylococcus aureus* (Mariathasan et al., 2006), but bacterial pathogens such as *Salmonella typhimurium*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *Francisella tularensis* activate caspase-1 independently of the Nlrp3 inflammasome (Lamkanfi and Dixit, 2009, Kanneganti et al., 2007b). Notably, the Nlrp3 inflammasome plays a critical role in the host response against influenza virus (Thomas et al., 2009, Ichinohe et al., 2009, Allen et al., 2009) and the fungal pathogen *Candida albicans* (Gross et al., 2009, Hise et al., 2009, Joly et al., 2009). The Nlrp3 inflammasome also drives the inflammatory response in skin keratinocytes exposed to various skin irritants such as ultraviolet B irradiation and chemicals inducing contact hypersensitivity (Sutterwala et al., 2006, Feldmeyer et al., 2007).

Activation of the Nlrp3 inflammasome in cultured macrophages is also achieved with millimolar concentrations of ATP provided the cells are pre-exposed to TLR ligands such as lipopolysaccharide (LPS), to bacterial or viral nucleic acids, or to fungal cell wall components (Kanneganti et al., 2006, Mariathasan et al., 2006, Sutterwala et al., 2006, Kanneganti et al., 2007a, Muruve et al., 2008, Lamkanfi et al., 2009). ATP triggers opening of the non-selective cation channel of the purinergic P2X₇ receptor. The shellfish toxin maitotoxin and the bacterial ionophore nigericin can substitute for ATP in the activation of caspase-1 via Nlrp3 (Mariathasan et al., 2006).

Alzheimer's disease-associated amyloid deposits and medically-relevant crystals such as monosodium urate (MSU), calcium pyrophosphate dihydrate (CPPD), crystalline asbestos and silica all induce Nlrp3-dependent activation of caspase-1 in LPS-primed macrophages (Kanneganti et al., 2007b, Lamkanfi and Dixit, 2009). Also aluminium adjuvants activate the Nlrp3 inflammasome in LPS-pretreated macrophages, suggesting that the Nlrp3 inflammasome may mediate antibody production with alum-containing vaccines (Lamkanfi and Dixit, 2009).

Clinical implications

Polymorphisms in regulatory elements that cause decreased Nlrp3 expression and IL-1 β production were recently linked with increased susceptibility to Crohn's disease in humans (Villani et al., 2009). In addition, gain-of-function mutations in and around the Nlrp3 NACHT domain (Figure 1) cause three auto-inflammatory conditions of which the primary symptoms are urticarial skin rashes and prolonged episodes of fever in the absence of any apparent infection. These hereditary periodic-fever syndromes are MWS, FACS and CINCA (Hoffman et al., 2001, Feldmann et al., 2002). Patients suffering from one of these disorders – which are collectively referred to as the Cryopyrin/Nlrp3-associated periodic syndromes (CAPS) – can further present with arthralgia, headaches, elevated spinal fluid pressure, cognitive deficits, sensorineural hearing loss and renal amyloidosis (Hoffman et al., 2001, Feldmann et al., 2002). The CAPS-associated mutations result in constitutively active forms of Nlrp3, which drive continuous caspase-1 activation (Dowds et al., 2004). As a consequence, mononuclear cells from CAPS patients spontaneously secrete IL-1 β and IL-18, resulting in the clinical manifestations of these syndromes (Agostini et al., 2004). Analysis of mice expressing CAPS-associated Nlrp3 mutations confirmed the role of the inflammasome and showed that CAPS symptoms are partially dependent on IL-1 β (Brydges et al., 2009, Meng et al., 2009).

Until recently, the therapeutic options for patients with CAPS disorders have been limited. Thanks to substantial advances in our understanding of the molecular mechanisms underlying these disorders, the efficacy of new therapeutic strategies targeting IL-1 production and signalling are underway. The safety and efficacy of the IL-1 receptor antagonist anakinra (Kineret, Amgen), the human IL-1 β neutralizing monoclonal antibody canakinumab (Ilaris, Novartis) and rilonacept (Arcalyst, Regeneron), a fusion protein of the ligand-binding domains of human IL-1 receptor (IL-1R1) and IL-1 receptor accessory protein (IL-1RAcP) with the F_c portion of a human immunoglobulin G1 (IgG1), have already been demonstrated (Hawkins et al., 2003, Hoffman et al., 2004, Hoffman et al., 2008, Lachmann et al., 2009). Caspase-1 inhibitors represent a fourth mechanism to prevent overproduction of IL-1 β in CAPS patients. VX-765 (Vertex Pharmaceuticals) is an orally active caspase-1 inhibitor that inhibited IL-1 β secretion from LPS-stimulated peripheral blood mononuclear cells of FCAS patients (Stack et al., 2005). Thus, the Nlrp3 research field represents a prime example of how basic research on the mechanisms governing immunity pave the way for new therapies.

Abbreviations

ASC	apoptosis-associated speck-like protein containing a CARD
BIR	baculovirus IAP repeat
CAPS	Cryopyrin/Nlrp3-associated periodic syndromes
CARD	caspase recruitment domain
CLR	C-type lectin receptor
Ig	immunoglobulin
IL	interleukin
IL-1R1	IL-1 receptor 1
IL-1RAcP	IL-1 receptor accessory protein
LPS	lipopolysaccharide
LRR	leucine-rich repeat
NLR	NOD-like receptor
NOD	nucleotide-binding and oligomerization domain
PRR	pattern recognition receptor
TLR	Toll-like receptor
RLR	RIG-I-like receptor

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Figure 1.

Structural organization of Nlrp3. The human *cias1* gene encoding Nlrp3 is localized on chromosome 1q44. The positions of the neighboring genes encoding members of the olfactory receptor family 2 (*or2b11*, *or2w5*, *or2wc3*) and the zinc finger protein ZNF496 is shown to scale. Nlrp3 is produced from 9 coding exons (shown as purple bars), of which the first and last also encode the 5' and 3' untranslated regions, respectively (shown as white bars). Nlrp3 is expressed as a protein of 1036 amino acids consisting of an N-terminal pyrin domain, a central NACHT domain and 12 C-terminal LRR motifs. The positions of the CINCA- FCAS- and MWS-associated point mutations in Nlrp3 are indicated.

Figure 2.

Overview of Nlrp3 signalling pathways. Assembly and activation of caspase-1 within the cytosolic Nlrp3 inflammasome complex is induced by several stimuli including binding of ATP to the purinergic receptor P2X₇, the ionophores nigericin and maitotoxin, β-amyloid and crystalline substances. Nlrp3 has been proposed to sense potassium efflux, the generation of ROS by the NADPH oxidase complex and lysosomal membrane disruption in response to these stimuli. Once activated in the Nlrp3 inflammasome, caspase-1 cleaves its substrates IL-1β, IL-18 and caspase-7 into the biologically active forms.