Recognition of Bacteria by Inflammasomes

Jakob von Moltke, Janelle S. Ayres, Eric M. Kofoed, Joseph Chavarria-Smith, and Russell E. Vance

Department of Molecular & Cell Biology, Division of Immunology and Pathogenesis, University of California, Berkeley, California 94720; email: rvance@berkeley.edu

Keywords
caspase, NLR, innate immunity, pyroptosis

Abstract

Inflammasomes are cytosolic multiprotein complexes that assemble in response to a variety of infectious and noxious insults. Inflammasomes play a critical role in the initiation of innate immune responses, primarily by serving as platforms for the activation of inflammatory caspase proteases. One such caspase, CASPASE-1 (CASP1), initiates innate immune responses by cleaving pro-IL-1β and pro-IL-18, leading to their activation and release. CASP1 and another inflammatory caspase termed CASP11 can also initiate a rapid and inflammatory form of cell death termed pyroptosis. Several distinct inflammasomes have been described, each of which contains a unique sensor protein of the NLR (nucleotide-binding domain, leucine-rich repeat-containing) superfamily or the PYHIN (PYRIN and HIN-200 domain-containing) superfamily. Here we describe the surprisingly diverse mechanisms by which NLR PYHIN proteins sense bacteria and initiate innate immune responses. We conclude that inflammasomes represent a highly adaptable scaffold ideally suited for detecting and initiating rapid innate responses to diverse and rapidly evolving bacteria.
**INTRODUCTION**

The term inflammasome was first coined by Tschopp and colleagues (1) in 2002 to describe a high-molecular-weight protein complex that forms in the cytosol and serves as a platform for the recruitment and autoproteolytic activation of certain caspases, notably CASP1, CASP4, and CASP5 (Figure 1). These caspases, along with CASP11 and CASP12 in the mouse, are termed the inflammatory caspases and are distinct from the caspases involved in the induction of apoptosis. CASP1 is the best characterized of the inflammatory caspases and was first described as the protease required for the proteolytic cleavage of pro-IL-1β and pro-IL-18, resulting in their functional maturation and release (2, 3).

In the decade since the initial description of an inflammasome, it has become clear that there are in fact multiple distinct inflammasomes, each of which is activated by unique stimuli that can include infectious agents as well as noninfectious stimuli. The formation of each inflammasome is dictated by a unique scaffolding protein (Figure 2). Most of these scaffolding proteins contain a nucleotide-binding domain (NBD) and leucine-rich repeats (LRRs) and are thus members of the NBD-LRR (NLR) superfamily. The LRRs are believed to have two functions (Figure 1). First, LRRs are thought to be required to maintain NLRs in an autoinhibited state because deletion of LRRs generally leads to constitutive activation. Second, by analogy to the well-characterized ligand binding function of LRR domains in Toll-like receptors (TLRs), the LRRs of NLRs are believed to mediate recognition of pathogen-derived (or potentially self-derived) ligands. As discussed below, however, evidence for ligand recognition by NLRs is weak. The NBD is believed to mediate the assembly of NLRs into an oligomerized state that is critical for the induction of downstream signaling. In NLRs, signaling is initiated by CARDs (caspase activation and recruitment domains) that can recruit CASP1 directly or by PYRIN domains that recruit CASP1 via the CARD-PYRIN-containing adaptor protein ASC (Figure 2). Once recruited to an oligomerized inflammasome, CASP1 is activated via dimerization and autoproteolysis (Figure 1). Interestingly, non-NLR proteins such as PYRIN-HIN-200 domain–containing (PYHIN) proteins have also been shown to mediate CASP1 activation (4–8). Although PYHIN proteins lack NBDs and LRRs, they may nevertheless exhibit the properties of autoinhibition and ligand-induced oligomerization (9).

It has become clear that inflammasomes not only have diverse mechanisms of activation but can also have diverse functions that extend beyond cytokine processing. Most notably,

---

**Figure 1**

Generic model of inflammasome activation. Cytosolic nucleotide-binding domain, leucine-rich repeat (NBD-LRR) proteins are maintained in a autoinhibited state until ligand recognition and ATP binding drive oligomerization via the NBD domain. Recruitment of CASP1 to form the inflammasome complex occurs directly via CARD-CARD interactions or indirectly via the adaptor ASC. Monomeric CASP1 is believed to be activated by proximity-induced autoproteolysis (autoprocessing), leading to downstream effector functions such as pyroptosis and processing of pro-IL-1β and pro-IL-18. In some cases, unprocessed CASP1 can also initiate pyroptosis. Similar mechanisms are postulated for the other inflammatory caspases (CASP4, CASP5, CASP11, and CASP12) but have not been well characterized. Abbreviations: CARD, caspase activation and recruitment domain; PYD, PYRIN domain.
inflammasome activation is frequently associated with a rapid and lytic form of host cell suicide called pyroptosis (10). Inflammasome activation plays a critical role in mediating host defense, but inappropriate or excessive inflammasome activation can also be detrimental to host health. Inflammasomes are therefore a complex and surprisingly diverse set of cytosolic sensors that must be stringently regulated. Here we focus on inflammasome activation by bacteria. We survey what is known about how bacteria activate inflammasomes and ask whether it is possible to discern any general principles that govern inflammasome activation or function in host defense.

The discovery of NLRs in mammals was predated by work demonstrating that innate immunity in plants depends largely on diverse NBD-LRR proteins (reviewed in 11, 12). Just as inflammasomes induce a pyroptosis response, plant NBD-LRR proteins often induce a rapid cell death response, termed the hypersensitive response, that is believed to restrict pathogen replication at sites of infection. Despite the strikingly similar domain architectures and functions of mammalian and plant NBD-LRR proteins, evolutionary analyses strongly suggest that NBD-LRR proteins were absent in the common ancestor of plants and animals and thus evolved independently (convergently) in each lineage (13, 14). The independent evolution of plant and animal NBD-LRR proteins raises a key question: What about the conjunction of an NBD and an LRR is so fundamentally advantageous that evolution would strike at least twice upon this protein architecture as a solution to the problem of effective innate immune defense?

Figure 2
Murine inflammasome proteins and their domains. NBD-LRR family members can be categorized according to the unique domains they encode: 1 NLRP1: FIIND; 2 all other NLRP proteins: PYRIN domain (PYD); 3 NLRC: CARD; 4 NAIP: BIR domains; 5 AIM2 belongs to the PYHIN family of proteins and uses the HIN-200 domain for ligand recognition. 6 NLRP10 is the only family member lacking an LRR domain. 7 ASC is an adaptor required by NLRP proteins for recruitment of CASP1. 8 CASP1 contains a CARD and a protease domain that autoprocesses into p10 and p20 subunits. CASP11 and CASP12 are additional inflammatory caspases in mice; CASP4 and CASP5 are additional inflammatory caspases in humans. Other important differences between murine and human proteins are listed (below). (See text for abbreviations.)
By articulating the key principles underlying inflammasome activation by bacteria, this review attempts to address this question.

**RECOGNITION OF BACTERIA BY INFLAMMASOMES**

**The NAIP/NLRC4 Inflammasome**

**Activation of NLRC4.** NLRC4 (also known as IPAF, CLAN, and CARD12) was identified on the basis of similarity to apoptotic protease-activating factor-1 (APAF-1) and was shown to recruit and activate CASP1 (15–17). Initial studies of *Nlrc4*−/− mice demonstrated that *Nlrc4* is essential for CASP1 activation by *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) (18). Since then, CASP1 activation by a large number of additional pathogens, including *Legionella pneumophila* (19–21), *Pseudomonas aeruginosa* (22–24), and *Shigella flexneri* (25), has been shown to depend on NLRC4. Activation of the NLRC4 inflammasome by bacteria depends largely on bacterial flagellin expression. Indeed, the cytosolic presence of the flagellin protein—or just its C-terminal 35 amino acids—is sufficient to activate NLRC4 (26–29). C-terminal truncation of the flagellin protein (20) or mutation of three conserved C-terminal hydrophobic amino acids (27) abolished recognition of flagellin by NLRC4 (20, 26, 27, 30–33). The C terminus comprises part of the D0 region of flagellin and is distinct from the D1 region, which is recognized by the TLR5 cell-surface flagellin receptor (34, 35). Importantly, direct binding of flagellin to NLRC4 has not been demonstrated, and in fact, flagellin activation of NLRC4 appears to be indirect (see below).

Activation of NLRC4 by flagellated bacteria also requires bacterial expression of specialized secretion systems, such as the *S. Typhimurium* SPI-1 type III secretion system (T3SS) or the *L. pneumophila* Dot/Icm type IV secretion system (T4SS). The physiological role of these secretion systems is to translocate bacterial virulence factors into the host cell cytosol, but considerable evidence suggests they can also translocate flagellin (27, 36). Cytosolic translocation of flagellin is presumably inadvertent, because flagellin has no known function in the host cell cytosol. The natural hosts of *L. pneumophila* are amoebae, not mammals. Thus *L. pneumophila* may not have experienced selective pressure to evade NLRC4, whereas other mammalian-adapted pathogens (e.g., *S. Typhimurium* and *Listeria monocytogenes*) appear to have evolved regulatory mechanisms that limit flagellin expression in host cells (see below).

Interestingly, several flagellin-deficient bacteria, most notably *Shigella*, are also capable of activating NLRC4 (22, 25, 31, 32, 37). Activation of NLRC4 by these pathogens requires expression of a T3SS (22, 25, 31, 32, 37). Miao and colleagues (31) provided an explanation for the flagellin-independent activation of NLRC4 by demonstrating that the inner rod proteins of several bacterial T3SSs are capable of activating NLRC4. Like the D0 domain of flagellin that activates NLRC4, the T3SS inner rod proteins are believed to adopt an α-helical secondary structure and oligomerize to form a protein-translocation channel (31, 38). However, despite analogous functional and structural properties, flagellin and T3SS inner rod proteins share little primary amino acid sequence similarity. The question of how NLRC4 could respond to structurally diverse ligands has recently been resolved by the discovery that cytosolic recognition of flagellin and inner rod proteins is mediated not directly by NLRC4 but instead by the NAIP subfamily of NLRs (32, 37). According to the current model, NAIP5 and NAIP6 directly recognize flagellin, whereas NAIP2 recognizes T3SS rod proteins (Figure 3). Epistasis analysis indicates that activated NAIPs induce downstream oligomerization and activation of NLRC4 (37), which appear to be required for the downstream recruitment and activation of CASP1 by NLRC4. In addition, CASP1 activation by mouse NLRC4 requires phosphorylation of NLRC4 on serine 533. PKCδ may be the kinase that phosphorylates NLRC4, but other...
Figure 3
Activation of NAIP/NLRC4 inflammasomes by bacteria. NAIP/NLRC4 inflammasomes are activated following detection of flagellin or T3SS rod proteins secreted into the host cytosol during bacterial infection by *Legionella pneumophila*, *Salmonella enterica* serovar Typhimurium, *Shigella flexneri*, and *Pseudomonas aeruginosa*. Ligand specificity is determined by the NAIP proteins: NAIP5 and NAIP6 recognize flagellin, and NAIP2 recognizes the T3SS rod. The CARD-containing NLRC4 serves as an adaptor for recruitment of CASP1 downstream of the NAIPs. Phosphorylation of NLRC4 is required for NLRC4 function. NAIP, NLRC4, and CASP1 are sufficient to initiate pyroptosis, but processing of the IL-1β and IL-18 cytokines requires recruitment of the ASC adaptor into the complex. Abbreviations: T3SS, type III secretion system; T4SS, type IV secretion system; CARD, caspase activation and recruitment domain.

kinases may also be involved (39). It remains unclear how NLRC4 kinases are activated and whether this occurs downstream of ligand recognition by NAIPs.

**NAIPs.** Naip5 was originally identified as a gene that mediates resistance of B6 mouse macrophages to intracellular replication of *L. pneumophila* (40, 41). By contrast, macrophages from the A/J mouse strain carry a partially defective *Naip5* allele and are thus highly permissive to *L. pneumophila* replication. Naip5 is one of several tandemly repeated *Naip* genes in mice, but Naip5 is the only *Naip* gene essential for the restriction of *L. pneumophila* replication, despite the expression in B6 mice of functional *Naip1*, 2, 5, and 6 transcripts (40, 41). Among different inbred mouse strains, the *Naip* locus contains variable numbers of *Naip* paralogs. Individual *Naip* genes also vary from strain to strain; for example, the A/J *Naip5* allele encodes 14 amino acid polymorphisms compared with the B6 allele and appears to be expressed at lower levels (41). However, the precise polymorphism(s) in *Naip5* responsible for the permissiveness of A/J remains unknown (42). The requirement for *Naip5* in restriction of *L. pneumophila* replication was confirmed by the generation of *Naip5*−/− mice on the B6 background (27).

NAIP proteins contain a central NBD and C-terminal LRRs, but they differ from other NLRs in that they contain three N-terminal baculovirus inhibitor-of-apoptosis repeats (BIRs). The BIRs appear critical for NAIPs to activate NLRC4 (37), but the mechanism of **BIR:** baculovirus inhibitor-of-apoptosis repeat
activation is completely unknown. Consistent with the proposed role of the NBD in mediating protein-protein oligomerization, mutation of the NBD abolishes the ability of NAIP5 to induce NLRC4 oligomerization (37). The LRRs of NAIPs appear to be responsible for ligand recognition (E. Kofoed, J. Tenthorey, and R. Vance, unpublished observations). Physical association of bacterial flagellin to NAIP5 and NAIP6 (but not NAIP2) has been shown recently using a variety of biochemical approaches (32, 37). Similarly, T3SS inner rod proteins associate with NAIP2 (but not NAIP5). These biochemical studies were supported by knockout and knockdown studies. For example, Naip5−/− macrophages exhibit a selective defect in the response to flagellin but respond normally to inner rod proteins (37, 43). Conversely, knockdown of Naip2 selectively affects NLRC4 activation in response to inner rod proteins but does not affect flagellin recognition (32, 37). Taken together, these observations suggest a receptor-ligand model for activation of the NAIP/NLRC4 inflammasomes (Figure 3) but do not rule out the involvement of additional proteins.

**Human NAIP.** In humans, *L. pneumophila* is the causative agent of a severe pneumonia known as Legionnaires' disease (44). Human alveolar macrophages and monocytes cultured ex vivo generally support robust *L. pneumophila* replication, which contrasts with the restricted replication seen in most mouse macrophages (45, 46). This difference between human and mouse macrophages is likely explained by the recent report that human NAIP (hNAIP) does not respond to flagellin or T3SS inner rod proteins but instead responds specifically to the T3SS needle protein from *Chromobacterium violaceum* (32). Because *C. violaceum* is not a significant human pathogen, it is important that T3SS needle proteins from a variety of clinically important pathogens, such as *Shigella*, *Salmonella*, and *Burkholderia*, are also detected by hNAIP (32). Whether hNAIP detection of T3SS needle proteins is relevant during infection by these or other common bacterial pathogens warrants further study.

It is striking that the needle proteins recognized by hNAIP are, like the mouse NAIP ligands, α-helical proteins that oligomerize to form a protein-translocation channel. Because these ligands share little in the way of primary sequence, a tempting hypothesis is that all NAIP ligands share a unique secondary structural characteristic that renders them suitable as targets of innate immune recognition. Unlike the tandemly duplicated arrays of *Naip* genes in mice, the human *Naip* gene locus is believed to harbor only a single full-length functional ortholog; however, copy number variation has been reported between geographic human populations (47, 48). It is therefore possible that in humans, as in mice, NAIP function is variable among individuals.

**The NLRP1 Inflammasome**

NLRP1 was a key constituent of the originally identified inflammasome (1), but the physiological signals that activate NLRP1 were initially unknown. It has since become apparent that many mammals have NLRP1 orthologs, and some species, such as mice, harbor multiple paralogs exhibiting high allelic variation (49, 50). Here we focus on rodent NLRP1 proteins because their activation and in vivo roles are best understood.

**Activation of NLRP1B by anthrax lethal toxin.** One of the main virulence factors of *Bacillus anthracis*, the causative agent of anthrax, is a bipartite protein toxin termed lethal toxin (LeTx). LeTx is composed of protective antigen (PA) and lethal factor (LF). PA forms a channel that translocates LF, a zinc metalloprotease, into the host cell cytosol (51). The best-characterized proteolytic targets of LF are the MAP kinase kinases (MAPKKs) 1–4 and 6–7. LF-mediated cleavage of MAPKKs results in their inactivation, thereby impairing numerous host defense pathways including neutrophil chemotaxis, cytokine and chemokine production, antigen presentation, and costimulation (reviewed in 52).
to disrupting MAPKK signaling, LeTx can also cause pyroptosis of macrophages from specific strains of mice (50, 53, 54); this responsiveness to LeTx was genetically mapped to the Nlrp1b gene (50). Although NLRP1B is sufficient to confer LeTx sensitivity, its requirement was only recently verified through the creation of an Nlrp1b knockout mouse line (55). Rat macrophages also undergo pyroptosis in response to LeTx; this responsiveness was genetically mapped to a locus containing the Nlrp1 gene, suggesting that NLRP1 activation is mechanistically conserved between mice and rats (56, 57). It is worth noting that LeTx can also induce a slower cell death in murine and human macrophages that is independent of the NLRP1B inflammasome and is instead related to disruption of p38 MAP kinase signaling downstream of TLRs (58, 59).

The mechanism of NLRP1B activation by LF is not fully resolved. NLRP1B activation requires the proteolytic activity of LF and is not due to recognition of LF protein alone, as catalytically dead LF does not induce pyroptosis (54, 60). As mentioned above, the known proteolytic substrates of LF are MAPKKs 1–4 and 6–7. Although plant NLRs respond to disrupted kinase signaling pathways, no strong connection between MAPKK cleavage and NLRP1B activation has been made. Recently it was shown that LF cleaves the N terminus of the responsive rat NLRP1 allele (from CDF rats) but does not cleave the nonresponsive allele (from Lewis rats). Importantly, cleavage of NLRP1 was suggested to be required for its activation, as mutations that made NLRP1 cleavage resistant abolished its ability to activate CASP1 (61). Although it is likely that the LeTx-sensitive alleles of mouse and rat NLRP1 share a conserved mechanism of activation, the site cleaved in rat NLRP1 does not appear to be conserved in any mouse NLRP1B allele. Thus it remains to be determined whether mouse NLRP1B is also directly cleaved by LF. Additionally, it is unknown whether cleavage by LF is sufficient for rat or mouse NLRP1B activation; it is possible that other LF substrates participate. Supporting this latter possibility, several groups have shown that proteasome activity is required for NLRP1B activation and that this activity is a specific requirement for activation of NLRP1B but not of NLRP3 or NLRC4 (54, 62, 63). In addition, inhibition of the N-end rule degradation pathway blocks NLRP1B activation (64). Taken together, these observations suggest a model in which LF cleaves cellular substrates that act as negative regulators of NLRP1B, and these substrates become destabilized according to the N-end rule degradation pathway (Figure 4).

**Human NLRP1.** Human NLRP1 does not respond to LeTx, and human macrophages do not undergo pyroptosis in response to LeTx (58, 65). Use of a cell-free reconstituted system suggested that muramyl dipeptide (MDP), a fragment of bacterial peptidoglycan, might be an agonist of human NLRP1 (66). However, although MDP has been reported to activate CASP1 in human macrophages, the requirement for NLRP1 is only partial, and the effect of MDP may result primarily from its established ability to activate NOD2-dependent priming of pro-IL-1\(\beta\) and NLRP3 (67–69). Thus, the function of human NLRP1 remains poorly understood.

**A unique domain: FIIND.** Both the human and mouse NLRP1 have a unique domain, termed the FIIND (domain with function to find), that is not found in other NLRs (70). Recently, investigators suggested that this domain resembles the ZU-5 and UPA domains found in proteins such as PIDD and UNC5B. In PIDD, the ZU-5/UPA domains exhibit an unusual ability to undergo autocatalytic cleavage at a conserved HFS site (49, 71). Similar spontaneous cleavage was observed in the FIINDs of human NLRP1 and murine NLRP1B (49, 72). Mutations that inactivate this self-cleavage disrupt the ability of NLRP1B to respond to LeTx (72). Thus, self-cleavage of NLRP1B within the FIIND, which occurs independently of LeTx, appears to be a maturation step required for NLRP1B function.
Figure 4
Activation of the NLRP1B inflammasome by anthrax lethal toxin. *Bacillus anthracis* secretes lethal factor (LF) and protective antigen (PA), which together form lethal toxin (LeTx). PA binds to the toxin receptor on host cells, leading to PA processing, PA oligomerization, and binding of LF. LeTx is then endocytosed, and in an acidified endosome LF is translocated into the host cell cytosol. LF rapidly cleaves and inactivates MAP kinase kinases (MAPKKs), interfering with many host defense pathways. NLRP1 must undergo autoproteolytic processing in the FIIND domain prior to LF activation. LF cleaves the N terminus of sensitive rat NLRP1 alleles, but it is unknown whether this cleavage is sufficient for activation. As the activity of the proteasome is required for NLRP1 activation, LF may also cleave unidentified substrates that become destabilized and degraded by the proteasome and can therefore no longer act as negative regulators of NLRP1. Once NLRP1B is activated, it can recruit CASP1 directly and induce pyroptosis; upon further recruitment of ASC, the NLRP1B inflammasome can also process IL-1β and IL-18 cytokines.

The NLRP3 Inflammasome

**NLRP3 agonists.** Unlike the *Nlrp1b*, *Nlrp4*, and the *Naip* genes, *Nlrp3* is not constitutively expressed in most resting cells. Activation of the NLRP3 inflammasome therefore requires two signals: (α) NF-κB-dependent transcriptional induction downstream of TLR or NOD ligands or inflammatory cytokines and (β) an agonist...
to induce oligomerization. The first signal, the transcriptional priming, represents a critical checkpoint: Once primed, the NLRP3 inflammasome is activated by an astonishing array of stimuli, at least in vitro. These stimuli include, but are not limited to, crystalline or particulate matter (e.g., uric acid crystals, asbestos, alum) (73–75), ATP signaling through the P2X₇ receptor (76), viral infections (77), fungal products (78), bacterial RNA and DNA (79–81), and numerous bacterial toxins and effector proteins (Supplemental Table 1; see the Supplemental Material link in the online version of this article or at http://www.annualreviews.org).

NLRP3 also appears to be activated in response to the gut microbiota (see below). Although structurally distinct, many of the bacterial products that activate NLRP3 (e.g., nigericin, listeriolysin O, hemolysins) share pore-forming activity, suggesting that this "pattern of pathogenesis" (82) is somehow detected by the NLRP3 inflammasome.

In evaluating NLRP3 agonists, it is important to distinguish their relative contributions to priming versus oligomerization of NLRP3. In some contexts, notably human blood monocytes and THP-1 cells, secretion of autocrine ATP may cause NLRP3 to oligomerize spontaneously once primed (83). Accordingly, stimuli such as lipopolysaccharide (LPS) and MDP, which in most cells [e.g., murine macrophages (76)] merely prime NLRP3, appear sufficient for NLRP3 oligomerization in human monocytes (84, 85).

Mechanism of activation. Given the variety of NLRP3 agonists, it is generally accepted that they do not act as direct ligands for NLRP3 but instead all converge on one or more disruptions of host physiology that are sensed by the NLRP3 inflammasome. A unifying mechanism for NLRP3 activation remains elusive (Figure 5). In the case of crystalline and particulate NLRP3 stimuli, the common mechanism may be lysosomal destabilization resulting in cytosolic release of lysosomal enzymes, such as cathepsin B, that somehow activate NLRP3 (86). For noncrystalline NLRP3 stimuli—including bacterial pathogens, ATP, and nigericin—guanylate-binding protein 5 (GBP5) is required in interferon (IFN)-γ-primed macrophages for robust NLRP3 oligomerization, although the mechanism of GBP5 and NLRP3 interaction remains unclear (87). Importantly, NLRP3 is readily activated in the absence of IFN-γ priming; therefore, it seems GBP5 mostly functions to amplify NLRP3 signaling.

Another unifying model of NLRP3 activation postulates a role for reactive oxygen species (ROS) generated by ATP signaling through the P2X₇ receptor (88), by NADPH-oxidase during phagocytosis of particulate stimuli (75), or by mitochondria (89, 90). How ROS might promote NLRP3 oligomerization remains unresolved. One report suggested that ROS can dissociate thioredoxin-interacting protein (TXNIP) from thioredoxin, allowing TXNIP to act as a ligand for NLRP3 (91); however, more recent data demonstrating that NLRP3 signaling is unchanged in Txnip⁻/⁻ macrophages strongly contradict this model (92). The ROS hypothesis also remains controversial, as ROS inhibitors may only block NLRP3 priming (93). Furthermore, macrophages deficient in NADPH-oxidase subunits respond normally to NLRP3 agonists (94–96). A model of NLRP3 ligand delivery through large membrane hemichannels formed by pannexin-1 (PANX1) (97) was similarly undermined by data from knockout mice demonstrating that Panx1⁻/⁻ macrophages activate the NLRP3 inflammasome normally (98).

NLRP3 activation by all agonists can be blocked by high extracellular potassium (K⁺), suggesting that efflux of intracellular K⁺ is a downstream convergence point (99); however, high extracellular K⁺ also blocks NLRP1B oligomerization (62). It seems, therefore, that additional mechanisms must exist to explain the activation of these distinct inflammasomes by distinct stimuli; the characterization of these mechanisms remains a central challenge for the field. One emerging model suggests that NLRP3 agonists converge on the mobilization of calcium (Ca²⁺), which in turn causes...
AIM2: absent in melanoma 2

**Figure 5**
Activation of the NLRP3 inflammasome by bacteria. Activation of the NLPR3 inflammasome requires two signals: Transcriptional induction of *Nlrp3* (Signal 1) and an agonist to drive NLRP3 oligomerization (Signal 2). The mechanism underlying the second signal remains unresolved. The model shown here postulates an endogenous ligand for NLRP3 that is activated downstream of one or more disruptions in host physiology, including generation of reactive oxygen species (ROS), potassium (K+) efflux, calcium (Ca^{2+}) influx, and/or phagolysosomal rupture or leakage. It is unclear how bacteria cause these disruptions, although in many cases activation of NLRP3 requires secreted bacterial effectors or toxins. In IFN-γ-stimulated macrophages, guanylate-binding protein 5 (GBP5) acts to promote NLRP3 oligomerization. Because NLRP3 lacks a CARD, the adaptor ASC is required for recruitment and activation of CASP1. Abbreviation: TLR, Toll-like receptor.

The AIM2 Inflammasome
Like the NLRP3 inflammasome, the AIM2 inflammasome requires transcriptional priming, but this priming occurs downstream of type I IFN signaling rather than NF-kB signaling. This pathway was first uncovered in studies of *Francisella tularensis* activation of CASP1 (103), which required signaling through the IFN receptor but not through any of the known NBD-LRR inflammasomes. Around the same
time, a DNA-sensing inflammasome was hypothesized on the basis of CASP1 activation in macrophages after double-stranded DNA (dsDNA) is delivered to the cytosol (81). These two observations were unified by the identification of AIM2 as the inflammasome that detects DNA released into the cytosol upon lysis of several pathogens, including *F. tularensis* (4–8).

AIM2 belongs to a family of IFN-inducible PYHIN proteins that contain PYRIN and HIN-200 DNA-binding domains. Like the NLRP proteins, AIM2 includes a PYRIN domain for recruitment of CASP1 via ASC; however, AIM2 lacks an NBD, and a HIN-200 domain replaces the LRR. Thus, AIM2 is structurally distinct from the other known inflammasomes. Despite these differences, AIM2 can nonetheless orchestrate the oligomerization of a large inflammasome complex (8), and the downstream pathways (cytokine processing and pyroptosis) are indistinguishable from those of NBD-LRR proteins. Another member of the PYHIN superfamily, IFI16, has also been proposed to function in CASP1 activation in response to Kaposi’s sarcoma–associated herpesvirus (104).

The generation of Aim2−/− mice confirmed that AIM2 is indeed the *F. tularensis*–sensing inflammasome (8, 105, 106) and that AIM2 can also detect *L. monocytogenes* (105, 107–110). The marked susceptibility of Aim2−/− mice to *F. tularensis* provides one of the best examples of the critical role inflammasomes can play in host defense against bacteria. In response to both *L. monocytogenes* and *F. tularensis*, activation of AIM2 results from bacterial lysis and release of DNA that occur upon bacterial entry into the cytosol (8, 106, 107, 111) (Figure 6). Recently, it was suggested that *L. pneumophila* that lacks its effector protein SdhA might also activate AIM2 via release of bacterial DNA into the host cell cytosol (112).

AIM2 is the only inflammasome for which direct interaction of sensor and ligand has been visualized in an infected cell (8, 106) or analyzed crystallographically (9). The optimal ligand for AIM2 activation is dsDNA of a sufficient length (>80 bp). Modeling suggests an 80-bp dsDNA molecule could accommodate up to 20 AIM2 HIN-200 domains (9). It was therefore proposed that instead of using an NBD to mediate oligomerization and downstream signaling, AIM2 multimerizes on a DNA template. Thus, oligomerization may be a conserved principle of inflammasome signaling despite the significant differences in the protein domains found in NLR versus PYHIN inflammasomes.

### Other NBD-LRR Proteins

Most members of the NBD-LRR protein family remain poorly characterized. NLRP12 was found to activate CASP1 even before the term inflammasome was coined (113), and polymorphisms in NLRP12, like those in NLRP3, are associated with hereditary inflammatory disorders in humans (114). Studies of Nlrp12−/− mice have uncovered diverse roles for NLRP12 in immunity and disease. Two groups described a role for NLRP12 in colonic inflammation (see below) (115, 116); additionally, in a model of contact hypersensitivity, NLRP12 appears to be required for migration of myeloid cells (117). There are also reports of a role for NLRP12 in negative regulation of NF-κB (115). Interestingly, a recent report also found a clear role for NLRP12 in immune defense against bacterial pathogens. Nlp12−/− mice show increased bacterial load and decreased survival when infected with *Yersinia pestis* (118). NLRP12 appears to provide protection via induction of IL-18, which in turn induces IFN-γ; accordingly, Il18−/− and Ifnγ−/− mice are also susceptible to *Y. pestis*. Although the immune defect in Nlp12−/− mice appears selective for *Yersinia*, it remains unclear whether NLRP12 detects the pathogen directly or by monitoring disruptions in host homeostasis. NLRP7, which is absent in mice, assembles an inflammasome in response to microbial acylated lipopeptides (119). Lastly, it should be noted that several NBD-LRR proteins (e.g., CIITA and NLRC5) seem to have diverse non-inflammasome-related functions (e.g., in regulation of gene expression). One further example may be NLRP6, which has been
suggested to negatively regulate NF-κB and MAP kinase signaling downstream of TLR activation in a manner apparently independent of CASP1 (120). At the same time, the role of NLRP6 in regulating intestinal inflammation appears to be IL-18-, CASP1-, and ASC-dependent (121, 122), suggesting that NLRP6 can form an inflammasome. Although it is surprising that NLRP6 exhibits such diverse functions, it is important not to assume that all NBD-LRR proteins act via inflammasome formation.

**Noncanonical Inflammasomes**

CASP1 is not the only inflammasome effector caspase. After confirming earlier reports (123) that the available Casp1−/− mice are actually Casp1/11−/− double knockouts, investigators showed that pyroptosis induced by *Escherichia coli* and *Vibrio cholerae* is CASP11 dependent and CASP1 independent (124). Interestingly, IL-1β processing and secretion downstream of *E. coli* and *V. cholerae* require NLRP3 and CASP1 in addition to CASP11 (123, 124), suggesting that CASP11 can induce pyroptosis directly but can only induce IL-1β via downstream activation of NLRP3 and CASP1 (125, 126, 127).

Surprisingly, pyroptosis induced by the noncatalytic B subunit of cholera toxin (CTB) also activates CASP11, although it is not clear how CTB would be sensed (124). One possible explanation is that CTB’s ability to activate CASP11 may result from LPS contamination of the B-subunit preparation, though the trafficking function of the B subunit may also play a role. The idea that LPS can activate CASP11 is supported by recent reports that CASP11 activation by gram negative bacteria requires TLR4, TRIF, and the type I IFN receptor.
CASP11 can also be activated by purified LPS (125, 126) or type I IFN (125), and Casp11-/- mice are resistant to LPS-induced toxic shock (124, 127). Interestingly, mice deficient in both Casp1 and Casp11 were more resistant to S. Typhimurium compared with Casp1 singly deficient mice (126). As a result, Casp11-dependent pyroptosis was proposed to be effective at restricting bacterial replication only if accompanied by a Casp1-dependent recruitment of neutrophils, which eliminate extracellular bacteria.

It remains unclear how CASP11 is activated. Pyroptosis downstream of known inflammasomes (NAIP/NLRC4, AIM2, NLRP1B) does not appear to require CASP11 (124, 128). Mechanistically, it is possible that a novel non-canonical inflammasome serves as a platform for CASP11 activation. Alternatively, because CASP11 expression is inducible (125–127), CASP11 may autoactivate upon expression, though one report suggests expression alone is insufficient for CASP11 activation (126). There is no direct ortholog of CASP11 in humans; instead, humans express CASP4 and CASP5 (which are absent in mice). Although CASP5 associates with the NLRP1 inflammasome in humans (1), it is not clear whether its functional role in the response to LPS in humans is similar to that of CASP11 in mice.

Accumulating evidence also suggests that inflammasome complexes include more than just NLR/PYHIN proteins, ASC, and CASP1. For example, the inhibitor of apoptosis (IAP) proteins cIAP1 and cIAP2 regulate inflammasomes, although published reports disagree about whether this interaction is activating or inhibitory (129, 130). The double-stranded RNA-dependent protein kinase PKR has also been proposed to regulate multiple inflammasomes (131).

**EFECTOR FUNCTIONS OF INFLAMMASOMES**

The development of knockout mice deficient for key inflammasome components has been critical for studying the contribution of inflammasomes to host defense and disease pathogenesis in vitro and in vivo. In some cases [notably *Mycobacterium tuberculosis* (132, 133)], in vitro activation of an inflammasome by a bacterial pathogen does not clearly correlate with an in vivo phenotype, but overwhelmingly, disruption of the relevant inflammasome leads to an increase in bacterial replication (colony-forming units) and a decrease in survival following bacterial infection of mice. Conversely, forced expression of inflammasome ligands or deletion of inflammasome inhibitors in bacterial pathogens that normally evade inflammasome detection leads to bacterial restriction (22, 30, 134, 135). Therefore, inflammasomes are an important component of innate immunity to bacterial pathogens, as summarized in **Table 1**. The control by inflammasomes of bacterial infections results from the inflammasomes’ downstream effector functions, of which pyroptosis and the processing and secretion of pro-IL-1β/pro-IL-18 are the best characterized.

**Pyroptosis**

Pyroptosis is thought to result from osmotic pressure generated by CASP1-dependent formation of membrane pores (136) and is characterized by the rapid release of cytosolic contents. Pyroptosis is not associated with the formation of characteristic apoptotic membrane blebs and does not require the apoptotic caspases (e.g., CASP3, 7, 9). Pyroptosis has been described in macrophages and dendritic cells but may also occur in other cell types. Surprisingly, cell lysis downstream of some NLRP3 agonists is largely undetectable or occurs hours after CASP1 autoproteolysis and IL-1β/IL-18 processing (74, 137). It is unclear why CASP1 activated via NLRP3 activates pyroptosis only inefficiently, whereas CASP1 activated downstream of NLRC4 or NLRP1B induces pyroptosis within minutes of inflammasome oligomerization in nearly all cells. The CASP1 substrate(s) required for pyroptosis remains elusive (138–140).

Autoproteolysis has long been considered an essential step in activation of CASP1. Yet,
Table 1  Contribution of inflammasome responses to bacterial restriction and host survival

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>CASP1</th>
<th>ASC</th>
<th>NBD/PYHIN</th>
<th>Cytokines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasma phagocytophilum</td>
<td>CFU+</td>
<td>CFU+</td>
<td>Nlrc4: CFU+</td>
<td>IL1b: CFU+</td>
<td>199</td>
</tr>
<tr>
<td>Aeromonas veronii</td>
<td>CFU+</td>
<td>CFU+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>SVL−</td>
<td></td>
<td>Nlpr1b: CFU+, SVL−</td>
<td>IIIR: SVL−</td>
<td>149, 150</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td></td>
<td></td>
<td>Nlrc4: CFU+, SVL−</td>
<td>IIIR: CFU+</td>
<td>201</td>
</tr>
<tr>
<td>Burkholderia pseudomallei</td>
<td>CFU+, SVL−</td>
<td>CFU =, SVL−</td>
<td>Nlrc4: CFU+, SVL−</td>
<td>IIIR: CFU =, SVL+</td>
<td>153</td>
</tr>
<tr>
<td>Burkholderia thailandensis</td>
<td>CFU+</td>
<td></td>
<td></td>
<td>IIIR/II18: CFU =</td>
<td>30</td>
</tr>
<tr>
<td>Chlamydia muridarum</td>
<td>CFU =/+</td>
<td></td>
<td>Nlrc4: CFU =</td>
<td>IIβ: CFU =/+</td>
<td>202</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>CFU+, SVL−</td>
<td></td>
<td>Nlrc4: CFU =</td>
<td>IIIR: CFU =</td>
<td>203, 204</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>CFU =</td>
<td></td>
<td></td>
<td>IIIR: CFU =</td>
<td>205, 206</td>
</tr>
<tr>
<td>Citrobacter rodentium</td>
<td>CFU+</td>
<td></td>
<td>Nlrc4: CFU+</td>
<td>IIβ: CFU+</td>
<td>207</td>
</tr>
<tr>
<td>Escherichia coli (O21:H4+)</td>
<td>CFU =, SVL+</td>
<td></td>
<td>Nlrc4: CFU =, SVL+</td>
<td>IIβ: CFU =, SVL+</td>
<td>196</td>
</tr>
<tr>
<td>Francisella tularensis</td>
<td>CFU+, SVL−</td>
<td></td>
<td>Nlrc4: CFU =, SVL = Aim2: CFU+, SVL−</td>
<td>8, 106, 208</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>CFU =, SVL−</td>
<td></td>
<td>Nlrc4: CFU =</td>
<td>IIIR: CFU =</td>
<td>209</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>CFU+</td>
<td>CFU =</td>
<td>Nlrc4: CFU+</td>
<td>IIIR: CFU+</td>
<td>19, 21, 30, 143, 210, 211</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>CFU =/+, SVL−</td>
<td></td>
<td>GBP5: CFU+</td>
<td>IIIR: CFU =, SVL =</td>
<td>87, 120, 135, 212, 213</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>CFU+</td>
<td></td>
<td>Nlrc4: CFU+, SVL =</td>
<td>IIIR: CFU−</td>
<td>22, b 24, 148, 217</td>
</tr>
<tr>
<td>Salmonella enterica serovar Typhimurium</td>
<td>CFU+, SVL−</td>
<td></td>
<td>Nlrc4: CFU =, SVL−</td>
<td>IIβ: CFU =</td>
<td>120, 157, 218–221</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>CFU+</td>
<td></td>
<td>Nlpr3: CFU =, SVL−</td>
<td>IIIR: SVL =</td>
<td>154</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>CFU+</td>
<td></td>
<td></td>
<td>IIIR: SVL =</td>
<td>222, 223</td>
</tr>
</tbody>
</table>

(Continued)
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>CASP1</th>
<th>ASC</th>
<th>NBD/PYHIN</th>
<th>Cytokines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus agalactiae</em> (Group B)</td>
<td>CFU+, SVL−</td>
<td>CFU+, SVL−</td>
<td>Nlrp3: CFU+/−, SVL−</td>
<td>IL1R: SVL−</td>
<td>224</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>CFU+, SVL−</td>
<td>Nlrp3: CFU+/−, SVL−</td>
<td>IL1β: SVL−</td>
<td>225, 226</td>
<td></td>
</tr>
<tr>
<td><em>Yersinia pestis</em></td>
<td>Nlrp12: CFU+, SVL−</td>
<td>Nlrp3: CFU+, SVL−</td>
<td>IL1β/IL18: CFU+</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td><em>Yersinia pseudotuberculosis</em></td>
<td>CFU+</td>
<td>CFU+</td>
<td>Nlrp3: CFU=</td>
<td>134</td>
<td></td>
</tr>
</tbody>
</table>

*This table summarizes changes in colony-forming units (CFU) and/or host survival (SVL) from in vivo models of bacterial pathogenesis in mice deficient for inflammasome-related genes (column headings). (+) increase; (−) decrease; (=) no change.

1CFU difference in Reference 22 seen only in the absence of ExoU.

2Salmonella enterica serovar Typhimurium results vary depending on status of Nramp1 gene and route of infection (intraperitoneal versus oral).

3CFU and survival differences observed only with *Y. pestis* expressing hexaacylated lipopolysaccharide.

4CFU differences enhanced in absence of inhibitory *Y. pseudotuberculosis* effectors.

in the absence of the adaptor protein ASC, CASP1 fails to undergo autoproteolysis (18, 25, 141) but can nevertheless induce pyroptosis in a manner requiring CASP1 protease activity (128). Induction of CASP1-dependent, ASC-independent pyroptosis occurs in response to activation of NLRC4 and NLRP1B (which contain CARDs) but not AIM2 or NLRP3 (which lack CARDs). The CARDs were proposed to mediate direct recruitment, dimerization, and activation of CASP1 without a requirement for ASC, which appears necessary only for CASP1 autoproteolysis and IL-1β/IL-18 processing. In fact, pyroptosis is accelerated in the absence of ASC, suggesting an inhibitory role for this adaptor (141). The molecular mechanisms that allow uncleaved CASP1 to induce pyroptosis but not cytokine processing remain uncertain. Inflammasomes lacking CARDs (e.g., NLRP3, AIM2) require ASC for both pyroptosis and cytokine processing (4, 76).

Interestingly, in many cases, innate restriction of bacterial replication is completely IL-1β/IL-18 independent. For example, replication of *L. pneumophila* in IL-1β/IL-18−/− bone marrow–derived macrophages is indistinguishable from that in wild-type cells (142). Similarly, *L. pneumophila, Burkholde-

**Cytokine Processing**

Despite appearing dispensable for innate restriction in some in vivo models, the CASP1-dependent cytokines IL-1β and IL-18 are critical inflammasome effectors in other models, although each cytokine contributes very differently. IL-18 is known principally for its ability to induce IFN-γ production, which in turn helps to restrict intracellular pathogens. By contrast, IL-1β is critical for inflammation
(e.g., neutrophil recruitment) and helps shape adaptive immune responses. Signaling through the widely distributed IL-1 receptor rapidly induces the expression of hundreds of inflammatory genes—including those encoding IL-6, IL-8, IL-12, COX-2, numerous chemokines, and antimicrobial peptides—as well as the IL-1β transcript itself. In the liver, IL-1β, together with IL-6, drives the acute phase response, and on CD4+ T cells, IL-1 can promote Th17 or Th2 differentiation depending on the cytokine context (145). Although IL-1β production often depends on CASP1, CASP1-independent IL-1β processing has been reported (146, 147, 148).

The NLRP1B-mediated response to B. anthracis provides an interesting case study for the role of cytokines and neutrophils in bacterial restriction downstream of inflammasomes. B6 mice carrying a responsive Nlrp1b allele exhibit increased resistance to B. anthracis spore challenge compared with nonresponsive nontransgenic B6 mice, even though macrophages in the transgenic mice are susceptible to LeTx-mediated lysis (149). Similar results were obtained in B6 mice congenic for a responsive allele of Nlrp1b; these mice survived spore and vegetative bacterial challenges in a manner dependent on Nlrp1b and Casp1. This restriction required the IL-1 receptor and recruitment of neutrophils to the site of infection (150), suggesting that inflammasome-dependent maturation of IL-1β is critical for restriction of B. anthracis. Therefore, although LeTx is important for B. anthracis virulence (151), likely through its inactivation of MAPKKs, the activity of LeTx is counteracted by NLRP1B-mediated detection of the toxin. Indeed, the release of intracellular pathogens by pyroptosis and the recruitment of neutrophils by IL-1β likely synergize to restrict bacterial replication in vivo.

The recruitment of neutrophils also carries with it the risk of significant damage to host tissues. Therefore, in circumstances in which neutrophils fail to restrict the microbe, neutrophil recruitment is associated with severe pathology and even increased bacterial dissemination (30, 152–154). For example, II1R−/− mice infected with Burkholderia pseudomallei, which can replicate in neutrophils, are protected compared with wild-type mice (153).

Signaling through the IL-18 receptor induces proinflammatory cytokines and, depending on the cytokine context, can drive CD4+ T cells to Th1 and perhaps even Th2 and Th17 phenotypes (145). However, IL-18 was first identified as an IFN-γ-inducing factor, and this remains its primary contribution to innate restriction of bacterial pathogens. For example, restriction of B. pseudomallei is lost in II18−/− mice but can be restored by exogenous IFN-γ (153). In the spleen, memory CD4+ T cells secrete IFN-γ in response to IL-18 produced by CD8α+ dendritic cells that activate the NLRC4 inflammasome in response to S. Typhimurium (155). Surprisingly, even purified flagellin can activate this response, so it was suggested that cross-presentation pathways in these CD8α+ cells deliver flagellin to the cell cytosol.

The contribution of IL-1β and IL-18 to T cell differentiation and the potential adjuvanticity of endogenous factors released during pyroptosis suggest a link between inflammasome activation and the adaptive immune response. Although evidence for this link exists in influenza infections (77), relatively little work has examined the requirement of inflammasomes for adaptive immunity to bacterial pathogens. Protective immunity to Helicobacter infections requires IL-1 receptor but is independent of IL-18 (156). Conversely, mice infected with a strain of L. monocytogenes engineered to hyperinduce the NAIP5/NLRC4 inflammasome fail to develop protective immunity (135). Further work is needed to clarify how inflammasome effector functions, such as cytokine processing, regulate adaptive immune responses.

Other Effector Functions

Although certain inflammasomes (notably NAIP/NLRC4 and NLRP1B) can be activated within minutes without priming, IL-1β and to a lesser extent IL-18 require de novo transcription and translation before they can be processed by CASP1. Although this transcriptional priming could occur constitutively (157), some
data suggest that inflammasomes are connected to additional fast-acting signaling outputs. Indeed, activation of NAIP5/NLRC4 by systemic cytosolic delivery of flagellin can kill mice in less than 30 min (158). This rapid response appears not to require IL-1β/IL-18 or pyroptosis but instead depends on the biosynthesis of inflammatory signaling lipids termed eicosanoids—e.g., prostaglandins and leukotrienes—that induce vascular leakage and massive fluid loss into the gut and peritoneum (158). Of note, NAIP5/NLRC4-dependent eicosanoid biosynthesis occurs specifically in resident peritoneal macrophages and is completely absent in bone marrow-derived macrophages commonly used to study inflammasome responses (158). Interestingly, administration of purified LeTx into rats can also cause rapid (<1 h) death that is related to vascular shock and pleural (rather than peritoneal) fluid accumulation; this death appears to require NLRP1, though a role for eicosanoids is not established (56). In mice, activation of NLRP1B by purified LeTx also induces rapid (but nonlethal) vascular leakage that is ameliorated in the absence of prostaglandin biosynthesis (149, 158). During infection, localized vascular leakage resulting from inflammasome-induced eicosanoids produced by tissue-resident macrophages might provide complement, antibodies, and leukocytes access to the site of infection, though this process remains to be shown.

Like the cytosolic flagellin model described above, the LPS-induced model of sepsis is also inflammasome dependent [requiring ASC, CASP11, and at some doses NLRC3 (124, 159)] but IL-1β/IL-18 independent (160). Although LPS-mediated lethality requires days, not minutes, it is interesting that lethality is delayed when prostaglandin synthesis is decreased (161). High-mobility group B1 (HMGB1) protein, which is released from cells during pyroptosis and has proinflammatory effects, has also been linked to LPS-mediated inflammation and pathology downstream of the inflammasome (160).

HMGB1, IL-1β, and IL-18 are all members of a group of proteins lacking canonical secretion signals that rely on CASP1 for their release (162). In addition, IL-1α—a cytokine that signals through the same receptor as IL-1β and thus has essentially the same in vivo effects—lacks a signal sequence and can in some cases be secreted in a CASP1-dependent manner (163). These proteins are passively released during pyroptotic cell lysis, but there is also evidence for an active secretion process that occurs before or even in the absence of readily detectable cell lysis (74, 137). Current models for this pathway, which is calcium dependent (164–166), include microvesicle shedding and lysosome exocytosis (165–167). The relative contributions of active and passive processes to CASP1-dependent protein release likely vary by cell type, inflammasome, and agonist; it will be particularly important to investigate these processes in vivo.

Another suggested effector function of CASP1 is the transcriptional induction of lipogenic genes to promote membrane biosynthesis and counteract cell death (168). This transcriptional response requires several hours and is apparently inadequate to counteract pyroptosis by CASP1, which can occur in 1–2 h. The in vivo relevance of the link between CASP1 and lipogenic genes is uncertain, and the response may be unique to the nonhematopoietic cells used in the study.

In a departure from the view that pyroptosis is the dominant mechanism that restricts intracellular bacterial replication via elimination of the intracellular niche, some studies have suggested that pyroptosis-independent pathways may also restrict bacterial replication. For example, Naip5-defective A/J macrophages are highly permissive to L. pneumophila replication (see above) despite the fact that some studies (169) (but not all; see Reference 20) show normal induction of pyroptosis in A/J macrophages in response to L. pneumophila. Restriction of L. pneumophila replication in Naip5-sufficient B6 macrophages may be due to delivery of L. pneumophila phagosomes to lysosomes for degradation (19, 144, 170, 171). This activity has been reported downstream of CASP1 through its processing of CASP7 (142) and also downstream of CASP11 (172).
Whether pyroptosis or altered phagosome trafficking causes bacterial restriction has been difficult to determine because there is currently no way to selectively eliminate either of these effector functions of the inflammasome.

In summary, inflammasomes seem to trigger more downstream outputs than previously appreciated, although many of these outputs need further evaluation. In particular, it will be important to understand their individual contributions to host immunity and pathology in vivo.

INHIBITION/EVASION OF INFLAMMASOMES BY BACTERIA

If an innate immune response plays an important role in host defense, then it is likely that bacterial pathogens will evolve to evade the response. The inflammasome is no exception to this general rule. *L. monocytogenes* and *S. Typhimurium*, for example, downregulate flagellin expression inside cells to evade detection by NAIP5/NLRC4 (173, 174). Enforced expression of flagellin by these bacteria in vivo results in their severe attenuation; this attenuation is reversed in *Nlrc4*−/− mice, demonstrating that the attenuation is indeed due to inflammasome activation (30, 135).

*Staphylococcus aureus* meanwhile modifies its cell wall to prevent degradation by lysozymes, and this modification indirectly reduces detection by NLRP3 (175). Examples of bacterial inflammasome inhibitors include the *M. tuberculosis*-secreted metalloprotease Zmp1 and the *P. aeruginosa*-secreted phospholipase ExoU (22, 176). The various *Yersinia* species are perhaps best equipped with an armament of inflammasome inhibitors. *Y. enterocolitica*, for example, blocks CASP1 activation through the inhibitory effect of its secreted effector YopE on Rho GTPases, in particular Rac-1 (177). Which inflammasome is targeted by this activity and how endogenous Rho GTPases contribute to CASP1 activation remain unresolved. Using a YopK-deficient strain of *Y. pseudotuberculosis*, Brodsky et al. (134) uncovered the activation of both the NLRC4 and NLRP3 inflammasomes by the *Yersinia* T3SS. YopK secreted by wild-type bacteria normally blocks this host pathway by binding the T3SS, and this inhibition is critical for host colonization. Bacterial load is attenuated in wild-type mice infected with the YopK-deficient *Y. pseudotuberculosis* strain compared with that in mice infected with the wild-type strain, but this attenuation is reversed in mice that are deficient for components of the inflammasome. Meanwhile, a YopJ isoform with enhanced MAP kinase and NF-κB inhibitory activity that is expressed in the *Y. pestis* KIM strain also leads to enhanced CASP1 activation and IL-1β secretion. *Y. pestis* CO92 and *Y. pseudotuberculosis*, in contrast, secrete a less potent YopJ isoform, suggesting another possible mechanism of inflammasome evasion (178).

Lastly, a study (118) recently demonstrated that production of a nonstimulatory tetraacylated LPS allows *Y. pestis* to evade TLR4- and inflammasome-dependent production of protective IL-18. Evasion or inhibition of the inflammasome by bacterial pathogens means that, in many cases, inflammasome deficiency should not be expected to result in heightened susceptibility to infection. Indeed, dramatic in vivo phenotypes are not always observed in inflammasome-deficient mice (Table 1).

INFLAMMASOME RECOGNITION OF THE MICROBIOTA

The mammalian intestine harbors a complex microbial ecosystem, termed the microbiota, that is composed of trillions of microbes representing hundreds of species. The microbiota provides numerous benefits to its host but also poses serious challenges to the mucosal immune system in the intestinal tract. On the one hand, the immune system must detect and provide a barrier against the microbiota and incoming pathogens. On the other hand, the immune system must avoid inappropriate pathological responses to the microbiota. Disruption of the host-microbiota equilibrium can lead to severe pathology, including inflammatory bowel disease (IBD) and sepsis.

The first evidence suggesting that inflammasomes may in part mediate host-microbiota
interactions stemmed from genome-wide association studies of IBD patients. Polymorphisms in the Nlrp3 gene have been linked to an increased risk of developing Crohn’s disease (CD), a form of IBD (179, 180). However, this link was not confirmed in a larger study of ~13,000 CD patients, a finding that may be explained by regional differences in genetic backgrounds (181). Additionally, polymorphisms in the genes encoding IL-1β, IL-18, and IL-18 receptor accessory protein have been associated with an increased risk of developing CD (182–184). Together, these studies, and others discussed below, suggest that inflammasomes may contribute to the pathogenesis of IBD and, more broadly, may play a variety of roles in regulating homeostasis in the intestinal tract (Figure 7).

The Inflammasome, Inflammation, and Tissue Repair

Oral administration of dextran sulfate sodium (DSS), which causes mucus erosion and cytotoxicity to colonic epithelial cells, provides a useful model of colitis-like disease in mice. Initial studies examining a role for the inflammasome in intestinal homeostasis found that CASP1-deficient mice were less susceptible to DSS-induced colitis compared with wild-type mice, suggesting that the inflammasome contributes to disease pathogenesis (185). Consistent with this notion, administration of an IL-1β neutralization antibody to wild-type mice protected against disease (185). However, more recent studies demonstrated that the inflammasome may actually provide protective responses to DSS-mediated colitis. Three groups demonstrated that mice deficient for functional CASP1 or ASC exhibit greater disease severity (186–188). Disease was associated with increased epithelial barrier permeability, resulting in increased extraintestinal dissemination of the microbiota (188). The increased barrier permeability was suggested to result from defective cell proliferation and repair of the epithelium. Il18−/− mice exhibit pathologies similar to those in Casp1−/− mice, and administration of recombinant IL-18 to Casp1−/− mice reduced disease severity (187–189). Which inflammasomes control CASP1 in the context of DSS-induced colitis? Two independent studies (121, 190) reported that Nlrp3−/− mice are partially protected from DSS. By contrast, other studies have reported that Nlrp3−/− mice exhibit increased disease severity, suggesting that NLRP3 may protect against the development of DSS-induced colitis. Inconsistencies among DSS studies may result from differences in the microbiota among mouse strains and/or colonies. Hirota et al. (191) showed that Nlrp3 deficiency is associated with reduced β-defensin antimicrobial peptide production in the colon. Zaki et al. (188) demonstrated that disease in Nlrp3−/− mice is associated with increased gut barrier permeability, defective epithelial proliferation, and increased extraintestinal invasion of the microbiota. In support of a role for the microbiota in triggering disease, antibiotic administration to Nlrp3−/− mice attenuates disease progression. Thus, NLRP3 may be required for the tissue-regenerative function of IL-18 in response to DSS-induced intestinal injury. However, it remains to be shown whether the levels of colonic IL-18 in Nlrp3−/− mice are impaired and whether administration of recombinant IL-18 to Nlrp3−/− mice is protective. Additional reports have suggested that NLRP6 and NLRP12 are also important for attenuation of colonic inflammation. Impaired NLRP12 signaling in both hematopoietic and nonhematopoietic cells results in increased inflammation in the DSS model (115, 116). NLRP6-deficient mice exhibit increased intestinal permeability and inflammation and impaired IL-18 production (192, 193). Interestingly, one study (121) proposed that NLRP6 also regulates responses to DSS via regulation of the microbiota composition (see below). These alternate roles for NLRP6 are not mutually exclusive.

Chronic inflammation associated with IBD is recognized as a major risk factor for the pathogenesis of colitis-associated colon cancer (CAC). As in colitis, the inflammasome reportedly plays a negative regulatory role in
Proposed roles for inflammasomes in host-microbiota interactions. ⓡ Repair: Activation of inflammasomes by unknown signals leads to production of IL-18, which in turn leads to regeneration of epithelial cells by an unknown mechanism. Deficiencies in inflammasome signaling and IL-18 production are associated with impaired repair of the colonic epithelial barrier. ⓢ Inflammation: Disruption of the epithelial barrier and microbial translocation result in localized and systemic inflammasome activation and inflammation driven by IL-18 and IL-1β. ⓣ Regulation of the microbiota composition: Upon recognition of unknown ligands, NLRP3 and NLRP6 lead to production of IL-18, contributing to regulation of the microbiota composition through an unknown mechanism. Disruption of inflammasome signaling is associated with a dysbiotic microbiota with proinflammatory properties.

The Inflammasome and Intestinal Dysbiosis

It is now well recognized that maintaining a properly balanced microbial composition is crucial for intestinal homeostasis. Disruption of the development of CAC. In a mouse model of CAC, Casp1−/− and Asc−/− mice present with increased tumor burdens and reduced levels of IL-18. Furthermore, roles for NLRP3, NLRP6, and NLRP12 in defense against CAC have also been established (186, 188, 192, 193).
Casp1

This composition (dysbiosis) can result in the expansion of pathobionts—constituents of a normal microbiota that are typically harmless to the host but can become pathogenic when homeostasis is disrupted (194, 195). Recent studies have revealed complex roles for the inflammasome in regulating the microbiota composition. For example, Nlrp6−/− mice, which are more sensitive to DSS than are wild-type mice, were found by 16S rRNA deep sequencing of the intestinal microbiota to harbor an overrepresentation of bacteria from the phyla Bacteroidetes (Prevotellaceae) and TM7 (121). An expansion of these phyla was also found in (Prevotellaceae) and TM7 (121). An expansion of these procolitogenic-associated bacterial species are the causative agents of disease in this model. An alternative model is that their expansion is the indirect consequence of the host response to other members of the microbiota.

In addition to producing local pathogenic effects, intestinal dysbiosis associated with impaired inflammasome signaling can trigger inflammatory disorders in distal organs. For example, the inflammasome is critical for protection against diet-induced liver inflammation, and this protection is associated with the intestinal microbiota composition (122). Asc−/− and Casp1−/− mice fed a methionine- and choline-deficient diet (MCDD) developed more severe liver damage compared with wild-type mice, as well as enhanced hepatic steatosis and increased levels of immune cells populating the liver. Disease progression appears to depend in part on the NLRP3 inflammasome, as MCDD-fed Nlrp3−/− and I118−/− mice exhibit increased liver damage and hepatic inflammation (122). Consistent with previous findings (121), Asc−/− mice also exhibited increased susceptibility to MCDD-induced liver inflammation, and this susceptibility was associated with specific alterations in the microbiota of Asc−/− mice (122). It remains to be shown whether MCDD-fed Nlrp3−/−, Nlrp6−/−, Casp1−/−, and I118−/− mice exhibit microbiota compositions similar to those in Asc−/− mice and what microbial species are the causative agents of disease.

The above studies demonstrate that the inflammasome plays an important beneficial role in controlling the microbiota composition. A recent study (196) provides evidence that inflammasomes can also mediate disease pathogenesis triggered by intestinal dysbiosis. Colony-born mice treated with a broad-spectrum antibiotic cocktail followed by a DSS challenge developed a sepsis-like syndrome, rather than colitis, that was associated with extraintestinal bacterial dissemination. Antibiologic treatment induced the expansion of an antibiotic-resistant E. coli O21:H+ pathobiont in the intestinal tracts of mice. Genome sequencing of the E. coli O21:H+ isolate revealed that this strain encodes several potential virulence factors associated with septicemic clinical E. coli isolates, including a
The authors demonstrated that the flagellin from this *E. coli* can activate the NAIP5/NLRC4 inflammasome. Furthermore, mice deficient for *Naip5*, *Nlrc4*, *Casp1*, and *Il1β* function exhibited an attenuated disease phenotype when treated with antibiotics/DSS or systemically challenged with *E. coli* O21:H+, despite having similar levels of microbial colonization of extraintestinal tissues (196), leading to the proposal that aberrant activation of the NAIP5/NLRC4 inflammasome by *E. coli* O21:H+ triggers IL-1β-mediated immunopathology. Thus, taken together, the studies on recognition of the microbiota by inflammasomes indicate that inflammasomes can have both beneficial and harmful regulatory effects, and the question of how appropriate responses are maintained will be important to address in future studies.

Inflammasome recognition of the microbiota remains a nascent field of research, and as such, the best practices and research tools are still emerging. One particular challenge is analysis of the complex microbiota. Although cost effective, basic laboratory culturing techniques fail to uncover this complexity, as the majority of the microbiota cannot be cultured by standard laboratory techniques. The commonly applied technique of 16S rDNA qPCR also has important caveats because 16S rDNA is a variable multicopy gene, and its abundance therefore does not correlate directly with bacterial colonization. Techniques such as microarray-based applications, 16S full-length sequencing, and pyrosequencing provide better alternative approaches to characterize the microbiota ecology with greater taxonomic resolution. Furthermore, novel culturing techniques or in vivo imaging are needed to identify how and where inflammasome activation occurs in the gut.

**IMPLICATIONS AND PERSPECTIVE**

Although the diversity both of inflammasomes and of the mechanisms by which they detect and respond to infection resists simplification, we propose that inflammasomes exhibit several essential properties that may account for their broad roles in host defense. First, inflammasomes exhibit the important property of autoinhibition. This prevents the unwanted initiation of inappropriate and potentially harmful inflammatory signals when no pathogen is present. For NLRs, autoinhibition is thought to be mediated by the LRR domain, which blocks NBD-mediated oligomerization through an unknown mechanism. Interestingly, a conceptually similar autoinhibitory function has been proposed for the HIN-200 domain of the non-NLR AIM2 inflammasome (9), implying that autoinhibition might be a general and essential feature of inflammasomes. Second, inflammasome activation appears highly cooperative, capable of transitioning from a fully off state to a fully on state across a narrow range of agonist concentration. This binary mode of activation contrasts with most signaling through cell surface receptors such as the TLRs, in which signaling outputs scale across a wide range of ligand concentration. The binary behavior of inflammasomes is a consequence of their highly cooperative oligomerization into a single cytosolic complex. For example, time-lapse movies of fluorophore-tagged ASC strikingly illustrate its complete oligomerization within minutes (197). Functionally, cooperative inflammasome oligomerization allows for rapid and decisive high-amplitude responses; such responses may be critical in host defense. Interestingly, although the non-NLR protein AIM2 lacks an NBD, it has nevertheless been proposed to be activated by a DNA-dependent multimerization step (9), suggesting that cooperative oligomerization is a common feature of inflammasomes. Third, inflammasomes must be able to evolve rapidly to keep pace with pathogen evasion mechanisms. The LRR domain appears to be a structurally robust domain that can tolerate mutations, particularly on its concave β-sheet surface, without loss of hydrophobic core stability (198). A final unifying property of inflammasomes is that they are all localized in the host cell cytosol. This localization implies that inflammasomes will respond to pathogens, which generally access the cytosol...
(directly or indirectly) as part of their virulence strategy, but will ignore harmless microbes, which are generally relegated to the extracellular space (82). Cytosolic localization, and the consequent ability to discriminate pathogens from nonpathogens, is a critical feature of inflammasomes that distinguishes them from TLRs, which detect extracellular-derived ligands from both pathogens and nonpathogens. Indeed, we suggest that the above properties of the NBD-LRR architecture, taken together, may explain why proteins with this architecture have been maintained alongside TLRs in animal evolution for the purposes of host defense. The unique properties of the NBD-LRR architecture may also explain why it has been deployed as the basis for much of the immune system of plants. Superimposed upon the above-mentioned core properties, inflammasomes have evolved numerous and surprisingly diverse mechanisms of activation and action, many of which we likely have yet to appreciate.

**FUTURE ISSUES**

1. Other than IL-1β and IL-18, what are the relevant protein substrates of CASP1 that mediate pyroptosis and other potential functions of the inflammasome?
2. What are the self or microbial ligands that interact with inflammasomes? With the exception of AIM2 and the NAIPs, ligands for inflammasomes remain unknown.
3. What are the molecular mechanisms of inflammasome activation? The biochemical mechanism of activation of even the intensively studied NLRP3 inflammasome remains poorly understood. How are inflammasomes maintained in an autoinhibited state and how does ligand binding, nucleotide binding, and hydrolysis regulate inflammasome assembly?
4. Do specific cell types in vivo exhibit unique inflammasome functions? Inflammasomes have been studied primarily in hematopoietic cells (particularly monocytes and macrophages), although recent work has hinted that inflammasomes can also have important roles in nonhematopoietic cells in vivo. Even different macrophage populations can have unique inflammasome signaling outputs (e.g., eicosanoid production). How many additional functions may be discovered in vivo?
5. What microbial constituents of the microbiota are recognized by inflammasomes? How and where are they recognized?
6. Do inflammasome responses play an important role in the induction of adaptive immunity?
7. How are inflammasome responses terminated? Most studies have focused on the activation of inflammasomes, but presumably termination of inflammasome activation is also critical for the maintenance of homeostasis.

**DISCLOSURE STATEMENT**

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

**ACKNOWLEDGMENTS**

This work was supported by investigator awards from the Cancer Research Institute (R.E.V.) and the Burroughs Wellcome Fund (R.E.V.), by a grant from the University of California Cancer
Research Coordinating Committee (J.v.M.), by a National Science Foundation Graduate Research Fellowship (J.C.S.), by National Institutes of Health Ruth L. Kirschstein National Research Service Award Fellowship AI091068 (J.S.A.), and by National Institutes of Health R01 grants AI063302, AI075039, and AI080749 (R.E.V.).

LITERATURE CITED


### Contents

**Years in Cologne**  
*Klaus Rajewsky* ................................................................. 1

**The Biology of Recent Thymic Emigrants**  
Pamela J. Fink ................................................................. 31

**Immunogenic Cell Death in Cancer Therapy**  
*Guido Kroemer, Lorenzo Galluzzi, Oliver Kepp, and Laurence Zitvogel* .............................................. 51

**Recognition of Bacteria by Inflammasomes**  
*Jakob von Moltke, Janelle S. Ayres, Eric M. Kofoid, Joseph Chavarría-Smith, and Russell E. Vance* ............................................. 73

**The Immunology of Fibrosis**  
*Georg Wick, Cecilia Grundtman, Christina Mayerl, Thomas-Florian Wimpissinger, Johann Feichtinger, Bettina Zeiger, Roswita Sgonc, and Dolores Wolfram* ........................................... 107

**Memory T Cell Subsets, Migration Patterns, and Tissue Residence**  
*Scott N. Mueller, Thomas Gebhardt, Francis R. Carbone, and William R. Heath* ........... 137

**Control of Human Viral Infections by Natural Killer Cells**  
*Stephanie Jost and Marcus Altfeld* .................................................. 163

**Functional T Cell Immunodeficiencies (with T Cells Present)**  
*Luigi D. Notarangelo* ................................................................. 195

**Controlling Natural Killer Cell Responses: Integration of Signals for Activation and Inhibition**  
*Eric O. Long, Hun Sik Kim, Dongfang Liu, Mary E. Peterson, and Sumati Rajagopalan* ................................................................. 227

**Metabolic Regulation of T Lymphocytes**  
*Nancie J. Macler, Ryan D. Michalek, and Jeffrey C. Ratmell* ................................................................. 259

**Mesenchymal Stem Cell: Keystone of the Hematopoietic Stem Cell Niche and a Stepping-Stone for Regenerative Medicine**  
*Paul S. Frenette, Sandra Pinho, Daniel Lucas, and Christoph Scheiermann* ................................................................. 285

**Interleukin-4- and Interleukin-13-Mediated Alternatively Activated Macrophages: Roles in Homeostasis and Disease**  
*Steven J. Van Dyken and Richard M. Locksley* ................................................................. 317

**Brain-Reactive Antibodies and Disease**  
*B. Diamond, G. Honig, S. Mader, L. Brimberg, and B.T. Volpe* ................................................................. 345
Immunology of the Maternal-Fetal Interface
Adrian Erlebacher ................................................................. 387

Regulation of Ligands for the NKG2D Activating Receptor
David H. Raulet, Stephan Gasser, Benjamin G. Gowen, Weiwien Deng,
and Heiyoun Jung ............................................................... 413

Pathways of Antigen Processing
Janice S. Blum, Pamela A. Wearsch, and Peter Cresswell .................. 443

The Immune Response in Tuberculosis
Anne O’Garra, Paul S. Redford, Finlay W. McNab, and Chloe I. Bloom,
Robert J. Wilkinson, and Matthew P. R. Berry .......................... 475

The Adaptable Major Histocompatibility Complex (MHC) Fold: Structure
and Function of Nonclassical and MHC Class I–Like Molecules
Erin J. Adams and Adrienne M. Luoma .................................... 529

The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and
Their Subsets in the Steady State and the Inflamed Setting
Miriam Merad, Priyanka Sathe, Julie Helft, Jennifer Miller, and Arthur Mortha ... 563

T Cell–Mediated Host Immune Defenses in the Lung
Kong Chen and Jay K. Kolls ................................................... 605

Human Hemato-Lymphoid System Mice: Current Use and Future Potential
for Medicine
Anthony Rongvaux, Hitoshi Takizawa, Till Strowig, Tim Willinger,
Elizabeth E. Eynon, Richard A. Flavell, and Markus G. Manz ............. 635

Signaling by the Phosphoinositide 3-Kinase Family in Immune Cells
Klaus Okkenhaug ............................................................... 675

Broadly Neutralizing Antiviral Antibodies
Davide Corti and Antonio Lanzavecchia .................................... 705

Molecular Control of Steady-State Dendritic Cell Maturation and Immune
Homeostasis
Gianna Elena Hammer and Averil Ma .................................... 743

Indexes

Cumulative Index of Contributing Authors, Volumes 21–31 ..................... 793
Cumulative Index of Articles Titles, Volumes 21–31 .......................... 800

Errata

An online log of corrections to *Annual Review of Immunology* articles may be found at
http://immunol.annualreviews.org/errata.shtml