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Mechanisms of inflammasome activation: recent advances and novel insights

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Abstract

Inflammasomes are cytosolic multiprotein platforms assembled in response to invading pathogens and other danger signals. Typically inflammasome complexes contain a sensor protein, an adaptor protein and a zymogen, procaspase-1. Formation of inflammasome assembly results in processing of inactive procaspase-1 into an active cysteine protease enzyme, caspase-1, which subsequently activates proinflammatory cytokines, IL-1 β and IL-18, and induces pyroptosis, a highly pyrogenic inflammatory form of cell death. Studies over the last year have unveiled exciting new players and regulatory pathways that are involved in traditional inflammasome signaling, some of them even challenging the existing dogma. This review outlines these new insights in inflammasome research and discusses areas that warrant further exploration.

Keywords

Inflammasomes; NLRs; ALRs; non-canonical inflammasome; caspase-1

Introduction

Inflammasomes play a central role in maintaining the sanctity of the cytosol. These multimeric complexes comprise a sensor protein belonging to the AIM2-like receptor (ALR) or the nucleotide-binding domain (NBD) and leucine-rich-repeat-(LRR)-containing (NLR) family, an adaptor protein, apoptosis-associated speck-like protein containing a CARD (ASC), and an inactive zymogen, procaspase-1 [1, 2]. Formation of inflammasomes in response to microbial or danger signals leads to cleavage of procaspase-1 into active

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caspase-1 enzyme, which further cleaves proforms of the inflammatory cytokines, IL-1 β and IL-18, into their active forms. Inflammasome activation also results in pyroptosis, an inflammatory form of cell death [2, 3].

Inflammasomes are essential components of host defense and they guard the host robustly from the assault of microbial pathogens and endogenous danger signals. Despite their cytosolic location, they are capable of launching an effective immune response against extracellular, vacuolar and intracellular bacteria, fungi, and viruses. Inflammasomes also sense crystalline substances such as silica and alum and endogenous danger signals such as ATP and mount appropriate immune responses [2, 4]. Although optimal inflammasome activation is highly beneficial to the well-being of the host, dysregulation of inflammasome activation can lead to exacerbation of symptoms in infectious diseases and development of autoimmune and inflammatory disorders [5]. Inflammasome research has witnessed outstanding advancements, and this review aims to summarize and update the reader of the key findings from the past few years.

Molecular mechanisms of inflammasome activation

With a few exceptions, members of the NLR family typically have a tripartite structure with a carboxy-terminal leucine-rich-repeat (LRR) domain, a central NACHT domain and an amino-terminal caspase recruitment and activation (CARD) or pyrin (PYD) domain [6]. In contrast, members of the ALR family have a PYD and up to two HIN-200 domains [7]. The HIN-200 domains directly bind to double stranded-DNA leading to the activation of ALRs [2]. Despite the NLRs being categorized as PRRs, no direct interaction between NLRs and their activating stimuli has been formally demonstrated. Once activated the NLRs and ALRs typically oligomerize through their NACHT and HIN-200 domains, respectively, resulting in recruitment of the adaptor protein, ASC, through interaction between the PYD of ASC and the PYD of the NLR/ALR [8]. Further, ASC recruits procaspase-1 into the complex via its CARD domain.

ASC is believed to function as a molecular platform for protein-protein interactions during inflammasome assembly and recent studies have shed light on the ultrastructure of these platforms in detail [9, 10]. These studies reported that activation of NLRP3 and AIM2 inflammasomes results in the interaction of their PYD with the PYD of ASC, an event that precipitates ASC prion nucleation. Subsequently, more ASC molecules are recruited into this structure and the ASC prion self-perpetuates leading to the generation of large stable filaments, which are necessary and sufficient for inflammasome activation. Importantly this ASC prion-like structure provides a platform for caspase-1 activation and subsequent cytokine processing. Together, these two landmark studies have revealed the existence of an assembly mechanism for ASC-dependent activation that unifies our understanding of the activation mechanisms of the NLR and ALR inflammasomes. Furthermore, recent studies have demonstrated that ASC prions also function as a cell-to-cell communication signal [11, 12]. Following inflammasome-induced cell death, ASC oligomers accumulate in the extracellular space and continue to process extracellular IL-1 β . Ingestion of ASC specks by macrophages results in lysosomal damage and IL-1 β production indicating their ability to act as a danger signal. Together, these studies point at a mechanism whereby inflammasome

triggers of minimal intensity can induce an immensely amplified response; the trigger activates relatively few sensor molecules, however, that is sufficient for polymerization of a large number of ASC-caspase-1 prions, which then through self-perpetuation and cell-to-cell propagation lead to a highly magnified inflammasome response [13].

Canonical inflammasomes: NLR and ALR inflammasomes

NLRs such as NLRP1b, NLRP3 and NLRC4 as well as the ALR, AIM2, constitute the most well characterized inflammasomes (Fig. 1) [2]. Among these, NLRP3 is still the most intensively investigated NLR, and it is activated by a vast array of microbial- and host-derived triggers. Conversely, NLRP1b is activated by anthrax lethal toxin and NLRC4 by bacterial type III secretion system components and flagellin. In contrast, AIM2 is activated by bacterial or viral double stranded DNA in the cytosol. Interestingly recent studies have identified novel pathways and triggers associated with these inflammasomes as well as novel inflammasomes formed by sensors such as a non-NLR protein, Pyrin, and NLRs such as NLRP6 and NLRP12 that have significant roles in microbial infections (Fig. 2). These advances are described in detail below.

NLRP1b inflammasome

NLRP1b, the first inflammasome to be characterized, is activated in a physiologically relevant manner only by a single signal, the anthrax lethal toxin (LeTx). LeTx is composed of protective antigen (PA) and lethal factor (LF). LF, a putative metalloprotease, has a zinc metalloprotease-like consensus sequence that is responsible for NLRP1b activation [14]. The presence of LeTx in the cytosol leads to the assembly of NLRP1b inflammasome, which is essential for defense against *B. anthracis* spores in mice [15, 16]. LeTx cleaves NLRP1b close to its N terminus, which is essential and sufficient for NLRP1b activation [17]. An interesting aspect of NLRP1b is that it contains a Function-to-Find domain (FIIND) and autoprocessing of NLRP1b within this FIIND is a prerequisite for its activation [18]. There have been several contrasting reports in recent years concerning the requirement of ASC in NLRP1b inflammasome activation [19–21]. However, using mice of two genetic backgrounds a recent study showed that while ASC mediates LeTx-driven caspase-1 autoproteolysis and speck formation these events are dispensable for activation of the NLRP1b inflammasome [22, 23]. Rather than autoproteolysis, ubiquitination of caspase-1 was associated with LeTx triggered NLRP1b activation independent of ASC. Consequently LeTx-induced caspase-1 activation, IL-1 β and pyroptosis proceed normally in ASC-deficient cells [22, 23].

NLRP3 inflammasome

NLRP3 still remains the best-studied inflammasome owing to its involvement in immunity to a multitude of bacterial, viral and fungal pathogens and its expanding roles in sterile inflammation and metabolic diseases such as Type 2 Diabetes. The exact mechanism by which these stimuli activate NLRP3 however remains unclear, but studies have revealed that NLRP3 can be activated through distinct canonical and non-canonical pathways [24]. We focus on the canonical mechanism here and discuss the noncanonical mode of activation in more detail later.

The canonical NLRP3 inflammasome is activated by Gram-positive bacteria such as *Staphylococcus aureus* and Group B *Streptococcus* viruses such as Influenza virus, pore-forming toxins such as hemolysin and pneumolysin as well as endogenous ligands and crystalline substances such as ATP, silica and alum. It is believed that rather than interacting with NLRP3 directly, NLRP3 activators induce one or more downstream cellular events or perturbations that lead to its activation [2, 3, 5]. The generally agreed upon events that activate NLRP3 include potassium efflux [25], generation of mitochondrial reactive oxygen species (ROS) [26], cathepsin release as a result of phagolysosomal membrane destabilization [27], release of mitochondrial DNA or cardiolipin [28–30], and translocation of NLRP3 to mitochondria through the adaptor molecule, MAVS [26, 31–33]. In addition, dsRNA-activated protein kinase, PKR, may also play a role in NLRP3 activation, but this remains controversial. One study reported that PKR was essential for activation of inflammasomes such as NLRP3 [34], however, further studies found no link between NLRP3 activation and PKR [35, 36]. Most recently, FADD and caspase-8 have been convincingly linked to NLRP3 inflammasome activation during both fungal infection and Gram-negative bacterial infection [39–42]. However, there has been dispute regarding the exact role of caspase-8 in NLRP3 activation in other scenarios. While one demonstrated a negative regulatory role for caspase-8 in NLRP3 activation in dendritic cells in response to LPS [43], another study showed that FADD and caspase-8 play a role in priming of the NLRP3 inflammasome [39]. Furthermore, CD36 has a role in NLRP3-mediated sterile inflammation. Specifically, CD36 nucleates soluble ligands such as oxidized low-density lipoprotein (LDL) into crystals or fibrils that promote NLRP3 inflammasome activation [44]. In addition, the deubiquitination of NLRP3 is essential for its activation [45–47]. The NLRP3 inflammasome also has a unique downstream effector function in Gram-positive bacterial infections; NLRP3-mediated caspase-1 activation regulates acidification of phagosomes by affecting buffering by NADPH oxidase, NOX2 [48].

In addition to the microbial and endogenous activators mentioned above, RNA and mitochondrial DNA are also activators of NLRP3 [30, 49, 50]. A recent study showed that RNA viruses induce formation of a complex of serine-threonine kinases RIP1 and RIP3. This complex initiates the activation and translocation of GTPase DRP1 to mitochondria resulting in mitochondrial damage and NLRP3 activation [51]. Recently, RNA:DNA hybrids have also been identified as a third class of nucleic acids that elicit NLRP3 activation during bacterial infection [36]. RNA:DNA hybrids represent bacterial replication intermediates. During infection with enterohemorrhagic *Escherichia coli* (EHEC), an extracellular bacteria that gets killed in the phagolysosome, both bacterial RNA and RNA:DNA hybrids gain access to the cytosol. The cytosolic RNA:DNA hybrids colocalize with active NLRP3 inflammasome specks and delivery of synthetic RNA:DNA hybrids into macrophages is sufficient to induce NLRP3-dependent caspase-1 and IL-1 β processing. Furthermore, cytosolic delivery of RNase H, which degrades RNA:DNA hybrids, abrogated EHEC-induced IL-1 β production [36]. These observations expand the collection of PAMPs that are associated with vitality of a microbe (vita-PAMPs) proposed in an earlier study [50]. While it is clear that these nucleic acids activate NLRP3, the question remains whether they interact with NLRP3 directly or induce its activation through intermediate nucleic acid binding proteins.

NLRP3-induced IL-1 β has also been suggested to have a role in the pathogenesis of Type 2 diabetes [37]. Recent studies have provided some mechanistic insights into this phenomenon. One study reported that oligomers of islet amyloid polypeptide (IAPP), a protein that forms amyloid deposits in the pancreas, induces NLRP3 inflammasome activation [37]. Another study showed that a protein associated with insulin resistance, thioredoxin (TRX)-interacting protein (TXNIP), binds to NLRP3 and contributes to its activation [38]. Further studies are required to define the specific players and signaling pathways involved in NLRP3 activation and type 2 diabetes.

NLRC4 inflammasome

Like NLRP1b, NLRC4 has a differential requirement for the adaptor protein, ASC; ASC is essential for NLRC4-mediated caspase-1 and IL-1 β processing, but is dispensable for NLRC4-mediated pyroptosis [22, 23]. NLRC4 is typically activated by a more streamlined set of ligands; bacterial flagellin and components of the bacterial type III secretion system. Unlike other inflammasomes, NLRC4 activation requires another NLR protein, NAIP, which functions as a receptor for the NLRC4 triggers. There are four NAIP proteins in C57BL/6 mice and among them, NAIP1 binds to needle proteins of the type III secretion system, NAIP2 binds to the *Salmonella* SPI-1 basal rod component PrgJ, and NAIP5 and NAIP6 sense flagellin [52–55]. In contrast, humans only express one NAIP protein, which binds the *Chromobacterium violaceum* needle protein, CprI [55]. This finding indicates that in the context of human infectious disease the role of NLRC4 may be specific for the type III secretion system components and it may not be important in mounting an inflammasome response against flagellin.

A recent study analyzed the molecular basis of ligand specificity of NAIPs using a panel of chimeric NAIPs [56], and found that the LRR domain was not involved in differential sensing of ligands. This finding was surprising because the LRR is traditionally considered the sensing component of NLRs. Instead, a region in the NBD containing several α -helical domains dictates ligand specificity. Ligand binding is required for co-oligomerization of NAIPs with NLRC4 and its subsequent activation [56]. Another factor that contributes to NLRC4 activation is the phosphorylation of the Ser⁵³³ residue in NLRC4. This phosphorylation is important in *Salmonella typhimurium* infection [57]; however, it is not clear whether this post-translational modification has a role in NLRC4 activation following NAIP-ligand binding.

NLRP12 inflammasome

Although earlier studies identified NLRP12 as an NLR with the capability of forming an ASC-associated inflammasome, infectious or endogenous triggers that induce this inflammasome were not defined. The exact role of NLRP12 in innate immunity also remained unclear as contrasting reports attributed both proinflammatory and anti-inflammatory properties to this NLR. Interestingly, *Yersinia pestis* infection induces NLRP12 inflammasome activation leading to production of IL-18, which is crucial for clearance of the infection [58]. Further, NLRP12 deficient mice are highly susceptible to *Y. pestis* infection. NLRP12 and NLRP3 inflammasome complexes are also present in monocytes of malaria patients, suggesting that along with the NLRP3 inflammasome, the

NLRP12 inflammasome may play a critical role in IL-1 β production and hypersensitivity to secondary bacterial infections following malaria [59]. Further understanding of the NLRP12 inflammasome in malaria and other infectious diseases is needed. Collectively, these studies establish a biologically relevant role for the NLRP12 inflammasome in innate immune responses against pathogens; however, the identity of the specific factor that is being sensed by NLRP12 remains to be known.

AIM2 and IFI16 inflammasomes

ALR inflammasomes are another class of inflammasomes that function to induce caspase-1 activation and IL-1 β cytokine maturation. However, unlike NLR inflammasomes, ALR inflammasomes directly bind their ligand, dsDNA, via HIN-200 domains. AIM2 and IFI16 do not contain CARD domains and hence require recruitment of ASC through their PYD for inflammasome activation [7]. AIM2 typically senses double stranded DNA in the cytosol from DNA viruses such as mouse cytomegalovirus and vaccinia virus as well as cytosolic bacteria such as *Francisella tularensis* and *Listeria monocytogenes* [60–62]. In contrast, IFI16, which is located in the nucleus, senses DNA from Kaposi's sarcoma-associated herpes virus (KSHV) [63]. AIM2 does not appear to differentiate cytosolic DNA based on its origin or sequence, but a minimum sequence length of 80 base pairs is required for effective AIM2 activation [2]. AIM2 has also been shown to contribute to the adjuvanticity of DNA vaccines [64] and is suggested to have a role in the development of autoimmune disorders such as systemic lupus erythematosus (SLE) through recognition of host DNA [65]. However, further studies are required to delineate the contribution of AIM2 in detecting endogenous DNA and in SLE pathogenesis.

Pyrin inflammasome

Pyrin is a non-NLR protein expressed primarily in immune cells such as monocytes and dendritic cells [66]. Pyrin contains an N-terminal PYD domain, two central B-box zinc-finger and coiled coil domains and a C-terminal B30.2/rfp/SPRY domain [67]. Pyrin has historically been associated with human autoinflammatory diseases as mutations in the gene encoding pyrin, *MEFV* cause familial Mediterranean fever [68]. Although Pyrin has also been shown to form an inflammasome complex with ASC and activate caspase-1 in in vitro reconstitution assays, the triggers that activate the Pyrin inflammasome and the biological relevance of this inflammasome in host immunity remained unknown [67, 69]. Interestingly, unlike other pattern recognition receptors that detect pathogen-associated molecular patterns (PAMPs), Pyrin is activated by pathogen-mediated modifications of host proteins, specifically Rho GTPases (Fig. 2) [66, 70]. A number of toxins produced by bacterial pathogens induce Rho GTPase modifications: Rho-glycosylation by cytotoxin TcdB of *Clostridium difficile*, FIC-domain adenylation by VopS and IbpA of *Vibrio parahaemolyticus* and *Histophilus somni* respectively, ADP-ribosylation by *C. botulinum* C3 toxin and deamination by *Burkholderia cenocepacia* (Fig. 2). Through a series of biochemical approaches and experiments with Pyrin deficient cells, the above-mentioned Rho-modifications are all detected by the Pyrin inflammasome [66]. It is important to note that all of these modifications occur in the switch-I residue of Rho subfamily members providing a common mechanism for their detection by Pyrin. Additionally, pertussis toxin induces the Pyrin inflammasome through its ADP-ribosyltransferase activity [70]. Pyrin

deficiency also leads to increased bacterial load in macrophages and decreased lung infection in *B. cenocepacia* infected mice pointing to an important role for the Pyrin inflammasome in immune responses against bacterial infections [71]. Together these studies have identified a novel inflammasome that senses pathogen-induced cellular changes and alerts the host to induce appropriate immune responses.

NLRP6 inflammasome

NLRP6 is a functionally unique inflammasome because it has downstream effects that are not typically associated with traditional inflammasome activation. A number of studies have shown that NLRP6 has a crucial role in maintaining intestinal health and homeostasis [72–74]. Deficiency of NLRP6 in mice results in alteration in the composition of colonic microbiota due to expansion of colitogenic bacterial phyla such as *Bacterioidetes* (*Prevotellaceae*) and TM7. This change in microbial ecology has important pathophysiological consequences such as development of spontaneous intestinal hyperplasia, inflammatory cell infiltration, and increased susceptibility to dextran sodium sulfate (DSS) colitis. Surprisingly, cohousing precipitated transmission of this aberrant microbiota from NLRP6 deficient mice to wild type mice resulting in a colitogenic phenotype in wild type [73]. Although it was found that NLRP6 regulates intestinal microbiota through IL-18, the exact mechanism by which NLRP6 deficiency modifies the intestinal microbial ecology remains unknown. A recent study provided further insights into the mechanisms of NLRP6 function in the gut [75], and showed that NLRP6 deficiency results in decreased autophagosome formation leading to impaired mucin granule exocytosis from goblet cells (Fig. 2). Defective mucus secretion compromises intestinal barrier integrity culminating in increased susceptibility to persistent infection. While this study presented compelling evidence for a link between inflammasome-mediated regulation of autophagy and maintenance of the intestinal mucus barrier, the mechanisms by which these events lead to an altered microbiota in NLRP6 deficient mice is still not clear. Another aspect that needs further investigation is the details of NLRP6 activation, specifically the identity of the triggers that induce NLRP6 inflammasome assembly in the intestine.

Non-canonical NLRP3 inflammasome activation by cytosolic LPS

Until recently, caspase-1 was considered the sole caspase responsible for inflammasome-dependent maturation of IL-1 β and IL-18. Caspase-11, an inflammatory caspase closely related to caspase-1, has since been implicated. Caspase-11 activation leads to pyroptotic cell death and the release of endogenous danger or alarmin molecules such as high mobility group box-1 (HMGB-1) in a caspase-1-independent fashion [76].

Unlike caspase-1, caspase-11 is dispensable for NLRP3-dependent caspase-1 activation by ATP, nigericin and other inflammasome ligands. Caspase-11 is activated in response to Gram-negative bacterial but not Gram-positive bacterial infections [77]. Using the 129 strain of mice, which naturally lack caspase-11, caspase-11 was found to be required for the activation of caspase-1 by *E. coli* and other enteric microbes. Consistent with this notion, the microbial trigger for caspase-11 activation was recently found to be the Gram-negative endotoxin lipopolysaccharide (LPS). However, the innate immune sensing of LPS that controls caspase-11 activation does not require any of the known components involved in

the recognition of LPS at the cell surface such as TLR4, MD2, and CD14. Instead, LPS must enter the cytosol to trigger caspase-11 [24, 78, 79]. The structural features of LPS that are required for the activation of this intracellular pathway and for TLR4-MD2 activation are similar. Hexaacylated lipid A represents the active moiety of LPS required for optimal activation in both cases. LPS from *Helicobacter pylori* and *Rhizobium galegae* that antagonize TLR4 activation also block cytosolic LPS sensing and caspase-11 activation. Nonetheless, a major difference in these pathways is that lipid IVa, a weak agonist of mouse TLR4, is unable to activate caspase-11 in the mouse [24, 78].

Normally, the recognition of microbial products such as bacterial flagellin and DNA in the cytosol by the innate immune system leads to the assembly of canonical inflammasomes and the recruitment of caspase-1, triggering the autocatalytic activity of caspase-1. However, caspase-11 activation seems to occur independent of all known inflammasomes including NLRP3, NLRC4, and NLRP6 [24, 76]. Recently, an alternative multiprotein platform was identified to assemble in cells exposed to cytosolic LPS [80]. Caspase-11 itself was found to be a direct receptor for cytosolic LPS (Fig. 3). Indeed, caspase-11 expressed in Sf21 insect cells purifies as a monomer, whereas that from *E. coli* purifies as an oligomer of higher molecular weight. This intriguing observation suggests that an *E. coli* derived product promotes caspase-11 oligomerization. Additional biochemical studies demonstrated that caspase-11 binds LPS directly, which in turn leads to its activation. Site-directed mutagenesis found that caspase-11 binding to LPS occurs via positively charged lysine residue motifs in its CARD domain [80]. This sensing of LPS by caspase-11 is reminiscent of recent evidence in the DNA sensing field, which found that the STING adapter protein functions as a receptor for cyclic-di nucleotides and is paradigm shifting for several reasons. First, caspases are not known to bind PAMPs. Second, the recruitment of caspases to an upstream molecular scaffold is widely believed to be a prerequisite for the activation of caspases. Classic examples for this idea include activation of caspase-1 by the inflammasome and caspase-9 activation by the apoptosome. However caspase-11 appears to be an exception to this rule. Direct binding of LPS by caspase-11 triggers its self-oligomerization and activation without a requirement for a preassembled molecular scaffold. It would be of interest to determine if this caspase-11 complex contains additional proteins. Since humans lack caspase-11, the relevance of this cytosolic LPS sensing pathway was unclear. However, a recent study suggests caspase-4 and -5 function as the human counterpart of caspase-11. These caspases directly bind LPS and trigger pyroptotic cell death similar to caspase-11 [80]. It appears that caspase-11 can substitute for caspase-4 and -5 in human cells and vice versa for the recognition of LPS and induction of pyroptosis. Caspase-4 is required for cytosolic LPS sensing and pyroptosis in human cells. Though caspase-5 can bind LPS, whether caspase-5 is required for cytosolic LPS sensing in humans remains unknown.

The molecular requirements for the activation of the caspase-11 pathway are just beginning to be unraveled. Earlier work from linked TRIF signaling and type I interferon production as prerequisites for caspase-11 dependent responses during infection with Gram-negative bacteria (Fig. 1) [77, 81, 82] [79, 83, 84]. Several independent studies have since confirmed key roles for type I interferons in pyroptosis induction and caspase-11 dependent caspase-1

activation in Gram-negative bacterial infections. Type I interferon seems to regulate caspase-11 by controlling its expression. Unlike other caspases, caspase-11 is expressed at very low levels under steady state conditions. Although NF- κ B can induce caspase-11 expression, type I interferon-mediated activation of STAT1-IRF9 is the predominant mechanism up-regulating caspase-11 expression in physiologically relevant conditions such as bacterial infection [77]. Recent in vivo findings also reinforce the importance of type I interferons for the caspase-11 pathway; pretreatment with an interferon inducing signal such as polyI:C, which triggers type I interferon production via TLR3 and/or MAVS, is required for caspase-11 activation in response to cytosolic LPS in TLR4-deficient mice [24, 78]. In addition to this priming mechanism, type I IFN modulates the caspase-11 pathway via its effects on guanylate binding proteins (GBPs), a family of type I IFN inducible proteins involved in host immunity and antimicrobial defenses (Fig. 3). The expression of GBPs in response to type I interferon signaling is essential for caspase-11 activation [85, 86]. It appears that the concerted action of more than one GBP is required to mediate optimal caspase-11 activation. GBPs have been reported to disrupt the pathogen containing vacuole thus enabling bacteria to enter the cytosol and activate caspase-11 [86]. However, one study claims that GBPs do not regulate the integrity of the pathogen containing vacuole but instead act downstream to activate caspase-11 [85]. The precise mechanism by which GBPs license the cytosolic LPS driven caspase-11 pathway remains to be fully solved.

While it is clear that caspase-11 coordinates cell death and caspase-1 maturation downstream of the NLRP3 inflammasome, exactly how caspase-11 mediates these events is still unclear. Caspase-11 interacts with caspase-1 during Gram-negative bacterial infections yet the assembly of the NLRP3-ASC complex appears to occur without the involvement of caspase-11. A cationic channel subunit transient receptor potential channel 1 (TRPC1) has recently been identified as a substrate for caspase-11 [87]. The activation of caspase-11 results in the degradation of TRPC1, which is required for the optimal release of active IL-1 β but not for the activation of caspase-1. TRPC1 deficient cells secrete elevated levels of IL-1 β compared to wild type cells not only in response to caspase-11 activators such as *E. coli* but also caspase-1 activators such as ATP suggesting that TRPC1 may also be a substrate of caspase-1 and be globally involved in inflammasome-dependent IL-1 β secretion. More studies are needed to identify the substrates of caspase-11 and understand how caspase-11 mediates its effector responses, particularly cell death.

Caspase-11 activation by cytosolic LPS sensing has profound biological implications. Caspase-11 deficient mice are significantly protected from the lethal effects of endotoxic shock. While the caspase-11 dependent activation of IL-1 cytokines appears to be dispensable for lethality in endotoxemia, how caspase-11 promotes the adverse host reaction culminating in lethality is poorly defined. In contrast, caspase-11 plays a protective role in systemic infections with *Burkholderia* spp, which may stem from its ability to induce pyroptosis rather than IL-1 β and IL-18 release [79]. These findings indicate that dysregulation of caspase-11 activation is detrimental for the host.

Concluding remarks

Interest in the inflammasome remains high. Important studies in recent years have clarified a number of mysteries that were prevalent in inflammasome research. However, as with all fields new discoveries also raise important new questions that remain to be answered (Box 1). New inflammasomes such as Pyrin and NLRP6 have been characterized, novel microbe-associated molecules such as RNA:DNA hybrids and cytosolic LPS that activate inflammasomes have been identified and novel signaling pathways integral to fine-tuning inflammasome responses (eg. ubiquitination) have emerged. Addressing the intricacies of these interesting proteins is likely to keep the research community engaged for many years to come.

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Box 1. Outstanding questions

- What is the exact mechanism by which NLRP3 is activated by diverse activators?
- Are there mechanisms to fine-tune or repress non-canonical inflammasome responses?
- What are the activators/ligands of NLRP6 and NLRP12 inflammasomes?
- How does caspase-11 activate caspase-1 and contribute to cell death?

Highlights

1. New inflammasomes and new microbial triggers of inflammasomes have been identified.
2. Cytosolic LPS is detected by caspase-11 resulting in its activation.
3. A novel pyrin inflammasome responds to pathogen-induced modifications of host proteins
4. Identification of a new NLRP6 pathway controlling mucus production.

Figure 1. Canonical inflammasomes

Canonical inflammasomes contain sensors belonging to the NLR or ALR family. NLRC4 is activated by bacterial flagellin and T3SS components, NLRP1b is activated by anthrax lethal toxin and AIM2 is activated by cytosolic dsDNA. NLRP3 is activated by a wide variety of signals including pore-forming cytotoxins, ATP, uric acid and alum. Once activated the receptors form an inflammasome complex with or without the adaptor, ASC, and recruits procaspase-1, which is subsequently cleaved into active caspase-1. Caspase-1 cleaves preforms of IL-1 β and IL-18 into their active forms as well as induces cell death.

Figure 2. Pyrin and NLRP6: newly characterized inflammasomes with atypical activators and downstream effects

Unlike classical inflammasomes Pyrin senses pathogen-induced alterations in cellular machinery such as modifications in Rho GTPases. The activator of NLRP6 is not yet identified. In addition to inducing caspase-1 activation and subsequent IL-1 cytokine production, NLRP6 inflammasome sustains intestinal homeostasis. NLRP6 regulates autophagosome formation, which is essential for mucin granule exocytosis from goblet cells and maintenance of intestinal barrier integrity.

Figure 3. Non-canonical inflammasome activation by cytosolic LPS

Gram-negative bacterial infections lead to the activation of TLR4-TRIF-type I IFN pathway as well as assembly of NLRP3 inflammasome. Type I IFN induces the expression caspase-11. Caspase-11 oligomerizes upon binding with cytosolic LPS from bacteria and this active caspase-11 controls NLRP3 inflammasome-dependent cleavage of caspase-1 through an unknown mechanism.