

# Evasion of inflammasome activation by microbial pathogens

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**Activation of the inflammasome occurs in response to infection with a wide array of pathogenic microbes. The inflammasome serves as a platform to activate caspase-1, which results in the subsequent processing and secretion of the proinflammatory cytokines IL-1 $\beta$  and IL-18 and the initiation of an inflammatory cell death pathway termed pyroptosis. Effective inflammasome activation is essential in controlling pathogen replication as well as initiating adaptive immune responses against the offending pathogens. However, a number of pathogens have developed strategies to evade inflammasome activation. In this Review, we discuss these pathogen evasion strategies as well as the potential infectious complications of therapeutic blockade of IL-1 pathways.**

## Introduction

The innate immune system plays a critical role in host defense against invading pathogens through the activation of pattern recognition receptors (PRRs) by highly conserved pathogen-associated molecular patterns (PAMPs) or host-derived danger-associated molecular patterns (DAMPs). PRRs include TLR, RIG-I-like receptors (RLR), C-type lectin receptors (CLR), nucleotide-binding domain leucine-rich repeat-containing family (NLR), and those belonging to the Pyrin and HIN200 domain-containing (PYHIN) family.

The human NLR family comprises over 23 structurally related proteins, the functions of many of which remain unknown (1). A number of NLRs and the PYHIN family member AIM2 form multi-protein complexes called inflammasomes, which play key roles in regulating both innate and adaptive immune responses. The assembly of an inflammasome results in a platform consisting of an NLR or AIM2, in most cases, the adaptor protein apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (CARD), known as ASC, and the cysteine protease caspase-1 (Figure 1 and ref. 1). Inflammasome activation results in the release of potent proinflammatory mediators and thus is a tightly regulated process, as their inadvertent release could cause collateral tissue damage. Inflammasome activation is generally a two-step process. The priming step results in the transcription of pro-IL-1 $\beta$ , pro-IL-18, and certain inflammasome components (2). The second signal, which can be initiated by a variety of stimuli, results in the activation of the inflammasome (2). The two-step process for inflammasome activation is clearly required for NLRP3 inflammasome activation; however, the requirement for a separate priming step is less clear for NLRP1, NLRC4, and AIM2 inflammasomes. Once activated, the inflammasome complex serves as a platform for the autocatalytic cleavage of pro-caspase-1 into its

mature activated form. Caspase-1 in turn cleaves pro-IL-1 $\beta$  and pro-IL-18 into their mature secreted forms. Caspase-1 activation is also required for the initiation of an inflammatory programmed cell death pathway termed pyroptosis. In addition, inflammasome activation is associated with the rapid release of eicosanoids that drive further inflammation and vascular permeability (3).

NLRP1, NLRP3, NLRC4, and AIM2 are the best-characterized sensors capable of forming inflammasome complexes (Figure 1). Recently, NLRP2, NLRP6, NLRP7, RIG-I, pyrin, and IFI16 have been implicated in the formation of unique inflammasome complexes (4–8); however, additional investigation will be required to establish their precise roles in inflammasome formation and activation. Pyrin, mutations in which cause the autoinflammatory disease familial Mediterranean fever, can also form a caspase-1-activating inflammasome in concert with ASC (9, 10). Interestingly, a recent study demonstrated that the Pyrin inflammasome is activated through the sensing of bacterial modification and inactivation of Rho GTPases (11).

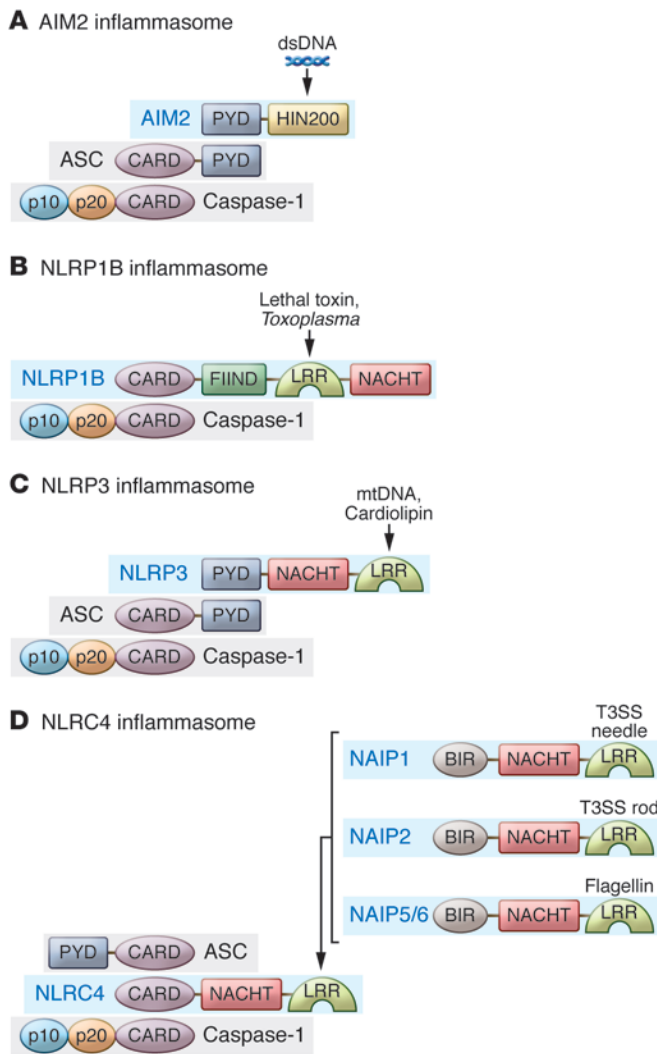
Noncanonical inflammasome activation promotes activation of caspase-11, which is important for caspase-1 activation, IL-1 $\beta$  secretion, and pyroptotic cell death in response to *Escherichia coli*, *Citrobacter rodentium*, and *Vibrio cholerae* (12). Activation of caspase-11 is triggered by the detection of cytosolic acylated lipid A, which is a component of LPS that is present in many Gram-negative bacteria. Of note, intracellular LPS or acylated lipid A is capable of activating caspase-11 independently of TLR4; however, the identity of the receptor that recognizes cytosolic LPS remains unclear (13, 14). A recent study demonstrated that human caspase-4 and caspase-5 and mouse caspase-11 were capable of directly binding to LPS and lipid A, resulting in their activation and the initiation of cell death (15).

## Pathogen-mediated inflammasome activation

A single *NLRP1* gene is present in humans; in contrast, mice possess three NLRP1 orthologs, *Nlrp1a*, *Nlrp1b*, and *Nlrp1c*. An important structural difference between human NLRP1 and its murine orthologs is that mice lack the N-terminal Pyrin domain (PYD) that

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**Figure 1. Schematic of AIM2, NLRP1B, NLRP3, and NLRC4 inflammasomes.** (A) The AIM2 inflammasome detects the presence of cytosolic dsDNA via its HIN200 domain. AIM2 then recruits ASC through its N-terminal PYD, which recruits caspase-1 via its CARD domain. (B) *B. anthracis* lethal toxin and *T. gondii* can induce the activation of the NLRP1B inflammasome; it is thought that they ultimately lead to mitochondrial dysfunction, resulting in mtDNA and cardiolipin interactions with NLRP3, which leads to its activation. NLRP3 interacts with ASC through an N-terminal PYD, which then recruits caspase-1. (C) A diverse array of agonists can activate the NLRP3 inflammasome; it is thought that they ultimately lead to mitochondrial dysfunction, resulting in mtDNA and cardiolipin interactions with NLRP3, which leads to its activation. NLRP3 interacts with ASC through an N-terminal PYD, which then recruits caspase-1. (D) NAIP1, NAIP2, and NAIP5/6 bind to the T3SS needle and rod proteins and bacterial flagellin, respectively. The NAIP proteins in turn activate the NLRC4 inflammasome. FIIND, domain with function to find; NACHT, nucleotide-binding and oligomerization domain; LRR, leucine-rich repeats; BIR, baculovirus IAP repeat domain; HIN200; HIN200 domain.

detects the cytosolic presence of these agonists; rather, it probably responds to a cellular stress signal induced by the infectious agents. Recent studies suggest that mitochondrial dysfunction leading to the release of mitochondrial DNA (mtDNA) and the phospholipid cardiolipin triggers activation of the NLRP3 inflammasome (25, 26); the current understanding of the mechanism of NLRP3 activation is reviewed in detail elsewhere (20).

The NLRC4 inflammasome is activated by a number of Gram-negative bacteria that possess either a type III (T3SS) or type IV (T4SS) secretion system, including *Pseudomonas aeruginosa*, *Salmonella enterica*, *Legionella pneumophila*, and *Shigella flexneri* (27). NLRC4 is activated in response to the detection of cytoplasmic flagellin or specific components of the bacterial T3SS or T4SS secretion systems. Activation of the NLRC4 inflammasome requires the involvement of the neuronal apoptosis inhibitor protein (NAIP) subfamily of NLR proteins. Murine NAIP1 binds to the needle protein of the T3SS; NAIP2 recognizes the basal rod structure of the T3SS; NAIP5 and NAIP6 bind to cytosolic flagellin (27). There is only one human NAIP homolog, which binds to the needle protein of the T3SS (27).

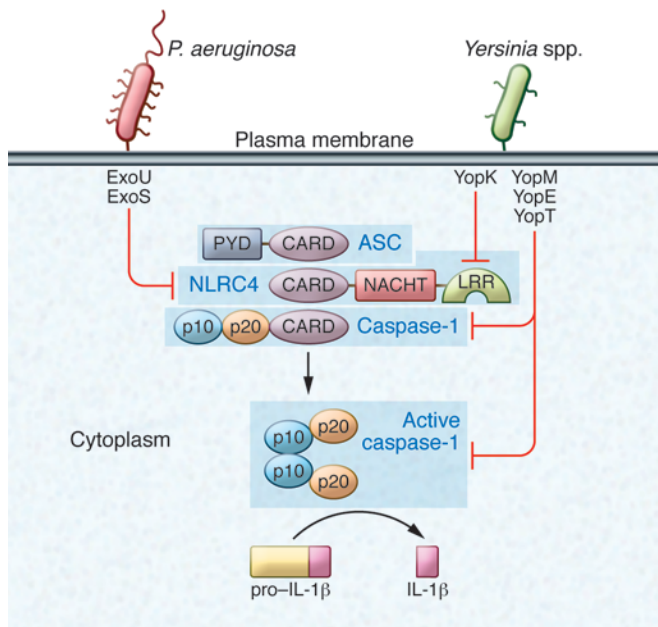
The AIM2 inflammasome plays a role in host defense through the recognition of dsDNA within the cytosol. This occurs through direct binding of DNA to the HIN200 domain of AIM2. AIM2 inflammasome activation occurs in response to infection with a number of intracellular bacterial pathogens (*Francisella tularensis*, *Mycobacterium tuberculosis*, *L. monocytogenes*, and others) and viruses (cytomegalovirus, vaccinia virus, and others) (28).

### Bacterial effector molecules that facilitate evasion of inflammasome activation

Given the role of the inflammasome in controlling a wide array of microbial pathogens, it is not surprising that a number of organisms have evolved specific strategies to avoid activation of this innate immune pathway. A number of pathogenic Gram-negative bacteria utilize a T3SS or T4SS to inject effector molecules into the cytoplasmic compartment of the host cell. The T3SS and T4SS are complex macromolecular structures that span both bacterial membranes and include a long, needle-like structure through which the effector molecules pass into the cytoplasm of the eukaryotic host cell. These effector molecules are capable of altering host cell functions, including inflammasome activation.

is present in human NLRP1 (1). The murine NLRP1B inflammasome regulates macrophage cell death in response to anthrax lethal toxin (16). NLRP1B also plays an important role in host defense against *Bacillus anthracis* in vivo (17). Recent studies have demonstrated that the *Nlrp1* locus is also required for host defense against the intracellular protozoan parasite *Toxoplasma gondii* (18, 19).

The NLRP3 inflammasome has been associated with numerous pathologic states, including infectious, autoinflammatory, and autoimmune disorders. As such, a wide array of agonists are capable of activating the NLRP3 inflammasome, including those derived from microbes (PAMPs) or from endogenous or environmental sources (DAMPs) (20). Microbial activators of the NLRP3 inflammasome include both Gram-positive and Gram-negative bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and others) (21), fungi (*Candida albicans*, *Aspergillus fumigatus*, *Microsporium canis*, and others) (22), RNA and DNA viruses (influenza virus, adenovirus, respiratory syncytial virus [RSV], and others) (23), and parasitic pathogens (*Plasmodium chabaudi*, *Leishmania amazonensis*, and *Schistosoma mansoni*) (24). Given the large number of chemically and structurally diverse agonists that are capable of activating the NLRP3 inflammasome, it is unlikely that NLRP3 directly



**Figure 2. Bacterial effector molecule-mediated inhibition of the NLRC4 inflammasome.** *Y. pseudotuberculosis* YopK is secreted into the host cell through the T3SS; YopK interacts with the translocon structure of the T3SS and effectively masks it from recognition by NAIP/NLRC4. *Y. pseudotuberculosis* YopM directly inhibits caspase-1 by binding to its catalytically active site. *Y. enterocolitica* YopE and YopT inhibit caspase-1 activation through an unknown mechanism. *P. aeruginosa* ExoU and ExoS inhibit NLRC4 inflammasome and caspase-1 activation through an unknown mechanism.

*S. enterica* serovar *typhimurium* requires two T3SSs for virulence, and the *Salmonella* pathogenicity islands SPI-1 and SPI-2 respectively encode these T3SSs. Intracellular *S. typhimurium* activates both NLRP3 and NLRC4 inflammasomes (29). SPI-1 T3SS-mediated injection of flagellin and the PrgJ rod protein into the macrophage cytoplasm results in NLRC4 inflammasome activation (30–32). During the systemic phase of infection, SPI-1 and flagellin expression are downregulated, allowing *S. typhimurium* to evade NLRC4 inflammasome activation (33). To promote replication within macrophages, *S. typhimurium* instead relies on the SPI-2-encoded T3SS, whose rod protein, SsaI, is not recognized by the NLRC4 inflammasome (30). *S. typhimurium*-induced NLRP3 inflammasome activation occurs in a T3SS-independent manner; however, the specific stimuli has yet to be identified (29). *S. typhimurium* has also developed mechanisms to evade NLRP3 inflammasome activation by utilizing the TCA enzymes aconitase (*acnB*) and isocitrate dehydrogenase (*icdA*). Interestingly, *acnB* and *icdA* mutants induced rapid NLRP3 inflammasome activation through a process that resulted in elevated bacterial citrate and increased mitochondrial reactive oxygen species (34).

In contrast to *S. typhimurium*, *Yersinia* spp., *P. aeruginosa*, and *Vibrio parahaemolyticus* have all been shown to utilize secreted effector molecules to subvert inflammasome activation (35–40); we will discuss *Yersinia* spp. and *P. aeruginosa* in greater detail below.

*P. aeruginosa*. One of the first reports of inflammasome inhibition by a pathogen described the ability of exoenzyme U (ExoU) expressing *P. aeruginosa* to inhibit NLRC4 inflammasome activation (Figure 2 and ref. 36). *P. aeruginosa* has four

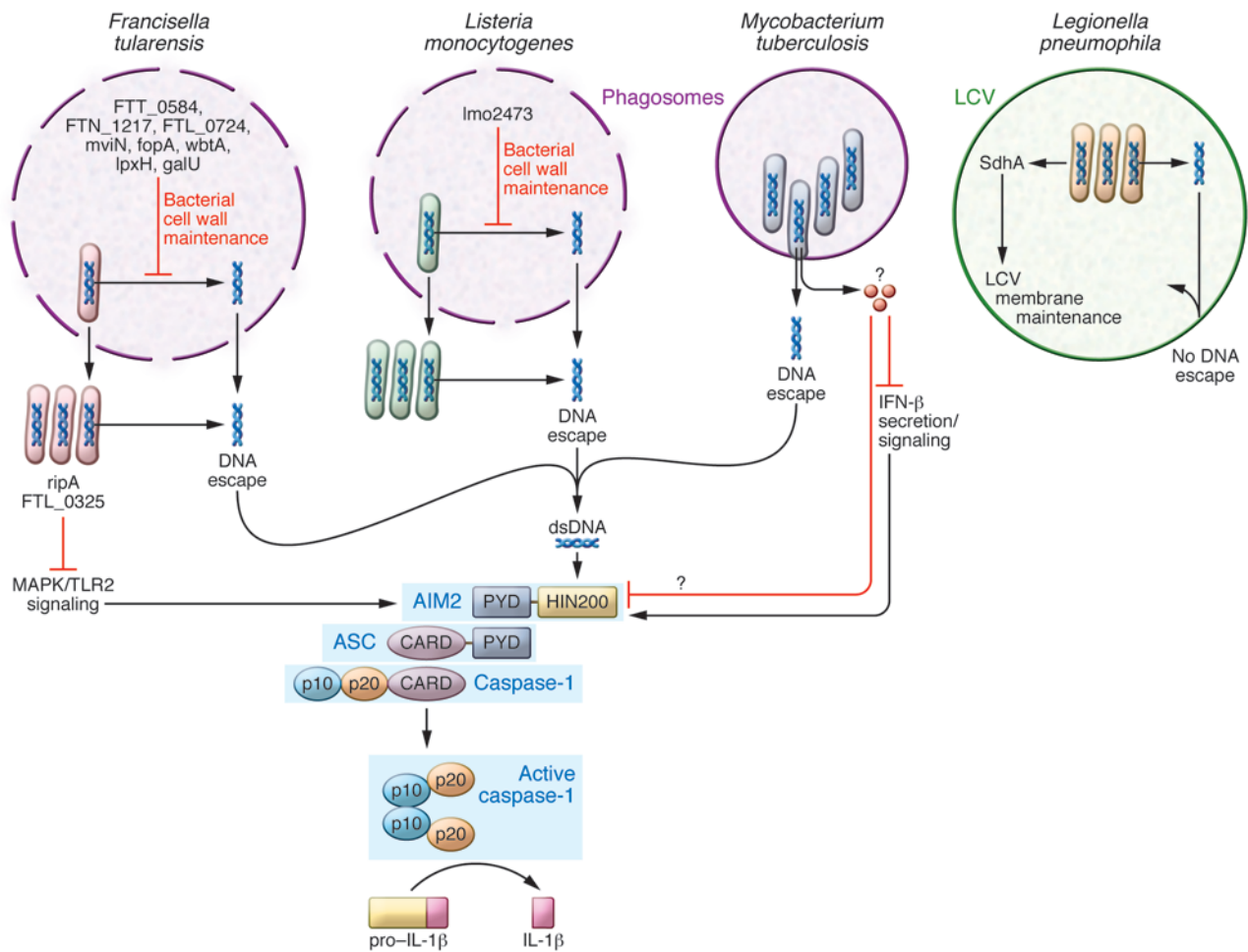
known effector molecules, ExoS, ExoT, ExoU, and ExoY, which can be secreted into the host cell. ExoU has phospholipase A2 activity, which is required to suppress caspase-1 activation by an as-yet-unknown mechanism (36).

ExoS, another *P. aeruginosa* effector molecule, is also translocated into host cells through the T3SS (Figure 2). Interestingly, although most *P. aeruginosa* strains carry ExoT and ExoY, the presence of ExoS and ExoU appears to be mutually exclusive (41). ExoS is a Rho GTPase/ADP-ribosyltransferase (ADPR) protein. ExoS-competent bacteria have been shown to inhibit *P. aeruginosa*-induced IL-1 $\beta$  maturation (37). ExoS also appears to effectively switch the mode of death of *P. aeruginosa*-infected cells from proinflammatory caspase-1-dependent pyroptosis to a comparably noninflammatory caspase-3-dependent apoptotic cell death (37). It is not known whether ExoS-induced caspase-3 activation is an active process or a consequence of caspase-1 inhibition. However, the strategy of inducing cellular death dependent upon caspase-3 may allow the organism to “silently” replicate prior to activation of the innate immune response. The direct link between in vivo virulence and inhibition of inflammasome activation by ExoS- and ExoU-containing *P. aeruginosa* strains is an area that requires further investigation.

*Yersinia* spp. Pathogens belonging to the genus *Yersinia* also have effector proteins capable of modulating caspase-1 activity. A number of species from the genus *Yersinia* possess the effector molecule YopM that in *Y. pseudotuberculosis* inhibits caspase-1 activity by binding to the active site of caspase-1 (Figure 2). This was mediated by a four-amino acid sequence in an exposed loop of YopM, which bears similarity to caspase-1 substrate YVAD. The interaction between YopM and caspase-1 has been proposed to both limit the activity of caspase-1 and inhibit the assembly of the complete inflammasome complex (39). Consistent with this innate immune evasion function of YopM, *Y. pseudotuberculosis* strains bearing mutated YopM are highly attenuated in vivo. Further, this attenuation has been demonstrated to be dependent upon caspase-1, thus implicating the ability to subvert the activation of the inflammasome as being an important survival strategy in the pathogenesis of YopM-competent *Yersinia* (39).

YopE and YopT are *Yersinia* effector proteins that target the actin cytoskeleton of the host cell and limit phagocytosis of the bacteria through targeting of Rho GTPase family members. YopE and YopT from *Y. enterocolitica* have also been demonstrated to inhibit pro-caspase-1 oligomerization and maturation when overexpressed (Figure 2). Overexpression of YopE and YopT interferes with Rac-1 activation and significantly inhibits caspase-1 activation (40). This inhibition of caspase-1 activation by YopE and YopT resulted in diminished macrophage caspase-1-mediated processing of pro-IL-1 $\beta$  into its mature secreted form (40). In a separate study, it was determined that *Y. enterocolitica* strains with mutant YopE were moderately attenuated in vivo (42). However, *Y. enterocolitica* YopT mutants did not display any defects in virulence (42). To date, no study has investigated a role for inflammasome activation or IL-1 $\beta$  secretion in the virulence of this mutation in vivo; therefore, additional studies are warranted to determine the role of the inflammasome in response to YopE- or YopT-deficient *Yersinia* spp.

The final identified mechanism by which *Yersinia* spp. subverts inflammasome activation is through the activity of YopK (Figure 2). YopK from *Y. pseudotuberculosis* was shown to bind to the T3SS



**Figure 3. Evasion of AIM2 inflammasome activation by intracellular pathogens.** *F. tularensis* and *L. monocytogenes* express a number of proteins involved in maintenance of bacterial cell wall integrity. In their absence, increased damage to a subpopulation of bacteria occurs while in the macrophage phagosome, resulting in leakage of bacterial DNA. Once *Francisella* and *Listeria* escape into the cytosol of the cell, this DNA is also released into the host cell cytosol, where it interacts with and activates the AIM2 inflammasome. *F. tularensis* ripA and the gene products of *FTL\_0325* inhibit MAPK and TLR2 signaling, respectively, and interfere with priming of the AIM2 inflammasome. *L. pneumophila* secretes the effector molecule into the LCV, where it maintains LCV membrane integrity and prevents *Legionella* DNA from entering the cytosol and being recognized by AIM2. *M. tuberculosis* secretes DNA into the cytosol via the ESX-1 secretion system concurrently with an unknown inhibitor of IFN-β and also possibly AIM2.

translocon, effectively masking it from recognition by NLRP3 (38). In the absence of YopK, *Y. pseudotuberculosis* activates caspase-1 in an NLRP3/NLRC4/ASC-dependent manner, resulting in increased bacterial clearance of the YopK mutant in vivo (38).

### Preventing DNA release to avoid AIM2 inflammasome activation

*F. tularensis*, *L. monocytogenes*, and *L. pneumophila* all express proteins that allow the bacteria to evade robust activation of the AIM2 inflammasome. *L. monocytogenes* is a Gram-positive facultative intracellular pathogen that rapidly replicates within host cells. Following internalization, *L. monocytogenes* escapes the phagosome and enters the host cell cytosol by a mechanism that is specialized in its ability to prevent the associated induction of host cell death. Mutation of *lmo2473*, a gene that encodes a protein of unknown function, resulted in *L. monocytogenes* that hyperactivated the AIM2 inflammasome independently of both NLRC4 and NLRP3 (Figure 3 and ref. 43). This increased AIM2 inflammasome activation

was associated with increased IL-1 secretion and pyroptotic cell death. Hyperactivation of the AIM2 inflammasome was linked to impaired cell wall integrity of the *lmo2473* mutant, driving an increase in its intracellular lysis. Enhanced bacterial lysis resulted in an increase in DNA release into the cytosol that in turn triggered augmented AIM2 inflammasome activation (43). Therefore, the maintenance of normal bacterial cell wall integrity while in the host cell cytosol, dependent in part upon expression of *lmo2473*, allowed the bacteria to subvert robust AIM2 activation.

*F. tularensis* is a virulent Gram-negative bacterium that, after its phagocytosis by the macrophage, escapes the phagosome into the cytosol where it replicates. Although this escape from the phagosome is critical to the survival of *F. tularensis*, it is thought to be associated with damage to a small population of bacteria. As in the case of *L. monocytogenes* lysis described above, damage of *F. tularensis* is associated with release of dsDNA, resulting in the activation of the AIM2 inflammasome. AIM2 inflammasome activation in turn leads to the secretion of IL-1β and IL-18 and

the induction of macrophage pyroptosis (44, 45). Mutation of the *F. tularensis* live vaccine strain (LVS) putative lipid II flippase, *mviN*, resulted in highly attenuated bacteria in in vivo infection models (46). The attenuation of the *mviN* mutant strain was dependent on the inflammasome, as mice deficient in ASC or caspase-1, but not wild-type mice, succumbed to infection with the *mviN* mutant strain. The *mviN* mutant strain also hyperactivates the AIM2 inflammasome in vitro (Figure 3). In addition to *mviN*, a number of additional *F. tularensis* LVS and *F. novicida* mutant strains have been identified that also result in increased macrophage cytotoxicity and elevated IL-1 $\beta$  secretion (Figure 3 and refs. 47–50). Importantly, Peng and colleagues demonstrated that these mutations generally result in defects in membrane-associated proteins or in genes involved in O-antigen or LPS biosynthesis (47). This results in increased intracellular lysis of the mutant bacteria, leading to increased bacterial DNA release into the host cell cytosol, which triggers AIM2 inflammasome activation (47). Therefore, the maintenance of bacterial membrane stability is required as a strategy for *F. tularensis* to avoid AIM2 inflammasome activation. In addition, mutations in *ripA* and *FTL\_0325* in *F. tularensis* LVS resulted in hypercytotoxicity and increased IL-1 $\beta$  secretion. These gene products were not involved in maintaining bacterial cell wall integrity, but instead interfered with MAPK- and TLR2-signaling pathways and hence interfered with the priming step required for AIM2 inflammasome activation (48, 51).

*L. pneumophila* is a Gram-negative intracellular bacterium that activates the NLRC4 inflammasome via its Dot/Icm T4SS. *L. pneumophila* resides within a structure called a *Legionella*-containing vacuole (LCV) that avoids fusion with lysosomes, thereby maintaining a replicative niche for this pathogen within the macrophage. The Dot/Icm-translocated effector molecule SdhA is required for *L. pneumophila* intracellular growth. Mutant bacteria deficient in SdhA induced elevated IL-1 $\beta$  secretion and macrophage pyroptosis that was dependent on AIM2 inflammasome activation, but independent of the flagellin-sensing NLRC4 inflammasome pathway (52). Interestingly, SdhA was required to maintain LCV membrane integrity, and thus its absence drove *Legionella* DNA release into the cytosol and increased AIM2 but not NLRC4 inflammasome activation (52).

*M. tuberculosis* possesses an ESX-1 secretion system through which bacterial DNA gains entry into the host cell cytosol. Interestingly, the DNA that enters the host cell does not result in AIM2 inflammasome activation. ESX-1-competent *M. tuberculosis*, but not other closely related species of *Mycobacterium*, inhibits secretion of IFN- $\beta$  by host cells as well as IFN- $\beta$ -mediated signaling (53). This *M. tuberculosis*-mediated reduction in IFN- $\beta$  secretion was partially responsible for the ability of *M. tuberculosis* to inhibit AIM2 inflammasome activation, as type I IFN signaling is required for AIM2 inflammasome activation (54). Hence, the ESX-1-dependent cosecretion into the host cell cytosol of a putative IFN- $\beta$  inhibitor (and/or AIM2 inhibitor) along with *M. tuberculosis* DNA may allow *M. tuberculosis* to evade AIM2 inflammasome activation. The identity of the *M. tuberculosis*-derived inhibitor of IFN- $\beta$  and/or AIM2 remains unknown. The relevance of these findings to in vivo host defense against *M. tuberculosis* also remains to be addressed.

## Evasion of inflammasome activation by pathogen decoy proteins

An inflammasome evasion strategy that has been described in viruses is the expression of viral decoy proteins that attenuate inflammasome activation. Kaposi's sarcoma-associated herpes virus (KSHV) encodes an NLRP1 homolog that lacks PYD and CARD, interacts with host NLRP1, NLRP3, and NOD2, and inhibits virally induced IL-1 $\beta$  secretion. The authors suggest that this ability of KSHV to inhibit inflammasome activation may contribute to the establishment of long-term viral persistence (55).

Johnston et al. identified a poxvirus-encoded PYD-containing protein, M13L, which interacted with ASC and inhibited subsequent inflammasome activation. Deletion of *M13L* resulted in attenuation of myxoma virus in rabbits in vivo (56). Cowpox virus and other orthopox viruses encode a protein that can also inhibit caspase-1 activity (57). The cowpox-encoded cytokine response modifier A (CrmA) protein serves as a pseudo-target for active caspase-1 that, upon cleavage, covalently bonds with a cysteine in the active site of caspase-1, rendering it inactive. This inactivation is very potent, occurs at very low concentrations of CrmA, and is important to virulence, as cowpox viruses lacking CrmA are highly attenuated in vivo (57–59). These observations further illustrate the importance of inflammasome subversion mechanisms as a survival strategy for pathogens.

## Safety of IL-1 inhibitors

The clinical use of biologic agents to modulate specific inflammatory pathways has grown exponentially in the past decade. Given the clear overlap in the pathways that are critical in the control of microbial pathogens and those that drive pathologic autoimmune and autoinflammatory diseases, it is not surprising that therapeutic blockade of cytokines for the treatment of autoimmune and autoinflammatory diseases could result in severe infectious complications. This is exemplified by our experience with TNF- $\alpha$  antagonists, including etanercept, adalimumab, and infliximab. Postmarketing data revealed a dramatic increase in the number of cases of *M. tuberculosis* reactivation related to anti-TNF- $\alpha$  treatment (60, 61). Given that inflammasome pathways play such a critical role in the control of numerous pathogens and that a number of pathogens themselves have developed strategies to specifically evade this innate immune pathway, it seems likely that blockade of IL-1 $\beta$  would be accompanied by a significantly increased risk of both serious and opportunistic infections. Surprisingly, to date, antagonists of IL-1 have been shown to have an excellent safety profile that is better than nearly all other widely used biologics that inhibit inflammatory cytokines. The reason for the remarkable safety profiles of anakinra, rilonacept, canakinumab, and gevokizumab is not at all clear but will be considered below.

*Anakinra*. The majority of the safety data on inhibiting the IL-1 pathway comes from studies using anakinra, the first IL-1 inhibitor available on the market. Anakinra is a recombinant human IL-1 receptor antagonist (rIL-1Ra) that mimics the action of the natural antagonist IL-1Ra.

The earliest large-scale trials utilizing anakinra were performed in the 1990s in sepsis, where it was added to standard therapy in an attempt to improve outcomes and to decrease multorgan system failure (62–64). The striking feature of these trials

was that the treatment of over 1,000 actively septic patients with anakinra (intravenously and in high doses) resulted in no serious safety concerns (62–64). However, there was not a significant therapeutic benefit in sepsis (65).

The therapeutic benefit of anakinra use was more apparent in the treatment of rheumatoid arthritis (RA); anakinra was FDA approved for this indication in 2001. Campion et al. reported preliminary safety data in 175 patients with active RA treated with anakinra at varying doses (66). The trial lasted 7 weeks; the most common adverse event was injection-site reactions, causing 5% of the patients to withdraw from the study, followed by gastrointestinal symptoms. Sixteen percent had an infectious event. There was no placebo arm (66). Subsequent double-blind, randomized, placebo-controlled multicenter trials were performed; over 2,000 patients with RA were enrolled and treated with anakinra, with the study confirming its excellent safety profile (67, 68). There was no significant increase in serious infections (67–71); however, Fleischmann et al. studied 1,346 patients with RA who received anakinra in a 30-month open-label extension and reported an increase in exposure-adjusted event rate for serious infections in the anakinra-treated group (5.37 events/100 patient years) versus controls (1.65 events/100 patient years). However, much of the increased risk of serious infection in this open-label extension was attributed to concurrent corticosteroid use (72). Despite this very favorable safety profile when used as the only biologic, the safety profile is unfavorable with combination biologic therapy. Anakinra in combination with the TNF inhibitor etanercept resulted in more frequent and more severe infections with no added efficacy above etanercept alone (73). Therefore, the use of anakinra with another biologic cytokine-blocking agent should be avoided if at all possible.

Anakinra was subsequently approved by the FDA for use in neonatal onset multisystem inflammatory disorder (NOMID), which is a severe form of cryopyrin-associated periodic syndromes (CAPS) (74). Anakinra has been utilized in a number of other conditions, including other monogenic autoinflammatory disorders (75), such as systemic-onset juvenile idiopathic arthritis (76, 77), adult-onset Still disease (AOSD) (78, 79), gout (80), polyarticular juvenile idiopathic arthritis (sJIA) (81), diabetes (82), heart disease (83–85), and chronic granulomatous disease (86), among many others (87). In these disorders, anakinra was well tolerated except for injection-site reactions and a slight increase in nonserious viral respiratory infections. To date, the development of opportunistic infections in individuals taking anakinra has been exceedingly rare (87).

**Rilonacept.** Rilonacept is a dimeric fusion protein consisting of the ligand-binding domains of the human IL-1R extracellular domains (IL-1RI and IL-1 receptor accessory protein) linked to the Fc portion of human IgG1. It acts as a soluble decoy receptor by binding to IL-1 $\beta$  and preventing its interaction with IL-1R on the cell surface. In 2008, the FDA approved rilonacept for certain forms of CAPS, granting it orphan drug status.

Goldbach-Mansky et al. reported the result of a small open-label trial of rilonacept in 5 CAPS patients that showed clinical improvement; they did not observe any drug-related adverse events (88). That same year, Hoffman et al. published results on the efficacy and safety of rilonacept in 47 patients with CAPS (89). Rilonacept was generally well tolerated, with injection-site reac-

tions and nonserious viral upper respiratory infections being the most common adverse events (89, 90). There were 2 deaths during the study: a 71-year-old female who died after developing sinusitis and pneumococcal meningitis and a 37-year-old who died of a myocardial infarction. Subsequently, studies of rilonacept in other autoinflammatory disorders including gout (91, 92), sJIA (93, 94), AOSD (95), familial Mediterranean fever (96), and Schnitzler syndrome have been reported (97). Again, blockade of IL-1 with this longer-acting agent was well tolerated, with no increased risk of serious or opportunistic infections.

**Canakinumab and gevokizumab.** There are several anti-IL-1 $\beta$  monoclonal antibodies available, including canakinumab and gevokizumab (98, 99). Canakinumab is a fully humanized IgG1 anti-IL-1 $\beta$  monoclonal antibody that binds to human IL-1 $\beta$  with high specificity and neutralizes the bioactivity of this cytokine. These agents have been utilized to treat a number of inflammatory disorders, including CAPS (98, 100, 101), other monogenic autoinflammatory disorders (75), gout (102), type 1 diabetes (103), type 2 diabetes (104, 105), RA (106), sJIA (107), Schnitzler syndrome (108), and Behçet syndrome (109) as well as other inflammatory conditions (87).

Gevokizumab is a monoclonal anti-IL-1 $\beta$  antibody that negatively modulates IL-1 $\beta$  signaling through an allosteric mechanism. It decreases the binding affinity of IL-1 $\beta$  for the IL-1 receptor type I (IL-1RI) signaling receptor, but not the IL-1 counterregulatory decoy receptor (IL-1 receptor type II) (110). It does not interfere with IL-1Ra or block IL-1 $\beta$  binding to the soluble forms of the IL-1 receptors (111). Gevokizumab inhibits both the binding of IL-1 $\beta$  to IL-1RI and the subsequent recruitment of IL-1 accessory protein, primarily by reducing the association rates of these interactions; as such, it is a unique inhibitor of IL-1 $\beta$  signaling (110). Initial studies with this monoclonal antibody have been in the treatment of type 2 diabetes (99, 112) and Behçet disease (113).

The safety profile of the longer-acting IL-1-blocking agents resembles that of anakinra. However, these agents are newer and a smaller number of patients have been treated; therefore, the long-term safety profile has yet to be fully delineated.

## Concluding remarks

The inflammasome is a critical mechanism by which the innate immune system recognizes and limits pathogenic insults. The inflammasome signals and coordinates the response of a number of different cell types primarily through the cytokines IL-1 $\beta$  and IL-18 and through the initiation of pyroptotic cell death. It is evident that the modulation of inflammasome activity is an important strategy employed by a number of pathogens to subvert the normal innate immune response. With the recent introduction of a number of different therapies targeting IL-1 $\beta$  and its receptor, it has become increasingly important to understand the interplay of pathogens and host in the context of inflammasome activation. Surprisingly, the clinical use of inhibitors of IL-1, such as anakinra, rilonacept, canakinumab, and gevokizumab, has been associated with exceedingly few reported infectious complications. Inflammasome-driven pyroptosis and IL-18 production play nonredundant roles in host defense against pathogens that are not blocked by the inhibition of IL-1 signaling. It may be that the unexpected safety profile for IL-1

blockade is due to this narrow function. As additional therapeutic strategies to inhibit this pathway emerge, careful attention will be required to determine whether inhibition of inflammasomes in toto is similarly seemingly well tolerated. In addition, as the use of longer-acting IL-1 inhibitors increases, patterns of infectious complications may declare themselves in select patient populations. Finally, as new therapies are developed to treat infectious disease, especially therapies against pathogens that subvert inflammasome activation, it may prove beneficial to investigate the use of adjunctive treatments that trigger inflammasome activation to enhance the innate immune responses against invading pathogens.

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