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Review

The inflammasome: Learning from bacterial evasion strategies

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ABSTRACT

The innate immune system plays a critical role in defense against microbial infection and employs germline-encoded pattern recognition receptors to detect broadly conserved microbial structures or activities. Pattern recognition receptors of the nucleotide binding domain/leucine rich repeat (NLR) family respond to particular microbial products or disruption of cellular physiology, and mediate the activation of an arm of the innate immune response termed the inflammasome. Inflammasomes are multiprotein complexes that are inducibly assembled in response to the contamination of the host cell cytosol by microbial products. Individual NLRs sense the presence of their cognate stimuli, and initiate assembly of inflammasomes via the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and the effector pro-enzyme caspase-1. Inflammasome activation leads to rapid release of pro-inflammatory mediators of the IL-1 family as well as the release of intracellular alarmins due to a lytic form of programmed cell death termed pyroptosis. Over the past 15 years, a great deal has been learned about the mechanisms that drive inflammasome activation in response to infection by diverse pathogens. However, pathogens have also evolved mechanisms to evade or suppress host defenses, and the mechanisms by which pathogens evade inflammasome activation are not well-understood. Here, we will discuss emerging evidence on how diverse pathogens evade inflammasome activation, and what these studies have revealed about inflammasome biology. Deeper understanding of pathogen evasion of inflammasome activation has the potential to lead to development of novel classes of immunomodulatory factors that could be used in the context of human inflammatory diseases.
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1. Introduction

Pattern recognition receptors (PRRs) detect conserved microbial features and initiate innate immune responses to control infection [1,2]. Membrane-bound PRRs such as Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) detect structural features of both pathogenic and non-pathogenic bacteria in the extracellular space or within endosomes [3]. In contrast, members of the nucleotide binding domain/leucine rich repeat (NLR) family of PRRs reside within the cytosol. NLRs are therefore poised to detect the presence of microbial pathogens, which violate the sanctity of the cytosol by delivering microbial proteins, nucleic acids, metabolites, or other products into the target host cell through the activity of microbial virulence factors such as pore-forming toxins or microbial secretion systems [4–6]. In contrast to TLRs and CLRs, which mediate transcriptional responses through activation of NF-κB and MAPK cascades, a subset of NLRs mediate assembly of multiprotein complexes termed inflammasomes, which regulate post-translational processing of IL-1 family cytokines and a pro-inflammatory cell death known as pyroptosis. Thus, while specialized secretion systems or pore-forming toxins enable microbes to colonize host niches, these systems unavoidably expose microbes to innate immune detection, thereby creating a requirement to suppress or evade this additional level of innate host defense.

Engagement of specific NLRs by their cognate stimuli leads to inflammasome assembly and the recruitment of the adaptor protein ASC and pro-caspase-1 [6–9]. The pro-caspase-1 zymogen is generally activated by proximity-induced cleavage to generate an active enzyme, but can also be active in the absence of cleavage, depending on the particular NLR complex involved in caspase-1 recruitment. ASC contains N-terminal Pyrin and C-terminal CARD domains, allowing it to serve as a bridge between NLR-pyrin domain-containing NLRs (NLRPs) and caspase-1, and to contribute to inflammasomes containing caspase-1 and NLR-CARD-containing

Abbreviations: CARD, caspase recruitment domain; ASC, apoptosis-associated speck-like protein containing a CARD; NLR, nucleotide binding domain/leucine rich repeat; NLRP, NLR with pyrin domain; NLRC, NLR with CARD domain; MAPK, mitogen activated protein kinase; TRIF, TIR-domain containing adaptor inducing beta-interferon; TCA, tricarboxylic acid.

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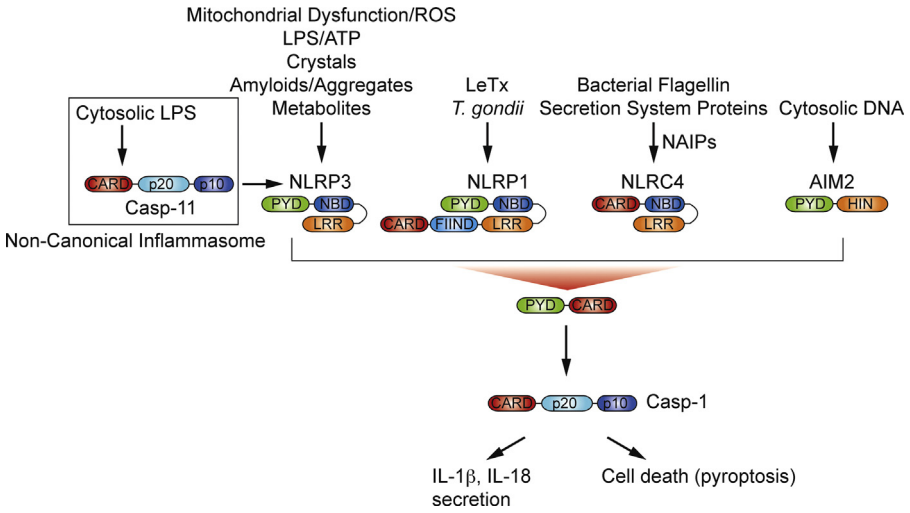

Fig. 1. Inflammasome components and stimuli. Individual NLR proteins and the ALR protein AIM2 are depicted with individual domains indicated. PYD – pyrin domain; CARD – caspase recruitment domain; NBD – nucleotide binding domain; LRR – leucine rich repeat domain; HIN – HIN200 domain; FIIND – function to find domain; p20, p10 – subunits of cleaved active caspase-1 and -11. Cytosolic LPS activates a non-canonical inflammasome involving caspase-11, which potentiates NLRP3-dependent caspase-1 activation.

NLRs (NLRCs). Fig. 1 describes our current understanding of the main inflammasome components and their interactions. Recent structural analyses of the NLRP3 inflammasome revealed that it assembles into a large filamentous structure, in which an initial nucleation of ASC, NLRP3, and caspase-1 induced the extension of multiple long caspase-1 filaments from a central structure [10]. Caspase-1 activation presumably occurs in these large filaments, and interference either with the initial nucleation of this structure or with the polymerization of these filaments may be a potential mechanism to negatively regulate inflammasome activation. Below, we briefly describe the most extensively studied inflammasomes and discuss our current knowledge of the mechanisms by which pathogens evade or suppress their activation, with a focus primarily on bacterial pathogens.

2. Mechanisms of inflammasome activation by NLRs

2.1. Induction of the NLRP3 inflammasome by diverse stimuli that induce mitochondrial dysfunction

Distinct NLRs are thought to mediate assembly of individual inflammasomes in response to specific stimuli. The NLRP3 inflammasome is triggered in response to a wide range of stimuli of both host and foreign origin. These include: a variety of organic and inorganic crystals; extracellular ATP; RNA; bacterial pore-forming toxins or secretion systems; and a variety of stimuli that disrupt mitochondrial membrane polarization and dynamics [11–20]. While mitochondrial ROS is important for NLRP3 inflammasome activation in response to many of these stimuli, certain stimuli can activate NLRP3 inflammasome without inducing mitochondrial ROS per se, and mitochondrial ROS alone is insufficient to induce NLRP3 inflammasome activation [21]. Potassium efflux is a key step in inducing NLRP3 inflammasome assembly [13,17,22], but how potassium efflux is triggered and how this leads to NLRP3 oligomerization remains a key question. The precise ligand or trigger of NLRP3 inflammasome activation remains undefined, but the finding that mitochondrial cardiolipin, which is normally present in the inner leaflet of the mitochondrial outer membrane, binds NLRP3 and induces NLRP3 inflammasome activation, provides an important link between mitochondrial dysfunction and NLRP3 activation [21].

2.2. Activation of NLRC4 inflammasomes by NAIP-mediated sensing of flagellin and related proteins

Whereas NLRP3 senses structurally diverse stimuli of both microbial and host origin, NLRC4 participates in inflammasome assembly triggered by cytosol bacterial flagellin or structurally related proteins from bacterial type III secretion systems (T3SS) [23–31]. Importantly, discrimination between flagellin and its structural analogs is made by members of the NLR apoptosis inhibitory protein (NAIP) subfamily of NLRs [32,33]. Thus, NLRC4 acts more analogously to an adaptor protein that is necessary for caspase-1 activation in response to sensing of flagellin by NAIP5 and 6, sensing of the T3SS inner rod by NAIP2, and sensing of the T3SS needle by NAIP1 [30–36]. Interestingly, processing of caspase-1 in NLRC4-containing complexes is not absolutely required for all caspase-1-dependent effector functions. While ASC is absolutely required for caspase-1-dependent functions downstream of NLRP3 inflammasome activation, ASC and caspase-1 cleavage are dispensable for induction of caspase-1-dependent pyroptosis in response to flagellin [37]. Whether other stimuli that engage NLRC4 also function similarly is currently unknown. Moreover, potential differences in the structures of NAIP-NLRC4-caspase-1 and NAIP-NLRC4-ASC-caspase-1 complexes, and how such complexes might differ from NLRP3-containing inflammasomes remains to be defined.

2.3. Activation of NLRP1 inflammasomes in response to lethal toxin and Toxoplasma infection

NLRP1 responds to the presence of *Bacillus anthracis* lethal toxin (LeTx), as well as to infection by the apicomplexan parasite *Toxoplasma gondii* [38–41]. NLRP1 possesses an N-terminal pyrin domain, and a C-terminal CARD, suggesting that NLRP1 may interact with caspase-1 directly via its CARD, or indirectly via ASC and the N-terminal pyrin domain. In contrast to other NLRs, NLRP1b-mediated sensing of LeTx involves cleavage of NLRP1b at its amino terminus [42,43]. Precisely how this cleavage enables NLRP1b inflammasome activation remains to be determined, but presumably this cleavage releases NLRP1b from an autoinhibited state and enables its oligomerization. Similarly to NLRC4, NLRP1b is capable of mediating caspase-1 activation even in the absence of caspase-1 autoprocessing [44]. However, in contrast to

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NLRC4-mediated activation of non-cleavable caspase-1, which can trigger pyroptosis but not IL-1β secretion, noncleavable caspase-1 could induce processing and release of IL-1β as well as pyroptosis in response to LeTx, even in the absence of ASC [37,44]. Different inflammasome activators and NLRs may therefore engage different functions of caspase-1, which perhaps occur in distinct subcellular compartments or domains. Variants in Nlrp1b among inbred mouse strains control the sensitivity of murine cells to LeTx-induced inflammasome activation and pyroptosis, and consequently the susceptibility to infection by *B. anthracis* [38]. Similarly, polymorphisms in NLRP1 in humans, and strain-specific differences in the rat ortholog NLRP1a have recently been linked to differences in susceptibility to toxoplasmosis [39,45]. Whether *T. gondii* is sensed by the NLRP1 inflammasome in a manner that also involves proteolytic processing of NLRP1, and whether caspase-1 autoprocessing is also dispensable for *T. gondii*-induced pyroptosis and IL-1β secretion remains to be determined.

2.4. A non-canonical inflammasome activates caspase-11 in response to Gram-negative bacterial pathogens

While initial studies of inflammasome activation focused on caspase-1 as the key effector enzyme of inflammasome activation, it was recently found that caspase-11 is activated in a non-canonical inflammasome that also contributes to pyroptosis and release of IL-1α specifically in response to Gram-negative bacteria [46]. This non-canonical inflammasome is primed by signaling through the TLR adaptor TRIF and subsequent type I IFN signaling [47–49]. The capacity of pathogens to access the cytosol is an important feature of non-canonical inflammasome activation [50–52]; notably, cytoplasmic LPS [47,48,49] is a key trigger for activation of caspase-11 [53,54]. Cytosolic access can be mediated by virulence-associated secretion systems in multiple bacterial pathogens [51], or by host factors, such as guanylate binding proteins (GBPs) that specifically permeabilize bacteria-containing vacuoles and release bacterial LPS into the cytosol [55,56]. Recognition of cytosolic LPS appears to involve direct binding of LPS to the CARD of caspase-11 as well as the human orthologs caspase-4 and -5 [57]. Importantly, caspase-11 is critical for defense against cytosolic bacterial pathogens, but also mediates endotoxic shock [53,54,58,59].

3. Suppression of inflammasome activation

A substantial amount of effort has been spent in identifying inflammasome activators of pathogens, and in defining the molecular mechanisms by which these activators induce inflammasome assembly (reviewed in Ref. [60]). Nevertheless, it has also become clear that like other host immune defenses, the inflammasome system imposes a selection pressure on microorganisms to develop avoidance or suppression mechanisms [61]. Below, we discuss several examples of microbial modulation of inflammasome responses. Our purpose here is not to describe exhaustively every instance of pathogen inhibition of inflammasomes, but rather to illustrate, using several key examples, two broad microbial strategies for inflammasome evasion – active suppression of inflammasome activation, and avoidance of inflammasome activation. These examples are summarized in Fig. 2, which highlights those mechanisms that fall into the category of inhibition and those that belong to the strategy of evasion. These broad microbial strategies to modulate inflammasome responses are an integral part of the interaction between eukaryotic hosts and microbial organisms that colonize them, and likely play an important role in shaping the outcome of the host-microbe conversation.

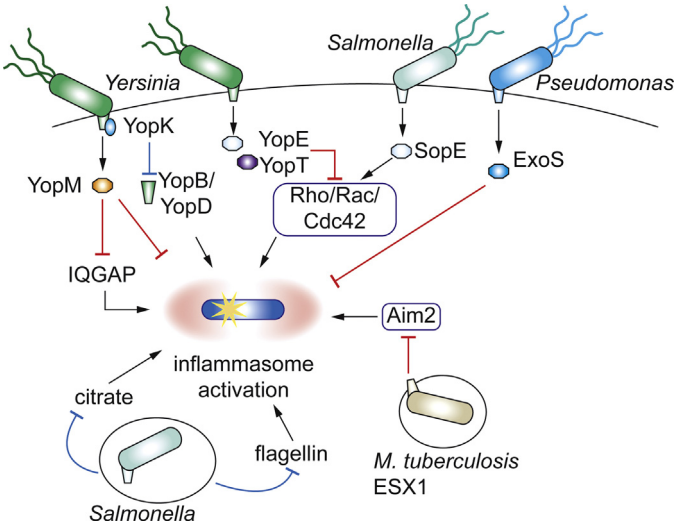

Fig. 2. Modulation of inflammasome activation by bacterial pathogens. Red pathways indicate active inhibition of inflammasome; blue pathways indicate mechanisms that prevent inflammasome activation by limitting levels of inflammasome triggers. *Yersinia* spp. utilize both mechanisms to interfere with inflammasome activation: YopM inhibits inflammasome activation by binding to IQGAP1 as well as to caspase-1 itself. Conversely, YopK prevents inflammasome activation by limiting injection of translocon proteins YopB and YopD. The Rho GAP protein YopE and the cysteine protease YopT both inhibit the activation of Rho family GTPases, which is critical for blockade of inflammasome activation. The Salmonella translocated effector SopE is a Rho-GEF, which enhances activity of small Rho GTPases, and therefore has the opposite effect on inflammasome actvation. Pseudomonas ExoS is a phospholipase whose activity is required to inhibit caspase-1 processing and IL-1b secretion. Although ExoS blocks caspase-1 activation and IL-1b secretion, this induces an undefined pathway of caspase-1 independent death. The *M. tuberculosis* ESX1 system inhibits AIM2 activation in response to avirulent Mycobacteria, while Salmonella enterica ser. Typhimurium evades inflammasome activation by down-regulating flagellin expression during systemic infection and limiting production of bacterial citrate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.1. Phospholipase activity and inflammasome activation

Among the earliest reports of inflammasome suppression by a specific bacterial effector is the *Pseudomonas aeruginosa* ExoU effector protein, which possesses phospholipase A2 activity [29]. ExoU phospholipase activity was found to inhibit caspase-1 processing in response to *Pseudomonas* infection. Interestingly, although ExoU inhibited caspase-1 processing and IL-1β secretion, cell death was unaffected or perhaps even slightly elevated. While cell death in response to *exoU* mutant *Pseudomonas* required NLRC4, (and was therefore likely pyroptosis), cell death in macrophages infected by wild-type *Pseudomonas* was NLRC4-independent, suggesting that it occurs in a manner that is independent of the inflammasome, potentially via programmed necrosis. This may be a common cellular response to inappropriate or unscheduled phospholipase activity, as PLA2 is also a major component of insect and snake venoms responsible for potent cytotoxicity and allergen-inducing activity [62]. Whether ExoU inhibits recruitment of caspase-1 to inflammasome complexes or modifies inflammasome components such as caspase-1 remains to be determined.

3.2. Modulators of actin cytoskeleton dynamics interfere with inflammasome activation

In addition to ExoU, *Pseudomonas* ExoS, a Rho GTPase-activating protein, interferes with caspase-1-dependent IL-1β secretion [63]. Intriguingly, the *Yersinia* effectors YopE and YopT, which inhibit Rho GTPase activity, also prevent caspase-1 activation [64]. The GAP activity of YopE was necessary to inhibit caspase-1

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oligomerization, and furthermore, dominant negative Rac1 inhibited ASC-dependent caspase-1 oligomerization [64]. These findings imply that stimulating the GTPase activity of small Rho GTPases that regulate actin polymerization prevents inflammasome activation. Conversely, the *Salmonella*-secreted effector protein SopE, which is a guanine nucleotide exchange factor for Rho GTPases and therefore promotes actin cytoskeleton rearrangements, induces inflammasome activation in a manner dependent on its enzymatic activity [65,66]. This activity appears to contribute not only to the ability of *Salmonella* to invade epithelial cells [67,68], but also to promote inflammation during the intestinal phase of *Salmonella* infection, which is important for the ability of *Salmonella* to compete with endogenous microbiota [69,70]. Collectively, these data suggest that the actin cytoskeleton is a central node in the regulation of inflammasome activation. Precisely how altering Rho GTPase function regulates inflammasome activation is unclear, and the NLR protein(s) involved in mediating inflammasome activation downstream of SopE are also unknown. Interestingly, cytochalasin D, an inhibitor of actin polymerization, inhibits inflammasome activation by sodium urate crystals, a finding that has been interpreted to indicate that phagocytosis and subsequent phagosome disruption is necessary for crystals to induce inflammasome activation [71]. Nevertheless, the finding that modulation of Rho GTPase activity impacts inflammasome activation suggests that the actin cytoskeleton may play a direct role in recruitment of assembly of inflammasome complexes, and targeting actin cytoskeleton dynamics may be a general mechanism by which pathogen effectors (or other inhibitors) modulate inflammasome activation.

Interestingly, SopE activity also triggers transcriptional activation of inflammatory responses in epithelial cells [72,73]. SopE induces MAPK and NFκB activation, as well as activation of gene expression, in a manner involving the NLR protein NOD1 and the Rho GTPases Rho, Rac1 and Cdc42 [72]. Intriguingly, this activation is independent of the RIPK2 signaling adaptor, which is required for inflammatory gene expression in response to NOD1-mediated sensing of microbial cell wall components in the cytosol [72]. In the setting of Rho GTPase activation, NOD1 formed a complex with SopE, Rac1, Cdc42, and Hsp90 in transfected HEK293 cells [73]. It is conceivable that NOD1 participates in inflammasome activation in response to SopE activity as well, although since NOD1 is not known to induce inflammasome activation on its own, another NLR could be involved.

3.3. *Inhibition by* Yersinia *YopM*

The YopM protein of pathogenic *Yersinia* species was recently reported to be a direct inhibitor of caspase-1 activation [74]. YopM is a member of a family of *Leucine Rich Repeat* (LRR)-containing virulence factors present in a number of pathogenic bacteria [75], and plays an important role in *Yersinia* virulence [76,77]. YopM was reported to inhibit recruitment of caspase-1 to ASC foci by binding to caspase-1 via a YLTD motif in the LRR domain that acts as a pseudo-substrate for caspase-1 [74]. However, YopM is highly polymorphic among strains of *Yersinia* [78] and a number of different YopM isoforms from *Yersinia pseudotuberculosis* and *Y. pestis* strains inhibit caspase-1 activation, but do not contain the YLTD motif [79]. YopM from *Y. pestis* strain KIM (which contains the YLTD motif) bound caspase-1, whereas YopM from *Y. pseudotuberculosis* strain 32777 did not, even though YopM from both strains inhibited caspase-1 activation [79]. Nevertheless, mutation of the key aspartate residue in the YLTD motif of $YopM^{KIM}$ did not affect its ability to inhibit caspase-1 activation. Interestingly, $YopM^{KIM}$ co-immunoprecipitated with IQGAP1, a Ras-GTPase-activating like protein, in *Yersinia*-infected cells, and IQGAP1-deficient cells exhibited a significant defect in *Yersinia*-induced inflammasome activation [79]. The YLTD motif may be dispensable for inhibition of caspase-1, or YopM isoforms lacking the YLTD motif may have an independent mechanism of inhibiting inflammasome activation. YopM may therefore act by binding to and inactivating a key regulator of caspase-1 activation. It is interesting to note that IQGAP1 is a scaffolding protein that has homology to GTPase activating proteins, and is involved in coordinating actin cytoskeleton rearrangements with cell cycle progression and dendritic cell migration [80,81]. Interestingly, YopM inhibits *Yersinia*-induced inflammasome activation preferentially in macrophages that have previously been activated by bacterial PAMPs [74,79]; in unprimed cells, YopJ-driven blockade of NF-κB and MAPK signaling induces caspase-1 activation via a newly described pathway involving the cell extrinsic death machinery [82,83]. The degree to which heterogeneity of the macrophage activation state impacts the overall level of inflammasome inhibition within tissues over the course of *Yersinia* infection remains to be further investigated.

3.4. *Inhibition of AIM2 by virulent* Mycobacterium tuberculosis

The Absent In Melanoma 2 protein (AIM2), which contains a pyrin domain and a HIN200-family DNA binding domain, induces inflammasome activation in response to cytosolic DNA in the context of a variety of bacterial and viral infections [84–91]. Avirulent Mycobacteria, such as *M. smegmatis*, induce both NLRP3- and AIM2-dependent caspase-1 activation and IL-1β secretion. However, co-infection with *M. smegmatis* and virulent *M. tuberculosis*, results in dramatically lower AIM2 inflammasome activation than in cells singly infected with *M. smegmatis* [92]. This *in trans* Inhibition of AIM2 requires the *M. tuberculosis* ESX-1 secretion system, suggesting that an ESX-1-secreted effector protein modulates AIM2 inflammasome activation in response to *M. tuberculosis* infection [92]. However, as with other bacteria secretion systems, ESX-1 itself also induces inflammasome activation [93–95], raising the question of how a system can both activate and inhibit the same pathway. As noted above, host sensing of conserved virulence activities such as pore formation and injection or release of microbe-derived products, including nucleic acids, into the cytosol, creates selection pressure for microbial pathogens to develop evasion mechanisms. In the case of the *M. tuberculosis* ESX-1 system, the effector(s) that mediate suppression of AIM2 inflammasome activation remain to be determined

3.5. *Viral inhibitors of inflammasome activation*

Inflammasome activation and pyroptosis provide an important avenue of host defense against viral infection by eliminating a cellular niche for viral replication and simultaneously releasing important anti-viral inflammatory cytokines. Accordingly, viruses have developed mechanisms to interfere with this arm of the innate immune response. Mammalian CARD-only and pyrin-only proteins that contain a single CARD or PYD domain are endogenous negative regulators that inhibit inflammasome activation by interfering with homophilic pyrin–pyrin or CARD–CARD interactions between inflammasome components [96]. Similarly, the myxoma virus encodes a pyrin domain-only protein, M013, which plays an essential role in virulence. This protein interferes with NLRP3 inflammasome activation by binding to ASC, and also interferes with other inflammatory responses by blocking NF-κB signaling [97,98]. Interestingly, the Kaposi's sarcoma-associated herpes virus Orf63 protein was recently identified as a viral homolog and inhibitor of NLRP1 [99]. Orf63 contains an NBD-LRR region, but does not contain a pyrin or CARD domain. Orf63 interacted with NLRP1 in a co-IP assay in THP1 cells, and prevented induction of IL-1β secretion in response to viral infection [99]. Moreover, THP-1 monocytic cells expressing Orf63 were also defective in activation of the NLRP3

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inflammasome in response to ATP or alum. Intriguingly, although ORF63 does not contain a pyrin or CARD, the NBD-LRR was able to interact with the NBD of NLRP1 and NLRP3, thus likely inhibiting the ability of these NLRs to oligomerize and nucleate an inflammasome complex. The viral triggers of inflammasome activation in these cases remain unknown, but could potentially involve a mechanism analogous to the M2 ion channel of influenza, which induces NLRP3 inflammasome activation [100].

3.6. Suppression of non-canonical inflammasome activation by Shigella

As in the case of other inflammasome pathways, the non-canonical inflammasome can also be subverted. While *Shigella flexneri* appears to cause NLRP3 inflammasome activation in macrophages, *S. flexneri* OspC3, a secreted effector of the *Shigella* T3SS, was found to bind to the p19 subunit of caspase-4, the human ortholog of caspase-11, and inhibit the assembly of the active form of the enzyme [101]. The basis for this binding is a motif, LSTD, in an exposed region of OspC3, a mechanism similar to that proposed for YopM inhibition of caspase-1 [74]. OspC3 binding to the p19 subunit of caspase-4 was highly specific, as it did not bind to mouse caspase-11, nor did it bind to human caspase-1. Additional inhibitors of non-canonical inflammasome activation likely also exist among other pathogens, and may function analogously to OspC3 and directly inhibit activation or assembly of active caspase-11. Alternatively, inhibition of the non-canonical inflammasome could be accomplished by altering the structure of LPS, a 'stealth'-based evasion mechanism discussed further below.

4. Avoidance of inflammasome activation

In contrast to the active inhibition mechanism described above, an alternative strategy is to limit or minimize the availability of inflammasome-triggering stimuli. This could occur by dynamic regulation of gene expression either in a temporal or spatial manner in accordance with the lifecycle of the pathogen; thus, a trigger of inflammasome activation may be expressed at one stage of infection, but not another. Alternatively, pathogens may modify the structure of an inflammasome-activating product; for example, the caspase-11-driven non-canonical inflammasome detects cytosolic hexa-acylated lipopolysaccharide from Gram-negative bacteria. However, a number of bacterial pathogens, including *Yersinia pestis* modify their LPS within the mammalian host such that it is tetra-acylated. This is a well-established strategy that enables *Yersinia* to avoid being recognized by TLR4 [102–104] (also discussed further in this issue of *Seminars in Immunology* by Sellge and Kufer). However, tetra-acylated LPS is an antagonist of the non-canonical inflammasome, as transfection of tetra-acylated LPS inhibits non-canonical inflammasome activation [53,54,57]. It is therefore possible that *Y. pestis*, as well as other Gram-negative bacteria that generate tetra-acylated LPS during infection, evade the non-canonical inflammasome activation by modifying the structure of their LPS. *Y. pestis* may therefore utilize LPS modification as a multi-pronged evasion strategy to evade both TLR4 and non-canonical inflammasome signaling. Below, we describe several examples of this 'stealth' approach for evading inflammasome activation that masks the presence of pathogen from the host.

4.1. Flagellin downregulation during systemic infection

During invasion of target cells, both *Salmonella* and *Legionella* express high levels of flagellin [105–107], and some of this flagellin is injected into target host cells by their respective secretion systems [23,26–28]. Notably, while *Salmonella* expresses high levels of flagellin and the pathogenicity island 1 (SPI-1) T3SS during intestinal infection, flagellin expression is repressed within systemic sites [108,109]. It is likely that the combined expression of SopE (briefly discussed above) as well as flagellin during the intestinal phase of infection promotes inflammasome activation. Indeed, intestinal resident macrophages express high levels of pro-IL1β and undergo rapid inflammasome activation in response to infection by invasive flagellin-expressing *Salmonella* [110]. Notably, forced expression of flagellin during the systemic phase of infection resulted in rapid NLRC4-dependent bacterial clearance, suggesting that downregulating flagellin enables *Salmonella* to evade immune clearance [111]. NLRC4-mediated pyroptosis resulted in externalization of bacteria that were then rapidly phagocytosed and killed by recruited neutrophils. In contrast to *Salmonella*, expression of flagellin by *Y. pseudotuberculosis* is inversely correlated with expression of its T3SS [112]. In *Y. pestis*, the flagellin subunit is a pseudogene [113]. Thus, downregulation of flagellin may be a general mechanism to avoid triggering NLRC4. Interestingly, *Salmonella* downregulates expression of NLRC4 in B cells, providing an example of a possible mechanism used to limit pathogen detection by downregulating a key component of the host sensing machinery [114]. Similarly, *Legionella pneumophila* has been suggested to downregulate the inflammasome components ASC and NLRC4 in human monocytes [115]. How the transcriptional regulation of NLRC4 limits rapid inflammasome activation that takes place upon flagellin sensing, and the specific mechanisms by which these bacteria downregulate expression of inflammasome components remain to be determined. Importantly, the host has multiple mechanisms of sensing secretion system activity – the T3SS inner rod and needle subunits themselves are injected and can also induce activation of the NLRC4 inflammasome. Additionally, sensing of T3SS-mediated pore formation or translocation activity also appears to be a signal for activation of the NLRP3 inflammasome, and is therefore another target of pathogen evasion strategies.

4.2. Evasion of inflammasome triggering by the T3SS translocon

In addition to proteins that comprise the external structure of the T3SS needle or rod structure, T3SS activities also have the potential to trigger inflammasome activation. Pathogenic *Yersinia* that lack all known secreted effectors trigger both an NLRP3- canonical inflammasome and a non-canonical inflammasome [51,116]. This activation nevertheless required the pore-forming proteins of the T3SS (YopB and YopD), and was inhibited by YopK [116]. YopK is a regulator of Yop translocation, and *yopK* mutants deliver elevated levels of other Yop proteins into target cells [117]. These data suggested that YopK limits the ability of the inflammasome to detect the presence of the bacteria by modulating translocation of a T3SS-dependent substrate. In support of this model, YopD mutants that are defective in translocation activity but not pore-forming activity per se were unable to trigger inflammasome activation, even in the absence of YopK [118,119]. Interestingly, both *Yersinia* and *Salmonella* inject the proteins that comprise the translocon pore into the target cell cytosol, raising the possibility that delivery of translocon proteins into target cells could be a mechanism for sensing bacterial pathogens [120,121]. Consistent with this possibility, microinjection of SipB, the *Salmonella* homologue of YopB, into cells is sufficient to induce caspase-1 activation [122]. Intriguingly, specific mutants in a chaperone protein that control the level of YopB/D expression, and reduce the levels of cytosolic YopB and D but do not affect translocation of *bona fide* T3SS effector proteins [123] also abrogate inflammasome activation [119]. Thus, YopK appears to control inflammasome activation by limiting the delivery of translocon proteins into the host cell. The molecular basis for YopK control of translocation is poorly defined, but could involve interactions with the scaffolding protein RACK1 or potentially other host proteins within the cytosol [124,125]. Alternatively

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YopK may act as a gate on the host side of the T3SS to control the translocation levels of T3SS client proteins. Tight control of effector delivery appears to be a generally important feature of the interaction between bacterial secretion systems and host immune responses, as altering the extent of translocation of a single effector is capable of dramatically altering bacterial virulence in the case of *Y. pestis* and *pseudotuberculosis* YopJ [126,127].

4.3. Inflammasome evasion by control of bacterial metabolites

Inflammasome activation can occur in response to virulence activities per se, but can also be triggered by the presence of viable bacteria within endosomal or cytosolic compartments [128–130]. The nature of these signatures of microbial viability and pathogen activities that are sensed by inflammasomes remains to be further defined, but microbe-derived small molecules and metabolites are becoming more appreciated as mediators of cellular responses to microbial infection or colonization. For example, short-chain fatty acids produced by the intestinal microbiota play an important role in modulating inflammatory responses of intestinal macrophages [131] as well as in promoting differentiation of intestinal regulatory T cells [132–134]. Similarly, control of bacterial production of the TCA cycle metabolite citrate by the intracellular pathogen *Salmonella* may enable these bacteria to evade NLRP3 inflammasome activation [135]. Enhanced inflammasome activation in response to bacterial mutants that produced elevated levels of citrate resulted in enhanced inflammasome-dependent clearance of bacterial infection in vivo [135]. Interestingly, aconitase, one of the TCA enzymes implicated in control of inflammasome activation, was also isolated in a transposon-based genome-wide screen for bacterial genes important for long-term persistence of *Salmonella* [136]. Both aconitase and isocitrate lyase contribute to inflammasome evasion in *Salmonella*, and isocitrate lyase contributes to persistent infection by *Salmonella* and *M. tuberculosis* [137,138]. Collectively, these findings indicate an important role for metabolite regulation in inflammasome evasion by *Salmonella* and suggest that other pathogens such as *M. tuberculosis* may evade inflammasome activation through a similar mechanism.

5. Future directions and unanswered questions

Inflammasome activation and downstream innate and adaptive responses present a strong host immune barrier to potential pathogens. Successful pathogens must therefore evolve mechanisms to overcome these barriers and examples of such mechanisms are presented here. It is likely that additional mechanisms and examples will be discovered in the near future. An area that remains unexplored is the potential for modulation of inflammasome activation by components of the commensal microbiota. Type III secretion systems are generally viewed as virulence factors, but they can serve to promote symbiotic colonization of plants and insects by diverse bacterial species [139]. Interestingly, a T3SS$^+$ strain of *E. coli* was recently isolated as a natural component of a murine microbial community that only caused disease in the setting of NLRC4 deficiency [140]. Commensal microbes may therefore also possess mechanisms that have yet to be described for dampening inflammasome activation. Such mechanisms may involve active suppression, but are more likely to involve modification of activating triggers, or limiting the availability of activating molecules. As additional mechanisms of inflammasome activation are revealed, it is likely that microbial modulators of these pathways will be identified. A potential area that is relatively unexplored as a location for microbial modulation of cell death pathways is the mitochondrion. Mitochondria are closely tied to inflammasome activation as well as other inflammatory signaling pathways [141]. The evolutionary history of mitochondria suggests that intracellular bacteria that maintain a persistent interaction with host cells evolve mechanisms to limit cell death by downregulating expression of inflammatory signals or inhibit assembly of protein complexes that transduce death-activating signals. Future studies in this area are likely to reveal new insights into the basis of inflammasome activation and may identify new ways to modulate these responses.

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