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Current Opinion in Immunology

Host inflammasome defense mechanisms and bacterial pathogen evasion strategies

Susan M Brewer[1], Sky W Brubaker[1] and Denise M Monack

Inflammasomes are a formidable armada of intracellular pattern recognition receptors. They recognize determinants of infection, such as foreign entities or danger signals within the host cell cytosol, rapidly executing innate immune defenses and initiating adaptive immune responses. Although inflammasomes are implicated in many diseases, they are especially critical in host protection against intracellular bacterial pathogens. Given this role, it is not surprising that many pathogens have evolved effective strategies to evade inflammasome activation. In this review, we will provide a brief summary of inflammasome activation during infection with the intent of highlighting recent advances in the field. Additionally, we will review known bacterial evasion strategies and countermeasures that impact pathogenesis.

Address
Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA

Corresponding author: Monack, Denise M (dmonack@stanford.edu)
[1] These authors contributed equally to this work.

Current Opinion in Immunology 2019, **60**:63–70

This review comes from a themed issue on **Host pathogens**

Edited by **Christina L Stallings** and **Michael S Glickman**

For a complete overview see the Issue and the Editorial

Available online 4th June 2019

https://doi.org/10.1016/j.coi.2019.05.001

Introduction

Inflammasomes are multiprotein complexes that consist of sensors, adaptors, and effectors that target downstream proteins for proteolytic cleavage (Figure 1). Sensor proteins determine a 'pattern' of infection by binding directly to bacterial ligands or through indirect mechanisms. The sensors recruit and activate effector proteins known as caspases, commonly with the help of the adaptor protein Apoptosis-associated Speck-like protein containing a CARD (ASC). The effector caspases control cell death and cytokine maturation using a cysteine protease activity that targets downstream proteins for cleavage (Figure 1). Recently, gasdermin D (GSDMD) was identified as a caspase target and the N-terminal product of GSDMD cleavage forms a pore in the plasma membrane leading to membrane rupture and cell death [1,2].

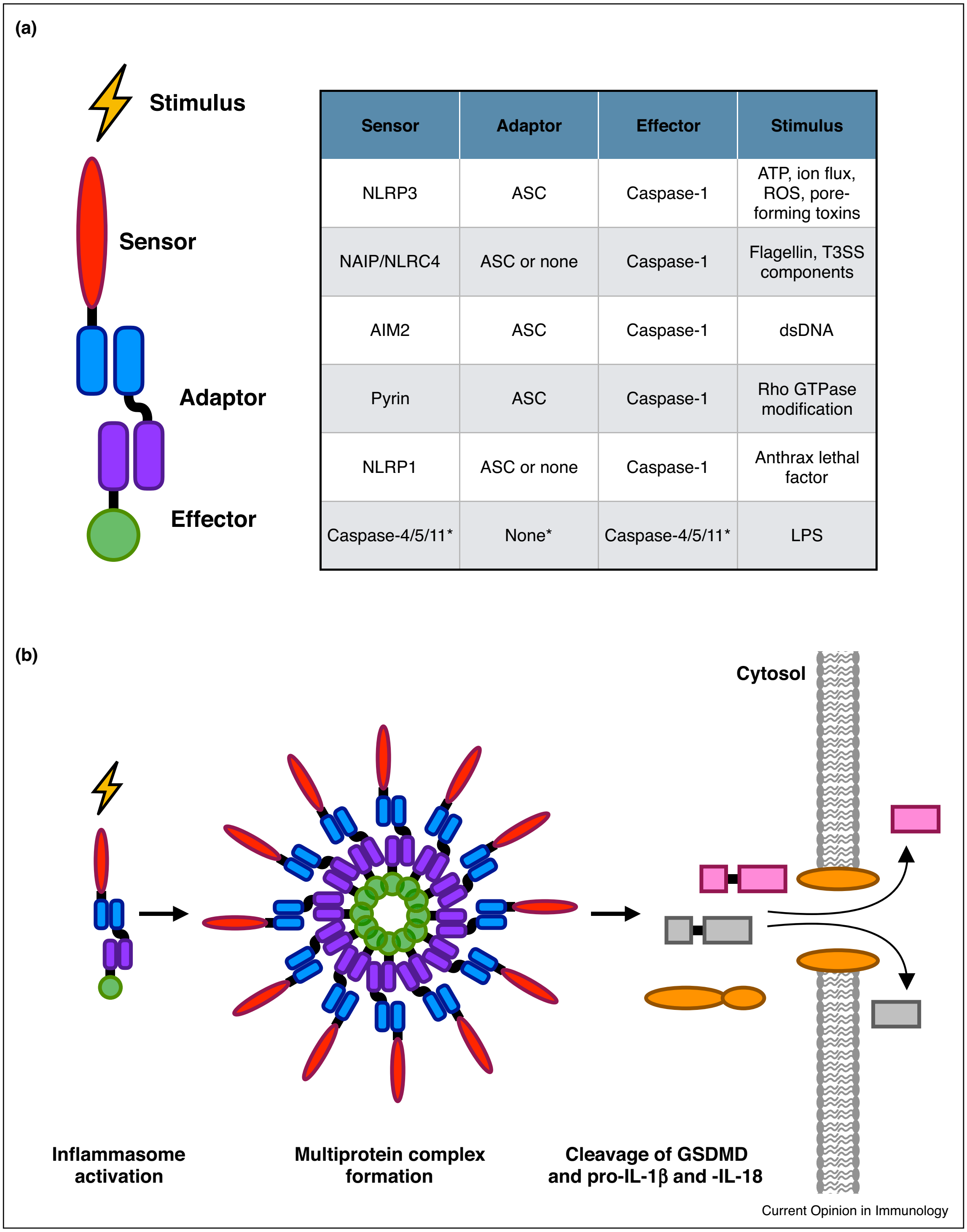
The inflammasome complexes are commonly referred to by the sensor protein that initiates complex formation. In this review, we will use this accepted convention and segregate information based on each individual inflammasome (although some mechanisms may broadly impact multiple inflammasomes). We should note that due to space limitations, we cannot provide a comprehensive review including all of the work that has been done to advance the field. Furthermore, key differences between human and animal models exist and more research is needed to characterize the distinctions that may impact therapeutic development. We hope that readers utilize other currently available review articles that discuss individual inflammasomes in more detail to understand these nuances. NLRP1 is not reviewed here as it is the specific topic of another article in this issue. Instead, we hope to inspire new investigations by specifically discussing knowledge gaps in the most recent findings. We also hope to spur an interest in the identification and characterization of bacterial evasion strategies and countermeasures against inflammasome activation (Table 1). A better understanding of the strategies employed by these intracellular pathogens is a useful tool for dissecting host cell biology and may lead to the development of novel therapeutics.

Pyrin

Only recently has the pyrin inflammasome been shown to protect against intracellular pathogens [3,4,5•]. Bacterial toxins and secreted effector molecules translocated to the host through the type 6 secretion system (T6SS) of *Burkholderia cenocepacia* initiate pyrin/ASC/caspase-1-dependent cell death and mature IL-1β release via modifications of Rho GTPases (Figure 1) [3,4,5•,6,7•]. Under homeostatic conditions, Rho GTPases activate the serine/threonine protein kinase C-related kinases (PKN), which phosphorylate pyrin and maintain an inactive state through the recruitment of 14-3-3 proteins [8]. During infection, bacterial inhibition of Rho GTPases prevents phosphorylation of pyrin, leading to its activation [4,5•,6,7•]. Thus, the pyrin inflammasome detects intracellular bacterial pathogens indirectly via perturbations to the Rho GTPase signaling pathway.

Despite this sophisticated host danger detection system, pathogens have evolved mechanisms to evade sensing by pyrin. Yersinia outer protein E (YopE) and YopT are two

Figure 1



Sensor	Adaptor	Effector	Stimulus
NLRP3	ASC	Caspase-1	ATP, ion flux, ROS, pore-forming toxins
NAIP/NLRC4	ASC or none	Caspase-1	Flagellin, T3SS components
AIM2	ASC	Caspase-1	dsDNA
Pyrin	ASC	Caspase-1	Rho GTPase modification
NLRP1	ASC or none	Caspase-1	Anthrax lethal factor
Caspase-4/5/11*	None*	Caspase-4/5/11*	LPS

Conceptual framework of inflammasome activation.
(a) Graphical depiction of the ‘Sensor, Adaptor, and Effector’ components and interactions that are required for inflammasome activation. On the right, a table lists the components for each inflammasome and the corresponding stimuli. Asterisk denotes uniqueness of caspase-4/5/11 in that it acts as both sensor and effector without requiring an adaptor.
(b) Multiprotein complex formation of inflammasome components occurs following stimulation. Formation of this large protein complex is required for caspase-dependent cleavage of downstream targets. Cleavage of Gasdermin D (GSDMD) generates an N-terminal protein product that forms pores in the plasma membrane and facilitates pyroptotic cell death and pro-inflammatory cytokine release. Several Interleukin 1 (IL-1) family cytokines are generated as a pro-form and must be cleaved into a mature cytokine to be fully active.

Table 1

Pathogen-encoded inflammasome countermeasures

Pathogen	Inflammasome activated by pathogen	Pathogenic countermeasure	References
Brucella spp.	Caspase-4/11 sensing of LPS and subsequent activation of caspase-1	TcpB induces ubiquitination and degradation of caspases 1, 4, and 11	[61]
Coxiella burnetii	Caspase-11 sensing of LPS	IcaA inhibits caspase-11 via unknown mechanism	[53]
Edwardsiella tarda	NAIP/NLRC4 and NLRP3 activation from T3SS	EvpP inhibits intracellular Ca^{2+} elevation, which inhibits Jnk signaling and ASC oligomerization	[40]
Francisella novicida	Caspase-11 sensing of LPS	Alteration of LPS acylation state	[60]
Legionella pneumophila	AIM2 detection of cytosolic DNA	SdhA maintains *Legionella*-containing vacuole	[33]
Listeria monocytogenes	Virulence protein p60 activates NLRP3	NLRP3 causes production of IL-10, which allows *Listeria* to disseminate within host	[43•]
Salmonella Typhimurium	NAIP/NLRC4 detection of T3SS and flagellin	Reduce flagellin expression and switch T3SS once intracellular	[14,23,24]
Shigella flexneri	Caspase-4 sensing of LPS	OspC3 prevents heterodimerization of cleaved caspase-4 subunits	[54•]
Yersinia spp.	YopE and YopT inhibition of Rho GTPases activates pyrin	YopM activates PKNs to phosphorylate pyrin and bypass RhoA inactivation	[6,7•]
	NAIP/NLRC4 detection of T3SS	YopK interacts with T3SS to dampen ligand translocation	[25]
	Multiple inflammasomes	YopJ inhibition of TAK1 prevents transcriptional upregulation of inflammasome genes	[41••]
	Caspase-11 sensing of LPS	Alteration of LPS acylation state	[60]

effectors that inhibit Rho GTPases, leading to pyrin activation [6,7•]. However, *Yersinia* spp. also inject the effector YopM, which mimics Rho GTPase PKN activation as a countermeasure against the inhibiting effects of YopE and YopT (Table 1). Specifically, murine model systems were used to demonstrate that YopM interacts with PKNs to induce pyrin phosphorylation and recruitment of 14-3-3 proteins [6,7•]. Thus, YopM bypasses one effect of Rho GTPase inhibition and allows intracellular *Yersinia* spp. to remain undetected by pyrin.

YopM is the only intracellular bacterial effector known to block pyrin activation. Further study is needed to determine if other bacterial species employ similar effectors that mimic the effects of Rho GTPase activity to inhibit pyrin activation.

NAIP/NLRC4

The NOD-containing, LRR-containing, and CARD-containing protein 4 (NLRC4) inflammasome detects T3SS structural proteins and flagellin, the monomeric protein subunit of flagella (Figure 1). These ligands are recognized by a family of murine NAIP genes or by a single NAIP gene in humans. Direct interactions have been demonstrated for murine NAIP1 with T3SS needle proteins, NAIP2 with T3SS inner rod proteins, and NAIP5 with the D0 domain of flagellin [9–12]. Ligand recognition by human NAIP has recently been clarified to demonstrate that the full-length protein isoform is sufficient to recognize all three of the distinct bacterial ligands [11,13,14•]. It is unclear whether gene duplication in mice or broad ligand recognition by a single gene in humans confers one advantage over the other.

Recent advances have been made toward a detailed mechanistic understanding of NLRC4 activation. Previous studies established NAIP-independent phosphorylation of NLRC4 as a critical activation step required for complete caspase-1 activation, cell death, and cytokine maturation [15,16]. The kinase activity at residue S533 of NLRC4 was initially attributed to PKCδ; however, a recent report identified a second kinase, LRRK2, that interacts with NLRC4 and controls the level of phosphorylation during inflammasome activation [15,17•]. It remains to be determined if the two kinases are functionally redundant or if there are circumstances that favor one over the other. In addition, structural studies demonstrate that a single ligand-bound NAIP protein induces a conformational change to NLRC4, exposing a region that can accept the next NLRC4 monomer, thus nucleating an oligomeric ring structure of NLRC4 [18–20]. Interestingly, the reports utilized different ligands to activate NLRC4 and found a 'closed' versus 'open' structure. Thus, it is tempting to speculate that different NLRC4 ligands initiate distinct structures to control different outcomes and that these structures may be specifically targeted pharmacologically.

The NLRC4 inflammasome provides protection against a broad range of intracellular pathogens. While most of the NLRC4 inflammasome characterization has previously been done in macrophages, the importance of NLRC4 activation in non-hematopoietic cell types is becoming more evident in the context of enteric infections. For example, *Salmonella enterica* serovar Typhimurium triggers an NLRC4-dependent protective response *in vivo* in mice that results in enterocyte expulsion of infected epithelial cells into the intestinal lumen [21••]. Mechanistically, the epithelial cell responses contribute to host defense and promote inflammation in a caspase-1 and caspase-8-dependent manner through cell expulsion, cell death, and the release of eicosanoids as well as IL-18 [21••,22].

Although many intracellular bacterial pathogens are flagellated and utilize effector secretion systems, not all are recognized by the NAIP/NLRC4 inflammasome. Indeed, several different bacterial pathogens have evolved unique mechanisms to evade detection by the NLRC4 inflammasome and promote pathogenesis. For example, the expression of ligands detected by NAIP proteins are notably downregulated during infection. Once established within a vacuole, *S.* Typhimurium reduces the expression of flagellin [23] and switches from using the T3SS rod protein PrgJ to one encoded by SsaI (Table 1), which is not detected by NAIP/NLRC4 in both murine [24] and human cells [14•]. In contrast to reducing the expression of a ligand, *Yersinia* spp. secrete YopK, an effector that directly interacts with the T3SS translocon, which is presumed to block ligand recognition (Table 1) [25].

AIM2

The absent in melanoma 2 (AIM2) inflammasome recognizes cytosolic DNA through a direct interaction and initiates ASC-dependent and caspase-1-dependent inflammasome activation (Figure 1). DNA might be considered an unsuitable determinant to distinguish self from non-self because it is a feature of both pathogens and host cells. However, mammalian cell compartmentalization enables a distinction by the innate immune system. Under normal circumstances DNA is spatially restricted within nuclear or mitochondrial membranes, but if DNA is detected outside these contexts it indicates cellular damage or infection.

A number of bacterial pathogens trigger AIM2 inflammasome responses [26], but the protective role of AIM2 is most dramatically observed using *Francisella* spp. infection. AIM2-deficient animals are highly susceptible to infection which is attributed to inflammasome activation in hematopoietic cell populations [27,28]. During macrophage infection, *Francisella* escape from a pathogen-containing vacuole utilizing a T6SS to replicate in the cytosol but are quickly recognized by AIM2. Strains with a non-functional T6SS fail to trigger the AIM2 inflammasome, demonstrating that access to the host cytosol is critical for recognition [28]. Efforts to identify bacterial countermeasures that block AIM2 have uncovered a number of mutants that induce stronger AIM2 responses [29–31]. These efforts did not uncover a bacterial effector that can directly manipulate host cell biology, but rather mutations in genes that maintain bacterial integrity and result in lysis during intracellular infection [31,32]. These findings suggest that disrupting an integral component of bacterial physiology allows for DNA release into the host cytosol and recognition by AIM2. In contrast to *Francisella*, *Legionella* maintain and replicate within a pathogen-containing vacuole. *Legionella* strains lacking the secreted effector SdhA, fail to maintain this vacuolar-niche and subsequently are recognized by AIM2 in the cytosol (Table 1) [33].

Studies unraveling the role of interferon-inducible factors during *Francisella* infection have expanded our understanding of how host proteins facilitate the lysis of cytosolic bacteria to promote inflammasome activation. Mechanistically, GBP2, GBP5, and IRGB10 are recruited to cytosolic bacteria and are required for bacterial lysis, release of DNA or LPS, and the activation of AIM2 or caspase-11, respectively [34,35,36,37•]. Like AIM2-deficient mice, GBP and IRGB10 knockout mice are highly susceptible to *Francisella* infection demonstrating that bacterial lysis and the release of microbial DNA is critical for AIM2-mediated host defense. Recently, the *Shigella flexneri* secreted effector IpaH9.8 was found to target host GBP proteins for degradation through Lys48-linked ubiquitination and promote bacterial replication [38,39•]. However, it remains to be determined whether IpaH9.8-mediated degradation of GBPs allow *S. flexneri* to evade the AIM2 inflammasome response or whether other bacterial pathogens employ similar GBP-targeting strategies. For a more in-depth discussion on the role of GBPs in host–pathogen interactions, please see the interferon-inducible GTPases review in this issue.

NLRP3

The NOD-containing, LRR-containing, and pyrin domain-containing protein 3 (NLRP3) inflammasome is the most well-studied of the inflammasomes and signals through ASC and caspase-1 (Figure 1). NLRP3 senses diverse stimuli, including ATP, ion flux, reactive oxygen species, and pore-forming toxins [26]. Although a unifying mechanism of activation remains unclear, NLRP3 can be characterized as a sensor of pathogen-mediated damage to host cells rather than a direct sensor of the pathogen itself [26].

NLRP3 has been implicated in protection against many intracellular bacterial pathogens [26]. In a recent example, the T3SS of *Edwardsiella tarda* was shown to trigger NLRP3 inflammasome activation as well as deliver the effector EvpP, which can suppress the same response (Table 1) [40•]. EvpP-deficient strains had a colonization defect that was ablated in NLRP3 knockout mice. Mechanistically, EvpP inhibits intracellular Ca^{2+} flux, prohibiting Jnk phosphorylation, which is necessary for ASC oligomerization and NLRP3 inflammasome activity [40•]. This study demonstrates the importance of NLRP3 in controlling infection and reveals a bacterial countermeasure against NLRP3 that impacts pathogenesis.

There are also several studies demonstrating that NLRP3 activation occurs subsequently to activation by other inflammasomes via GSDMD pore formation and potassium efflux. For example, TAK1 inhibition by the *Yersinia* spp. effector YopJ triggers caspase-8-mediated cleavage of GSDMD, which activates NLRP3, leading to ASC oligomerization, caspase-1 cleavage, and IL-1β secretion [41••]. During *Legionella pneumophila* infection, NLRP3 is triggered subsequently to AIM2, NLRC4, or caspase-11 activation. Of note in this study, the authors provide evidence that the processing or cleavage of caspase-1 is NLRP3-dependent and thus does not occur during initial AIM2 activation [42]. These results suggest nuanced regulation of inflammasome responses and that the regulated cleavage of inflammatory caspases may be important in dictating the magnitude of the innate response to intracellular pathogens. However, further study will be needed to tease apart these intricacies.

In contrast to the role inflammasomes play in bacterial restriction, recent work demonstrates that *Listeria monocytogenes* utilizes NLRP3 activation to establish systemic infection (Table 1) [43•]. The *L. monocytogenes* virulence protein p60 activates NLRP3 and causes release of IL-18, which stimulates NK cells to produce the anti-inflammatory cytokine IL-10 [43•]. Using knockout mice and bone marrow chimeras, the authors demonstrated that in the absence of NLRP3, IL-18, or IL-10, systemic expansion of *L. monocytogenes* is limited [43•]. Thus, *L. monocytogenes* can capitalize on NLRP3-dependent production of IL-10 for dissemination. This demonstrates that rather than evading inflammasome activation, some bacterial pathogens may actively trigger it as a mechanism of pathogenesis.

Non-canonical inflammasome

The noncanonical inflammasome is activated by cytosolic lipopolysaccharide (LPS)—an outer membrane component of Gram-negative bacteria [44]. Uniquely, the caspases of the non-canonical inflammasome (murine caspase-11 or human caspase-4 and caspase-5) function as both sensor and effector without the need of additional inflammasome components (Figure 1). Caspase-4/5/11 detect LPS through direct interaction with its CARD domain [45]. Upon detection, caspase-4/5/11 oligomerize and cleave GSDMD, leading to pyroptotic cell death. Additionally, caspase-11 activity can lead to the activation of caspase-1 via NLRP3/ASC (see above), causing maturation and release of IL-1β and IL-18 [1,46,47]. Recent evidence suggests that lipoteichoic acid from Gram-positive bacteria as well as host-derived lipids indirectly (via NLRP6) and directly activate caspase-11, respectively [48,49•]. Interestingly, these alternative caspase-11 activators fail to trigger cell death, suggesting complexities in caspase-11 regulated inflammasome activation that require further characterization. Exploitation of the differences in caspase-11 activation might be developed for therapeutic applications.

At the cellular level, *in vitro* studies demonstrate that several Gram-negative bacterial pathogens activate the noncanonical inflammasome. In murine macrophages caspase-11 is critical for IL-1β release and pyroptosis when infected with *S. flexneri*, *S.* Typhimurium, and *L. pneumophila* [50–53], whereas human monocyte-derived macrophages and epithelial cells activate caspase-4 in response to *L. pneumophila*, *Francisella novicida*, *S. flexneri*, and *S.* Typhimurium [52,54•,55•]. Interestingly, recent *in vitro* work utilizing murine macrophages, showed that hyperinjection of the *Yersinia* effectors YopB and -D in the absence of YopK leads to noncanonical inflammasome activation in a GBP-dependent manner [56]. However, further study is needed to describe the mechanism linking YopB/D hyperinjection with caspase-11 activation.

Several studies also demonstrate the importance of caspase-11 in defense against Gram-negative bacterial pathogens *in vivo*. For example, caspase-11 deficient mice have higher bacterial burdens and are more susceptible to infection compared to wild-type mice infected with *Burkholderia thailandensis* or *pseudomallei* [57,58]. Using bone-marrow transplants and intranasal infection with *B. thailandensis*, recent work demonstrated that caspase-1 is primarily responsible for inducing pyroptosis and release of IL-1β and IL-18 in macrophages, whereas caspase-11 mediates pyroptosis of lung epithelial cells [59••]. This provides an example of how multiple inflammasomes work together across cell types to coordinate host protection.

Another recent study showed that caspase-11−/− mice infected with *Brucella abortus* had higher splenic bacterial burdens than wild-type or caspase-1−/− mice at both one and two weeks post-infection [37•]. Furthermore, this defect in bacterial restriction may be attributed to a defect in splenic neutrophil recruitment in caspase-11-deficient mice. Together, these results indicate that caspase-11-dependent pyroptosis and subsequent immune cell recruitment is important to defend against *B. abortus* infection.

Several intracellular pathogens have been documented to evade detection or directly dismantle the caspase-4/5/11 detection system. *Yersinia* spp. and *F. novicida* modify the acylation state of their LPS to avoid detection by murine caspase-11 (Table 1) [60]. In human cells, caspase-4—but not caspase-5—can detect *F. novicida*'s tetra-acylated LPS, indicating species-specific differences in LPS sensing via the CARD domain of caspase-4/5/11 [55•]. In contrast, *Brucella* spp. secrete the effector TcpB into host cells, which causes ubiquitination and degradation of caspase-1, caspase-4, and caspase-11, preventing inflammasome activation and maintaining *Brucella*'s replicative niche (Table 1) [61]. However, it remains to be determined whether TcpB targeting of the noncanonical inflammasome impacts pathogenesis *in vivo*.

Other pathogens have evolved strategies that inhibit the steps following inflammasome activation. *S. flexneri* triggers caspase-4 activation in gut epithelial cells but counteracts this using the T3SS effector OspC3 (Table 1). OspC3 interacts with the catalytic site of the p19 subunit of activated caspase-4, preventing its heterodimerization with the p10 subunit and inhibits the proteolytic activity required for pyroptosis [54•]. *Coxiella burnetii* also secretes a caspase-11 inhibiting effector—IcaA—via its T4SS, but the mechanism of inhibition is unknown (Table 1) [53]. The extracellular enteric pathogens *Citrobacter rodentium*, enteropathogenic *Escherichia coli* (EPEC), and enterohemorrhagic *E. coli* (EHEC) encode functional homologs of OspC3 that counteract caspase-4/5/11 responses, demonstrating a role for the non-canonical inflammasome during infection with extracellular bacteria [62,63].

Conclusion

Many advances have been made toward a better understanding of inflammasome activation and the role this process plays in the defense against intracellular bacterial pathogens. Surprisingly, there is a relative paucity of information known about countermeasures or evasion tactics that pathogens use to cope with these host defense mechanisms. We highlighted many of the known examples here in this review, yet we speculate that many more exist and have yet to be identified. Future studies should continue to focus on elucidating the mechanisms intracellular bacterial pathogens employ to thwart inflammasome activation. We expect that a better understanding of these mechanisms may result in the development of useful therapeutics for the treatment of inflammasome-related disease.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors were supported by the following grants: T32AI00732831, R01AI095396, W911NF-16-C-0052D.

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