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Intracellular sensing of microbes and danger signals by the inflammasomes

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Summary

The cells of the innate immune system mobilize a coordinated immune response towards invading microbes and after disturbances in tissue homeostasis. These immune responses typically lead to infection control and tissue repair. Exaggerated or uncontrolled immune responses, however, can also induce acute or chronic inflammatory pathologies that are characteristic for many common diseases such as sepsis, arthritis, atherosclerosis or Alzheimer's disease. In recent years the concerted efforts of many scientists have uncovered numerous mechanisms by which immune cells detect foreign or changed self-substances that appear in infections or during tissue damage. These substances stimulate signaling receptors, which leads to cellular activation and the induction of effector mechanisms. Here, we review the role of inflammasomes, a family of signaling molecules that form multi-molecular signaling platforms and activate inflammatory caspases and IL-1 β cytokines.

Keywords

Monocytes/Macrophages < Cell Lineages and Subsets; Neutrophils < Cell Lineages and Subsets; Toll-like Receptors/Pattern Recognition Receptors < Molecules; Inflammation < Processes; Adapter proteins < Molecules; Lipopolysaccharide < Molecules

Introduction

Innate immune cells are able to mount an immediate response towards infectious microorganisms or sterile tissue insults by virtue of a set of germline-encoded receptor signaling molecules. In recent years, rapid progress has been made in our understanding of the molecular mechanisms of immune activation. Several families of pattern recognition receptors have been discovered that are engaged in surveillance of the extracellular and intracellular space for the presence of microbial products or endogenously derived danger signals. These families include the Toll-like receptor (TLR) and C-type lectin receptor (CLR) transmembrane proteins and the cytoplasmic nucleotide-binding domain, leucine-rich repeat containing receptors (NLRs) or Rig-I like receptors (RLRs) (see reviews: (1–3)). While most receptors evolved to modulate the transcriptional response of cells after activation, members of the NLR protein family can form multimolecular protein complexes termed inflammasomes. These inflammasomes activate a proteolytic cascade culminating in the maturation and release of pro-cytokines of the IL-1 β family of cytokines amongst other proteins.

Inflammasomes are typically formed by a ‘receptor’ protein, the adaptor molecule apoptosis-related speck-like protein (ASC) and the effector molecule pro-caspase-1. Whether the receptor part of the inflammasome actually interacts directly with a ligand has not yet been firmly established. Indeed, for some inflammasomes there is indication that other proteins can function in binding the activating ligand. The main function of inflammasomes appears to be in controlling the activation state of caspase-1 and thereby the activity of the main substrates, the members of the pro-IL-1 β family of cytokines.

The proximal ‘receptor’ part of inflammasomes is categorized by the domain structure of the proteins. The NLRP proteins (formerly termed NALPs) contain a pyrin (PYD) domain, while the NLRC proteins carry a caspase recruitment domain (CARD). Other members include the MHC class II transactivator (CIITA) and baculoviral IAP repeat-containing protein 1 (NAIP) (Fig. 1). Another protein that can form an inflammasome together with ASC is the absent in melanoma 2 (AIM2) protein, which lacks the typical NACHT domain of the NLR inflammasome proteins. Here, we review the biology and function of inflammasomes and their relevance in disease.

NLRP1

NLRP1 (NAC, DEFCAP, CARD7, NALP1) is the first NLR protein that was shown to form large structures akin to the apoptosome and since these were caspase-1 and IL-1 β activating platforms, the term ‘inflammasome’ was coined (4). The domain structure for human NLRP1 (containing the NLRP-specific PYD-, nucleotide-binding NACHT- and LRR-domains followed by a FIIND- and a CARD-domain) differs from the mouse Nlrp1, as the latter lacks the PYD-domain at the N-terminus. Moreover, the mouse genome contains three paralogue genes of human *NLRP1*, and there are five different strain-specific *Nlrp1b* alleles in inbred mice (5). Human CARD8 (CARDINAL, TUCAN) is highly similar to the C-terminus of mouse Nlrp1b, and since it is not found in the mouse genome, it was hypothesized that Nlrp1 might play a dual role in mice (6).

Microbial sensing

NLRP1 was identified as the cytosolic sensor for lethal toxin (LT) from *Bacillus anthracis* and for muramyl dipeptide (MDP) peptidoglycan from bacterial cell walls. *B. anthracis*, the causative agent of anthrax disease, secretes three proteins: the cell-binding and pore-forming protective antigen (PA), and two enzymatic proteins, edema (EF) and lethal factor (LF). LF is delivered to the endosomal compartment by binding to a PA heptamer/octamer, where acidification causes the receptors to form small pores and denaturation of LF, which, in turn, is secreted through the pore into the cytosol (7). LT infection of mouse macrophages isolated from an Nlrp1b-sensitive mouse strain have been shown to engage the Nlrp1 inflammasome leading to processing of pro-caspase-1 and -11, as well as pro-IL-18 (8). LT-induced lysosomal degradation and cathepsin B release with subsequent cell death, was also Nlrp1b-dependent, probably due to processing of the protein Bid (9). Interestingly, human fibroblasts reconstituted with Nlrp1b and pro-caspase-1 were susceptible to LT-infection. In contrast, the Nlrp1 CARD-domain alone was not rendering cells susceptible, however, together with some parts of the FIIND-domain (similar to CARD8 in humans) it was able to catalytically process pro-caspase-1 without the requirement for ASC (10).

Whilst MDP was shown to directly activate NLRP1 in cell-free systems (11), it is also known to be a ligand for NLRC2 (NOD2) (another member of the NLR family), *in vivo* (12). It was recently observed, that the direct interplay between these two receptors is responsible for caspase-1-dependent IL-1 β release upon *B. anthracis* infection and bacterial MDP (13). NLRC2 can directly interact with caspase-1 via its two N-terminal CARD-domains and can also induce NF- κ B via RIP2. NF- κ B-activation initiates the transcription

of pro-IL-1 β , which in turn is processed by the NLRC2-NLRP1 (but not the NLRP3) inflammasome.

Genetic disorders

Genetic variations in the *NLRP1* genomic region have been associated with high risk to generalized vitiligo, a multifactorial disease characterized by loss of skin pigmentation. Genetic analysis of chromosome 17 in patients with autoimmune and autoinflammatory diseases (generalized vitiligo, autoimmune thyroid disease, rheumatoid arthritis, lupus and Addison's disease) identified several single-nucleotide polymorphisms (SNPs) in the *NLRP1* gene (14). One SNP encoded an L155H non-synonymous amino acid change in an otherwise highly conserved region of NLRP1 and this was strongly associated with generalized vitiligo alone or together with other autoinflammatory diseases. Sequence variants in the promoter region of *NLRP1* were also identified, however, their exact role remains to be elucidated.

Mechanism of activation

NLRP1 was the first protein found to form inflammasomes that processed caspase-1 and -5 in an ASC-dependent manner (4). The minimal protein constituents for NLRP1 inflammasome formation were later characterized in cell-free reconstitution assays (11). NLRP1 directly interacted with pro-caspase-1 via CARD/CARD-domain interaction, and MDP-induced NLRP1 activation was sufficient for processing caspase-1 while the addition of ASC enhanced caspase-1 activity. NLRP1 activation follows a two-step mechanism upon MDP-treatment. MDP, as an effective ligand, could bind to the LRR-domain of NLRP1 to induce a conformational change in the protein, that allows for the binding of NTPs and subsequent oligomerization of NLRP1 via its NACHT-domain. Electron microscopic images of MDP- and ATP-activated NLRP1 showed a mixture of small and large particles, where alignment and averaging of the larger oligomer structures showed five- and seven-fold symmetries, not unlike structures found in the APAF-1 apoptosome. NLRP1 was also shown to interact with caspase-2 and -9 in an apoptosome and contribute to apoptosis; however, the exact mechanism remains unknown (15). Furthermore, the mechanism by which NLRP1 is activated is yet to be described.

Regulation

NLRP1 is not widely expressed, with the highest levels seen in T cells and Langerhans cells and at lower levels in stomach epithelial cells, gut and lungs (16). Interestingly, the anti-apoptotic BCL-2 and -X_L directly bind to the LRR-domain of NLRP1 and block caspase-1-activation (17). Furthermore, its short loop-domain is sufficient to inhibit NLRP1 ATP-binding and oligomerization (18). A viral NLR homolog, Orf63 (isolated from Kaposi's sarcoma-associated herpesvirus), was shown to block NLRP1-mediated caspase-1 and IL-1 β processing and interact with NLRP3 and NLRC2 (19).

NLRP3

Danger signal and microbial sensing

The NLRP3 inflammasome can be activated by a plethora of agonists, endogenous and exogenous, sterile or pathogen-derived. However, judging from the diverse nature of these activators, it is highly unlikely that these danger- and pathogen-associated molecular patterns (DAMPs and PAMPs) all directly activate or interact with NLRP3, and NLRP3 is rather considered as a sensor for changes in the cellular environment.

Potent danger signals in the extracellular matrix and tissue environment can be a result of cell death and elevated levels of metabolites, aggregate or crystalline material. These

molecules can be physiological constituents of functioning cells, that when displaced from their physiological locations or when aggregate can be inflammatory (20).

In vitro studies have shown that LPS-primed macrophages respond to extracellular ATP by turning on the NLRP3 inflammasome (21) via the purinergic receptor P2X7 (22). Conceptually, ATP can appear in extracellular space as a result of cell death following tissue damage and thus, NLRP3 activation may serve as a sensing mechanism for tissue damage. ATP may be derived from cytosolic contents that are released following cell disintegration. Additionally, ATP could be actively secreted by mitochondria expelled into the extracellular environment. Even though sensing of ATP in the extracellular environment occurs via the P2X7 receptor, ATP is also involved in the direct activation of NLRP3. There is evidence *in vitro* and in reconstitution assays, that ATP binds to the NACHT-domain of NLRP3 and is hydrolyzed by the ATPase activity of NLRP3. This activity appears to be necessary for the activation of NLRP3 (23).

Uric acid is a normal catabolic product of purine metabolism and it has higher solubility inside cells than outside cells, since the ion composition in these environments dramatically differs. During cell death, cytosolic uric acid is released into the extracellular environment where it can precipitate to form monosodium urate (MSU) crystals that activate NLRP3. Thus, NLRP3 can also sense cell death by recognizing the phase transition of uric acid. Uric acid crystals can also trigger the classic crystal-induced disease, gout.

Patients displaying elevated levels of uric acid in their blood (hyperuricemia), produce MSU crystals that result in an acute and painful inflammatory response in joints that can lead to chronic arthritis (24). Similarly, calcium pyrophosphate (CPPD) can form spontaneous crystal deposits in joints, leading to the acute arthritis known as pseudogout (25). Release of IL-1 β upon stimulation with MSU and CPPD crystals was shown to be dependent on the components of the NLRP3 inflammasome in mouse macrophages and human monocytes and this could be blocked by colchicine, a drug used for treatment of patients with gout (26).

Alzheimer's disease is an inflammatory disease associated with amyloid β deposits in so-called senile plaques. These plaques attract resident macrophage-like microglia cells and astrocytes (27). Phagocytosis of the fibrillar form of these aggregates can activate the NLRP3 inflammasome in microglia cells and the subsequent release of pro-inflammatory cytokines and neurotoxic factors could contribute to disease pathogenesis (28). IL-1 β is a known pro-inflammatory cytokine involved in the pathogenesis of type 2 diabetes mellitus (T2DM) (29) and atherosclerosis and recently, some of the mechanisms that govern IL-1 β production in these diseases have been described. Obesity is the most common underlying condition for many diseases in the Western world and hyperlipidemia-induced insulin resistance and pancreatic islet beta-cell dysfunction are the characteristic features of T2DM. IL-1 β was shown to induce cell death of beta cells (30) and hyperglycemia-induced IL-1 β production by the beta cells further aggravates this cytotoxic effect (31). Islet amyloid polypeptide (IAPP) is a peptide hormone that is secreted in low amounts together with insulin in the pancreas and probably has a role in glucose metabolism and gut function. Increased insulin secretion results in increased quantities of this peptide that can lead to the formation of oligomers and amyloid deposits and result in apoptosis of the beta cells (32). Recently, IAPP was also found to engage the NLRP3 inflammasome and produce IL-1 β in bone-marrow derived macrophages (BMDMs) that were primed by products of heightened glucose metabolism (33). Hence, increased production of insulin and amylin during peripheral insulin resistance states in T2DM could lead to the generation of NLRP3-dependent beta cell toxic factors. These factors could in turn, favor the development of insulin-dependent diabetes, a feature commonly observed in patients that have T2DM for a prolonged time. Intriguingly, it is also possible that by treating patients with drugs that

increase insulin production and thereby potentially also amylin secretion, the rate of amylin deposition may be iatrogenically increased.

It was recently shown that fatty substances are also directly involved in NLRP3 activation. Saturated, but not unsaturated fatty acids, can induce IL-1 β production in a *Nlrp3*- and *Asc*-dependent manner (34). The activation of NLRP3 by palmitate and LPS inhibited the AMP-activated protein kinase pathway, which led to impaired autophagocytosis and elevated mitochondrial ROS generation, and together, IL-1 β and TNF mediated insulin signaling and resistance *in vitro* and *in vivo*. Obesity and T2DM are two of several risk factors for atherosclerosis. This disease is characterized by thickening of the artery walls due to the accumulation of fatty metabolites and cholesterol. The subsequent invasion of macrophages elicits a chronic inflammatory response to the cholesterol crystals and since they cannot be cleared, the unstable atherosclerotic plaques can rupture and cause thrombosis. The appearance of cholesterol crystals in the plaques were thought to be a late sign of atherosclerotic lesions (35), however, utilizing a novel microscopic technique, it was revealed that crystal depositions could be found in the lesions as early as two weeks following a high cholesterol diet in mice prone to atherosclerosis (*ApoE*^{-/-}) (36). *In vitro* experiments showed that cholesterol crystals and oxidized LDL activated NLRP3 in a manner dependent on phagolysosomal rupture. LDL receptor knock-out mice reconstituted with bone marrow from mice lacking genes of the components of NLRP3 inflammasome also showed a reduced phenotype of cholesterol crystal-induced inflammation and atherosclerosis. Overall, there is a growing body of evidence suggesting that the severe inflammatory response to metabolic disorders resulting from crystal and aggregate formation is mediated by the NLRP3 inflammasome.

As in the case of endogenous crystal formation, exogenous inorganic fibrils and crystals from environmental hazards can engage the NLRP3 inflammasome in severe fibrosing disorders, like asbestosis and silicosis. *In vitro* studies in human monocytes and mouse macrophages showed that once silica crystals and asbestos engage NLRP3, the subsequent processing of pro-IL-1 β is dependent on the endocytic pathway and ROS production (37). Additionally, in an *in vivo* silicosis model, *Nlrp3*- and *Asc*-deficient mice showed decreased pulmonary pathology to inhaled crystalline silica (38). It was also shown that lysosomal destabilization might be a common trigger for NLRP3 activation as in the case of silica, aluminum salts and other lysosomal damaging agents (39).

Pathogens that can gain access to the cytosol and their associated PAMPs can also activate NLRP3. Several bacteria expressing a type III secretion system (T3SS) were shown to activate NLRC4 (see below), however, certain bacteria are able to engage NLRP3 directly and multiple NLRs in a redundant or cooperative fashion. The exact mechanism of how these various pathogens act is not yet clear, however, destabilization of the ion-milieu of various cellular compartments by pore-forming bacterial toxins seems to be a common theme. Studies in a human monocyte-like cell line and mouse macrophages primed with *S. aureus*-secreted lipoproteins showed recombinant hemolysins (exotoxins produced from *Staphylococcus aureus*) to produce pro-inflammatory cytokines (IL-1 β and IL-18) in an NLRP3-, ASC- and caspase-1-dependent manner (40,41). *Mycobacterium tuberculosis* causes tuberculosis that affects about one-third of the world population and kills 1.7 million people every year (42). *M. tuberculosis* has the ability to induce the exocytosis of lysosomes by its microbial toxins and secretion systems, which corresponded to increased release of IL-1 β and IL-18 in an NLRP3 inflammasome dependent manner (43). In an elegant RNA interference screen, multiple NLRs were identified to be involved in recognizing various elements of *M. tuberculosis* infection and NLRP3 was found to be the main constituent for IL-1 β -secretion (44). Granuloma formation in the lungs is an important mechanism for arresting chronic *M. tuberculosis* growth. While TLRs and NLRs are required for the initial

response against the bacteria, antigen-presenting T-cells of the adaptive immune system initiate secondary invasion of multiple cell types that results in granuloma formation. Interestingly, in mouse models, Asc but not Nlrp3 or caspase-1, was indispensable for containing bacteria in granulomas and promoting survival (45). For *Salmonella typhimurium* infection, both NLRC4 and NLRP3 were implicated in IL-1 β production at specific ASC-containing cytosolic loci, but while NLRC4 responds to flagellin, NLRP3 probably senses the bacteria in its quiescent state during the systemic phase of the infection (46). *Listeria monocytogenes* was found to activate the inflammasomes in a similar fashion (47). Recent findings have indicated that not only NLRP3, but also AIM2 and other NLRs are involved in IL-1 β production during *L. monocytogenes*-infection (48). *Clostridium difficile* can invade the intestines after antibiotic treatment and cause nosocomial diarrhea and colitis and although its associated toxins (TcdA and TcdB) are not pore-forming molecules, they show partial phenotypes in mouse macrophages deficient for various NLRs. Furthermore, toxin-induced cell death was fully dependent on Asc (49). Fungal pathogens can also induce IL-1 β secretion, which is indispensable for host survival. PAMPs from several species are detected by TLRs and CLRs, which turn on the canonical or Syk-mediated NF- κ B-signaling pathway for the transcription of pro-IL-1 β and *Candida albicans* activates NLRP3-mediated IL-1 β -cleavage via ROS production and K⁺ efflux (50,51).

The NLRP3 inflammasome has also been implicated in the recognition of viral RNA and DNA. *Nlrp3*-deficient mice showed reduced survival in influenza A infection, and *Nlrp3* inflammasome components were important for pro-inflammatory cytokine production in mouse models (52). However, a recent publication indicated that influenza A RNA is sensed by TLR7 to initiate transcription of pro-IL-1 β and NLRP3, and the ion-channel activity of the viral M2 protein, via acidification of the Golgi apparatus, is responsible for activation of the NLRP3 inflammasome (53). In light of these findings, the endosomal and cytosolic RNA and DNA receptors are more likely to play a direct role in recognition of viruses by the innate immune system.

Recently it was also shown that certain antibiotics (polymyxin B, gramicidin, tyrothricin and neomycin) could trigger *Nlrp3*-dependent IL-1 β -secretion in mouse BMDMs and this was dependent on K⁺ efflux, but independent of the P2X7 receptor (54).

Genetic disorders

Diseases associated with IL-1 β production are collectively called inflammasomopathies. These can be intrinsic, where the changes happen on the proteins that are part of the inflammasome itself, or alternatively, where the change affects proteins upstream or downstream of the IL-1 β activation platform (55). NLRP3 was previously named cryopyrin when it was found to be associated with cold-induced fevers in patients and to contain a pyrin domain, and now a range of genetic disorders in the *NLRP3* gene are called cryopyrin-associated periodic syndromes (CAPS) or cryopyrinopathies. These dominant or *de novo* mutations exhibit three illnesses with a broad spectrum of varying severity: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurologic cutaneous and articular (CINCA) syndrome or neonatal-onset multisystem inflammatory disease (NOMID). FCAS is the mildest form of the disease and presents with cold-induced fevers and rash. MWS patients show symptoms of fevers, rashes and hives, hearing loss and arthritis. The most severe form, CINCA/NOMID has a neonatal or early onset with fevers, skin lesions, chronic meningitis, joint inflammations, hearing loss and overgrowth of the epiphysis in the long bones (56).

The identified mutations that occur in protein-coding regions are mostly in the NACHT-domain and a few appear in the LRR-domain of NLRP3 (57). These mutations cluster around several loci on the *NLRP3* gene, one of which is next to the conserved ATPase motif,

but there isn't any clear correlation between sequence location of the mutation and disease severity. Interestingly, ATP was no longer required for inflammasome activation in monocytes isolated from CAPS patients (58) or in mutant *Nlrp3* knock-in mice (59,60); however, when both gain-of-function (R260W or A439V) and ATPase (Walker A) mutations were present, IL-1 β release was completely abrogated in reconstituted human monocytes (23). Based on crystal-structure homology models, it is hypothesized that the mutation clusters are located at a solvent-exposed surface of the NACHT-domain and destabilize the potential inactive closed conformation of the protein, thus, keeping it constitutively in its active form. Similarly, mutations in the hinge-region between the NACHT- and LRR-domain, or in the LRR-region itself (e.g. G755A/R), may render the protein more prone to change to an open conformation (61).

As inflammasomopathies are primarily linked to increased IL-1 β production, therapies that target IL-1 receptor or IL-1 β itself became the primary approach. Anakinra, an IL-1 receptor antagonist was shown to be effective for reducing symptoms even in the more severe disease phenotypes of NOMID patients (62). Recently, two anti-IL-1 β therapies have been evaluated (rilonacept (63) and canakinumab (64)), and their efficiency and better pharmacology holds great promise for future therapies in CAPS patients. However, there are still several open questions in the understanding of disease propagation and treatment procedures (65).

Mechanisms of action

In the last decade following the discovery of NLRP3, a wealth of information has been generated towards understanding the mechanisms involved in activation and regulation of the NLRP3 inflammasome. Even though the exact mechanisms are still unknown, there are a few hypotheses that illuminate parts of the processes. In general, at least two signals are required for a successful activation of the NLRP3 inflammasome. In resting macrophages the protein levels of NLRP3 are too low to support the activation of an inflammasome. Signal one is acting to transcriptionally induce NLRP3 to sufficient levels for its activation and this signal can be provided by innate immune receptors, cytokine receptors or other factors that act in 'priming' of macrophages (66,67). At the same time the substrate for caspase-1, that is, IL-1 β is also transcriptionally induced awaiting its cleavage after inflammasome activation. The second step of activation is much less clear. Crystalline substances can induce lysosomal damage leading to the translocation of the proteolytically active lysosomal content into the cytosol. Conceivably, this could lead to the cleavage of an as of yet unknown substrate involved in the activation of the NLRP3 inflammasome (28,36,39). It was also proposed that NADPH oxidase driven reactive oxygen species (ROS) production is involved in crystal mediated NLRP3 activation. Since crystal uptake can lead to both lysosomal leakage and ROS, a separation of these processes is not possible and both effects may in fact be necessary for the activation of the NLRP3 inflammasome as suggested by studies using pharmacological inhibitors (reviewed in (68,69)).

Recently, the source of ROS involved in the activation of the NLRP3 inflammasome was challenged as it was shown to be mitochondria rather than a NADPH oxidase (70–72). ROS appear to be important for IL-1 β production, but it has yet to be established whether they play a direct, or indirect role in the activation of the NLRP3 inflammasome. It has been shown that ROS act upstream of the induction of cytokines (73) and thus, it would appear possible that ROS influence the required priming step of the NLRP3 inflammasome. Moreover, caspase-1 can be covalently modified and thereby functionally inactivated under conditions of enhanced oxidative challenge which seemingly would argue that ROS decrease the production of active IL-1 β cytokines (74). A potential explanation for this apparent paradox was recently provided by the description that ROS activity may in fact be

required to initiate an antioxidative response, which may be important to counteract caspase-1 inhibition (75,76).

It seems that the activation of the NLRP3 inflammasome is very complex and potentially requires multiple signals that appear in a coordinated fashion for full activation. Given the dramatic inflammatory consequences of inflammasome activation for the host, it is not surprising that this process is well controlled and complex in nature.

Regulation

NLRP3 is tightly regulated at the transcriptional level and also in a tissue-specific manner. Most tissues do not express NLRP3 at the RNA level, except peripheral blood leukocytes, among which, monocytes express the highest levels, and granulocytes and T-cells show detectable expression levels (77). In a more comprehensive screen, organ- and tissue-specific mRNA levels of various pattern recognition receptors were compared in human and mouse, and NLRP3 expression was also found in the brain (from microglia), lungs and testis (78).

The induction of NLRP3 is also regulated by CARD8 as it inhibits NF- κ B induction by binding to the regulatory subunit of the I κ B kinase complex, NEMO (6) and it also regulates caspase-1 activation (79). A heterozygous polymorphism (C10X) in *CARD8* is a common variation (34%) in Caucasian populations, and the resulting truncated protein appears to be important for disease phenotype in an otherwise non-symptomatic *NLRP3* polymorphism (Q705K); however, its role in inflammatory bowel disease is highly debated (80). A separate analysis showed that functional CARD8 protein is present, even in people with homozygous C10X mutation due to a splice variant where the nucleotide change does not result in truncated protein (81).

NLRP10 (PYNOD) is an NLR protein that is missing the LRR-domain and is widely expressed in many tissues and organs (82). It was shown to inhibit pro-caspase-1 and pro-IL-1 β cleavage via its NACHT-domain in reconstitution assays, and its PYD-domain inhibited NLRP3- and ASC-mediated IL-1 β -release and ASC speck-formation *in vivo* (83). Additionally, NLRP7 (PYPAF3) is also a widely expressed NF- κ B inducible protein, which was able to inhibit NLRP3-mediated IL-1 β secretion (84).

Recently, NLRC5 has been shown to be indispensable for IL-1 β and IL-18 processing in RNA interference experiments and NLRC5 forms inflammasomes either with itself or cooperatively with NLRP3 (85).

NLRC4

NLRC4 (also known as IPAF, CARD12, and CLAN) was originally described in 2001 (86–88). It is the only known member of the NLRC family to form an inflammasome.

Microbial sensing

NLRC4 has been implicated in the sensing of a number of bacterial pathogens via flagellin recognition. The flagellin protein is a major component of bacterial flagella. *Salmonella typhimurium* was one of the first bacterial species demonstrated to activate an NLRC4-dependent inflammasome response (21,89). Interestingly, only *caspase-1*^{−/−} and not *Nlrp4*^{−/−} mice, showed enhanced susceptibility to oral infection by *S. typhimurium* (90). This seems to indicate redundant pathways for the detection of *S. typhimurium*. Purified *S. typhimurium* flagellin has since been shown to induce NLRC4-dependent, TLR5-independent inflammasome activation when delivered directly to the cytosol (91,92). Furthermore, *S. typhimurium* mutants lacking *fliC* and *fliB*, the genes that encode flagellin

monomers, are unable to induce caspase-1 autoproteolysis, IL-1 β secretion, and pyroptosis; thus, flagellin is essential for *S. typhimurium*-induced inflammasome activation via NLRC4.

Pseudomonas aeruginosa also activates caspase-1 processing, IL-1 β and IL-18 secretion, and cell death in a NLRC4- and flagellin-dependent manner (93,94). However, unlike with *S. typhimurium*, mice lacking functional *Nlrc4* were significantly more susceptible to *P. aeruginosa* peritonitis, as measured by increased IL-1 β and IL-18 secretion and bacterial loads in the nose and spleen (95).

Legionella pneumophila, the cause of Legionnaire's disease, has also been shown to be sensed by the NLRC4 inflammasome, and the resulting caspase-1 cleavage occurs in a flagellin-dependent manner (96–98). NLRC4-dependent caspase-1 activation is implicated in restriction of *L. pneumophila* growth. BMDMs from *Nlrc4*^{-/-} and *caspase-1*^{-/-} mice failed to arrest *L. pneumophila* replication by allowing *Legionella*-containing vacuole to avoid fusion with lysosomes (98). Subsequent experiments showed that caspase-7 is actually required for restriction of intracellular replication in mice (99). Similarly, *L. pneumophila* with mutated or removed *fla*, the gene responsible for encoding flagellin, was able to replicate in normally restrictive mouse B6 macrophages. Unlike in mice, *L. pneumophila* is able to replicate in human macrophages and lung epithelial cells, leading to a severe pneumonia known as Legionnaire's disease. However, NLRC4 does still play a role in inhibiting bacterial growth in humans, as siRNA silencing of *NLRC4* in human THP-1 macrophages allows enhanced bacterial growth, while overexpression of the gene restricts growth (100). As in mouse macrophages, flagellin-deficient *L. pneumophila* replicate more efficiently in human macrophages and epithelial cells. It appears that NLRC4 plays the same role in restricting *L. pneumophila* in both mice and humans; however, the human cells allow a much higher baseline replication rate than mouse cells.

L. pneumophila is unique among these three bacterial species in that *Naip5*, as well as *Nlrc4*, are required to form an immune response in mice. *Naip5* (also known as *Birc1*) was originally described in 2003 as the component required for resistance to *L. pneumophila*, and *L. pneumophila* replication in A/J mice was pinpointed to variations in the *Naip5* gene at the *Lgn* locus (101,102). When *L. pneumophila* flagellin was demonstrated to activate the inflammasome in a *Nlrc4*-dependent manner, flagellin recognition was also shown to be *Naip5*-dependent (96,97,103). NAIP, the closest *Naip5* homolog in humans, was also shown to be essential for *L. pneumophila* flagellin recognition (100). *Naip5* was not strictly necessary for flagellin-induced *Nlrc4* activation in *S. typhimurium* or *P. aeruginosa* infection (104). This differential requirement for *Naip5* will be further examined below.

The intracellular pathogen *Listeria monocytogenes* is also sensed by the NLRC4 inflammasome. *L. monocytogenes* pathogenesis includes escape from the macrophage phagosome into the cytosol, where it replicates (105). Once in the cytosol, *L. monocytogenes* activates caspase-1 processing and IL-1 β secretion through NLRC4 in a flagellin-dependent manner, and detection of flagellin resulted in bacterial clearance (47). *Naip5*-deficiency does not appear to greatly inhibit the detection of *L. monocytogenes* (94). Although the NLRC4 inflammasome was activated upon artificial delivery of purified flagellin to the cytosol and in *L. monocytogenes* infection, a functional type III (*S. typhimurium*, *P. aeruginosa*) or type IV (*L. pneumophila*) secretion system was also necessary for flagellin-mediated caspase-1 cleavage to other bacteria (91,92,94,96–98). The common consensus is that these secretion systems are responsible for mistakenly secreting small amounts of flagellin into the cytosol to be recognized by NLRC4, and this was later demonstrated to occur in the *S. typhimurium* T3SS (106). *Legionella parisiensis* and *Legionella tusconensis* do not induce pyroptosis or IL-1 β secretion in mouse macrophages, however, purified flagellin from these two species does induce pyroptosis and IL-1 β secretion when delivered directly to the cytosol (107).

These two species lack type IV secretion system-dependent characteristics, so it appears that lack of functional secretion prevents flagellin delivery to the cytosol and subsequent NLRC4 inflammasome activation. Thus, it appears that NLRC4 detection of flagellin can either indicate an intracellular pathogen is in the cytosol, or that an active type III or IV secretion system is present. Some bacterial species are able to activate NLRC4 in a flagellin-independent manner due to the direct detection of the T3SS, such as *Shigella flexneri*, the bacteria responsible for bacillary dysentery. It had been shown previously that *S. flexneri* infection of *caspase-1*^{-/-} mice does not result in inflammation and resolution of bacterial infection, due primarily to a failure to secrete IL-18 (108). THP-1 cells that overexpressed NLRC4 showed an increase in IL-1 β secretion in response to *S. flexneri* (89), but it was not until 2007 that the work of Suzuki and colleagues demonstrated that NLRC4-dependent recognition of *S. flexneri* was flagellin-independent but T3SS-dependent (109). Interestingly, they found that knockout of caspase-1 or *Nlrc4* greatly increased autophagy in *S. flexneri*-infected macrophages. Another group found caspase-1 promoted pyroptosis to be dependent on the T3SS-effector protein IpaB (110).

In addition to the previously described flagellin-dependent pathway, *S. typhimurium* and *P. aeruginosa* can also be detected via an NLRC4-dependent but flagellin-independent pathway. Although *S. typhimurium* activates NLRC4 primarily via flagellin, a flagellin-deficient strain of *S. typhimurium* was able to induce small amounts of T3SS-dependent IL-1 β secretion at a high multiplicity of infection (MOI) (91). Similarly, WT mouse macrophages infected with a flagellin-deficient mutant of *P. aeruginosa* secreted IL-1 β in a T3SS-dependent manner, however, not to the extent of that seen in macrophages infected with a flagellin-expressing strain (95). This T3SS-dependent, flagellin-independent activation of NLRC4 was later observed to be the result of NLRC4 detection of the T3SS basal body rod protein (111). Although the rod proteins sensed by NLRC4 are slightly different for *S. flexneri* (MxiI), *S. typhimurium* (PrgJ) and *P. aeruginosa* (PscI), they share a sequence motif that is required for detection by NLRC4, and this motif is related to the flagellin motif also sensed by NLRC4. In a subsequent review, the authors hypothesize that these rod proteins may reach the cytosol through accidental translocation or sloughing off during effector translocation (112). Thus, it is possible that *L. pneumophila* can only be recognized by the flagellin-dependent pathway because it lacks a T3SS.

Mechanism of action

Like NLRP1 and NLRP3, NLRC4 self-oligomerizes via homotypic interactions of its NACHT domain (113). It contains a CARD domain and is able to associate with pro-caspase-1 directly through CARD-CARD interactions (86–88), and it can also associate directly with ASC in the same manner (87).

The adaptor molecule ASC is required for NLRP3 activation of caspase-1 because it lacks a CARD domain, but NLRC4 is able to interact with pro-caspase-1 directly, casting doubt on the usefulness of ASC. In response to *Nlrc4* stimulation, *Asc*^{-/-} mouse macrophages generally cannot initiate caspase-1 autoproteolysis and they secrete significantly less IL-1 β and IL-18, however, they remain capable of undergoing pyroptosis at similar levels to *Asc*-expressing macrophages (93,95,109,114). Interestingly, *L. pneumophila* infection appears to inhibit *Asc* expression, thus inhibiting caspase-1 processing and allowing bacterial replication (115). A recent paper demonstrated that *Nlrc4*-dependent cell death and cytokine processing are actually mediated by different inflammasomes (116). In the *Asc*-containing inflammasome, a large *Asc* speck is formed, which then leads to the autoproteolysis of caspase-1 and IL-1 β /IL-18 secretion. In the absence of *Asc*, no large inflammasome structure is formed and caspase-1 fails to undergo autoproteolysis; however, caspase-1 is still

activated and can induce pyroptosis. This appears to answer the question of the role of ASC in NLRC4-mediated inflammasome activation.

As previously mentioned, Naip5 has been shown to play an essential role in Nlrc4-dependent recognition of *L. pneumophila*. NAIP and NLRC4 are grouped in the same clade within the NLR protein family based on their functional similarities (117). Naip5 and Nlrc4 do physically interact with each other (103). Naip5 was shown to be essential for the recognition of the 35 amino acids at the C-terminus of both *L. pneumophila* and *S. typhimurium* flagellin (104), and the same sequence is also necessary to activate Nlrc4. *S. typhimurium*- and *P. aeruginosa*-induced cell death is partially Naip5 dependent, but strictly Nlrc4 dependent. When *L. pneumophila* is forced to express the flagellin of *S. typhimurium*, detection of this strain is still dependent on Naip5 (118). This seems to indicate that it is the bacteria, not the flagellin, that results in differential Naip5 requirements for sensing. However, PrgJ activates Nlrc4 in a Naip5-independent manner. Lightfield and colleagues proposed a specificity model in which Naip5 is responsible for recognizing flagellin, while the Naip5-independent Nlrc4 activation is due to direct recognition of the T3SS.

Regulation

NLRC4 is expressed predominately in hematopoietic tissues (86), however, it is also expressed in the colon epithelia (119). The leucine-rich-repeat (LRR) domain appears to auto-repress NLRC4 activation in the absence of a ligand since NLRC4 protein lacking an LRR domain is constitutively active (86). The chaperone molecules SGT1 and HSP90 associate with NLRC4 and are essential for IL-1 β processing (120). It had previously been reported that increased levels of extracellular K⁺ ions do not inhibit NLRC4 inflammasome formation (121). A recent study showed that high extracellular K⁺ levels inhibited activation of the NLRC4 inflammasome to non-flagellated *P. aeruginosa* and *S. typhimurium* (122), however, the K⁺ levels were higher than those required to inhibit the NLRP3 inflammasome and that might actually inhibit other processes, for example bacterial uptake. Interestingly, mouse CD4⁺ effector and memory T cells suppress Nlrp1 and Nlrp3 signaling, but do not suppress the Nlrc4 inflammasome (123). As mentioned earlier, inhibition of caspase-1 has been shown to upregulate autophagy in macrophages infected with *S. flexneri*, while induction of autophagy lead to pyroptosis resistance (124). A recent review by Bortoluci and Medzhitov (125) proposes a mechanism in which inflammasomes and pyroptosis are used to clear pathogenic microorganisms, while autophagy is used for less dangerous microbes.

The NLRC4 inflammasome is also negatively regulated by multiple bacterial components. The *Yersinia* T3SS is partially recognized by NLRC4, but the effector protein YopK has been shown to interact with the T3SS translocon to inhibit its recognition (126). The *P. aeruginosa* PA103 strain does not stimulate caspase-1 cleavage or IL-1 β secretion due to the phospholipase activity of the effector molecule ExoU (95).

Genetic disorders

NLRC4 and NAIP have been implicated in a few genetic disorders. *NLRC4* and *NAIP* are two of the five genes upregulated in acute-phase Kawasaki disease, which is characterized by an increased activation of the immune system and upregulation of chemokines and cytokines (127). The disease is associated with vasculitis, damage to the coronary artery, and even heart attack and death. There is also evidence of a role for NLRC4 in atopic dermatitis, since a rare haplotype of the *NLRC4* gene is more frequent in atopic dermatitis patients (128). Spinal muscular atrophy has been linked to a failure in regulating motor neuron apoptosis, and the gene for neuronal apoptosis inhibitory protein (*NAIP*) is truncated in these patients (129).

NLRC4 deficiency has also been shown to play a role in inflammation-induced colonic tumorigenesis (119). *Caspase-1*^{-/-} mice demonstrate increased tumor formation in response to injury, and it was determined that caspase-1 was essential for regulation of colonic epithelial cell growth and death. NLRC4 is upregulated in colonic epithelial cells, and *Nlrc4*^{-/-} mice also showed increases in colonic tumor formation and tumor load. Thus, it appears that NLRC4-mediated caspase-1 activation is essential for regulating colonic epithelial cell response to injury.

AIM2

Microbial sensing

It has been known for some time that introduction of double-stranded DNA (dsDNA) into the cytosol can cause cell death in a sequence-independent manner (130). Single-stranded DNA does not activate the immune system when delivered into the cytosol (131). Subsequent studies have shown that delivery of viral, bacterial, and mammalian dsDNA into the cell leads to ASC-dependent cleavage of caspase-1 and IL-1 β secretion (132). The ASC-dependent nature demonstrated that there must be a receptor upstream that detects dsDNA, but no known NLRs were able to do so. Subsequently, four different groups identified AIM2 (absent in melanoma 2, PYHIN4) as the dsDNA receptor that was able to form an ASC-dependent inflammasome (133–136).

AIM2 has been demonstrated to play an essential role in the recognition of certain viruses by detecting viral DNA in the cytosol (135,137). *Aim2*-deficient mouse macrophages were impaired in their ability to recognize vaccinia virus and murine cytomegalovirus (mCMV) (136,137). Additionally, *Aim2*^{-/-} mice infected with mCMV secreted significantly lower levels of IL-18 and had fewer IFN- γ -producing NK cells when compared to *Aim2*^{+/+} mice, thus leading to increased viral loads in the spleen. However, thioglycollate-elicited macrophages from *Aim2*^{-/-} mice were not deficient in their response to herpes simplex virus type 1 (HSV-1) (137) and adenovirus recognition was dependent on the NLRP3 inflammasome (132), indicating that AIM2 is not able to recognize all DNA viruses. The inability of AIM2 to recognize these DNA viruses is most likely due to their infection and replication cycles, which seem to insulate viral DNA from the cytosol, and thus, from AIM2 entirely (138,139).

The highly infectious intracellular bacteria *Francisella tularensis*, the causative agent of tularemia, is also sensed by the AIM2 inflammasome (137,140). *F. tularensis* enters cells, primarily macrophages, through phagocytosis, but then quickly escapes the phagosome/endosome and replicates within the cytosol (141). It had been shown previously that *caspase-1*^{-/-} and *Asc*^{-/-} mice are significantly more susceptible to *F. tularensis* infection, however bacterial recognition was not *Nlrc4*-dependent (142). Following infection with *F. tularensis*, *Aim2*^{-/-} mouse macrophages were deficient in caspase-1 cleavage, IL-1 β secretion, and cell death, but not type I IFN secretion, and Aim2 and Asc were shown to colocalize with *F. tularensis* DNA in WT macrophages. Furthermore, *Aim2*^{-/-} mice had higher mortality rates and bacterial loads following *F. tularensis* infection, demonstrating that Aim2 is essential for sensing *F. tularensis* infection (143,144). The *F. tularensis* gene *mviN* was shown to be important for limiting Aim2 inflammasome activation, as *mviN* mutant strains of *F. tularensis* induced increased IL-1 β secretion and cell death, possibly due to increased lysis, in an Aim2-dependent manner (145).

As mentioned previously, *L. monocytogenes* are intracellular bacteria that are sensed at least partially, by NLRC4 and NLRP3. Studies using shRNA knockdown of Aim2 in mouse macrophages showed a decrease in IL-1 β secretion and cell death as compared to a controlled shRNA upon challenge with *L. monocytogenes* (146–148), and similar decreases

were shown in *Aim2*^{-/-} mouse macrophages. AIM2-dependent inflammasome activation increased when macrophages were infected with a *L. monocytogenes* strain engineered to undergo higher levels of bacteriolysis (147). Also, *L. monocytogenes* DNA was shown to colocalize with ASC specks (148) and induce IL-18 in an AIM2-dependent manner (149). Wu and colleagues demonstrated that AIM2 is essential for detecting *L. monocytogenes* that do not express flagellin in pre-primed macrophages, however, NLRC4 is essential for detecting *L. monocytogenes* in unprimed cells (150). Therefore, it was speculated that NLRC4 recognition of *L. monocytogenes* flagellin is upstream of NLRP3 and AIM2 signaling.

Mechanism of action

AIM2 activates an inflammasome complex by first binding to DNA via its HIN-200 domain (134–136). Nine members of the HIN-200 (hematopoietic IFN-inducible nuclear proteins with 200-amino acids) family have been described: four in humans (IFI16, IFIX, MNDA, AIM2) and five in mice (p202, p203, p204, Mndal, Aim2) (151). All members contain DNA-binding HIN-200 domains, but only AIM2 and p202 locate to the cytosol (133,135,152). AIM2 binds preferentially to dsDNA over ssDNA or RNA, and this binding can be diminished by a F165A point mutation in the HIN-200 domain. Recognition of dsDNA is non-specific, since bacterial, viral, or self-DNA can activate AIM2. Interestingly, the high-mobility group box (HMGB) proteins, which have been shown to bind to all nucleic acids, may also be necessary for AIM2 to detect DNA (153). AIM2 appears to self-oligomerize through the binding of AIM2 molecules to multiple sites on the dsDNA molecule. Once AIM2 binds dsDNA, it associates with ASC via PYD-PYD homotypic interactions, which then catalyzes the cleavage of caspase-1. Although all members of the HIN-200 family, except p202, contain a PYD domain, only the PYD domain of AIM2 can interact with the PYD domain of ASC.

Regulation

Human *AIM2* is expressed constitutively in the small intestine, spleen, and peripheral blood leukocytes (154). *AIM2* is also an interferon-inducible gene, as demonstrated by the fact that growth of HL60 cells in the presence of IFN- γ induces *AIM2* expression. Treatment of human THP-1 cells with IFN- β can also upregulate *AIM2* expression (134). Induction of *Aim2* can also affect Aim2 inflammasome responses *in vitro*, as it is upregulated in mouse BMDMs in a type I IFN-dependent manner following infection with *F. tularensis*, and failure to upregulate *Aim2* expression resulted in decreased IL-1 β secretion and cell death (144). Similarly, type I IFN signaling is important to form an inflammasome response in mouse macrophages infected with the cytosolic bacteria *F. tularensis* subspecies *novicida* and *L. monocytogenes*, however, is not important for infection with vacuole-located *S. typhimurium* (155), providing additional evidence for the importance of type I IFN in AIM2 inflammasome formation.

Other members of the HIN-200 family may also regulate AIM2. The p202 protein was able to bind dsDNA in the cytosol and siRNA knockdown of p202 induced caspase-1 cleavage in BMDM challenged with poly(dA):(dT) (133). Furthermore, p202 can heterodimerize with Aim2 (152) and inhibit Aim2 from clustering with Asc, so it appears that p202 can negatively regulate Aim2 responses by sequestering DNA or preventing Aim2-Asc oligomerization. IFI16 is the only human HIN-200 protein shown to heterodimerize with AIM2 (156), however it has not been shown to antagonize AIM2 inflammasome activation, most likely since it is predominately found in the nucleus.

Genetic disorders

Since AIM2 has been shown to recognize self-DNA in the cytosol, it has been postulated that it plays a role in systemic lupus erythematosus (SLE). The chromosomal location of the HIN-200 genes falls in a lupus susceptibility locus for both humans and mice (157). Additionally, mRNA transcripts for IL-1 β were elevated in many SLE patients when compared to controls, indicating possible AIM2 inflammasome activation (158). Conversely, a recent study showed the gene for p202, *Ifi202*, was upregulated in *Aim2*^{-/-} mouse macrophages, and lupus-susceptible mice expressed reduced levels of Aim2, but elevated levels of p202, when compared to healthy controls (159). It was speculated that this imbalance could inhibit Aim2 inflammasome-mediated cell death, allowing cells with cytosolic DNA to survive longer and secrete more type I IFNs, leading to lupus. The same group subsequently discovered that increased expression of p202 as a result of *Aim2*-deficiency suppressed the expression of the *Fcgr2b* (Fc γ RIIB receptor) gene, of which deficient expression is linked to lupus susceptibility (160). The results of these studies seem to indicate a role for Aim2 deficiency in susceptibility to lupus through its effect on p202 expression. However, humans lack the p202 protein, so it is unclear how these results translate to human SLE.

AIM2 has also been linked to psoriasis. Keratinocytes of psoriatic lesions have increased levels of both dsDNA and AIM2, and AIM2 can form an inflammasome and release IL-1 β in response to cytosolic DNA in cultured keratinocytes (161). The antimicrobial peptide LL-37 can reduce AIM2-dependent inflammasome activation in keratinocytes by binding to dsDNA. Thus, AIM2 appears to play a role in some autoinflammatory diseases.

Other Inflammasomes

Although the four inflammasomes previously discussed are by far the most studied ones, other NLRP proteins have recently been implicated in inflammasome formation. Here, we review the NLRP6 and NLRP12 inflammasomes.

NLRP6

It had been demonstrated that NLRP6 (also known as PYPAF5, NALP6) and ASC colocalize and induce caspase-1 dependent IL-1 β secretion when co-expressed in COS-7L cells (162). Recently, three different groups have elucidated the role of Nlrp6 in a mouse model of colitis. *Nlrp6*^{-/-} mice given dextran sodium sulfate (DSS) in their drinking water had exacerbated colitis when compared to WT mice (163), and this lead to increased tumorigenesis in the colon (164,165). Interestingly, *Nlrp6*^{-/-} mice have an altered gut microbiota, which together with the exacerbated colitis phenotype, can be transferred to cohoused WT mice and this appears to be due to the lack of inflammasome-induced IL-18 secretion by *Nlrp6*^{-/-} mice (163). Nlrp6 also appears to regulate epithelial cell proliferation and repair (164,165). Two independent studies have shown conflicting results as to whether Nlrp6 expression in the nonhematopoietic or hematopoietic compartment of the intestine is responsible for protection; one group found that the decreased colonic epithelial IL-18 production in *Nlrp6*^{-/-} mice was responsible for enhanced colitogenic microbiota in DSS-induced colitis (163), while the other group assessed that Nlrp6 in hematopoietic cells is essential for protection against colitis-associated tumorigenesis (164). Nevertheless, NLRP6 appears to be essential for preventing recurring colitis, and it is speculated that NLRP6-deficiency may contribute to IBD.

NLRP12

Like NLRP6, NLRP12 (PYPAF7, Monarch-1, NALP12) has also been shown to colocalize with ASC and induce caspase-1 dependent IL-1 β secretion when co-expressed in COS-7L

cells (166). However, NLRP12 was also shown to inhibit non-canonical activation of NF- κ B in human monocytes (167). When two non-ambiguous mutations in the *NLRP12* gene were found in three patients with CAPS symptoms but without *NLRP3* mutation, it was observed that both mutations decreased NF- κ B inhibition *in vitro* (168). It was later demonstrated that PBMCs from two of these patients with a nonsense mutation in the NACHT-domain spontaneously secreted dramatically more IL-1 β than control cells, implying that this mutation results in constitutive NLRP12 inflammasome activation (169). These patients initially responded well to anakinra, with clinical improvement and lowering of IL-1 β to near normal levels, however, they developed resistance in less than a year. A missense mutation (D294E) in the NACHT-domain of *NLRP12* was found in multiple members of one family presenting with sensitivity to cold exposure and this mutation accelerated the kinetics of PAMP-induced IL-1 β secretion *in vitro* (170). Two unrelated patients with genetically unexplained CAPS symptoms were found with a different missense mutation (R352C) in the NACHT-domain and this mutation had no effect on NF- κ B inhibition, but increased ASC speck formation and caspase-1 cleavage (171). Thus, the term *NLRP12*-associated disorders (NLRP12AD) was proposed for these diseases.

Conclusion

We have experienced a dramatic increase in our knowledge of how inflammasomes are formed and regulated, how they influence the production of key pro-inflammatory cytokines and what roles they play in infection control and tissue inflammation. While the gain in information has been rapid and has opened great promises for potential therapeutic manipulation of inflammatory conditions, many open questions remain. Most importantly, the exact molecular mechanisms of activation for most inflammasomes are still unclear. We will, undoubtedly, learn about many more activators of inflammasomes and their involvement in disease pathology and it is expected that this research will generate exciting novel future therapies needed for a variety of inflammatory pathologies.

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Figure 1. Inflammasomes

Upon their activation inflammasomes form multi-molecular protein complexes that typically consist of an NLR protein, or AIM2, the adapter molecule ASC and the effector pro-caspase-1. The different inflammasomes differ in their domain organization and composition as depicted in the figure. The NLRP1 inflammasome contains both a CARD and a PYD domain and can recruit several caspases, while the NLRC4 inflammasome can also contain NAIP5.

Figure 2. The NLRP3 inflammasome

Successful NLRP3 inflammasome formation requires multiple steps. In a priming step, transcriptionally active signaling receptors, such as PRRs or cytokine receptors, first induce NF-κB-dependent induction of NLRP3 itself as well as the caspase-1 substrates of the pro-IL-1β family. NLRP3 is thought to exist in a signaling incompetent conformation, which changes upon activity of a second signal, leading to the assembly of a multimolecular inflammasome complex together with the proteins ASC and caspase-1. Multiple signals which are potentially provided in combination, such as lysosome-derived molecules, reactive oxygen species (ROS) and potassium efflux can trigger the formation of an active inflammasome, which, in turn, leads to the cleavage and release of bioactive IL-1β cytokines.

Figure 3. Bacterial recognition by the Nlr4 inflammasome

The Nlr4 inflammasome is able to recognize Gram-negative bacteria in two distinct ways. (A) Cytosolic flagellin can activate Nlr4 in a Naip5-dependent manner. Flagellin from cytosolic *L. monocytogenes* can activate Nlr4 directly, however, flagellin-dependent activation by *S. typhimurium*, *P. aeruginosa*, and *L. pneumophila* requires a type III or type IV secretion system to secrete flagellin into the cytosol. (B) Nlr4 can recognize the rod protein from the type III secretion system of *S. flexneri*, *P. aeruginosa*, and *S. typhimurium* directly in a Naip5-independent manner. Once activated by either ligand, Nlr4 can recruit pro-caspase-1 directly through CARD-CARD interactions (Asc-independent pathway) or recruit Asc and pro-caspase-1 (Asc-dependent pathway). In the Asc-independent pathway, pro-caspase-1 does not undergo autoproteolysis, but it is adequately activated to promote cell death. In the Asc-dependent pathway, Asc is able to form a large speck through CARD-CARD and PYD-PYD interactions, and pro-caspase-1 can undergo autoproteolysis. Fully cleaved caspase-1 can then process IL-1 β and IL-18 for secretion.

Table 1
Inflammasome activators and associated diseases

Inflammasomes, their stimuli and potentially associated diseases are listed.

Inflammasome	Stimulus	Diseases
NLRP1	Lethal toxin from <i>B. anthracis</i> muramyl dipeptide (MDP)	vitiligo
NLRP3	bacterial pore-forming toxins ion channels crystalline and fibrous material protein aggregates	cryopyrinopathies (CAPS): FCAS MWS CINCA/NOMID
NLRC4	flagellin bacterial type III secretion system	Kawasaki disease, atopic dermatitis, IBD?
AIM2	double-stranded DNA	Psoriasis, SLE?
NLRP6	unknown	IBD?
NLRP12	unknown	NLRP12AD