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The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis

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The inflammasome is a multiprotein complex that mediates the activation of caspase-1, which promotes secretion of the proinflammatory cytokines interleukin 1β (IL-1β) and IL-18, as well as 'pyroptosis', a form of cell death induced by bacterial pathogens. Members of the Nod-like receptor family, including NLRP1, NLRP3 and NLRC4, and the adaptor ASC are critical components of the inflammasome that link microbial and endogenous 'danger' signals to caspase-1 activation. Several diseases are associated with dysregulated activation of caspase-1 and secretion of IL-1β. Thus, understanding inflammasome pathways may provide insight into disease pathogenesis that might identify potential targets for therapeutic intervention.

The eradication of invading microorganisms is essential for the survival of multicellular organisms, including humans. To ensure the removal of harmful pathogens, eukaryotic hosts have evolved an arsenal of defense mechanisms to sense and destroy invading microbes. The innate immune system is responsible for the initial task of recognizing and eradicating potentially dangerous microorganisms. Unlike the adaptive immune system, which relies on a diverse and specific repertoire of clonally selected lymphocytes, innate immune cells have broad antimicrobial functions that are activated rapidly after microbes are encountered[1]. A critical property of the innate immune system is its ability to discriminate microbes from 'self' through the recognition of conserved microbial structures called 'pathogen-associated molecular patterns' (PAMPs), such as lipopolysaccharide (LPS), peptidoglycan, flagellin and microbial nucleic acids[2]. The sensing of PAMPs is mediated by germline-encoded innate immune receptors, which include Toll-like receptors (TLRs) and Nod-like receptors (NLRs). Unlike membrane-bound TLRs, which sense PAMPs on the cell surface or in endosomes, NLRs recognize microbial molecules in the host cytosol[3,4]. After microbial recognition, both TLRs and NLRs induce the activation of host signaling pathways, which lead to innate and adaptive immune responses[1,5].

The NLR family is composed of 23 family members in humans, whereas the mouse genome contains at least 34 NLR-encoding genes[6]. Homologs of NLRs are present in plants (R genes) and animals, including phylogenetically more primitive organisms such as zebrafish and sea urchins, although they are not found in insects or worms[7,8]. Most NLRs have a tripartite structure that consists of a variable amino-terminal domain, a centrally located nucleotide-binding oligomerization domain (Nod) that mediates the formation of self oligomers, and a carboxy-terminal leucine-rich repeat that detects PAMPs. The amino-terminal domain includes several protein-interaction modules, such as a caspase-recruitment domain (CARD), pyrin domain or baculovirus inhibitor repeat, which are critical for 'downstream' signaling through the recruitment of adaptors or effector molecules[8]. Nod1 and Nod2 were the first NLRs to be identified[9]. Both Nod1 and Nod2 sense bacterial molecules produced during the synthesis, degradation and remodeling of peptidoglycan, a chief component of bacterial cell walls. Nod2 detects muramyl dipeptide (MDP), which is found in nearly all Gram-positive and Gram-negative organisms, whereas Nod1 recognizes peptidoglycan fragments containing γ-D-glutamyl-*meso*-diaminopimelic acid, from most Gram-negative bacteria and certain Gram-positive bacteria[10]. In response to their microbial agonists, Nod1 and Nod2 induce activation of the transcription factor NF-κB and mitogen-activated protein kinases, which drive the transcription of many genes encoding molecules involved in both innate and adaptive immune responses[11–13]. In contrast, several NLRs, including NLRC4 (also called IPAF), NLRP1 and NLRP3 (also called cryopyrin or NALP3), and neuronal apoptosis inhibitor proteins are involved in the assembly of a multiprotein platform called the 'inflammasome', which is responsible for caspase-1 activation[14]. In this review, we will discuss the recent studies of the inflammasome, including studies of its regulation by NLRs and its function in host defense and inflammatory disease.

The inflammasomes

Caspases are a family of intracellular cysteine proteases that cleave a limited number of substrates present after aspartic acid residues. The function of caspases has been studied extensively in apoptosis, in which they are essential in the activation and implementation of cellular demise[15]. However, several caspases, including human caspase-1, caspase-4 and

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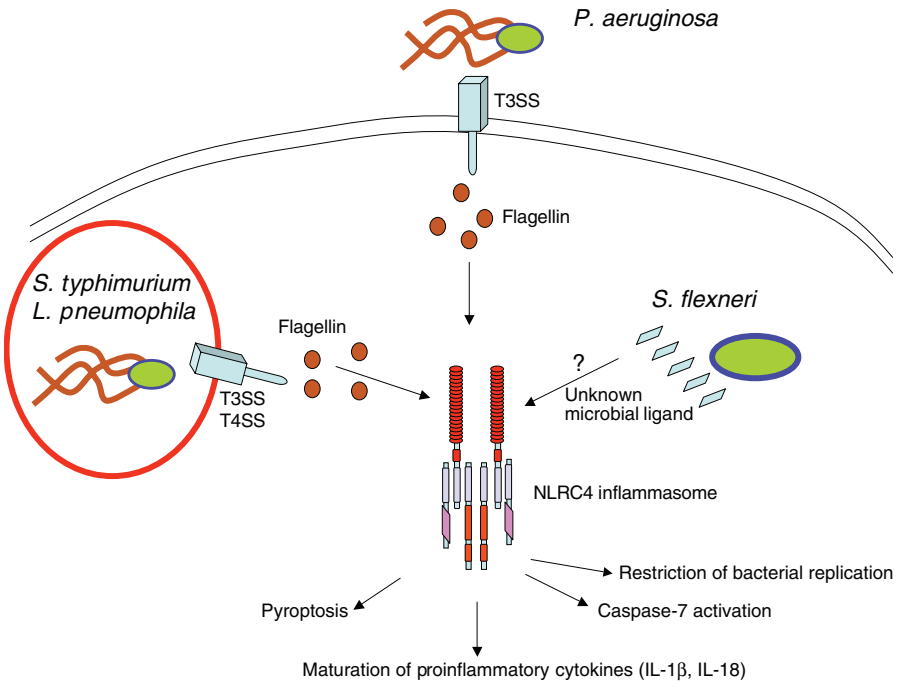

Figure 1 The NLRC4 inflammasome. Infection of macrophages with several Gram-negative bacteria, including *S. typhimurium*, *L. pneumophila* and *P. aeruginosa*, activates caspase-1 via NLRC4 and ASC. A critical step is the cytosolic delivery of flagellin through a bacterial T3SS or T4SS. *S. flexneri* activates the NLRC4 inflammasome independently of flagellin via an unknown microbial molecule. Activation of caspase-1 through NLRC4 leads to the processing and secretion of IL-1β and IL-18 as well as other activities.

caspase-5 and mouse caspase-1, caspase-11 and caspase-12, are involved in the processing and secretion of proinflammatory molecules and are often called 'proinflammatory caspases'[14,15]. Caspase-1, the first caspase to be identified, is present in the cytosol of phagocytic cells as an inactive zymogen[16,17]. After stimulation by myriad microbial and endogenous signals, the dormant procaspase-1 zymogen is self-activated by proteolytic cleavage into the enzymatically active heterodimer composed of two 10- and 20-kilodalton subunits[18]. Active caspase-1 is essential for the cleavage of pro–interleukin 1β (pro-IL-1β) and pro-IL-18 into their mature, biologically active forms. Mature IL-1β has been linked to many immune reactions, including the recruitment of inflammatory cells to the site of infection, whereas IL-18 is important for the production of interferon-γ and enhancement of the cytolytic activity of natural killer cells[19]. In addition, caspase-1 cleaves other cellular protein substrates, including pro-caspase-7, and mediates unconventional protein secretion as well as membrane repair and cell survival in response to bacterial pore-forming toxins by mechanisms that remain mostly undefined[20–22].

A critical step in caspase-1 activation is the assembly of large macromolecular complex through CARD-CARD and pyrin domain–pyrin domain protein-protein interactions to form a scaffold for the recruitment and activation of procaspase-1. This molecular platform for caspase-1 activation, which includes NLR family members and the adaptor ASC, has been called the 'inflammasome' as an analogy to the 'apoptosome' that drives caspase-9 activation by means of apoptotic protease activating factor-1 (Apaf-1) during apoptosis[23]. On the basis of the mechanism of caspase-9 activation induced by Apaf-1, it is postulated that NLRs drive caspase-1 activation through their formation of oligomers and induced proximity of caspase-1 molecules[8,23]. The inflammasome was initially described in extracts of human THP-1 monocytic cells and was found to contain NLRP1, caspase-1, caspase-5 and the adaptor proteins ASC and CARDINAL[23]. This initial inflammasome was induced by a temperature shift in buffer with a low concentration of potassium[23], but whether NLR-specific inflammasome formation occurs in response to physiological stimuli such as bacterial infection remains to be determined.

Three inflammasomes named after the NLR involved (NLRP1, NLRP3 and NLRC4) have been partially characterized. Common to these inflammasomes is the function of ASC as the adaptor protein that links these NLRs to caspase-1 (ref. 24). In humans, pyrin-containing NLRs such as NLRP3 and NLRP1 associate with caspase-1 through ASC[23,25–27]. In contrast, NLRC4 is thought to directly associate with caspase-1 through CARD-CARD interactions. However, genetic studies have shown conclusively that ASC is required for NLRC4-dependent caspase-1 activation[28–30]. These results suggest that ASC is somehow required for the interaction between NLRC4 and caspase-1 or that ASC mediates another critical step that is important for inflammasome activation. The NLRP1 inflammasome is the only caspase-1-activating platform that has been reconstituted *in vitro* with purified proteins[31]. The existence of NLRP3 and NLRC4 inflammasomes has been shown mainly by the ability of both NLRP3 and NLRC4 to activate caspase-1 in an ASC-dependent way in response to specific stimuli[28–30,32–35]. There is evidence that NLRP2 associates with procaspase-1 and promotes IL-1β production, but whether this is a true inflammasome that functions to activate caspase-1 in response to specific microbial stimuli remains to be determined[36]. Similarly, mouse Naip5 can act in concert with NLRC4 to promote caspase-1 activation in response to flagellin from *Legionella pneumophila*[37,38]. However, the mechanism by which Naip5 acts to control the NLRC4 inflammasome in response to *L. pneumophila* infection remains poorly defined.

The NLRC4 inflammasome

Several Gram-negative bacteria, including *Salmonella typhimurium*, *L. pneumophila*, *Pseudomonas aeruginosa* and *Shigella flexneri*, induce caspase-1 activation and the specific form of cell death called 'pyroptosis' by means of the NLRC4 inflammasome (**Fig. 1**). Activation of the NLRC4 inflammasome requires a functional type III secretion system (T3SS) for *S. typhimurium*[28,29], *S. flexneri*[39] and *P. aeruginosa*[40,41] or a type IV secretion system (T4SS) for *L. pneumophila*[35,37]. These bacterial secretion systems can form pores in host membranes and mediate the translocation of virulence factors (effector proteins) into the host cell cytosol[42]. Notably, *S. typhimurium*, *L. pneumophila* and *P. aeruginosa* seem to induce caspase-1 activation through the cytosolic delivery of flagellin, which triggers activation of the NLRC4 inflammasome. The activation of caspase-1 by means of the NLRC4 inflammasome can be recapitulated by the delivery of purified flagellin into the host cytosol with cationic liposomes or expression systems[28,29,38]. Thus, small amounts of flagellin that leak through the T3SS or T4SS into the host cytosol may trigger NLRC4 activation during bacterial infection. In agreement with that conclusion, the T3SS of *S. typhimurium* promotes

the translocation of flagellin into the macrophage cytosol[43]. However, it is also possible that the T3SS creates an organelle that permits the transport of flagellin into the host cytosol, perhaps by an endogenous host peptide-transport apparatus.

Caspase-1 activation by means of NLRC4 is TLR5 independent in that it proceeds unabated in TLR5-deficient macrophages[28,29,35]. Such results indicate that flagellin is recognized by at least two different sensors: TLR5 senses extracellular flagellin, whereas NLRC4 recognizes cytosolic flagellin. Evidence indicates that the NLR protein Naip5 is also important for recognition of the carboxy-terminal portion of flagellin from *L. pneumophila* and contributes to activation of the NLRC4 inflammasome[38]. It has been proposed that Naip5 physically associates with NLRC4 to form an inflammasome complex in response to *L. pneumophila*[37]. In contrast, caspase-1 activation induced by infection with *S. typhimurium* or *P. aeruginosa*, which is also triggered by cytosolic flagellin, is Naip5 independent[38]. Because Naip5 polymorphisms can influence susceptibility to *L. pneumophila* independently of caspase-1 activation[44], it will be important to investigate whether Naip5 has an additional function independent of NLRC4 in restricting the growth of *L. pneumophila* in macrophages. There is also evidence of a flagellin-independent pathway that activates the NLRC4 inflammasome after infection with certain aflagellated bacteria. For example, *S. flexneri* induces caspase-1 activation and IL-1β secretion in an NLRC4- and ASC-dependent way[39]. Similarly, *Mycobacterium tuberculosis*, another aflagellated bacterium, activates caspase-1 through NLRC4 (ref. 45). As a strategy for avoiding host defenses, *M. tuberculosis* inhibits inflammasome activation by producing Zmp1, a zinc metalloprotease, by a mechanism that remains poorly understood[45]. As many PAMPs can be recognized by the same TLR[46], it is likely that other microbial molecules distinct from flagellin can also induce NLRC4 activation. It has also been reported that *P. aeruginosa* may activate caspase-1 by an NLR4-dependent but flagellin-independent pathway[41]. Consistent with that, infection of macrophages with large numbers of *P. aeruginosa* or *S. typhimurium* mutants deficient in flagellin can induce weak but reproducible activation of caspase-1 through NLRC4 (ref. 28 and L.F., unpublished observations). These results suggest the existence of a minor alternative pathway that leads to caspase-1 activation in macrophages infected with flagellated bacteria, which can be elicited in certain experimental conditions. However, the importance of the different inflammasome pathways elicited by *P. aeruginosa* and *S. typhimurium in vivo* remains to be determined.

There is evidence that activation of the NLRC4 inflammasome functions as a host defense strategy against pathogenic infection. After infection with *S. typhymurium*, *P. aeruginosa* or *S. flexneri*, NLRC4-dependent activation of caspase-1 is accompanied by IL-1β secretion and the induction of pyroptosis. Unexpectedly, although both NLRC4 and ASC are required for caspase-1 activation and IL-1β secretion, NLRC4 is critical for the induction of pyroptosis, but ASC is not[30,40]. Thus, the cellular activities of NLRC4 and ASC can be dissociated. In agreement with that conclusion, NLRC4 can inhibit autophagy independently of ASC[39]. A possible explanation for these results is that ASC mediates cell survival thorough NF-κB activation independently of NLRC4 (ref. 47).

The NLRC4 inflammasome is also involved in restricting replication of *L. pneumophila*, an intracellular pathogen that causes Legionnaire's disease, a pneumonia that can be deadly, especially in older people. A key event in this disease pathogenesis is replication of *L. pneumophila* inside host macrophages, which requires the formation of a specialized vacuole that blocks fusion of the phagosome containing the bacterium to the lysosomes[48]. *L. pneumophila* induces NLRC4-dependent activation of caspase-1 through a T4SS and cytosolic flagellin[35,37]. The recognition of flagellin by means of Naip5 and the NLRC4 inflammasome is critical for phagosome maturation and restriction of *L. pneumophila* replication inside macrophages[35]. Thus, NLRC4-dependent caspase-1 activation promotes fusion of the *L. pneumophila*–containing phagosome to the lysosome for bacterial degradation[35]. Notably, mice deficient in NLRC4 develop a greater bacterial burden after pulmonary infection with *L. pneumophila*[35], which indicates that the inflammasome is important for disease pathogenesis. However, the mechanism by which caspase-1 activation controls phagosome maturation in response to *L. pneumophila* infection is unknown at present. One possibility is that caspase-1 targets substrates involved in phagosome maturation or *L. pneumophila* virulence factors required for lysosomal evasion.

The NLRP1 inflammasome

The human NLRP1 inflammasome was the first caspase-1-activating platform to be identified[23]. Studies using purified NLRP1, ASC and caspase-1 have shown that NLRP1 forms oligomers with caspase-1 in the presence of MDP[31]. Such studies suggest that caspase-1 is activated by a two-step mechanism whereby microbial MDP induces a conformational change in NLRP1, which in turn allows it to bind nucleotides and to form oligomers, thus creating a platform for caspase-1 activation[31]. Because there is no evidence that MDP binds NLRP1, the mechanism that triggers the formation of NLRP1 oligomers remains unclear. Notably, ASC is not essential for this process, although its addition augments NLRP1-mediated caspase-1 activation *in vitro*[31]. Consistent with the results of the human studies, ASC is not required for the caspase-1 activation mediated by NLRP1b in mouse macrophages[49], which suggests that ASC acts as an adaptor only for a subset of inflammasomes. Unlike humans, which have a single *NLRP1* gene, three *Nlrp1* paralogs, *Nlrp1a*, *Nlrp1b* and *Nlrp1c*, are present in the mouse genome. Genetic studies have identified *Nlrp1b* as the gene that encodes the molecule responsible for the pathology induced by lethal toxin, secreted by *Bacillus anthracis*[50]. Lethal toxin is a dimeric protein complex consisting of protective antigen, a pore-forming toxin, and lethal factor, a protease delivered by the protective antigen into the cytosol of infected cells[51]. Studies have shown that susceptibility to lethal toxin–induced macrophage death, a critical event in disease pathogenesis, is mediated by NLRP1b-dependent activation of caspase-1 (ref. 50). An important function for the NALP1b inflammasome in host defense is suggested by the observation that mice with the *Nlrp1b* 'susceptibility' allele are more susceptible to infection with *B. anthracis*[52]. The mechanism by which lethal factor, a metalloprotease, triggers activation of the NLRP1 inflammasome remains to be determined.

The NLRP3 inflammasome

Initial studies identified NLRP3 as a critical component of the inflammasome that is spontaneously formed in extracts of human monocytes after hypotonic lysis[23]. Follow-up studies have shown that the NLRP3 inflammasome is activated by a plethora of microbial stimuli, including LPS, MDP, bacterial RNA and the double-stranded RNA analog poly(I:C), as well as the imidazoquinoline antiviral compounds R837 and R848 (refs. 32,53–57). In contrast, activation of caspase-1 by cytosolic double-stranded DNA is not mediated by NLRP3 but involves the pyrin domain–containing protein AIM2, a member of the HIN-200 family (hematopoietic interferon-inducible nuclear protein), and ASC[58–61]. An important characteristic of the NLRP3 inflammasome is its activation by endogenous molecules such as urate crystals and ATP, bacterial pore-forming toxins and particulate matter, including asbestos and silica[14,33,34,62]. Important insight into the activation of the NLRP3 inflammasome has been provided by the observation that microbial ligands such as LPS induce robust caspase-1 activation and IL-1β secretion after brief stimulation with high concentrations of ATP[63]. Extracellular ATP activates the purinergic ATP-gated P2X7

receptor (P2X7R), which acts as a cation channel to rapidly induce complete collapse of normal ionic gradients, including the release of intracellular potassium[64]. Evidence indicates that extracellular ATP also results in the opening of a pore mediated by pannexin-1 (refs. 65–67), which induces the translocation of MDP from an intracellular vesicular compartment to the cytosol, where it induces caspase-1 activation via NLRP3 (ref. 55; **Fig. 2**). In contrast, activation of the NLRP3 inflammasome triggered by cytosolic delivery of microbial molecules with pore-forming bacterial toxins or by means of the lipophilic DOTAP delivery system is pannexin-1 independent[67]. Thus, the pannexin-1 pore activated by ATP through P2X7R may serve as a conduit for the delivery of microbial molecules to the host cytosol. Although MDP can mediate caspase-1 activation via NLRP3 (refs. 55,68), there is also evidence to support the idea of involvement of Nod2 and NLRP1 in caspase-1 activation induced by MDP and the lethal toxin of *B. anthracis*[49]. It has been shown that Nod2 interacts with NLRP1, and it has been suggested that the Nod2-NLRP1 complex mediates caspase-1 activation[49]. Further studies are needed to understand the discrepancies between these studies and the physiological relevance of the Nod2-NLRP1 complex in inflammasome activation.

Knowledge about the activation of the NLRP3 inflammasome in response to microbial infection is limited. NLRP3 has been linked to the activation of caspase-1 induced by viruses such as Sendai virus[54], Influenza virus[54] and certain adenovirus strains used as vectors for gene therapy[56], as well as by bacteria such as *L. monocytogenes* and *S. aureus*[34]. Initial reports indicated that *L. monocytogenes* induces caspase-1 activation through the NLRP3 inflammasome in a TLR2-independent way[69], which suggests a critical function for cytosolic recognition in caspase-1 activation. Accordingly *L. monocytogenes*–induced caspase-1 activation requires the bacterial pore-forming lysteriolysin O, which mediates escape of the bacterium from the vacuole to the cytosol[69,70]. Subsequent studies, however, have not confirmed an essential function for NLRP3 in the activation of caspase-1 induced by *L. monocytogenes* infection[71]. These differences in results may be explained at least in part by differences in the expression of bacterial factors that contribute to caspase-1 activation in different experimental conditions. Consistent with that possibility, studies have reported that *L. monocytogenes* induces caspase-1 activation through both the NLRC4 and NLRP3 inflammasomes[72]. Determination of the physiological relevance of these findings awaits studies comparing mice deficient in one or more inflammasomes with mice deficient in ASC or caspase-1.

'Danger' signals, crystals and the inflammasome

Another stimulus that activates the NLRP3 inflammasome is monosodium urate crystals. Uric acid has been identified as an endogenous 'danger' signal released by necrotic cells able to activate adaptive immune responses[73]. Subsequently, it has been shown that monosodium urate crystals and calcium pyrophosphate dehydrate crystals are potent activators of caspase-1 through the NLRP3 inflammasome[74]. Involvement of the inflammasome in inducing innate immune responses is suggested by the finding that mice deficient in ASC show impaired neutrophil recruitment after intraperitoneal injection of urate crystals[74]. In a similar model of uric acid–induced peritonitis, it has been shown that mice lacking IL-1R in non-myeloid cells have less recruitment of neutrophils[75]. The urate crystal–induced pathway of caspase-1 activation seems to be important for triggering gouty inflammation. Consistent with that observation, patients with gouty attacks improve clinically when treated with IL-1Ra, a molecule that inhibits IL-1R signaling[76]. However, necrotic cells do not stimulate the recruitment of neutrophils through the NLRP3 inflammasome, as caspase-1-deficient mice have unimpaired inflammatory responses to necrotic cells[77]. Further studies have shown that necrotic cells passively release IL-1α, which mediates the recruitment of neutrophils into the peritoneal cavity through IL-1R and induction of the chemokine CXCL1 (ref. 77). These results suggest IL-1R signaling is involved in mediating acute inflammation in response to necrotic cells, but the NLRP3 inflammasome is not.

The molecular mechanism whereby urate crystals induce activation of the NLRP3 inflammasome is not fully understood, but it may involve phagocytosis of the crystalline particles and the generation of reactive oxygen species[62]. Another set of stimuli that induce activation of the NLRP3 inflammasome is represented by silica and asbestos[62,78,79], aluminum hydroxide[80–83] and fibrilar amyloid-β[84], a molecule linked to the pathogenesis of Alzheimer's disease. These observations are potentially important, because inflammation is thought to contribute to the disease pathways

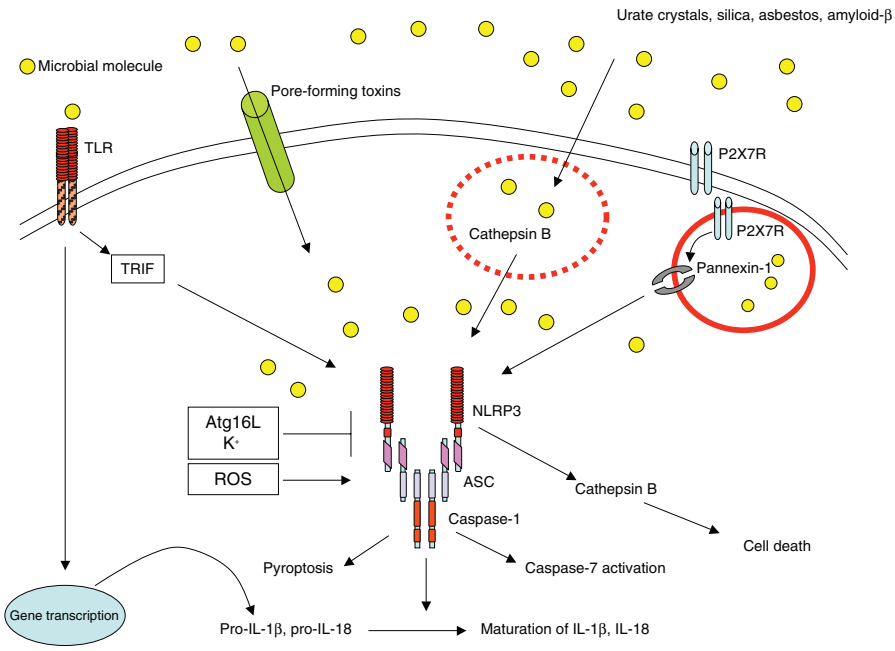

Figure 2 The NLRP3 inflammasome. The activation of caspase-1 through NLRP3 is induced by costimulation with microbial molecules such as LPS and P2X7R, pore-forming molecules or particulate matter (such as silica, asbestos, urate crystals and fibrilar amyloid-β). Stimulation of P2X7R by extracellular ATP induces the activation of a cation channel that mediates potassium efflux, an event that has been linked to inflammasome activation. In addition, P2X7R activation promotes opening of the pannexin-1 pore, which may mediate the cytosolic delivery of microbial molecules such as MDP. The mechanism of NLRP3 activation in the cytosol remains poorly understood. There is evidence that it may involve, at least in part, destabilization of the lysosomal membrane and activation of cathepsin B (in the case of silica, urate crystals and fibrillar amyloid-β). TLR stimulation also induces activation of the NLRP3 inflammasome via TRIF when the autophagy machinery is compromised (as in Atg16L or Atg7 deficiency). Reactive oxygen species (ROS) have been also linked to activation of the NLRP3 inflammasome.

elicited by these stimuli. The activation of the NALP3 inflammasome induced by some of these particulate stimuli seems to hinge in part on destabilization of the lysosomal membrane and the activation of lysosomal proteases[79] (**Fig. 2**). Notably, it has been shown that cathepsin B is important for the activation of caspase-1 induced by silica, whereas that induced by ATP is cathepsin B independent[79]. Conversely, NLRP3 has been linked to the activation of a caspase-1-independent cell-death pathway that involves cathepsin B[85]. Clearly, additional work is needed to understand the interaction between NLRP3 and cathepsin B in the regulation of innate immune responses.

Several groups have shown that aluminum hydroxide (alum), the most widely used adjuvant, activates caspase-1 and IL-1β through the NLRP3 inflammasome[86]. Notably, initial studies showed a critical function for the NLRP3 inflammasome in mediating antigen-specific adjuvant activity of immunoglobulin G1 elicited by alum[80,83]. However, other studies have not found any alteration in antigen-specific immunoglobulin G titers, including immunoglobulin G1, after intraperitoneal immunization of NLRP3-null mice[81,82]. The reason for this discrepancy is not clear, but it maybe explained at least in part by the different immunization protocols used. Collectively, these experiments suggest that NLRP3 is not essential for but can influence adaptive immune responses. As the production of antibodies induced by alum is independent of TLRs, IL-1R and IL-18R signaling[87], a principal challenge for future research will be elucidation of the pathway(s) by which alum induces adjuvant activity.

Mechanisms of inflammasome activation

The mechanism responsible for the induction of IL-1β secretion is complex, but for simplicity it can be divided into two separate steps: induction of pro-IL-1β, and activation of caspase-1. An initial layer of complexity is the fact that the pro-IL-1β amounts are usually low and upregulation of pro-IL-1β is required for IL-1β secretion. Induction of pro-IL-1β by microbial stimuli is mediated by stimulation of TLR or Nod2 and involves NF-κB activation independent of the inflammasome[30]. A second layer of complexity is the intricate mechanism of activation of the inflammasome itself. This is demonstrated by the NLRP3 inflammasome. Unlike activation of the NLRC4 inflammasome, which is triggered by cytosolic flagellin, stimulation with a microbial ligand alone is not sufficient to induce activation of the NLRP3 inflammasome. A notable exception is activation of the inflammasome by LPS in conditions in which the autophagic pathway is compromised[88] (**Fig. 2**). This TLR4-induced pathway of inflammasome activation, found in macrophages deficient in the autophagy regulators Atg16L or Atg7, is mediated by the adaptor protein TRIF but it remains poorly characterized[88]. In most cases, however, robust activation of caspase-1 requires an additional signal that can be provided by several molecules, including extracellular ATP, certain bacterial toxins and particulate agents such as silica or asbestos fibers. Activation of P2X7R by ATP induces substantial efflux of potassium, which may be critical for activation of the NLRP3 inflammasome but not the NLRC4 inflammasome[71]. Consistent with that, the bacterial toxins nigericin and maitotoxin, two potassium ionophores, trigger activation of the NLRP3 inflammasome[34]. Furthermore, incubation of cells in buffers containing high concentrations of potassium abolishes NLRP3-mediated caspase-1 activation[71,89]. However, stimulation with ATP alone, which induces potassium efflux, does not activate caspase-1, which indicates that less cytosolic potassium is not sufficient to drive activation of the NLRP3 inflammasome. Similarly, stimulation with silica, asbestos or aluminiun hydroxide does not activate the NLRP3 inflammasome unless macrophages are prestimulated with microbial ligands such as LPS[62,81,84]. These results collectively indicate that microbial molecules provide a necessary signal that acts in concert with P2X7R, bacterial toxins or crystalline structures to induce activation of the NLRP3 inflammasome. A possible unifying model is that microbial ligands are delivered via pores or membrane-damaging molecules to the cytosol, where they trigger inflammasome activation[67](**Fig. 2**). Consistent with that model, P2X7R stimulation induces the opening of an endogenous pore mediated by pannexin-1 that promotes the translocation of microbial molecules such as MDP to the cytosol[55]. In addition, particulate matter such as silica, aluminum hydroxide and fibrillar amyloid-β induces the rupture of lysosomal membranes, and this could facilitate the passage of microbial molecules into the cytosol[79,84]. Alternatively, microbial stimulation and the signals provided by P2X7R or membrane-damaging molecules may induce different signaling pathways, both of which are required for inflammasome activation. Because there is no evidence that microbial ligands bind to NLRs, it has been suggested that inflammasome activation is indirect. Such a mechanism is particularly plausible for the NLRP3 inflammasome, given that many molecules without obvious homology can induce the activation of caspase-1 via NLRP3. A possible model is that the different molecules induce a common activity in the host cytosol that mediates NLRP3 activation. Indeed, involvement of reactive oxygen species, calcium-independent phospholipase A_2 and cathepsin B is proposed for activation of the NLRP3 inflammasome[62,84,90,91]. The contribution of cathepsin B has been found to be stimulus specific in that it has been reported in caspase-1 activation induced by silica and amyloid-β but not LPS[84]. Additional work is needed to elucidate the mechanism by which different stimuli trigger activation of the NLRP3 inflammasome.

Function of the inflammasome in human disease

Given the importance of IL-1β in mediating inflammation, it is not unexpected that dysregulated inflammasome activation is linked to the pathogenesis of a variety of inflammatory diseases. In fact, mutations in genes encoding components of the inflammasome or inflammasome-associated pathways are linked to periodic fever syndromes, vitiligo and Crohn's disease. In addition, inflammasome-dependent production of IL-1β by specific disease-related stimuli seems to be involved in the pathogenesis of gout, pseudogout, asbestosis, silicosis and Alzheimer's disease.

Autosomal dominant mutations in *NLRP3* are associated with a group of rare autoinflammatory disorders, including familial cold autoinflammatory syndrome, Muckle-Wells syndrome and neonatal-onset multisystemic inflammatory disease[92]. Common features of these inherited syndromes include recurring episodes of fever, skin rash and arthropathy. The disease-associated *NLRP3* mutations result in enhanced activation of caspase-1 and secretion of IL-1β by causing constitutive activation of the NLRP3 inflammasome[93,94]. Monocytes from some patients with *NLRP3* mutations spontaneously produce IL-1β, which is enhanced by stimulation with LPS but not ATP[95]. These findings indicate that the requirement for ATP in inflammasome activation can be bypassed by expression of the disease-associated *NLRP3* mutations. Notably, treatment of these patients with an IL-1R antagonist reverses the clinical symptoms, which suggests a cause-effect relationship between IL-1β production and the development of disease[96,97]. As discussed above, NLRP3 detects monosodium urate crystals and therefore may be critical in the development of gout, which is triggered by the deposition of uric acid crystals in the joints. In a mouse model of monosodium urate crystal–induced inflammation with induction of peritonitis, IL-1β blockade results in impaired neutrophil influx in ASC-deficient mice[74]. Indeed, treatment of gouty patients who are refractory to conventional anti-inflammatory drugs with IL-1 receptor antagonist induces an effective response[98].

Vitiligo is an autoimmune disorder that involves the destruction of melanocytes, which results in patches of depigmented skin. Patients with vitiligo have a greater frequency of other autoimmune disorders, such as autoimmune thyroid disease, rheumatoid arthritis, diabetes and lupus. NLRP1 variants are associated with susceptibility to vitiligo[99], although the mechanism by which NLRP1 promotes skin depigmentation is unclear.

Deregulated activation of the inflammasome has been associated with Crohn's disease through genetic variation of *ATG16L1*, which encodes a critical component of the autophagic machinery[88]. After being stimulated with LPS, macrophages deficient in Atg16L1 show enhanced TRIF-dependent activation of caspase-1 and secretion of mature IL-1β and IL-18. The enhanced production of IL-1β noted in Atg16L1-deficient macrophages requires both potassium efflux and the generation of reactive oxygen species, which suggests that autophagy negatively regulates the NLRP3 inflammasome[88]. However, the mechanism by which the autophagy machinery regulates the inflammasome remains unclear. Furthermore, Atg16L1 also regulates the function of Paneth cells[100], a specialized type of epithelial cells located in the crypts of the small intestine that produce antimicrobial peptides. Additional studies are needed to determine which function of Atg16L1 is critically involved in the pathogenesis of Crohn's disease.

Conclusions and perspectives

The idea of the inflammasome was introduced less than 10 years ago. Since then, there have been tremendous advances in understanding of the activation, regulation and function of the inflammasome. A critical function for NLRs in inflammasome activation is now well established. Furthermore, microbial molecules that trigger the activation of specific NLR inflammasomes have been identified. A chief highlight has been the discovery of inherited diseases caused by constitutive activation of the NLRP3 inflammasome, which has rapidly led to specific and effective therapy for these disorders. However, many hurdles still must be overcome for full understanding of the inflammasome, including identification of the molecular mechanisms that control the assembly and activation of endogenous inflammasomes, the identification and confirmation of additional protein substrates of caspase-1 and better knowledge about the function of inflammasomes *in vivo*. In addition, the function of inflammasomes in several diseases to which caspase-1 activation has been linked, including gout and Alzheimer's disease, remains to be further clarified. Ultimately, the successful development of therapeutics will benefit from greater understanding of the mechanisms that govern inflammasome-associated signaling pathways.

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