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The Inflammasome: A Caspase-1 Activation Platform Regulating Immune Responses and Disease Pathogenesis

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Abstract

The inflammasome is a multi-protein complex that mediates activation of caspase-1 which promotes the secretion of the proinflammatory cytokines IL-1 β 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 by linking microbial and endogenous danger signals to caspase-1 activation. Several diseases are associated with the dysregulated activation of caspase-1 and IL-1 β secretion. Thus, understanding of inflammasome pathways may provide insights into disease pathogenesis that might serve as 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 that relies on a diverse and specific repertoire of clonally selected lymphocytes, innate immune cells display broad anti-microbial functions which are activated rapidly upon encountering microbes¹. 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 lipopolysaccharides (LPS), peptidoglycan (PGN), flagellin and microbial nucleic acids². The sensing of PAMPs is mediated by germ-line encoded innate immune receptors which include Toll-like receptors (TLRs) and Nod-like receptors (NLRs). Unlike membrane-bound TLRs that sense PAMPs on the cell surface or within endosomes, NLRs recognize microbial molecules in the host cytosol³. Upon microbial recognition, both TLRs and NLRs induce the activation of host signaling pathways which lead to innate and adaptive immune responses¹ 5.

The NLR family is composed of 23 family members in humans whereas the mouse genome contains at least 34 NLR genes⁶. Homologs of NLRs are present in plants (R genes) and animals including phylogenetically more primitive organisms such as the zebrafish and the sea urchin, although they are not found in insects or worms⁷ 8. Most NLRs exhibit a tripartite structure consisting of a variable N-terminal domain, a centrally located nucleotide-binding oligomerization (NOD) domain that mediates self-oligomerization and a C-terminal leucine-rich repeat that detects PAMPs. The N-terminal domain includes several protein interaction modules such as caspase recruitment domain (CARD), pyrin domain (PYD), or baculovirus inhibitor repeat (BIR) which are critical for downstream signaling through the recruitment of

adaptors or effector molecules⁸. NOD1 and NOD2 were the first identified NLRs⁹. Both NOD1 and NOD2 sense bacterial molecules produced during the synthesis, degradation, and remodeling of PGN, a major 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 γ -D-glutamyl-*meso*-diaminopimelic acid (iE-DAP)-containing PGN fragments from most Gram-negative bacteria and certain Gram-positive bacteria¹⁰. In response to their microbial agonists, NOD1 and NOD2 induce the activation of NF- κ B and MAPKs which drive the transcription of numerous genes involved in both innate and adaptive immune responses^{11–13}. In contrast, several NLRs including NLRC4 (also called IPAF), NLRP1, NLRP3 (also called Cryopyrin or NALP3) and NAIPs are involved in the assembly of a multi-protein platform, named the inflammasome, that is responsible for the activation of caspase-1¹⁴. In this review, we will discuss recent studies on the inflammasome including its regulation by NLRs and its role in host defense and inflammatory disease.

The inflammasomes

Caspases are a family of intracellular cysteine proteases that cleave a limited number of substrates after aspartic acid residues. The function of caspases has been extensively studied in apoptosis where they play an essential role in the activation and implementation of cellular demise¹⁵. However, several caspases including the human caspase-1, -4, and -5 and the murine caspase-1, -11 and -12 are involved in the processing and secretion of pro-inflammatory molecules and are often referred to as “pro-inflammatory 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}. Upon stimulation by a myriad of 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-kDa subunits¹⁸. Active caspase-1 is essential for the cleavage of pro-IL-1 β and pro-IL-18 into their mature, biologically active forms. Mature IL-1 β is implicated in multiple immune reactions including the recruitment of inflammatory cells to the sites of infection; whereas IL-18 is important for the production of interferon- γ and enhancement of the cytolytic activity of NK cells¹⁹. 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 through mechanisms that remain largely undefined^{20–22}.

A critical step in caspase-1 activation is the assembly of large macromolecular complex through CARD-CARD and PYD-PYD protein-protein interactions to form a scaffold for procaspase-1 recruitment and activation. This molecular platform for caspase-1 activation which includes NLR family members and the adaptor ASC has been termed the “inflammasome” as an analogy to the “apoptosome” which drives caspase-9 activation via Apaf-1 during apoptosis²³. Based on the mechanism of caspase-9 activation induced by Apaf-1, it is postulated that NLRs drive caspase-1 activation through their oligomerization and induced proximity of caspase-1 molecules^{8,23}. The inflammasome was initially described using extracts from human THP-1 monocytic cells and found to contain NLRP1, caspase-1, caspase-5, and the adaptor proteins ASC and CARDINAL²³. This initial inflammasome was induced by temperature shift using low K⁺ buffer²³, 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, that is, NLRP1, NLRP3, and NLRC4 have been partially characterized. Common to these inflammasomes is the role of ASC as the adaptor protein that links these NLRs to caspase-1²⁴. In humans, Pyrin-containing NLRs such as NLRP3 and NLRP1 associate with caspase-1 through the adaptor molecule ASC^{23,25–27}. In contrast, NLRC4 is thought to directly associate with caspase-1 via CARD-CARD interactions. However, genetic studies show 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 which is important for inflammasome activation. The NLRP1 inflammasome is the only caspase-1-activating platform that has been reconstituted *in vitro* with purified proteins³¹. The existence of the NLRP3- and NLRC4-inflammasome has been largely defined by the ability of both NLRP3 and NLRC4 to activate caspase-1 in an ASC-dependent manner in response to specific stimuli^{28–30, 32–35}. There is evidence that NLRP2 associates with procaspase-1 and promotes IL-1 β production, but whether this is a bonafide inflammasome that functions to activate caspase-1 in response to specific microbial stimuli remains to be determined³⁶. Similarly, mouse Naip5 can act in concert with Nlrc4 to promote the activation of caspase-1 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.

NLRC4 inflammasome

Several Gram-negative bacteria, including *Salmonella typhimurium*, *L. pneumophila*, *Pseudomonas aeruginosa*, and *Shigella flexneri* induce caspase-1 activation and pyroptosis via the NLRC4 inflammasome (Fig. 1). The 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 type IV secretion system (T4SS) in the case of *L. pneumophila*^{35, 37}. These bacterial secretion systems can form pores in host membranes and mediate translocation of virulence factors (effectors proteins) into the host cell cytosol⁴². Remarkably, *S. typhimurium*, *L. pneumophila* and *P. aeruginosa* appear to induce the activation of caspase-1 through the cytosolic delivery of flagellin which triggers the activation of the NLRC4 inflammasome. The activation of caspase-1 through 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 leaked via the T3SS or T4SS into the host cytosol may trigger NLRC4 activation during bacterial infection. In agreement with this observation, the T3SS of *S. typhimurium* promotes the translocation of flagellin into the macrophage cytosol⁴³. However, it is also possible that the T3SS creates an organelle that permits flagellin transport into the host cytosol, perhaps by an endogenous host peptide transport apparatus.

Caspase-1 activation via NLRC4 is TLR5 independent, in that it proceeds unabated in TLR5 deficient macrophages^{28, 29, 35}. These results indicate that flagellin is recognized by at least two different sensors; TLR5 senses extracellular flagellin whereas NLRC4 recognizes cytosolic flagellin. Recent evidence indicates that the NLR protein Naip5 is also important for the recognition of the C-terminal portion of flagellin from *L. pneumophila* and contributes to the activation of the NLRC4 inflammasome³⁸. It was 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 *S. typhimurium* and *P. aeruginosa* infection which is also triggered by cytosolic flagellin is Naip5 independent³⁸. Because Naip5 polymorphisms can influence the susceptibility to *L. pneumophila* independently of caspase-1 activation⁴⁴, it will be important to investigate whether Naip5 has an additional role independently of NLRC4 in restricting the growth of *L. pneumophila* in macrophages. There is also evidence for 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-1 β secretion in a NLRC4 and ASC-dependent manner³⁹. Similarly, *Mycobacterium tuberculosis*, another aflagellated bacterium, activates caspase-1 via NLRC4⁴⁵. As a strategy to avoid host defenses, *M. tuberculosis* inhibits inflammasome activation by producing *zmp1*, a Zn²⁺ metalloprotease, by a mechanism that remains poorly understood⁴⁵. As multiple PAMPs can be recognized by

the same TLR⁴⁶ it is likely that other microbial molecules distinct from flagellin can also induce the activation of NLRC4.

Recently, it was also reported that *P. aeruginosa* may activate caspase-1 through a NLR4-dependent, but flagellin-independent pathway⁴¹. Consistently, infection of macrophages with high number of *P. aeruginosa* or *S. typhimurium* mutants deficient in flagellin can induce weak, but reproducible activation of caspase-1 through NLRC4 (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 under 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 infections. In the case of *S. typhimurium*, *P. aeruginosa*, and *S. flexneri*, NLRC4-dependent activation of caspase-1 is accompanied by the secretion of IL-1 β and the induction of a specific form of cell death called pyroptosis. Surprisingly, while both NLRC4 and the adaptor molecule ASC are required for caspase-1 activation and IL-1 β secretion, NLRC4, but not ASC, is critical for the induction of pyroptosis^{30, 40}. Thus, the cellular activities of NLRC4 and ASC can be dissociated. In agreement with this, NLRC4 can inhibit autophagy independently of ASC³⁹. A possible explanation for these results is that ASC mediates cell survival through NF- κ B activation independently of NLRC4⁴⁷.

The NLRC4 inflammasome is also involved in restricting the replication of *L. pneumophila*, an intracellular pathogen that causes Legionnaire's disease, a pneumonia that can be deadly especially in older individuals. A key event in disease pathogenesis is the replication of *L. pneumophila* inside host macrophages which requires the formation of a specialized vacuole that blocks the fusion of the phagosome containing the bacterium to the lysosomes⁴⁸. *L. pneumophila* induces NLRC4-dependent caspase-1 activation via a T4SS and cytosolic flagellin^{35, 37}. The recognition of flagellin through the Naip5 and NLRC4 inflammasome is critical for phagosome maturation and restriction of *L. pneumophila* replication inside macrophages³⁵. Thus, NLRC4-dependent caspase-1 activation promotes the fusion of the *L. pneumophila*-containing phagosome to the lysosome for bacterial degradation³⁵. Importantly, mice deficient in *Nlrc4* exhibit an increased bacterial burden after pulmonary infection with *L. pneumophila*³⁵, indicating 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 presently unknown. A possibility is that caspase-1 targets substrates involved in phagosome maturation or *L. pneumophila* virulence factors required for lysosomal evasion.

NLRP1 inflammasome

The human NLRP1 inflammasome was the first caspase-1-activating platform to be identified²³. Studies with purified NLRP1, ASC and caspase-1 revealed that NLRP1 oligomerizes with caspase-1 in the presence of MDP³¹. These studies suggested that caspase-1 is activated via a two-step mechanism, whereby microbial MDP induces a conformational change in NLRP1, which in turn allows it to bind nucleotide and to oligomerize, thus creating a platform for caspase-1 activation³¹. Because there is no evidence that MDP binds NLRP1, the mechanism that triggers NLRP1 oligomerization remains unclear. Remarkably, the adaptor molecule ASC was not essential, although its addition augmented NLRP1-mediated caspase-1 activation *in vitro*³¹. Consistent with the human studies, ASC is not required for the activation of caspase-1 mediated by *Nlrp1b* in mouse macrophages⁴⁹, suggesting that ASC acts as an adaptor only for a subset of inflammasomes. Unlike humans that possess a single *NLRP1* gene,

three *Nlrp1* paralogs, named *Nlrp1a*, *-b* and *-c*, are present in the mouse genome. Genetic studies identified *Nlrp1b* as the gene responsible for the pathology induced by lethal toxin (LT), a toxin secreted by *Bacillus anthracis*⁵⁰. LT is a dimeric protein complex consisting of Protective Antigen (PA), a pore forming toxin, and Lethal factor, a protease that is delivered by the PA into the cytosol of infected cells⁵¹. Subsequent studies revealed that susceptibility to LT-induced macrophage cell death, a critical event in disease pathogenesis, is mediated by Nlrp1b-dependent activation of caspase-150. An important role for the Nalp1b inflammasome in host defense is suggested by the observation that mice harboring the Nlrp1b “susceptible” allele are more susceptible to infection with *Bacillus anthracis*⁵². The mechanism by which the Lethal factor, a metalloprotease, triggers Nlrp1 inflammasome activation remains to be determined.

NLRP3 inflammasome

Initial studies identified NLRP3 as a critical component of the inflammasome that is spontaneously formed in cell extracts of human monocytes after hypotonic lysis²³. Follow-up studies revealed that the NLRP3 inflammasome is activated by a plethora of microbial stimuli including LPS, MDP, bacterial RNA, the double-stranded RNA analog poly I:C as well as the imidazoquinoline antiviral compounds R837 and R848^{32, 53–57}. In contrast, activation of caspase-1 by cytosolic double-stranded DNA is not mediated via NLRP3, but it involves the Pyrin domain-containing AIM2, a member of the hematopoietic interferon-inducible nuclear protein HIN-200 family, and ASC⁵⁸. 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, 59}. Significant insight into the activation of the NLRP3 inflammasome comes from the observation that microbial ligands such as LPS induce robust caspase-1 activation and IL-1 β secretion after brief stimulation with high concentrations of ATP⁶⁰. Extracellular ATP activates the purinergic ATP-gated P2X7 receptor (P2X7R), which acts as a cation channel to rapidly induce a complete collapse of normal ionic gradients, including the release of intracellular K⁺⁶¹. Recent evidence indicates that extracellular ATP also results in the opening of a pore mediated by pannexin-1^{62–64} that induces the translocation of MDP from an intracellular vesicular compartment into the cytosol, where it induces the activation of caspase-1 via NLRP3⁵⁵ (Fig. 2). In contrast, the activation of the NLRP3 inflammasome triggered by cytosolic delivery of microbial molecules with pore-forming bacterial toxins or through the lipophilic DOTAP delivery system is pannexin-1 independent⁶⁴. Thus, the pannexin-1 pore activated by ATP via the P2X7R may serve as a conduit to deliver microbial molecules to the host cytosol. Although MDP can mediate caspase-1 activation via NLRP3^{55, 65}, there is also evidence supporting a role for NOD2 and NLRP1 in caspase-1 activation induced by MDP and the LT of *B. anthracis*⁴⁹. It was shown that NOD2 interacts with NLRP1 and suggested that the NOD2-NLRP1 complex mediates the activation of caspase-1⁴⁹. Further studies are needed to understand the discrepancies between these studies and the physiological relevance of the NOD2-NLRP1 complex in inflammasome activation.

Our knowledge about the activation of the NLRP3 inflammasome in response to microbial infection is limited. NLRP3 has been implicated in the activation of caspase-1 induced by viruses such as Sendai virus⁵⁴, Influenza virus⁵⁴, and certain Adenovirus strains used as vectors for gene therapy⁵⁶ as well as bacteria such as *L. monocytogenes* and *S. aureus*³⁴. Initial reports indicated that *L. monocytogenes* induces caspase-1 activation through NLRP3 inflammasome in a TLR2-independent manner⁶⁶, suggesting a critical role of cytosolic recognition for caspase-1 activation. Accordingly *L. monocytogenes*-induced caspase-1 activation requires the bacterial pore-forming lysteriolysin O (LLO) that mediates the escape of the bacterium from the vacuole into the cytosol^{66, 67}. Subsequent studies, however, could not confirm the essential role of NLRP3 in the activation of caspase-1 induced by *Listeria* infection⁶⁸. The difference

in results may be explained, at least in part, by differential expression of bacterial factors that contribute to caspase-1 activation under different experimental conditions. Consistent with this possibility, recent studies reported that *L. monocytogenes* induces the activation of caspase-1 via both the NLRC4 and NLRP3 inflammasomes⁶⁹. The physiological relevance of these findings awaits studies that compare 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 was identified by Rock and coworkers as an endogenous danger signal released by necrotic cells capable of activating adaptive immune responses⁷⁰. Subsequently, it was shown that monosodium urate crystals and calcium pyrophosphate dehydrate crystals are potent activators of caspase-1 via the NLRP3 inflammasome⁷¹. A role for the inflammasome in inducing innate immune response was suggested by the finding that mice deficient in ASC exhibit impaired neutrophil recruitment after intraperitoneal injection of urate crystals⁷¹. Using a similar model of uric acid-induced peritonitis, it was shown that mice lacking the IL-1R in non-myeloid cells have decreased recruitment of neutrophils⁷². The urate crystal-induced pathway of caspase-1 activation appears to be important for triggering gouty inflammation. Consistently, patients with gouty attacks improved clinically when treated with IL-1Ra, a molecule that inhibits IL-1R signaling⁷³. However, necrotic cells do not stimulate the recruitment of neutrophils via the NLRP3 inflammasome because caspase-1 deficient mice display unimpaired inflammatory responses to necrotic cells⁷⁴. Further studies revealed that necrotic cells passively release IL-1 α which mediates neutrophil recruitment in the peritoneal cavity via IL-1R and induction of CXCL1⁷⁴. These results suggest IL-1R signaling, but not the NLRP3 inflammasome, is involved in mediating acute inflammation in response to necrotic cells.

The molecular mechanism whereby urate crystals induces the 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 (ROS)⁵⁹. Another set of stimuli that induce the activation of the NLRP3 inflammasome is represented by silica and asbestos⁵⁹, 75, 76, aluminium hydroxide^{77–80} and fibrillar amyloid- β ⁸¹, a molecule implicated in the pathogenesis of Alzheimer disease. These observations are potentially important because inflammation is thought to contribute to the disease pathways elicited by these stimuli. The activation of the NALP3 inflammasome induced by some of these particulate stimuli appears to hinge, in part, on the destabilization of the lysosomal membrane and the activation of lysosomal proteases⁷⁶ (Fig. 2). Intriguingly, it was shown that cathepsin B is important for the activation of caspase-1 induced by silica, while that induced by ATP was cathepsin B independent⁷⁶. Conversely, NLRP3 has been implicated in the activation of a caspase-1-independent cell death pathway that involves cathepsin B⁸². Clearly, additional work is needed to understand the interplay between NLRP3 and cathepsin B in the regulation of innate immune responses.

Adjuvants, adaptive immunity and the inflammasome

Several groups have shown that aluminium hydroxide (Alum), the most widely used adjuvant, activates caspase-1 and IL-1 β via the NLRP3⁸³. Importantly, initial studies revealed a critical role for the NLRP3 inflammasome in mediating antigen-specific IgG1 adjuvant activity elicited by Alum^{77, 80}. However, other authors did not observe alteration in antigen-specific IgG titers including IgG1 following intraperitoneal immunization in Nlrp3-null mice^{78, 79}. 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, but it can influence adaptive immune responses. As the production of antibodies

induced by Alum is independent of TLRs, IL-1R, and IL-18R signaling⁸⁴, a major challenge for future research will be the elucidation of the pathway(s) by which Alum induces adjuvant activity.

Mechanisms of inflammasome activation

The mechanism responsible for the induction of IL-1 β secretion is complex, but for simplicity it can be divided into two separate steps, namely induction of pro-IL-1 β and activation of caspase-1. A first layer of complexity is represented by the fact that the proIL-1 β amounts are usually low, and up-regulation of proIL-1 β is required for IL-1 β secretion. Induction of pro-IL-1 β by microbial stimuli is mediated via TLR or NOD2 stimulation and involves NF- κ B activation independently of the inflammasome³⁰. A second layer of complexity is represented by the intricate mechanism of activation of the inflammasome itself. This is clearly demonstrated in the case of the NLRP3 inflammasome. Unlike the NLRC4 inflammasome that is triggered by cytosolic flagellin, stimulation with a microbial ligand alone is not sufficient to induce the activation of the NLRP3 inflammasome. A notable exception is the activation of the inflammasome by LPS under conditions in which the autophagic pathway is compromised⁸⁵ (Fig. 2). This TLR4-induced pathway of inflammasome activation, revealed in macrophages deficient in the autophagy regulators Atg16L or Atg7, is mediated via TRIF but it remains poorly characterized⁸⁵. In most cases, however, robust activation of caspase-1 requires an additional signal which can be provided by several molecules including extracellular ATP, certain bacterial toxins, and particulate agents such as silica or asbestos fibers. Activation of the P2X7R by ATP induces marked K⁺ efflux which may be critical for NLRP3, but not NLRC4, inflammasome activation⁶⁸. Consistently, the bacterial toxins nigericin and maitotoxin, two K⁺ ionophores, trigger the activation of the NLRP3 inflammasome³⁴. Furthermore, incubation of cells in buffers containing high concentrations of K⁺ abolishes NLRP3-mediated caspase-1 activation^{68, 86}. However, stimulation with ATP alone, which induces K⁺ efflux, does not activate caspase-1, arguing that reduction of cytosolic K⁺ is not sufficient to drive the activation of the NLRP3 inflammasome. Similarly, stimulation with silica, asbestos, and aluminium hydroxide does not activate the NLRP3 inflammasome unless macrophages are pre-stimulated with microbial ligands such as LPS^{59, 78, 81}. Collectively, these results indicate that microbial molecules provide a necessary signal that act in concert with the P2X7R, bacterial toxins or crystalline structures to induce the 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 activation⁶⁴ (Fig. 2). Consistent with this model, P2X7R stimulation induces the opening of an endogenous pore mediated by pannexin-1 that promotes the translocation of microbial molecules MDP to the cytosol⁵⁵. In addition, particulate matter such as silica, aluminium hydroxide and fibrillar amyloid- β induces the rupture of lysosomal membranes and this could mediate the passage of microbial molecules into the cytosol^{76, 81}. Alternatively, microbial stimulation and the signals provided through the 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 NLR proteins, it has been suggested that inflammasome activation is indirect. Such a mechanism is particularly attractive for the NLRP3 inflammasome given that multiple molecules without obvious homology can induce caspase-1 activation via NLRP3. A possible model is that the different molecules induce a common activity in the host cytosol which mediates NLRP3 activation. Indeed, a role for ROS, calcium-independent phospholipase A2, and cathepsin B is proposed for the activation of the NLRP3 inflammasome^{59, 81, 87, 88}. The contribution of cathepsin B was found to be stimulus-specific in that it was observed in caspase-1 activation induced by silica and β -amyloid but not LPS⁸¹. Additional work is needed to understand the mechanism by which different stimuli trigger the activation of the NLRP3 inflammasome.

Role of the inflammasome in human disease

Given the importance of IL-1 β in mediating inflammation, it is not surprising that dysregulated inflammasome activation is implicated in the pathogenesis of a variety of inflammatory diseases. In fact, mutations in the inflammasome or inflammasome-associated pathways is linked to periodic fever syndromes, vitiligo, or Crohn's disease. In addition, inflammasome-dependent IL-1 β production by specific disease-related stimuli appears to play a role 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 disease⁸⁹. The common features of these inherited syndromes include recurring episodes of fever, skin rash and arthropathy. The disease-associated NLRP3 mutations result in enhanced caspase-1 activation and IL-1 β secretion by causing constitutive activation of the NLRP3 inflammasome^{90, 91}. Monocytes from some patients harboring NLRP3 mutations spontaneously produce IL-1 β which is enhanced by LPS, but not by ATP stimulation⁹². These findings indicate that the requirement of ATP for inflammasome activation can be bypassed by expression of the disease-associated NLRP3 mutations. Remarkably, treatment of these patients with IL-1 receptor antagonist reverses clinical symptoms suggesting a cause-effect relationship between IL-1 β production and the development of disease^{93, 94}. As we discussed above, NLRP3 detects monosodium urate crystals and therefore, may play a critical role 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-1 β blockade resulted in impaired neutrophil influx in ASC deficient mice⁷¹. Indeed, treatment of gouty patients who were refractory to conventional anti-inflammatory drugs with IL-1 receptor antagonist induced an effective response⁹⁵.

Vitiligo is an autoimmune disorder that involves the destruction of melanocytes resulting in patches of depigmented skin. Patients with vitiligo have an increased frequency of other autoimmune disorders, such as autoimmune thyroid disease, rheumatoid arthritis, diabetes, and lupus. NLRP1 variants are associated with vitiligo susceptibility⁹⁶, although the mechanism by which NLRP1 promotes skin depigmentation is unclear.

The deregulated activation of the inflammasome has been associated to Crohn's disease via genetic variation of Atg16L1, a gene encoding a critical component of the autophagic machinery⁸⁵. Upon stimulation with LPS, macrophages deficient in Atg16L1 show enhanced TRIF-dependent activation of caspase-1 and secretion of mature IL-1 β and IL-18. The enhanced production of IL-1 β observed in Atg16L1-deficient macrophages required both K⁺ efflux and the generation of ROS, suggesting that autophagy negatively regulates the NLRP3 inflammasome⁸⁵. However, the mechanism by which the autophagy machinery regulates the inflammasome remains unclear. Furthermore, Atg16L1 also regulates the function of Paneth cells⁹⁷, a specialized type of epithelial cells located in the crypts of the small intestine that produce anti-microbial 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 concept of the inflammasome was introduced less than 10 years ago. Since then there has been tremendous advances in our understanding of the activation, regulation and function of the inflammasome. A critical role for NLRs in inflammasome activation is now well established. Furthermore, microbial molecules triggering the activation of specific NLR inflammasomes have been identified. A major highlight has been the discovery of inherited diseases caused by constitutive activation of the NLRP3 inflammasome which have rapidly

led to specific and effective therapy for these disorders. However, major hurdles in our understanding of the inflammasome remain including the molecular mechanisms controlling the assembly and activation of the endogenous inflammasomes, identification and validation of novel protein substrates of caspase-1, and better knowledge about the function of the inflammasomes *in vivo*. In addition, the role of the inflammasome in several diseases in which caspase-1 activation has been implicated including gout and Alzheimer's disease remains to be further clarified. Ultimately, successful development of therapeutics will be benefited from greater understanding of the mechanisms that govern inflammasome-associated signaling pathways.

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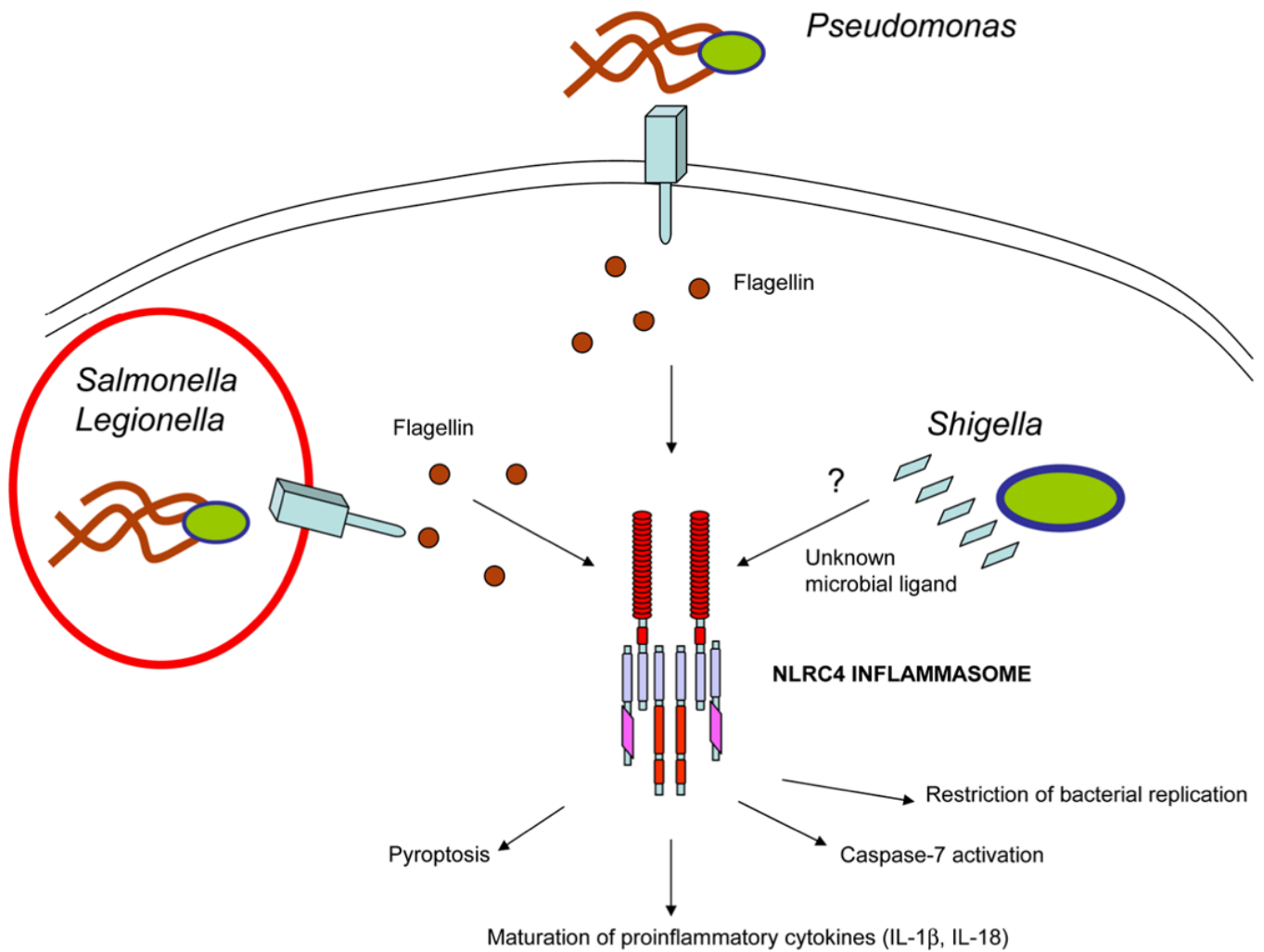


Figure 1. The NLRC4 inflammasome. Infection of macrophages with several Gram-negative bacteria including *Salmonella*, *Legionella* and *Pseudomonas* activates caspase-1 through NLRC4 and ASC. A critical step is the cytosolic delivery of flagellin via bacterial T3SS or T4SS. *Shigella* activates the NLRC4 inflammasome independent of flagellin through an unknown microbial molecule. Activation of caspase-1 via NLRC4 leads to processing and secretion of IL-1 β and IL-18 as well as other activities.

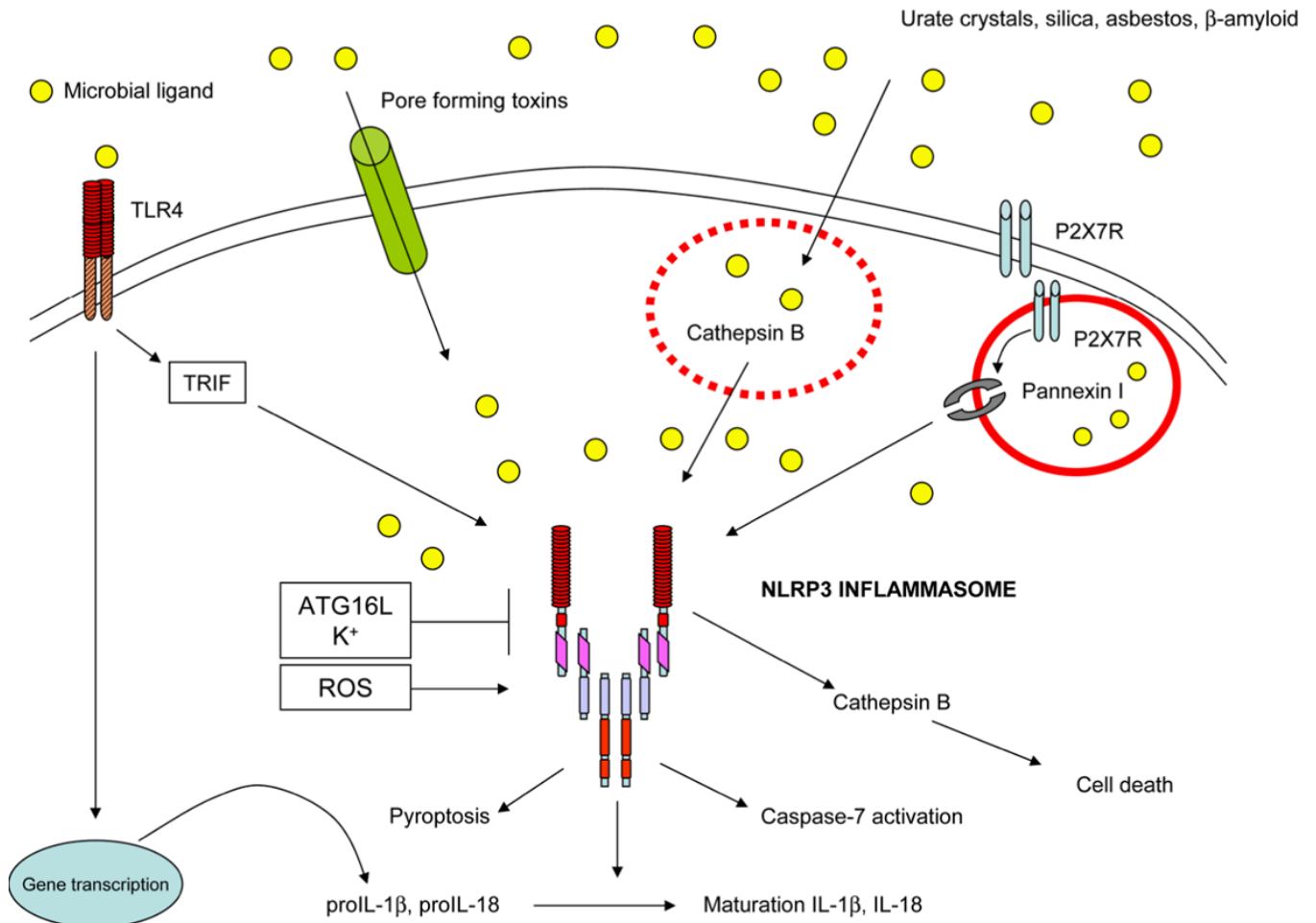


Figure 2.

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