HHS Public Access

Author manuscript

Semin Immunopathol. Author manuscript; available in PMC 2017 March 01.

Published in final edited form as:

Semin Immunopathol. 2017 February ; 39(2): 215–223. doi:10.1007/s00281-016-0581-1.

The role of NLRP3 and AIM2 in inflammasome activation during *Brucella abortus* infection

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Abstract

The innate immune system is essential for detection and elimination of bacterial pathogens. Upon inflammasome activation, caspase-1 cleaves pro-IL-1β and pro-IL-18 to their mature forms IL-1β and IL-18, respectively, and the cell undergoes inflammatory death termed pyroptosis. Here we reviewed recent findings demonstrating that *Brucella abortus* ligands activate NLRP3 and AIM2 inflammasomes which leads to control of infection. This protective effect is due to inflammatory response caused by IL-1β and IL-18 rather than cell death. *Brucella* DNA is sensed by AIM2 and bacteria induced mitochondrial reactive oxygen species is detected by NLRP3. However, deregulation of proinflammatory cytokine production can lead to immunopathology. Nervous system invasion by bacteria of the genus *Brucella* results in an inflammatory disorder termed neurobrucellosis. Herein we discuss the mechanism of caspase-1 activation and IL-1β secretion in glial cells infected with *B. abortus*. Our results demonstrate that the ASC inflammasome is indispensable for inducing the activation of caspase-1 and secretion of IL-1β upon infection of astrocytes and microglia with *Brucella*. Moreover, our results demonstrate that secretion of IL-1β by *Brucella*-infected glial cells depends on NLRP3 and AIM2 and leads to neurobrucellosis. Further, the inhibition of the host cell inflammasome as an immune evasion strategy has been described for bacterial pathogens. We discuss here that the bacterial type IV secretion system VirB is required for inflammasome activation in host cells during infection. Taken together, our results indicate that *Brucella* is sensed by ASC inflammasomes mainly NLRP3 and AIM2 that collectively orchestrate a robust caspase-1 activation and proinflammatory response.

Keywords

inflammasome; dendritic cells; AIM2; NLRP3; *Brucella*; neurobrucellosis

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Introduction

The first defense mechanism of the innate immune system is to sense pathogens through pattern-recognition receptors (PRR) and respond rapidly by producing cytokines, and antimicrobial intermediates [1]. Microbial recognition mediated by PRRs identifies the pathogen-associated molecular patterns (PAMPs), conserved microbial components [2], and host-derived danger signals (danger-associated molecular patterns, DAMPs) [3]. The most common PAMPs are bacterial lipoproteins, peptidoglycan, LPS, flagellin, and nucleic acids derived from bacteria, viruses, fungi, and protozoa [4].

In recent years, many receptors have been described to be responsible for this early recognition, such as Toll-like receptors (TLRs) [4], NOD-like receptors (NLRs) and the cytosolic RNA-sensing RNA helicases retinoic acid-inducible gene I (RIG-I) [5]. More recently several DNA sensors have been described, such as DNA-dependent activator of IRFs (DAI), absent in melanoma 2 (AIM2) [6,7]; cGAS (cyclic GMP-AMP synthase) [8] and STING (stimulator of interferon genes) [9]. These receptors participate in signaling pathways that induce the production of pro-inflammatory cytokines and type I IFN, crucial for host defense against various pathogens [10], such as *Mycobacterium tuberculosis* [11], *Listeria monocytogenes* [12,13], *Francisella tularensis* [12,14], and *Brucella abortus* [15,16].

The Gram-negative facultative intracellular bacterium *Brucella abortus* causes brucellosis, a systemic infectious zoonotic disease. In humans, *B. abortus* causes among others symptoms, undulant fever, endocarditis, arthritis, and osteomyelitis. In animals, it leads to abortion and infertility, resulting in serious economic losses [17,18]. The immune response against *B. abortus* is initiated with the recognition of the bacteria by antigen presenting cells (APCs) such as dendritic cells and macrophages and requires $CD4^+$ and $CD8^+$ T lymphocytes, Th1-type cytokines such as tumor necrosis factor (TNF-α) and interferon-γ (IFN-γ) [19,20].

On entering the host cells, *Brucella* interact with the early and late endosomes and acquire several markers, including Rab5, early endosome antigen (EEA) 1, and Rab7, resulting in the formation of a "*Brucella*-containing vacuole" (BCV) [21]. The BCVs then fuse rapidly with the lysosome in a controlled manner, as suggested by the presence of the lysosomal markers, lysosomal-associated membrane protein (LAMP), and CD63. After the first hours of infection, BCVs interact with endoplasmic reticulum (ER). Such interactions require the VirB operon encoding the type IV secretion system (T4SS) and lead to fusion between BCVs and the ER, generating an ER-derived organelle permissive for bacterial replication, thus preventing bacterial killing by the host [22].

Regarding *Brucella* agonists, the lipoproteins of *B. abortus* outer membrane Omp16 and Omp19 induce macrophages to produce TNF-α, IL-6, IL-10 and IL-12 dependent on TLR2 and TLR4 signaling [23,24]. Furthermore, Gomes et al, 2015 [25] revealed that CpG motifs derived from *Brucella* DNA are involved in activation of host innate immune response through the TLR9 receptor. TLR9 plays an important role in initial control of infection by *Brucella abortus* [26]. *Brucella* enters the host cell, prevents fusion of the phagosome with the lysosome by altering the intracellular traffic of the early phagosome vesicle being located in structures that resemble the ER. Therefore, DNA from dead *Brucella* is available

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in this endoplasmic reticulum-like organelle and/or escape to the cytosol compartment being available to bind to cytosolic DNA sensors. Furthermore, an endoplasmic reticulum resident transmembrane protein termed STING (stimulator of interferon genes) has been identified as an adaptor required to induce type I IFN in response to intracellular bacteria. By siRNA silencing, we have demonstrated that STING is an important mediator of IFN-β induced by *Brucella* or its DNA. Since STING was found to basally reside in the ER, similarly to *Brucella*, this cell compartment would be an important site that facilitates STING to signal during this bacterial infection. Additionally, there are recent findings in the literature that links cGAS/STING, activation of IRF-1 and type I IFN production with enhanced AIM2 sensing of cytosolic DNA [27].

In this review, we will describe how the sensors AIM2 and NLRP3 detect *Brucella abortus* and how these inflammasome receptors function to control infection and are involved in immunopatholgy related to this disease.

Dendritic cells sense *Brucella* DNA by AIM2 inflammasome

Inflammasome activation leads to the production of IL-1β, and we have shown this cytokine is protective against *B. abortus* infection [16]. The canonical inflammasomes are composed of at least three main components: an inflammatory caspase (caspase-1, caspase-11), an adapter molecule (such as ASC), and a sensor protein (such as NLRP1, NLRP3, NLRP12, NAIP1, NAIP2, NAIP5, or AIM2). The sensor molecule determines the inflammasome specificity by detecting specific microbial products or cell stress signals [28].

AIM2 is a cytosolic double-stranded DNA (dsDNA) receptor that contributes to the host defense against bacterial and viral pathogens. AIM2 belongs to the hematopoietic interferon-inducible nuclear HIN200 protein family characterized by an N-terminal pyrin (PYD) domain and a C-terminal hematopoietic interferon-inducible nuclear antigen with a 200 amino acid repeat (HIN200) domain. This sensor binds to DNA via its HIN200 domain and oligomerizes with ASC to initiate the formation of a caspase-1-activating inflammasome, leading to the secretion of proinflammatory cytokines, including IL-1β and IL-18 [12,7]. The dsDNA-AIM2 inflammasome pathway is important for host cells to detect stealth bacterial pathogens that lack highly stimulatory ligands such as flagellin and LPS as observed in the case of *Brucella* spp and *Francisella* spp.

The majority of *in vitro* inflammasome studies have been performed in murine macrophages, but there is a good reason to suspect that macrophages and DCs differ in their expression of inflammasome components and/or their responses to bacterial PAMPs. However, there are few studies describing the mechanisms of AIM2 activation triggered by bacterial infection in dendritic cells. One study performed with intracellular *Francisella tularensis* observed that this bacterium was able to activate the AIM2 inflammasome in dendritic cells (DCs) causing release of large amounts of IL-1β and host cell death [29]. Moreover, AIM2-deficient mice displayed increased susceptibility to *Francisella* infection compared to wild-type mice [14]. *Francisella* escapes the initial phagosome and replicates in the cytosol, where it is detected by the cytosolic DNA sensor AIM2 leading to activation of the inflammasome. Just as occurs in macrophages infected by *F. tularensis*, DCs derived from AIM2, ASC or caspase-1 knockout (KO) mice are defective for cell death, IL-1β

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secretion and caspase-1 processing in response to infection [30,29]. A Pathogenicity Island is required for *Francisella* escape and replication and for inflammasome activation in dendritic cells. Also, IFN-β is upregulated in DCs following *Francisella* infection, and the IFN-β signaling pathway is only partially required for inflammasome activation in this cell type [29,31,30].

Brucella replicates within dendritic cells and hinders their functional activation [32]. Similar to what has been observed for other cell types such as macrophages, *Brucella* is dependent on its VirB type IV secretion system (T4SS) to replicate within DCs in an ER-derived compartment that evades the lysosomal degradative pathway. *B. abortus* also inhibits the process of DC maturation leading to a reduction of cytokine secretion and antigen presentation [32]. The role of AIM2 in *B. abortus* infection was studied by our research group, and we have shown that ASC inflammasomes, mainly AIM2 sensing DNA and NLRP3, were essential for the secretion of caspase-1-dependent IL-1β and for the resistance of mice to *Brucella* infection [16]. *In vitro* assay showed that *Brucella* DNA induced caspase-1 activation and IL-1β secretion in wild-type cells but not in AIM2 KO, ASC KO and caspase-1 KO macrophages. We also observed a significant reduction in IL-1β secretion and caspase-1 activation in AIM2 KO macrophages infected with *B. abortus*, indicating an important role of AIM2 in recognizing *B. abortus* genomic DNA and further caspase-1 activation and IL-1β secretion. Consistent with the *in vitro* findings, AIM2, ASC, and caspase-1 KO mice showed a reduced resistance to *B. abortus* at four weeks postinfection, determined by their increased bacterial burden in the spleens. Therefore, these studies establish AIM2 as an important antimicrobial sensor and a key determinant of protective immunity to bacterial pathogens [16]. Similar to what was observed in macrophages, DCs from AIM2 KO mice infected with *B. abortus* or transfected with *Brucella* DNA showed a major reduction in caspase-1 activation and impaired secretion of IL-1β (unpublished data). We also observed that engagement of the AIM2 inflammasome by infection with *B. abortus* required the transcription factor IRF-1 in DCs. Additionally, IRF-1 and type I IFN signaling were required for robust expression of guanylate-binding proteins (GBPs), which are involved in intracellular killing of *Brucella* (unpublished results). We suggest that GBPs lyse the *Brucell*a-containing vacuole (BCV) releasing bacterial components to the cytoplasm such as DNA to activate the AIM2 inflammasome as demonstrated in Figure 1. Taken together, we have identified a specific requirement for IRF-1, type I IFN and GBPs in *B. abortus*-induced activation of the AIM2 inflammasome. Finally, we have demonstrated that AIM2 acts as an important sensor of *Brucella* DNA in DCs and plays a critical role in caspase-1-mediated cytokine processing and control of infection.

In contrast, AIM2 is not critical to other bacterial pathogens. *Mycobacterium tuberculosis* inhibits IFN-β production and signaling, which was partially responsible for the inhibition of AIM2-inflammasome activation. This AIM2 inhibition by *M. tuberculosis* was dependent on the presence of a functional ESX-1 secretion system [33]. This study suggests that the same secretion system that is responsible for introducing AIM2 ligands into the host cytosol may also transfer an effector that inhibits AIM2 activation. This novel immune evasion mechanism may allow *M. tuberculosis* to replicate in host cells. Additionally, *Pseudomonas aeruginosa* infection induces AIM2 mRNA expression in macrophages and in the lungs of mice. Interestingly, *P. aeruginosa* infection induced a similar level of IL-1β production in

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wild-type and AIM2-deficient mice. Similarly, no significant differences in bacterial clearance, neutrophil infiltration and NF-kB activation were observed between wild-type and AIM2 KO animals following *P. aeruginosa* lung infection [34]. These data suggest that the AIM2 inflammasome is dispensable for host defense against *P. aeruginosa* infection. In conclusion, there is a growing list of important human bacterial pathogens that have developed strategies to avoid or manipulate the activation the AIM2 inflammasome.

NLRP3 activation during *Brucella* infection

The molecular platform that triggers the activation of inflammatory caspases, termed inflammasome, was first described in 2002 [35]. There was an observation that patients suffering from Muckle-Wells syndrome were associated to NLRP3 gain of function-mutations in the NACHT domain [36]. This syndrome is characterized by increased levels of inflammatory cytokines like IL-1β, suggesting that NLRP3 deregulation activates proinflammatory caspases resulting in sporadic fever episodes. In that context, NLRP3, was initially identified as a novel PYRIN-containing domain protein that interacts selectively with the PYRIN domain of ASC [37]. In addition, the NLPR3-ASC complex interacts with procaspase-1 through the CARD domain of ASC. The recruitment of procaspase-1 induces its activation by a proximity mechanism leading processing of pro-IL-1β and secretion of this cytokine in the mature form [38]. Thus, the NLRP3 activated inflammasome complex is a three part structure assembled by NLRP3-ASC-caspase-1 proteins [39].

The activation of NLRP3 inflammasome usually occurs through two signaling steps. The priming signal is considered the first step, resulting in NF-κB pathway activation generally induced by TLR recognition of PAMPs [40]. This signal evokes not only the pro-IL-1β synthesis, but is also crucial to upregulate the expression of NLRP3 [41]. The second signal is required to promote the NLRP3-ASC-caspase-1 assembly, and is initiated by a variety of damage-associated molecular patterns (DAMPs) that activates this multi-protein complex [40]. One of these NLPR3 activation mechanisms is the increase in cellular K^+ efflux, promoted by pore-forming toxins such as nigericin and extracellular ATP, that activates the purinergic $P2X_7$ receptor [42]. Phagocytosis of particulate matter like monosodium urate (MSU), amyloid-β, silica and asbestos also promotes NLRP3 inflammasome activation [43]. This activation is correlated with lysosome rupture promoting the leakage of active enzymes to the cytosolic compartment [44]. Recently, other activators of the NLRP3 inflammasome such as cation flux, ER stress, and mitochondrial dysfunction arose as novel pathways [45]. In addition, generation of reactive oxygen species (ROS) induces thioredoxin (TRX)-interacting protein (TXNIP) interaction with NLRP3 leading its activation [46].

Initially, our group demonstrated that the pathogenic bacteria *B. abortus* induces IL-1β production through ASC activation of caspase-1. Moreover, ASC- or caspase-1-deficient mice were more susceptible at 4 weeks post-infection with this bacterium [16]. Furthermore, NLRP3 was associated with IL-1β secretion and host resistance against this pathogen. The activation of NLRP3 was correlated with mitochondrial ROS production induced by *B. abortus* infection that was inhibited by Mito-TEMPO, a scavenger specific for mitochondrial ROS [16]. Hence, secretion of IL-1β through NLRP3 activation by *Brucella* is partially dependent on mitochondrial generation of ROS as shown in Figure 1.

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Recently, a novel intricate pathway emerged suggesting the mechanism of NLRP3 activation by *Brucella*. Endoplasmic reticulum stress can be promoted by infection of *Brucella abortus* [47]. The unfolded protein response (UPR) generally senses ER stress with the participation of the ER membrane-spanning proteins called inositol-requiring enzyme 1α (IRE1α), protein kinase R-like ER kinase (PERK), and activating transcription factor 6 (ATF6) [48]. In the case of *Brucella*, the ER stress induced during the infection activates IRE1α pathway leading TXNIP and NLRP3 recruitment to the mitochondria where they engage mitochondrial ROS in a mechanism independent of ASC and caspase-1 [47]. Indeed, NLRP3 association upstream of mitochondrial damage activates caspase-2, rather than caspase-1, leading to the processing of the BH3-only protein Bid. The truncated active form of Bid induces pore formation in the mitochondrial membrane releasing mitochondrial DNA which binds to cytosolic NLRP3 forming the NLRP3-ASC-caspase-1 protein complex [49]. Thus, during *B. abortus* induction of IL-1β secretion, NLRP3 acts upstream and downstream of mitochondrial damage, linking UPR, mitochondrial ROS and inflammasome assembly.

Inflammasome activation of the central nervous system (CNS)

Innate immune activation in the central nervous system (CNS) can be triggered by numerous pathways after recognition of invading pathogens and/or tissue damage by pattern recognition receptors. In recent years, much attention has been focused on a two-signal model mediated by TLR and NLR. These responses play key roles in CNS infectious diseases, primarily by eliciting pro-inflammatory cytokine production leading to pathogen clearance [50]. Paradoxically, the activation of this same innate immunity may also have detrimental effects on neurons, glial cells and the brain microvascular endothelium [51].

CNS invasion by bacteria of the genus *Brucella* results in an inflammatory disorder termed neurobrucellosis. The precise mechanism whereby the bacterium leaves the bloodstream and gains access to the CNS remains unclear. Regardless of mechanism, it is clear that once the bacterium reaches the CNS it induces a pathological pro-inflammatory response [52]. The direct presence of *B. abortus* within the brain parenchyma of mice induces an inflammatory response that leads to astrogliosis and a perivascular infiltrate of neutrophils [53]. *In vitro*, infection of astrocytes and microglia induced the secretion of IL-6, IL-1β and TNF-α and chemokines such as CCL2 and CXCL1. These responses were induced by *B. abortus* lipoproteins via TLR2 [53]. Likewise, rhesus macaques aerosol-infected with *B. melitensis* displayed astrogliosis together with an increased expression of TLR2 on astrocytes [54].

The triad of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 have been shown to be induced in CNS under many pathological conditions by astrocytes and microglia [55,56]. These mediators trigger 2 types of events: i) they activate neighboring cells and amplify local innate immune responses, and ii) they modify the integrity of the blood-brain barrier (BBB) and attract immune cells from the peripheral circulation, giving rise to adaptive immune responses. During *Brucella*-induced astrogliosis, IL-6 contributes to, and TNF-α determines astrocyte proliferation and apoptosis, respectively [53]. TNF-α is also involved in the secretion of metalloproteinases by infected astrocytes [57]. Although we have described a major role for TNF-α and IL-6 in the immunopathogenesis of neurobrucellosis [53,57], the role of IL-1β has never been investigated until recently [58].

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IL-1β exerts numerous functions within the CNS, including modulation of the BBB permeability, activation of resident glia, and indirectly affecting leukocyte recruitment by augmenting chemokine and adhesion molecule expression at the BBB [59]. In the context of CNS *B. abortus* infection, IL-1β plays a direct role in activating the BBB endothelium and modifying its integrity. IL-1β secreted by *B. abortus*-infected microglia and astrocytes was able to activate human brain microvascular endothelial cells (HBMEC). Secretion of IL-1 β by glial cells and concomitant HBMEC activation requires caspase-1 and ASC, indicating that a NLR is involved in the production of IL-1β and the subsequent activation of HBMEC by *B. abortus* [58].

Inflammasomes have emerged as critical signaling molecules of the innate immune system in the CNS, and the function of NLR in neuroinflammation is a rather recent discovery [60]. Caspase-1 activation and IL-1β secretion in glial cells infected with *B. abortus* depends on the ASC inflammasomes NLRP3 and AIM2, confirming and extending previous work of murine macrophages [16]. *B. abortus*-induced glial production of IL-1β that leads to activation of HBMEC is dependent on the adapter molecule Mal/TIRAP and TLR2-mediated [58]. These findings are congruent with our previous observations indicating that TLR2/MyD88 signaling is crucial for inflammatory responses induced by *B. abortus* [61]. We hypothesize that *Brucella* lipoproteins would be at least, as demonstrated for other cell types [61,23,62], the first signal employed by *Brucella* to trigger the inflammasome activation and the release of IL-1β that leads to activation of HBMEC via the TLR2 signaling pathway. With respect to signal 2, recent data demonstrate that *Brucella* DNA is involved in caspase-1 activation via AIM2 inflammasome and that NLRP3 inflammasome activation depends on mitochondrial ROS induced by *Brucella* [16].

A defining characteristic of the cytokine-induced inflammatory response in the CNS is the destabilization of the BBB which results in an increased vascular permeability. In this context, IL-1β signaling seems to have a major role. *In vitro*, the activation of HBMEC by *B. abortus*-infected glial cells correlates with an increased capacity of the microvascular endothelial cells to promote the transmigration of neutrophils and monocytes. This phenomenon depends on the secretion of IL-1β upon the activation of AIM2 and NLRP3 inflammasomes [58]. This suggests that the interaction of *Brucella* with innate immunity *in vivo* may result in an increased transmigration of phagocytes to the brain parenchyma which would explain the pleocytosis observed in neurobrucellosis patients [63,64]. Inflammasomes would dictate this cellular migration. Accordingly, the perivascular neutrophilic infiltrate induced by *B. abortus* in the brain of mice was reduced in mice lacking AIM2 or NLRP3 [58].

Finally, as with many infectious neurological disorders, inflammation is a key contributor to the pathogenesis of neurobrucellosis. As *Brucella* invades the CNS, inflammatory responses elicited by this organism upon its interaction with astrocytes and microglia would not only lead to the observed reactive gliosis, with astrocyte proliferation and apoptosis, but also would contribute to the activation of the brain microvascular endothelium and the infiltration of immune cells from the periphery as demonstrated in Figure 2. In this process, TLR and ASC-dependent inflammasomes play a major role.

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Brucella escape mechanisms of innate immunity

Pathogenic bacteria use a variety of virulence factors to overcome the immune system facilitating their survival in host cells. During *Brucella* infection in humans, patients have a latency period of 2-4 weeks before becoming symptomatic. Therefore, during passage of mucosal surfaces and systemic dissemination, these bacteria must be able to evade detection by innate immunity.

Brucella spp. evade proper sensing via TLR4 by producing a poorly recognized form of lipid A. *Brucella* lipid A contains a much longer fatty acid residue compared to enterobacterial LPS and this modification greatly reduces its endotoxic activity [65]. Further, *Brucella* has a flagellin that lacks the TLR5 agonist domain and, therefore, escapes TLR5 recognition. As a result, *Brucella* flagellin is a poor inducer of TLR5-mediated inflammatory responses [66]. Additionally, *Brucella* can actively suppress innate immune signaling via bacterial Btp1 or TcpB molecule. This is a TIR domain-containing protein that inhibits both TLR2 and TLR4 signaling by inducing ubiquitination and degradation of the adaptor molecule MAL [67]. This TLR-inhibitory activity reduces both maturation of dendritic cells following infection and their ability to induce IL-12 and TNF-α [32].

Additionally, several Gram-negative and Gram-positive bacteria are also equipped with a protein secretion apparatus known as the type IV secretion system (T4SS). The T4SS is a specialized macromolecule that translocates DNA and protein substrates to bacterial or eukaryotic target cells generally by a mechanism dependent on direct cell-to-cell contact [68]. The T4SS can be found in bacterial pathogens, such as *Agrobacterium tumefaciens, Helicobacter pylori*, *Brucella abortus*, *Bordetella pertussis* and *Legionella pneumophila,* among others [69].

The composition of T4SS between the bacteria is generally conserved but variations can be observed [70]. Studies with *Agrobacterium tumefaciens* VirB revealed that the system comprises three functional groups: the pilus at the bacterial surface (virB2 and virB5), a pore traversing the two membranes (virB3 and virB6-10) and two ATPases (virB4 and virB11) on the cytoplasmic side of the inner membrane that provide power to the system, and transporting substrates [71]. Most encoded VirB proteins show similarity to components of the T4SS in other bacteria. In *Brucella*, T4SS is encoded by the *virB* operon, consisting of 12 genes (*virB1–12*) located on chromosome II, conserved in all sequenced *Brucella* species [72,73]. *B. abortus* virB1 and virB2 are considered to be located on the bacterial surface, and virB2 forms a similar structure of pilus, through which it can interact with the host cells. VirB1 and virB2 are essential for intracellular replication of *B. abortus* in J774 macrophages, and the virB2 mutant was unable to promote persistence of infection in the murine model demonstrating the essential role of virB2 and type IV secretion during infection [73]. Furthermore, mice infected with a VirB mutant were unable to induce proinflammatory responses, demonstrating that the T4SS was required to trigger innate immunity [74].

T4SS is an essential mechanism associated with virulence and important for intracellular *Brucella* survival. Thus, the encoded proteins by the VirB operon of *Brucella* are directly related to intracellular survival, acting in the maturation of the vacuole containing the

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bacteria, as well as transportation to the site of replication. A study of host responses induced by T4SS during early infection revealed that VirB mutants with a deficient T4SS did not induce expression of genes related to inflammation and immunity, suggesting that T4SS function triggers innate immune responses [74]. To address the role of *Brucella* T4SS on inflammasome activation, we infected C57BL/6 macrophages with wild-type bacteria or VirB mutant strain and determined IL-1β secretion. *Brucella* lacking the T4SS induced much lower secretion of IL-1β in C57BL/6 macrophages compared to wild-type bacteria [16]. These findings reveal that *Brucella* uses the T4SS to translocate effector proteins or DNA into host cytosol to further activate the ASC inflammasome.

Conclusions

Brucella infects the host and shields itself from PRR recognition such as TLRs. However, this stealth pathogen after entering the host cell and manipulating its intracellular fate via the T4SS, releases bacterial components that trigger inflammasome activation. *Brucella* induces activation of the adaptor molecule STING that triggers type I IFN and IRF-1 leading to GBPs expression. GBPs interact with *Brucella*-containing vacuoles resulting in lysis of the compartment and escape of the bacteria and its components to the cytosol. This cargo rich in inflammasome ligands such as DNA reaches the host cell cytosol and activates AIM2. Additionally, bacterial-induced ROS mediates NLRP3 activation. However, uncontrolled inflammasome activation may lead to inflammatory conditions related to this disease such as neurobrucellosis.

Acknowlegdments

This study was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG), CNPq/CONICET, CAPES/PVE, CAPES/PNPD, CNPq/CT-Biotec, CNPq/CBAB, Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT-Argentina), Universidad de Buenos Aires and National Institute of Health R01 AI116453.

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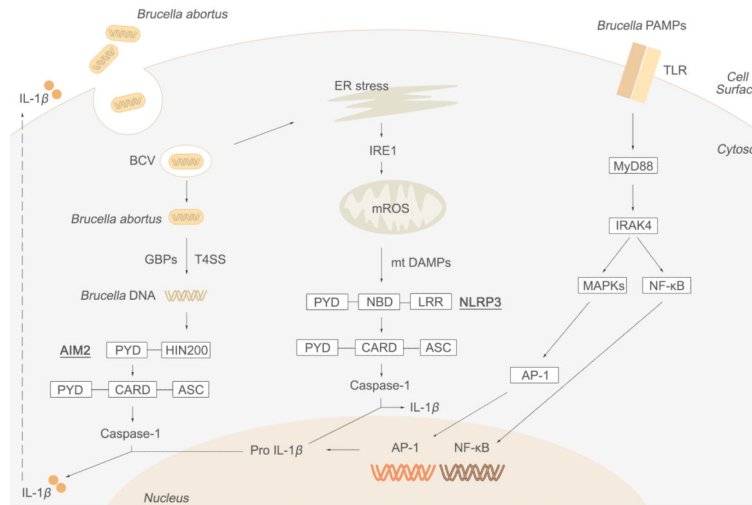

Figure 1. Working model of AIM2 and NLRP3 inflammasome activation by *B. abortus*

B. abortus PAMPs trigger MAPK and NF-κB signaling pathways in a MyD88 and IRAK-4-dependent manner which is important in the induction of the first signal and generate inactive form of pro-IL-1β. *B. abortus* ensures its survival by forming the *Brucella*-containing vacuole (BCV), which traffics along the endocytic pathway. It is believed that DNA can be released by the type IV secretion system or by the action of GBPs. GBPs mediate bacterial killing resulting in abundant release of bacterial DNA for recognition by AIM2. The released bacterial DNA is then sensed by the cytosolic AIM2 inflammasome. In parallel, infection of host cells with *B. abortus* can generate ER stress and consequent activation of the sensor IRE1. This sensor induces an increase in mitochondrial ROS and culminates in the release of mitochondrial DAMPs. NLRP3 and AIM2 activation triggers assembly of inflammasomes containing ASC and caspase-1, leading to pro-IL-1β processing and secretion of mature IL-1β.

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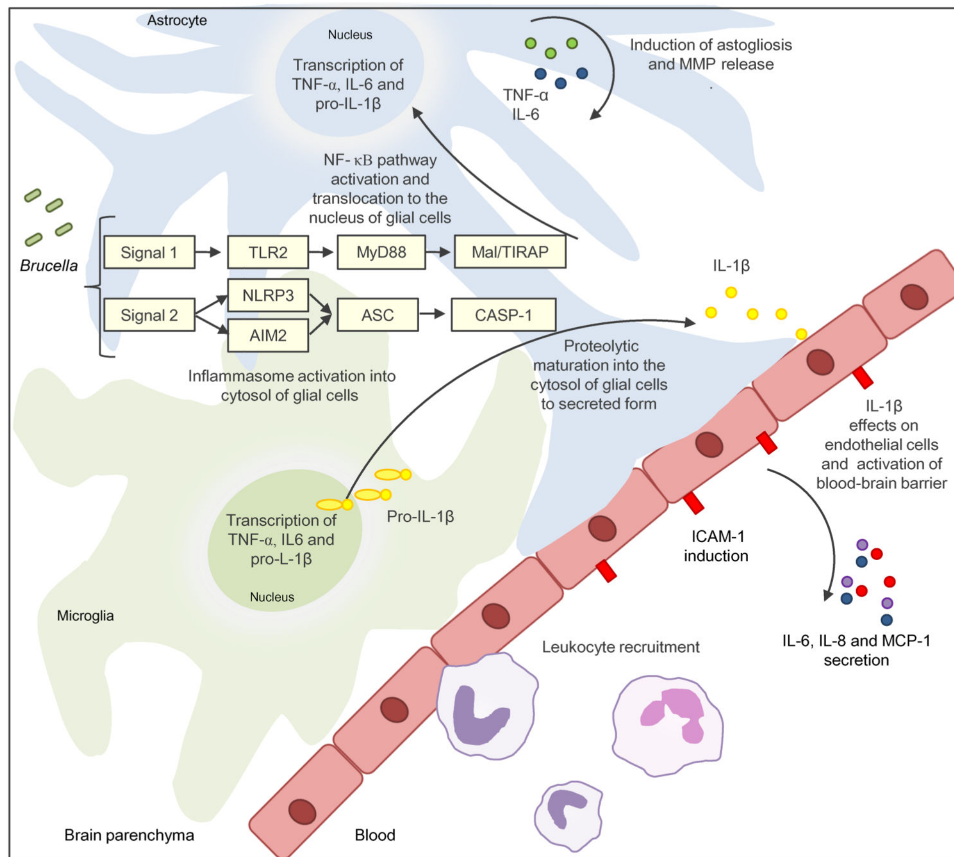

Figure 2. *Brucella* activation of CNS innate immunity

Brucella invades the CNS and triggers two signals in glial cells. A *Brucella* PAMP (i.e. lipoproteins) interacts with TLR2 and through the recruitment of MYD88 and Mal/TIRAP induces the activation of the NF-kB pathway. The translocation of NF-kB to the nucleus of glial cells, both astrocytes and microglia, induces the transcription and secretion of TNF and IL-6. These cytokines have an autocrine effect on glial cells and produces astrogliosis (proliferation and apoptosis simultaneously of astrocytes) and secretion of MMP-9. On the other hand, the interaction with TLR2 triggers the transcription of pro-IL-1β. A second signal results in the assembly of the ASC inflammasomes NLRP3 and AIM2. These in turn activate caspase-1 that induces the proteolytic maturation of the secreted form of IL-1β. This cytokine activates microvascular endothelium of the blood-brain barrier and results in the recruitment of leukocytes to the CNS.