Review

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Lose the battle to win the war: bacterial strategies for evading host inflammasome activation

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The inflammasome is composed of nucleotide-binding, oligomerization domain (NOD)-like receptor (NLR) proteins, and leads to caspase-1 activation and subsequent secretion of the proinflammatory cytokines interleukin 1β (IL-1β) and interleukin-18 (IL-18). After certain pathogenic bacteria infect host cells, such as macrophages, NLR-mediated inflammasome activation is triggered to form part of the host defenses against the invading pathogens. However, recent evidence has shown that bacteria have strategies for evading inflammasome activation in host cells. In this review, we focus on NLR-mediated inflammasome activation and bacterial evasion of the inflammasome as part of the battle between the host defenses and pathogens.

Inflammasome formation and activation

Inflammasomes are multiprotein complexes that induce caspase-1 activation and consist of NLRs such as NLR family, pyrin domain containing 3 (NLRP3), NLR family, CARD domain containing 4 (NLRC4), and Pyrin and HIN-200 domain-containing (PYHIN) proteins including absent in melanoma 2 (AIM2). NLRs and PYHIN proteins function as sensors of exogenous pathogen-associated molecular patterns (PAMPs) or endogenous danger-associated molecular patterns (DAMPs), which are released by injured tissues or necrotic cells [1–5]. After recognizing these stimulators, the inflammasomes are assembled allowing caspase-1 activation. After activation, caspase-1 causes the proteolytic processing of pro-IL-1β and pro-IL-18, which results in their functional maturation and secretion. Biologically active IL-1β or IL-18 broadly affect the inflammatory process including activation of phagocytes, neutrophil migration, or tissue repair. Activation of caspase-1 is tightly regulated by inflammasomes and caspase-1 is synthesized as an inactive zymogen. Inflammasome-mediated caspase-1 activation is also associated with a rapid and lytic form of cell death known as pyroptosis (Box 1).

Inflammasome activation plays a critical role in regulating host defenses against pathogen infections, but excessive or inadequate activation may have detrimental effects on host health. Recent studies have shown that inflammasome activation is also associated with multifaceted diseases such as metabolic syndrome, inflammatory bowel diseases, vitiligo, gouty arthritis, and type II diabetes [4]. Mutations in components of inflammasome complexes have also been linked with less common autoinflammatory disorders such as cryopyrinopathies. It is therefore crucial that inflammasomes are sufficiently activated to counteract pathogens, but also this activation must be carefully regulated to prevent excessive activation leading to disease. Here, recent progress on how inflammasomes are activated via NLRC4, NLRP3, and AIM2 in response to bacterial infection is reviewed. Furthermore, recent studies have shown that an increasing number of pathogenic virulence factors prevent inflammasome activation and IL-1β processing. We also review how pathogens such as bacteria and viruses evade or modulate inflammasome function to ensure their survival in the host. Other NLRs such as NLRP1, NLRP6, NLRP12, and PYHIN protein interferon gamma-inducible protein 16 (IFI16) have been linked to inflammasome activation after pathogen infections or inflammatory disorders but they are not the focus of this review [1–3,5].

NLRC4 inflammasome

NLRC4 consists of an N-terminal caspase activation and recruitment domain (CARD), a central NOD, and a leucine-rich repeat (LRR) in its C terminus. Gram-negative bacteria harboring type III (T3SS) or type IV (T4SS) secretion systems can activate the NLRC4 inflammasome. Activation of the NLRC4 inflammasome occurs as part of the innate immune response during infections with *Salmonella* spp., *Legionella* spp., *Pseudomonas* spp., *Yersinia* spp., *Shigella* spp., *Aeromonas veronii*, and *Burkholderia pseudomallei* [5,6]. The flagellin monomer, a component of bacterial flagella, is released into the host cytoplasm via T3SS or T4SS after infection and it triggers the NLRC4 inflammasome. The inner rod protein, a component of T3SS such as the PhoPQ-repressed gene (Prg) J protein in *Salmonella* spp., is also capable of triggering the NLRC4 inflammasome [7]. Although the function of triggering NLRC4 is analogous, there is little similarity in the amino acid sequence of the flagellin and rod proteins. Two recent papers have resolved this issue by showing that flagellin

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Box 1. Inflammasomes

Inflammasomes are intracellular multiprotein complexes that mediate the activation of caspase-1. Inflammasome-mediated caspase-1 activation occurs in response to pathogen infections, such as bacterial, viral, fungal, and parasitic infections. Inflammasomes are activated in response to stimulation by PAMPs and endogenous DAMPs such as ATP after exposure to crystalline substances, for example, MSU, cholesterol, fatty acids, and particles such as silica, asbestos, and alum adjuvants [1–5,96]. Inflammasome formation is dependent on various stimuli involving NLRs and PYHIN proteins. Most NLRs contain a nucleotide-binding domain (NBD) and LRRs. Analogous to TLRs, LRRs are thought to function during ligand binding/sensing and autoinhibition. The NBD mediates the assembly of the inflammasome by inducing an oligomerized state, which is critical for activation. PYHIN proteins lack NBDs and LRRs, but they may contain the equivalent functional domains of NLRs. In CARD-containing NLRs, such as NLRC4 and NLRP1, signaling is initiated by homophilic interactions between CARDs and complexing with pro-caspase-1. In PYD-containing NLRs and PYHIN proteins such as NLRP3 and AIM2, respectively, inflammasome assembly with pro-caspase-1 involves bridging a bipartite protein with an apoptosis-associated speck-like protein containing a CARD (ASC, also known as PYCARD), which comprises CARD and PYD. ASC has also a key role in inflammasome assembly with CARD-containing NLRs, although the precise role of ASC is disputed [11,12,90,97,98].

and rod proteins can interact with different members of the NLR family, apoptosis inhibitory protein (NAIP) subfamily of NLRs but not with NLRC4 directly [8,9]. Thus, flagellin is recognized by NAIP5 and NAIP6 whereas rod proteins are recognized by NAIP2. Ligand-bound NAIP5, NAIP6, or NAIP2 then interact with NLRC4 to facilitate inflammasome activation. Therefore, NLRC4 inflammasome activation is differentially regulated by NAIP proteins in response to bacterial components.

It is notable that the *Naip* genes are located in a genomic locus that contains seven highly homologous genes (*Naip1–7*) and four of these genes (*Naip1*, *Naip2*, *Naip5*, and *Naip6*) have transcripts in C57BL/6 mice. However, humans only have one NAIP protein and its behavior during NLRC4 inflammasome activation is quite different from those in mice. Human NAIP does not respond to flagellin or rod proteins such as BsaK from *Burkholderia thailandensis* or PrgJ from *Salmonella* spp. Instead, human NAIP appears to recognize the T3SS needle protein CprI from *Chromobacterium violaceum* and other needle proteins from pathogenic bacteria such as enterohemorrhagic *Escherichia coli* (EHEC), *B. thailandensis*, *Pseudomonas aeruginosa*, *Shigella flexneri*, and *Salmonella enterica* serovar Typhimurium, but not enteropathogenic *E. coli* (EPEC) or *Vibrio parahaemolyticus* [9]. The mechanism of differential recognition by human NAIP for NLRC4 activation is an important issue and it should be clarified in future studies from the perspective of host immune systems resisting bacterial infections (Figure 1).

The T3SSs of *Yersinia pseudotuberculosis* and *A. veronii* trigger caspase-1 activation via several inflammasomes including NLRC4 and NLRP3 [10,11]. Furthermore, their T3SSs may even trigger the NLRP3 inflammasome, although the pathway leading to this is unknown.

NLRC4 contains a CARD domain allowing it to directly interact with pro-caspase-1. Indeed, activation of NLRC4 inflammasomes dependent on and independent of ASC (apoptosis-associated speck-like protein containing a CARD) after *Aeromonas* infection suggests that some bacteria with T3SSs can simultaneously activate two signaling pathways, an NLRC4–ASC–caspase-1 and an NLRC4–caspase-1 lineage [11]. However, there is currently no direct evidence that bacteria induce two NLRC4 inflammasomes. When ASC-deficient macrophages are infected with NLRC4-activating bacteria such as *Salmonella*, *Legionella*, or *Shigella*, defective caspase-1 activation and processing/release of IL-1β occurs. However, pyroptosis in ASC-deficient macrophages caused by NLRC4 inflammasomes is unaffected, suggesting that cell death pathways downstream of NLRC4 are independently regulated by ASC. Furthermore, activation of the NLRC4 inflammasome can occur in an ASC-dependent and ASC-independent manner [12]. In addition, cell death with insufficient IL-1β processing continued to occur in a caspase-1 mutant unable to induce autoproteolysis. Taken together, these data support the idea that bacterial pathogens can use different routes to activate the NLRC4 inflammasome [13].

In addition, the function of NLRC4 is regulated by phosphorylation in host cells. The phosphorylation of NLRC4 on serine 533 by protein kinase C δ (PKCδ) and possibly other kinases is required to activate the NLRC4 inflammasome [14]. It is not known how the phosphorylation of NLRC4 is regulated during bacterial infections.

NLRP3 inflammasome

Proteins from the NLRP subfamily, including NLRP3, have an N-terminal pyrin domain (PYD), a central NOD, and an LRR in their C terminus. Interaction with ASC activates the NLRP3 inflammasome and causes caspase-1 activation. Diverse molecules with unrelated structures from both nonpathogenic and pathogenic sources can activate the NLRP3 inflammasome. Detailed descriptions of nonpathogenic and nonbacterial stimulators of the NLRP3 inflammasome can be found in previous reviews [5,15,16].

Bacterial pathogens can stimulate the NLRP3 inflammasome and this is most commonly achieved through the secretion of pore-forming bacterial toxins. Activation of the NLRP3 inflammasome is promoted by aerolysins from *Aeromonas hydrophila* [17] or *A. veronii* [11], adenylate cylase toxin from *Bordetella pertussis* [18], listeriolysin O from *Listeria monocytogenes* [19–21], pneumolysin from *Streptococcus pneumoniae* [22–24], streptolysin O from *Streptococcus pyogenes* [25], β-hemolysin from *Streptococcus agalactiae* [26], hemolysins from *Staphylococcus aureus* [27–29], and the hemolysins and multifunctional repeat-in-toxins secreted by *Vibrio vulnificus* or *Vibrio cholerae* [30]. Not only do *Clostridium difficile* toxins activate the NLRP3 inflammasome, but this contributes to *C. difficile*-associated pathogenesis [31].

In addition to bacterial toxins, infections with intracellular bacteria can activate the NLRP3 inflammasome. In mycobacteria, the Esx-1 (type VII) secretion system facilitates NLRP3 inflammasome activation [32,33]. *Yersinia* outer membrane protein (Yop) J from the *Yersinia pestis* KIM strain is also involved in the activation of the NLRP3 inflammasome [34,35]. Recent reports showed that the surface protein of *Treponema denticola* interacts with

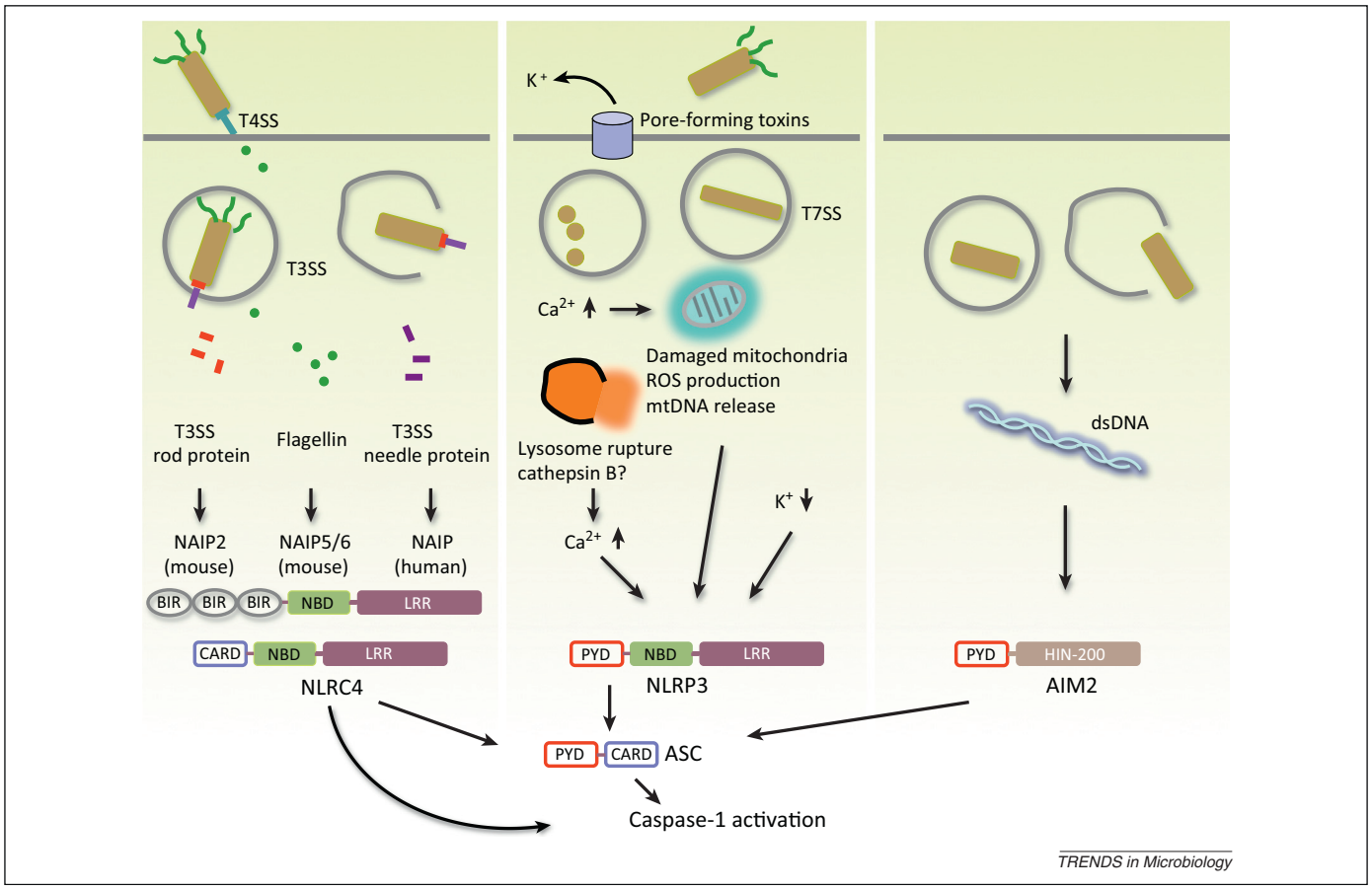

Figure 1. Model of nucleotide-binding, oligomerization domain (NOD)-like receptor (NLR) family, CARD domain containing 4 (NLRC4), NLR family, pyrin domain containing 3 (NLRP3), or absent in melanoma 2 (AIM2)-mediated caspase-1 activation by bacteria. NLRC4 responds to flagellin injected by type III secretion system (T3SS) or type IV secretion system (T4SS), as well as the rod or needle proteins of T3SS. In the mouse, NLR family, apoptosis inhibitory protein 2 (NAIP2) binds to T3SS rod proteins and NAIP5/6 binds to flagellin, whereas human NAIP can only interact with the needle proteins of T3SS. Abbreviations: BIR domain, unknown function baculovirus inhibitor of apoptosis (IAP) repeat. The NLRP3 inflammasome is triggered by the pore-forming toxins secreted from bacteria. NLRP3 activation may also be triggered by reactive oxygen species (ROS) production from damaged mitochondria or lysosome rupture after its induction by bacterial infection. AIM2 responds to dsDNA derived from intracytoplasmic bacteria. Activation of the NLRP3 and AIM2 inflammasomes are dependent on ASC (apoptosis-associated speck-like protein containing a CARD), whereas the NLRC4 inflammasome can assemble without ASC. Adapted from [6].

integrin α5β1 and the glycolipoprotein from *Leptospira interrogans* lead to the activation of the NLRP3 inflammasome [36,37]. The activation of NLRP3 inflammasome is triggered in the infections with *Chlamydia trachomatis* [38], *Chlamydophila pneumoniae* [39–41], *Brucella abortus* via T4SS-independent [42], *C. rodentium* via T3SS-independent [43,44], *Klebsiella pneumoniae* [45], *Porphyromonas gingivalis* [46], *Neisseria gonorrhoeae* [47], and *Y. pseudotuberculosis* via T3SS [10]. However, which specific bacterial factors utilized during bacterial infection that activate the NLRP3 inflammasome are currently unknown.

The NLRP3 inflammasome process activates caspase-1 in two steps. In the first step, nuclear factor-kappa B (NF-κB) mediates transcriptional induction of NLRP3, which is sufficient to facilitate inflammasome assembly and expression of pro-IL-1β. Priming consists of NF-κB-driven transcriptional activation via the pathogen–Toll-like receptor (TLR; or Nod1/2) interaction or cytokine signaling, such as by tumor necrosis factor-α (TNF-α) [30,48,49]. However, recent reports have shown that lipopolysaccharide (LPS) rapidly primes NLRP3 in a manner distinct from transcriptional activation [50–52]. The caspase-1 activation occurs by simultaneous activation with LPS and the NLRP3 stimulator ATP. The de-ubiquitination of NLRP3 is involved in the transcription-independent priming. In the second step, the NLRP3 inflammasome is assembled following cellular signaling.

The response to diverse NLRP3 stimulators, including intracellular activators, may demand a more general mechanism for the cellular signaling required for NLRP3 inflammasome formation. In the first model, potassium efflux and depletion through ATP-induced P2X7 nonselective cationic channels or pathogen-derived pore-forming toxins acts as a signal for assembly of the NLRP3 inflammasome. The second model for NLRP3 inflammasome activation is via reactive oxygen species (ROS) production. NLRP3 activators such as ATP, monosodium urate (MSU), and silica trigger the generation of ROS and treatment with various ROS scavengers blocks the NLRP3 inflammasome [53]. Another study showed that ROS was required to prime NLRP3, but not to activate the NLRP3 inflammasome [54]. Damaged mitochondria could also generate ROS [55,56]. Nakahira *et al.* proposed that ATP-driven mitochondrial (mt)DNA release is critical for NLRP3 activation and this release is dependent on ROS generation [55]. A recent report found that the mtDNA released from damaged mitochondria is capable of interacting with NLRP3 directly to trigger inflammasome activation [57]. The third model for NLRP3 inflammasome

activation involves lysosomal destabilization after the phagocytosis of crystals such as MSU and alum, which leads to activators of the NLRP3 inflammasome being leaked from the cytoplasm. The lysosomal enzymes cathepsin B or L have been examined to see if they activated inflammasomes, but mice lacking cathepsin B or L had similar levels of NLRP3 inflammasome activation after stimulation as wild type mice [58,59]. However, recent reports indicate that anti-cancer drugs, that is, 5-fluorouracil (FU) and gemcitabine, induce lysosomal damage and release of cathepsin B, which binds to NLRP3 and promotes inflammasome activation [60] (Figure 1).

It is important to determine the common intracellular events induced by all NLRP3 stimulators, potassium efflux, ROS production, or lysosome rupture from damaged mitochondria to clarify the status of different models and to reconcile these activation models. Recent reports have shown that calcium mobilization is a key factor involved in NLRP3 inflammasome [61–63]. Murakami *et al.* also reported that calcium mobilization is required for ATP-driven mtDNA release from damaged mitochondria. Calcium mobilization by ATP could also be suppressed in the presence of high extracellular potassium, whereas lysosome rupture-mediated NLRP3 activation was blocked by a calcium signaling inhibitor [61].

Overall, the exact mechanisms whereby the NLRP3 inflammasome assembles are currently unclear. It is possible that several cellular pathways could be activated, and even regulated, in response to different types and levels of stimulation. Our current understanding of the molecular mechanism for various stimuli to activate the NLRP3 inflammasome remains in a chaotic state.

Noncanonical NLRP3 inflammasome activation regulated by caspase-11 was proposed recently [43]. Conventional, widely-used caspase-1-deficient mice are actually caspase-1, 11-double deficient. In contrast to the canonical activation induced by ATP or MSU, NLRP3 inflammasome activation by *E. coli*, *Citrobacter rodentium*, and *V. cholerae* are caspase-11-dependent. The cell death of infected macrophages due to these bacteria is dependent on caspase-11 activation but not NLRP3 inflammasome components [43]. The caspase-11-dependent NLRP3 inflammasome activation by the noncatalytic B subunit of cholera toxin (CTB) has also been observed, but the mechanism of the activation by CTB remains unclear. Caspase-11-dependent NLRP3 activation is also induced by other Gram-negative bacteria where the signaling requires TLR4, Toll/IL-1 receptor (TIR) domain-containing adapter inducing IFN-β (TRIF), and the type I interferon (IFN) receptor [64,65]. Recent evidence that bacterial mRNA triggered the NLRP3 inflammasome may provide a clue to the understanding of noncanonical activation via caspase-11 [66]. The rupture of the intracellular vacuole formed by invading bacteria triggers caspase-11 activation and cell death [67]. So far, it is unknown as to how caspase-11 is activated and how it regulates the NLRP3 inflammasome after specific bacterial infections. It is notable that the detection of caspase-11-dependent inflammasome activation caused by bacteria requires a long incubation period of more than 10 h with antibiotics to kill extracellular multiplying bacteria. This makes it difficult to interpret whether the degraded, surviving, or killed extracellular bacteria are most important in inflammasome activation.

The NLRP3 inflammasome is negatively regulated by endogenous signaling in host cells. Basal autophagy in the unstimulated state prevents excessive activation of the NLRP3 inflammasome by ROS and mtDNA from damaged mitochondria [55,56,68,69]. Leaky and depolarized mitochondria are thought to be removed by autophagy, that is, a type of house-keeping autophagy. The nitric oxide (NO) induced in infected cells triggers thiol-nitrosylation of NLRP3, which negatively regulates the NLRP3 inflammasome to modulate destructive innate inflammatory responses during chronic infections [70,71]. In addition, $CD4^+$ T cells inhibit the activation of the NLRP3 and NLRP1 inflammasomes but not that of the NLRC4 inflammasome. This suppression is mediated by TNF family ligands such as CD40L [72].

PYHIN protein AIM2 inflammasome

AIM2 is composed of an N-terminal PYD and HIN-200 domain (HIN) in its C terminus. The AIM2 inflammasome interacts with ASC to become activated, leading to caspase-1 activation via the cytosolic sensing of double-stranded (ds)DNA from viruses and bacteria, including *B. abortus* via T4SS [42], *Francisella tularensis* [73,74], *L. monocytogenes* [20,74–76], and *Mycobacterium tuberculosis* [77]. Recently, a *Legionella pneumophila* mutant lacking the T4SS effector succinate dehydrogenase (Sdh) A was found to trigger the AIM2 inflammasome via the release of bacterial DNA into the cytoplasm of host cells, which suggests that AIM2 recognizes the DNA of invading *L. pneumophila* [78] (Figure 1).

Evasion of inflammasome activation by pathogens

The innate immune system plays an important role in host defense against pathogens so it is possible that many pathogens need to establish a replicative niche by evading this response. Successful bacteria manipulate inflammasome activation in various ways. Downregulation of the expression of PAMPs is one bacterial strategy for avoiding detection by NLRs or other innate immune receptors. For example, *Salmonella* downregulates the expression of flagellin and *Salmonella* pathogenicity island (SPI)-1 T3SS during systemic infections [79,80]. *S. aureus* modifies its cell wall to prevent degradation by lysosomes in infected host cells and this modification also contributes indirectly to the reduction in sensing by the NLRP3 inflammasome [81].

The modulation of inflammasome signaling was first reported in viral infections [3,82]. For example, poxviruses produce homologs of serpins, which are endogenous serine protease inhibitors in mammalian cells. The cowpox virus protein cytokine response modifier (CrmA) and its homologs in orthopoxviruses such as vaccinia, ectromelia, and rabbitpox viruses directly target caspase-1 activity by acting as pseudosubstrate inhibitors of caspase-1 [83]. Furthermore, some virus proteins target inflammasome assembly rather than caspase-1 as viral decoy proteins. Kaposi's sarcoma-associated herpesvirus (KSHV) open reading frame 63 (Orf63), a viral NLRP1 homolog that lacks CARD motifs, interacts with human NLRP1 and

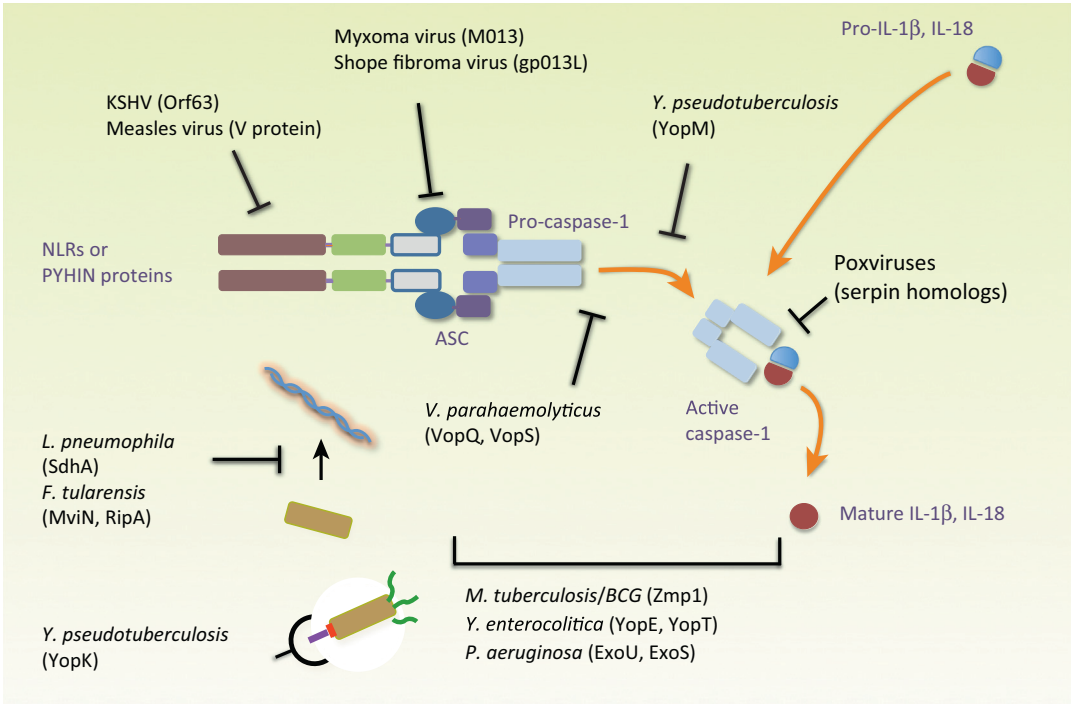

Figure 2. Pathogen virulence factor-mediated interference with the inflammasome. Viruses and bacteria have an array of strategies for interfering with inflammasome activation. The Kaposi's sarcoma-associated herpesvirus (KSHV) open reading frame 63 (Orf63) protein interacts with human nucleotide-binding, oligomerization domain (NOD)-like receptor (NLR) family, pyrin domain containing 1 (NLRP1) and NLRP3 to prevent inflammasome activation. Measles virus V protein directly targets NLRP3 to inhibit inflammasome activation. Myxoma virus and Shope fibroma virus produce the PYRIN domain-only protein (POP)-like proteins M013 and gp013L, respectively, which bind ASC (apoptosis-associated speck-like protein containing a CARD) to interfere with inflammasome assembly. Poxviruses produce homologs of serpins, which block the catalytic activity of caspase-1. The *Yersinia pseudotuberculosis Yersinia* outer membrane protein (Yop) K effector inhibits NLR family, CARD domain containing 4 (NLRC4) and NLRP3 inflammasomes by acting as a gatekeeper for type III secretion system (T3SS) to modulate the leakage of flagellin or pathogen-associated molecular patterns (PAMPs). YopM can also bind caspase-1 directly and prevent its activation. The YopE and YopT effectors secreted by the T3SS of *Yersinia enterocolitica* inhibit caspase-1 activation. The effector proteins Exoenzyme (Exo) U and ExoS from *Pseudomonas aeruginosa* block NLRC4 inflammasome activation. *Vibrio* outer protein (Vop) Q and VopS from *Vibrio parahaemolyticus* inhibit speck formation by ASC in the NLRC4 inflammasome. Zinc metalloprotease (Zmp) 1 from *Mycobacterium tuberculosis* and *M. bovis* bacillus Calmette Guérin (BCG) targets the NLRP3 inflammasome. The *Legionella pneumophila* type IV secretion system (T4SS) effector succinate dehydrogenase (Sdh) A prevents activation of the absent in melanoma 2 (AIM2) inflammasome by inhibiting the release of bacterial DNA into the cytoplasm of host cells. The gene products of mouse virulence (*mvi*) N or required for intracellular proliferation factor (*rip*) A from *Francisella tularensis* may also prevent DNA release from bacteria.

NLRP3 to prevent inflammasome activation [84]. A recent study showed that measles virus V protein directly targets NLRP3 to inhibit inflammasome activation [85]. The proteins M013 from myxoma virus and gp013L from Shope fibroma virus are pyrin-only decoy proteins, which are similar to mammalian cellular PYRIN domain-only protein (cPOP) proteins, and they bind ASC to prevent inflammasome activation [86,87] (Figure 2).

There is no evidence that bacteria directly modulate the signaling involved in inflammasome activation. However, a recent report showed that *Y. pseudotuberculosis* YopM, a bacterial effector injected into the host cells via T3SS, can directly bind caspase-1 and prevent its activation. This activity is similar to that of the viral CrmA protein [88]. Interestingly, YopM-expressing macrophages exhibited lower activation of caspase-1, which was triggered by simultaneous agonists of NLRC4, NLRP3, or NLRP1. In another mechanism of inflammasome inhibition, the YopK effector inhibits NLRC4 and NLRP3 inflammasomes by acting as a gatekeeper for T3SS, thereby modulating the leakage of flagellin or PAMPs in the host cell cytoplasm [10]. YopK- or YopM-mediated inhibition of inflammasomes is critical for bacterial colonization of the host because the bacterial burden is attenuated in wild type mice infected with YopK or YopM mutants whereas the attenuation is reversed in inflammasome component-deficient mice. YopE and YopT are secreted via T3SS in *Y. enterocolitica* and they inhibit caspase-1 activation and IL-1β release [89]. YopE and YopT are negative regulators Rho GTPases and the Rho-mediated cytoskeleton process. The inhibition of inflammasome activation by Yop effectors may be due to indirect effects that trigger the disruption of the actin cytoskeleton and the inhibition of phagocytosis.

P. aeruginosa strain PA103 secretes T3SS effector exoenzyme (Exo) U, which has a phospholipase A2 activity that inhibits the NLRC4 inflammasome in infected macrophages [90]. It is unclear how ExoU activity contributes to the inhibitory effect on caspase-1 activation. In addition, the ExoS effector interferes with inflammasome-mediated IL-1β production. An ExoS deficiency triggers apoptosis of macrophages but not pyroptosis so the effect of ExoS on caspase-1 activation is likely to be indirect.

F. tularensis live vaccine strain mutants lacking *mviN* or *ripA* genes have been reported to enhance the inflammasome activation triggering that is mediated by AIM2 [91,92]. The mechanisms underlying inflammasome activation enhancement after infections with mutants have not been elucidated, but the high amounts of bacterial DNA released from these mutants in the cytosol of infected cells might be a powerful trigger of AIM2 inflammasome

activation [1]. Mouse virulence (Mvi) N or required for intracellular proliferation factor (Rip) A may prevent DNA release from invading bacteria. Similarly, the *L. pneumophila* T4SS effector SdhA prevents activation of the AIM2 inflammasome by inhibiting the release of bacterial DNA into the cytoplasm of host cells [78]. *Salmonella* T3SS effector *Salmonella*-induced filament (Sif) A and *L. pneumophila* T4SS effector SdhA are involved in maintenance of vacuole stability to prevent activation of caspase-11 [67]. In addition, the T3SS rod protein PrgJ of the SPI1 T3SS in *Salmonella* is sensed by the NLRC4 inflammasome, but another rod protein (secretion system apparatus (Ssa) I) of SPI2 T3SS does not trigger the NLRC4 inflammasome, suggesting that *Salmonella* is capable of evading the NLRC4 inflammasome [7].

Infections with *M. tuberculosis* or vaccine strains of *Mycobacterium bovis* do not induce high levels of IL-1β production. However, an *M. bovis* mutant lacking the *zmp1* gene encoding a putative Zn^{2+} metalloprotease triggered caspase-1 activation in infected macrophages [93]. Zinc metalloprotease (Zmp) 1 also appears to inhibit phagosome–lysosome fusion and prevents mycobacterial clearance, so further analysis may uncover the functional role of Zmp1 in preventing inflammasome activation and lysosome maturation. In addition, the Gram-negative pathogen *Legionella* interferes with transcriptional upregulation of the adaptor protein ASC, which supports bacterial proliferation in infected human monocytes [94] (Figure 2).

Recently, Higa *et al.* provided insights into novel mechanisms of T3SS effector-mediated inhibition of inflammasome activation during infections with *V. parahaemolyticus* [95]. The NLRP3 and NLRC4 inflammasomes are activated by thermostable direct hemolysins (TDHs) and T3SS1, one of two sets of T3SS, in response to *V. parahaemolyticus* infections. T3SS1 effectors, *Vibrio* outer protein (Vop) Q and VopS, had selective inhibitory effects on NLRC4 inflammasome activation. Consequently, the data suggested that VopQ-mediated autophagosome accumulation and VopS inactivation of Rho GTPases were involved in NLRC4–ASC-mediated speck formation, which is an essential step during caspase-1 activation in infected mouse macrophages. Autophagy is generally believed to protect NLRP3 inflammasome activation by preventing the release of mitochondrial DNA. However, this study suggests that VopQ-mediated autophagy targets the NLRC4 inflammasome, although much less than NLRP3. Inflammasome activation by *V. parahaemolyticus* is also independent of the phagocytic activities of macrophages, so the mechanisms of VopS-mediated NLRC4 inhibition via the induction of Rho GTPases inactivation may be different from the actions of YopE or YopT from *Y. enterocolitica*. How this cellular signaling is triggered by two effectors to regulate speck formation remains to be elucidated. Further analysis is also necessary to clarify the molecular basis of effector-mediated inflammasome inhibition by *V. parahaemolyticus* (Figure 2).

Concluding remarks

Several members of the NLR and PYHIN proteins are key molecules in the innate immune response to bacterial pathogens. Inflammasome activation by NLRs and PYHIN proteins is triggered by the detection of components of pathogens that are essential for infection, colonization, and survival in the host. However, the mechanisms of how bacterial components stimulate inflammasome activation and how NLRs recognize stimulators including cellular pathways and antibacterial function in host defense require further investigation.

Growing evidence suggests that viruses and bacteria have developed an array of strategies to interfere with inflammasome activation based on displaying molecules that target inflammasome components or the catalytic activity of caspase-1. In these types of bacterial infections, inflammasome deficiency is not expected to affect the host susceptibility to infection or colonization with wild type bacteria. Indeed, inflammasome-deficient mice do not always exhibit more sensitivity to infection with pathogens *in vivo* [5]. Because evidence on inflammasome-evading strategies of pathogens has been accumulating, it is possible that we might fail to determine how pathogens evade the inflammasome if we continue to use wild type pathogens in studies. Further investigations into the interactions between bacteria and NLRs should contribute to our understanding of immune evasion mechanisms of pathogenic bacteria and provide new insights into the targets for the development of therapeutic strategies.

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