Brief Reviews

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Modulation of Inflammasome Pathways by Bacterial and Viral Pathogens

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Inflammasomes are emerging as key regulators of the host response against microbial pathogens. These cytosolic multiprotein complexes recruit and activate the cysteine protease caspase-1 when microbes invade sterile tissues or elicit cellular damage. Inflammasome-activated caspase-1 induces inflammation by cleaving the proinflammatory cytokines IL-1β and IL-18 into their biologically active forms and by releasing the alarmin HMGB1 into the extracellular milieu. Additionally, inflammasomes counter bacterial replication and clear infected immune cells through an inflammatory cell death program termed pyroptosis. As a countermeasure, bacterial and viral pathogens evolved virulence factors to antagonize inflammasome pathways. In this review, we discuss recent progress on how inflammasomes contribute to host defense against bacterial and viral pathogens, and we review how viruses and bacteria modulate inflammasome function to their benefit. ***The Journal of Immunology*****, 2011, 187: 597–602.**

Inflammasomes: caspase-1–activating platforms

The innate immune system relies on a limited set of germline-encoded pattern recognition receptors (PRRs) to detect and mount defensive responses against invading microbes (1). PRRs are expressed on a variety of host cells, among them epithelial cells in tissues with mucosal surfaces and immune cells of the myeloid lineage. PRRs comprise, but are not limited to, members of the TLRs, C-type lectin receptors, retinoic acid-inducible gene-I–like receptor, HIN-200 proteins, and nucleotide binding and oligomerization domain-like receptors (NLRs) (2–4). TLRs and C-type lectin receptors detect microbes on the cell surface and in endosomes, whereas retinoic acid-inducible gene-I–like receptors, HIN-200 proteins, and NLRs sense microbial components in intracellular compartments (3). PRR activation may result from direct binding of conserved microbial proteins, polysaccharide structures, and nucleic acids such as flagellin and components of the bacterial cell wall or viral envelope (3). Alternatively, PRRs may respond indirectly to invading pathogens by monitoring changes in the concentration or the subcellular localization of endogenous danger signals such as uric acid and HMGB1 as proxies for the tissue damage elicited by microbes (5). PRR activation rapidly targets invading pathogens and infected host cells for elimination through immune cell recruitment, phagocytosis, and autophagy. Moreover, PRRs induce transcriptional and posttranslational programs leading to the production of inflammatory mediators (3).

A subset of PRRs belonging to the NLR and HIN-200 families contributes to pathogen clearance by mediating activation of caspase-1. This conserved metazoan cysteine protease is produced as an inactive zymogen that is recruited and activated by cytosolic PRR-containing multiprotein complexes known as inflammasomes (6). Genetic studies in mice distinguish at least four inflammasomes of distinct composition, namely those containing the NLRs Nlp1b, Nlrp3, and Nlrc4 and a recently characterized inflammasome complex assembled around the HIN-200 protein absent in melanoma 2 (AIM2) (Fig. 1). Once activated, caspase-1 modulates inflammatory and host defense responses by processing the proinflammatory cytokines IL-1β and IL-18 into their biologically active forms, which is a perquisite for their secretion (7–10). These related cytokines mediate a wide variety of local and systemic immune responses to infection including the induction of fever, transmigration of leukocytes into sites of injury or infection, and activation and polarization of Th1, Th2, and Th17 responses (11–13). In addition to secreting IL-1β and IL-18, caspase-1 contributes to host defense through incompletely understood mechanisms such as unconventional protein secretion and an inflammatory cell death program known as pyroptosis that occurs in myeloid cells infected with bacterial pathogens such as *Salmonella typhimurium*, *Francisella tularensis*, and *Bacillus anthracis* (14–17).

Pathogen-specific activation of inflammasomes during bacterial, viral, and fungal infection

Intriguingly, inflammasome assembly and caspase-1 activation occur in a pathogen-specific manner (Fig. 1), although the

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Abbreviations used in this article, AIM2, absent in melanoma 2; CARD, caspase recruitment domain; CrmA, cytokine response modifier A; Exo, exoenzyme; KSHV, Kaposi's sarcoma-associated herpesvirus; NLR, nucleotide binding and oligomerization domain-like receptor; POP, pyrin-only protein; PRR, pattern recognition receptor.

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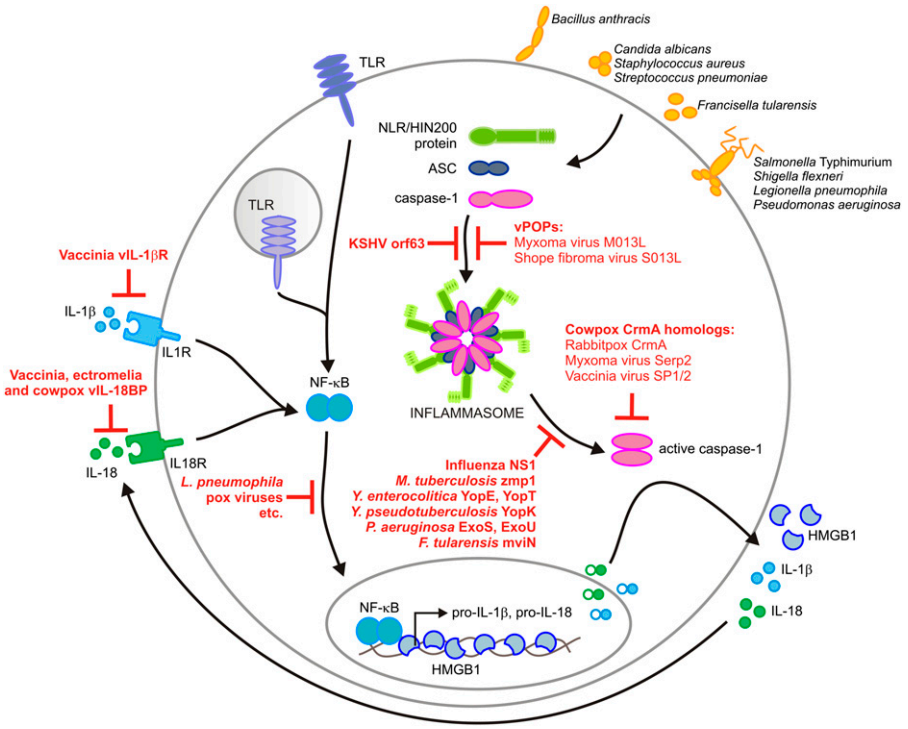

FIGURE 1. Modulation of inflammasome pathways by bacterial and viral effectors. Infection of macrophages and dendritic cells with bacterial, viral, and fungal pathogens induces assembly of inflammasome complexes. These multiprotein complexes drive the proximity-induced activation of caspase-1, which results in the extracellular release of IL-1β, IL-18, and HMGB1, as well as the induction of pyroptosis. Viral and bacterial pathogens have evolved a number of mechanisms (shown in red) to interfere with inflammasome assembly and activity. For example, inhibition of NF-κB translocation and transactivation may result in defective synthesis of Nlrp3 and the inflammasome substrates IL-1β and IL-18. Additionally, orthopoxvirus pyrin-only decoy proteins (vPOPs) scavenge the inflammasome adaptor ASC to prevent caspase-1 recruitment. Similarly, KSHV Orf63 encodes a viral Nlrp1 decoy protein that interacts with human Nlrp1 and Nlrp3 to prevent assembly of their respective inflammasomes. Viruses also target the enzymatic activity of caspase-1 directly with serpins such as CrmA and homologous protein produced by myxoma and vaccinia virus. Finally, they interfere with downstream activation of IL-1β and IL-18 receptors through scavenger receptors for secreted IL-1β (vIL-1βR) and IL-18. The N-terminal RNA-binding domain of influenza virus NS1 protein inhibits caspase-1 activation and secretion of IL-1β and IL-18 through an unknown mechanism. The *Yersinia* effector proteins YopE and YopT and the *Pseudomonas* virulence factor ExoS may prevent caspase-1 activation indirectly by interfering with Rho GTPase-mediated cytoskeletal changes, whereas *Yersinia* YopK masks the bacterial type III secretion system. *Pseudomonas* ExoU makes use of phospholipase A_2 activity to block caspase-1 activation in inflammasomes, whereas *Francisella* mviN prevents activation of the AIM2 inflammasome.

different inflammasomes may have redundant roles during infection (6, 18). The Nlrp1b inflammasome recognizes the cytosolic presence of the *B. anthracis* lethal toxin (19), and mutations in the *Nlrp1b* gene were identified as the key susceptibility locus for anthrax lethal toxin-induced macrophage death (16, 19). Notably, Nlrp1b inflammasome-induced pyroptosis confers resistance to infection with *B. anthracis* spores in vivo (16), highlighting the importance of pyroptosis for host defense against pathogens. Activation of the NLR family member Nlrp3 comprises a two-step process that requires priming with TLR and NLR ligands to enhance NF-κB–driven transcription of Nlrp3, followed by exposure of macrophages to microbial toxins and ionophores such as nigericin and maitotoxin, or endogenous alarmins such as ATP and uric acid to induce assembly of the Nlrp3 inflammasome (6, 20). Alternatively, the Nlrp3 priming and activation steps may be combined during infection of macrophages with bacterial, viral, and fungal pathogens such as *Staphylococcus aureus*, *Streptococcus pneumonia,* influenza virus, and *Candida albicans*, respectively (6, 20). Similar to the role of the Nlrp1b inflammasome in *B. anthracis* infection (16), defective activation of the Nlrp3 inflammasome renders mice hypersusceptible to candidiasis (21–23). The Nlrc4 inflammasome detects bacterial flagellin and the basal body rod component of bacterial types III and IV secretion systems of *Salmonella*, *Pseudomonas*, *Legionella*, and *Shigella* spp. (6, 24, 25). The Nlrp3 inflammasome also contributes to host defense during systemic *S. typhimurium* infection when flagellin expression is inhibited (18). In addition to the secretion of IL-1β and IL-18, the induction of pyroptotic cell death was recently established as a critical in vivo mechanism by which the Nlrc4 inflammasome clears flagellin-expressing bacteria such as *Legionella pneumophila* and *Burkholderia thailandensis* (15). Pyroptosis is thought to expose intracellular bacteria to extracellular immune surveillance, thus allowing their destruction by antimicrobial peptides, Igs, and the complement system, as well as their uptake by neutrophils and other immune cells. Finally, AIM2 responds to *F. tularensis, Listeria monocytogenes*, and certain DNA viruses such as CMV and vaccinia virus to induce caspase-1 activation (26–31). The increased susceptibility of caspase-1–deficient mice to infection with *F. tularensis*, the causative agent of tularaemia, illustrates the critical role the AIM2 inflammasome plays in host defense responses to microbial pathogens (32). Apart from AIM2, which appears to directly bind microbial dsDNA in the cytosol of infected immune cells, the molecular mechanisms leading to activation of the Nlrp1b, Nlrc4, and Nlrp3 inflammasomes are less clear. Sensing pathogens indirectly by

monitoring changes in the production or posttranslation modifications of a "secondary messenger" in infected host cells appears especially attractive to explain activation of the Nlrp3 inflammasome by a diverse set of microbial components. Although still debated, activation of this inflammasome may involve several mutually nonexclusive mechanisms including K^+ efflux, the generation of reactive oxygen species, lysosomal destabilization, and the translocation of microbial ligands into the host cytosol (20, 33).

Modulation of inflammasome activation by bacterial virulence factors

Given the importance of inflammasomes in controlling replication and dissemination of microbial pathogens, it is not surprising that bacteria have evolved a set of intriguing mechanisms to counter inflammasome assembly and to interfere with the induction of caspase-1 effector mechanisms (34). For instance, enteropathogenic *Yersinia enterocolitica* injects virulence factors called Yop proteins into the host cell cytosol through a dedicated type III secretion system. Among these effector proteins, YopE and YopT inhibit caspase-1 activation and the subsequent secretion of mature IL-1β (35). These Yop proteins are well-known negative regulators of Rho GTPases and Rho-mediated processes such as cytoskeletal reorganization and phagocytosis. YopE keeps Rho GTPase family members in the inactive GDP-bound state by increasing the rate of GTP hydrolysis. In contrast, YopT inactivates Rho GTPases by proteolytically removing the C-terminal prenyl membrane anchor by which they are attached to the plasma membrane. Although it is currently unclear how Yop effector proteins and cytoskeletal processes may interfere with inflammasome signaling, inactivation of the Rho GTPase Rac1 with dominant-negative proteins and chemical inhibitors suggested a critical role for this Rho GTPase in caspase-1 activation and IL-1β secretion (35). Nevertheless, the precise molecular events linking YopE and YopT to inflammasome inhibition await further analysis. Intriguingly, *Yersinia pseudotuberculosis* employs a third effector protein named YopK to mask the bacterial type III secretion system and to prevent its recognition by the Nlrp3 and Nlrc4 inflammasomes (36). This results in increased bacterial survival in host macrophages, illustrating the importance of inflammasomes in controlling intracellular proliferation of invasive *Yersinia* spp. *Pseudomonas aeruginosa* isolates expressing the virulence factor exoenzyme (Exo)U use a different strategy to inhibit caspase-1 activation in human phagocytes. This Gram-negative pathogen encodes an enzyme with phospholipase A_2 activity named ExoU that inhibits Nlrc4 inflammasome-driven secretion of IL-1β and IL-18 from infected macrophages (37). However, the precise molecular mechanism by which ExoU impacts inflammasome activation and its contribution to bacterial virulence in vivo remains to be determined. ExoS is another *Pseudomonas* virulence factor that interferes with inflammasome-induced IL-1β production. This effector protein inhibits caspase-1 activation through an incompletely characterized process that involves its ADP-ribosyl transferase activity (38). *L. pneumophila*, the causative agent of Legionnaire's disease, represents yet another example of how Gram-negative pathogens interfere with inflammasome signaling. The Nlrc4 inflammasome restricts *Legionella* growth in in vitro-cultured macrophages and in the lungs of infected mice (39, 40). This pathogen interferes with the transcription upregulation of the inflammasome adaptor ASC to safeguard its proliferation in human monocytes (41). *F. tularensis* makes use of the putative lipid II flippase *mviN* to dampen activation of the AIM2 inflammasome (42). Infection of mice with *mviN* mutant strains resulted in compromised in vivo virulence as a consequence of enhanced AIM2 inflammasome-mediated IL-1β secretion and macrophage pyroptosis (42). Also Gram-positive pathogens such as *Mycobacterium tuberculosis* have evolved mechanisms to interfere with inflammasome function. Inhibition of Nlrc4 inflammasome activation and IL-1β secretion by the putative Zn^{2+} metalloprotease named Zmp1 contributes to the ability of this causative agent of human tuberculosis to proliferate in myeloid cells (43). *Mycobacterium bovis* mutants lacking Zmp1 failed to prevent caspase-1 activation, which resulted in enhanced maturation of *Mycobacterium*-containing vacuoles and rapid mycobacterial clearance from infected macrophages and in the lungs of aerosol-infected mice (43). Further characterization of the mechanism by which Zmp1 inhibits caspase-1 activation may reveal currently unknown checkpoints controlling inflammasome activation.

Inhibition of inflammasome functions by viral pathogens

Caspase-1 inhibition by orthopoxvirus-encoded serpins. Modulation of inflammasome signaling is not limited to bacterial pathogens. In fact, viruses provide some of the best characterized mechanisms by which microbial pathogens hijack the inflammasome. Indeed, the cowpox virus protein cytokine response modifier A (CrmA) and its homologs in orthopoxviruses such as vaccinia, ectromelia, and rabbitpox virus directly target the enzymatic activity of caspase-1 (44–47). CrmA inhibits caspase-1 activity with an inhibition constant of 0.01 nM, rendering it one of the most effective caspase-1 inhibitors. This is accomplished by acting as a pseudosubstrate inhibitor of caspase-1, which entails the cleavage of CrmA in a first step that is followed by the formation of a permanent covalent bond with the active site cysteine of caspase-1 to render the protease inactive (44, 48, 49). Notably, CrmA has two homologs in rodents (50) and shares 54% amino acid identity with the human serpin PI-9 (51). The observation that PI-9 prevents caspase-1–mediated processing of IL-1β and IL-18 in vascular smooth muscle cells illustrated that this inhibitory mechanism is evolutionary conserved (52).

The importance of CrmA and other serpins in augmenting poxvirus virulence is demonstrated by the observation that deletion of CrmA attenuated virulence in intranasally and intracranially infected BALB/c and C57BL/6 mice (53–55). Moreover, CrmA deficiency reduced both the size and number of lesions on the chorioallantoic membrane of developing chick embryos (49, 54). Similarly, viral titers were dramatically reduced in rabbits infected with myxoma virus mutants lacking the CrmA homolog Serp2 (56). In contrast, deletion of the CrmA homologs SPI-1 and SPI-2 in vaccinia virus failed to affect virulence in intranasally infected BALB/c mice (57). This may be explained by the deployment of (partially) redundant effector mechanisms by which vaccinia virus interferes with inflammasome signaling. For example, the vaccinia genome encodes for a scavenger receptor named virus-encoded IL-1β receptor, of which the protein product neutralizes secreted IL-1β (58). Additionally, vaccinia, ectro-

melia, and cowpox viruses produce soluble IL-18–binding proteins that prevent cytokine-induced activation of the IL-18 receptor (59). Thus, orthopoxviruses increase virulence though the combined inhibitory action of serpins and scavenger receptors on caspase-1 activity and downstream inflammasome effectors, respectively.

Inhibition of inflammasome assembly by viral decoy proteins. In addition to directly targeting the enzymatic activity of caspase-1 and interfering with ligation of the IL-1 and IL-18 receptors, viruses deploy molecules that prevent inflammasome assembly altogether to further enhance viral virulence. This is best illustrated by the action of Kaposi's sarcoma-associated herpesvirus (KSHV) Orf63, a viral Nlrp1 homolog that lacks the pyrin and caspase recruitment domain (CARD) motifs found at the amino-terminal and carboxyl-terminal of human Nlrp1, respectively (60). KSHV Orf63 interacts with human Nlrp1 and Nlrp3 to prevent assembly of their respective inflammasomes and the subsequent induction of caspase-1–dependent innate immune responses. Transcriptional downregulation of KSHV Orf63 expression reduced the rate of viral replication as a consequence of enhanced Nlrp1- and Nlrp3-mediated IL-1β secretion and pyroptosis induction in KSHV-infected human monocytes and 293T cells (60). Pyrin-only proteins (POPs) such as myxoma virus M013L and Shope fibroma virus S013L represent another example of inflammasome inhibition by viral decoy proteins (61, 62). Viremia of myxoma virus mutants lacking the gene encoding M013L was dramatically reduced as a result of increased host inflammatory responses and attenuated viral replication, thus underscoring the importance of viral POPs during myxomatosis (61). The inhibitory mechanism used by these viral pyrin-only decoy molecules to inhibit inflammasome assembly is thought to resemble that employed by endogenous POPs (63, 64). In analogy to viral POPs, human cellular POP1 and cellular POP2 interact with the pyrin motif of the inflammasome adaptor ASC and with pyrin domains found in inflammasome-assembling NLRs and in HIN-200 member AIM2 to prevent ASC-mediated recruitment of caspase-1 in inflammasomes (63). Additionally, the human CARD-only proteins ICEBERG, COP, INCA, and $CASP12_S$ are thought to interfere with inflammasome assembly by scavenging caspase-1 through homotypic CARD interactions with the CARD motif in the prodomain of caspase-1 (65–69). Unlike viral POPs, however, viral counterparts of human CARD-only proteins remain to be identified.

Inhibition of inflammasome signaling by influenza virus. Interestingly, influenza virus uses a mechanism unrelated to those of the orthopoxviruses described above to prevent caspase-1 activation and interfere with inflammasome signaling. Mutant viruses of human influenza A/PR/8/34 (H1N1) in which the influenza NS1 gene was deleted triggered secretion of significantly increased levels of IL-1β and IL-18 from infected host cells and failed to prevent caspase-1 maturation in macrophages (70). These mutant viruses were attenuated in vitro, but how much caspase-1–dependent and –independent mechanisms contribute to this phenotype is not clear. Influenza NS1-driven inhibition of caspase-1 activation appears to rely solely on the N-terminal RNA binding/dimerization domain of NS1, whereas the carboxyl-terminal effector domain was dispensable for inhibition of IL-1β and IL-18 secretion (70). Further analysis of the molecular mechanism by which influenza NS1 inhibits caspase-1 activation may unveil interesting new mechanisms by which viruses target inflammasomes.

Conclusions

It is becoming clear that caspase-1 activation by inflammasomes contributes in a variety of ways to protective host responses against invading microbes. Inflammasome activation induces inflammation through secretion of IL-1β and IL-18, mediates the release of alarmins such as HMGB1, and triggers pyroptosis of infected host cells to eliminate the microbial pathogen. Consequently, recent studies showed that orthopoxviruses, influenza H1N1 virus, and a variety of bacterial pathogens all evolved a variety of strategies to interfere with inflammasome activation and downstream signaling cascades. Further understanding of the molecular mechanisms by which these and other microbes interfere with inflammasome pathways will undoubtedly shed light on new and intriguing mechanisms by which bacterial and viral pathogens aim to silence the inflammasome, and it may open up new windows of opportunity to develop much needed therapies against infectious agents.

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