Canonical and Non-Canonical Inflammasome Pathway in Ehrlichiosis

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Ehrlichia is an obligately intracellular bacterium which is responsible for causing human monocytic ehrlichiosis (HME), a potentially lethal disease similar to toxic shock syndrome and septic shock syndrome. Several studies have indicated that canonical and non-canonical inflammasome activation is a crucial pathogenic mechanism that induces dysregulated inflammation and host cellular death in the pathophysiology of HME. Mechanistically, the activation of canonical and non-canonical inflammasome pathways affected by virulent *Ehrlichia* infection is due to a block in autophagy.

non-canonical inflammasome PAMPs inflammasome DAMPs

1. Canonical Inflammasome Pathway (s) in Ehrlichiosis

The research has shown that canonical and non-canonical inflammasomes get activated in ehrlichiosis [1][2][3]. The increased expression of inflammasomes, such as NLRP3, NLRP1, NLRC4, NLRP12, and AIM2, and caspase 1 and caspase 11 activation has been linked with fatal ehrlichiosis in mice [2][4][5]. Mice deficient in caspase 1 and infected with virulent IOE died from an infection early in comparison to wild-type mice [5]. Caspase 1 deficient mice were less effective at clearing the *Ehrlichia* infection and developed extensive liver injury compared to wild type mice and mice deficient in NLRP3 [5]. These data suggest that NLRP3 activation via non-canonical pathways is a key mediator of immunopathology following lethal *Ehrlichia* infection. However, the enhanced susceptibility of caspase 1 deficient mice to fatal ehrlichiosis indicates that caspase 1 plays a protective role in the effective antimicrobial host's defense against *Ehrlichia* [5]. This antimicrobial effect of caspase 1 could be due to caspase 1-mediated pyroptosis. In infections with *Salmonella* and *Burkholderia* species, caspase 1 induced pyroptotic cell death in addition to the clearance of bacteria by reactive oxygen species in neutrophils, and moreover, this occurred without the release of IL-1b and IL-18 [6]. Lethal *Ehrlichia* infection induces the activation of neutrophils and their migration to the site of the infection including the liver [7]. Thus, it is possible that caspase 1-induced pyroptosis leads to a release of intracellular *Ehrlichia* from the infected macrophages, the main target cells. These extracellular bacteria can then be phagocytosed by activated neutrophils and killed via ROS, as suggested in other infection systems [8]. As described above, neutrophils are resistant to Gasdermin-mediated pyroptosis in response to certain inflammasome activators. Thus, the link between caspase 1 activation at the sites of infection and the subsequent killing by neutrophils may account for the host-protective function of caspase 1.

Apart from the potential antimicrobial effect of caspase 1, the finding that caspase $1^{-/-}$ mice develop extensive liver damage following *Ehrlichia* infection suggests that caspase 1 is likely hepatoprotective [7]. This conclusion is

supported by studies showing that caspase $1^{-/-}$ deficiency mice developed systemic inflammation and liver damage in a hemorrhagic shock model [7]. Interestingly, patients who survive sepsis have increased expression of caspase 1, which correlates with a decreased expression of caspase 3 [9]. Similarly, macrophages lacking caspase 1 that are infected with *Francisella* express elevated levels of caspase 3 and undergo apoptosis, suggesting a causal link between caspase 1 and caspase 3 [10]. As researchers detected an inverse relationship between the caspase 1 and caspase 3 expressions, researchers examined whether the potential hepatoprotective role of caspase 1 is due to the inhibition of caspase 3.

2. Non-Canonical Inflammasome Pathways and Their Regulation by Type I- IFN in Fatal Ehrlichiosis

Unlike caspase 1, researchers have found that non-canonical inflammasome signaling is likely a pivotal inducer of liver injury during severe and fatal ehrlichiosis [2][3]. Furthermore, researchers have showed that caspase 11 activation in fatal ehrlichiosis is regulated by type I interferon (IFN-I), and that IFN-I mediated caspase 11 activation accounts for immunopathology and fatal outcomes following infection with virulent IOE [3]. In comparison to wild type mice, IFN-I receptor deficient mice were resistant to fatal *Ehrlichia* infection [11]. IFNAR-I deficient mice had a significant reduction in the activation of caspase 11, and moreover, the production of IL-1b, which highlights the importance of IFN-I mediated regulation of non-canonical inflammasomes in fatal ehrlichiosis [11]. Using murine bone marrow chimera, it was found that the expression of IFN-I receptor (IFNAR) on non-hematopoietic cells during fatal *Ehrlichia* infection is essential for a fatal outcome following IOE infection. Recently, researchers demonstrated that virulent IOE infects primary murine hepatocytes and that IFNAR signaling on hepatocytes promotes bacterial replication and positively regulates caspase 11 activation and inflammation [3]. IFNAR-mediated regulation of caspase 11 activation in hepatocytes resulted in the secretion of IL-1b, IL-1a, and HMGB1, as well as pyroptosis/inflammatory cell death [3]. In addition to IFNAR-mediated positive regulation of non-canonical inflammasome during fatal IOE infection, IFNAR signaling also results in the loss of bone marrow and a reduction of hematopoietic stem and progenitor cells (HSC/HSPCs) via lower expression and activity of caspase 8 [12]. The latter prevents the cleavage of RIPK1 and leads to RIPK1-kinase-dependent cell death [12].

How IFN-I regulates the non-canonical inflammasome pathways during *Ehrlichia* infection remains elusive. IFNAR can promote non-canonical NLRP3 inflammasome activation by increasing the abundance of the caspase 11 protein [1]. Alternatively, as suggested in other studies, infection with Gram-negative bacteria triggers an IFN-I response, which results in the increased expression of genes that encode guanylate binding proteins (GBPs) [13]. GBPs promotes bacterial lysis to release cell wall or bacterial components, which can lead to the activation of non-canonical NLRP3 inflammasome pathways [1]. In addition, GBPs open pores in the phagosomes in which bacteria reside, enabling access for bacterial LPS from the phagosome into the cytoplasm and the subsequent activation of caspase 11 [1]. As *Ehrlichia* lack LPS, there is the possibility for other pathogen-associated molecular patterns (PAMPs) to be released into the cytosol, that may lead to the activation of IFNAR signaling. Recent research showed that mice lacking Caspase 11 can survive longer than wild-type mice when infected with SARS-CoV-2, indicating that targeting this pathway could be a promising therapeutic approach [14].

3. Potential Pathogen-Associated Molecular Patterns and Danger-Associated Molecular Patterns That Trigger Inflammasome (s) Activation in Ehrlichiosis

As *Ehrlichia* lacks LPS as well as peptidoglycan, it is less likely that LPS-like molecules can trigger the IFN-I-caspase11 axis. However, other *Ehrlichia* PAMPS may trigger inflammasome activation in ehrlichiosis. Like other intracellular bacteria, *Ehrlichia* utilizes the type IV secretion system (T4SS) to secrete proteins that might be PAMPs or toxins, which access the cytosol and may activate the immune system via activating inflammasome signaling [15]. Studies have shown that *Helicobacter pylori* secrete CagA (Cytotoxin-associated gene A) into the epithelial cells to activate the NLRP3/caspase 1 axis to secrete IL-1β, which hastens inflammation in atherosclerosis [16]. Similarly, *Legionella pneumophila*, an intracellular bacterium that is known to exploit the inflammasome pathway, especially non-canonical inflammasomes, employ a type IV secretion system (T4SS) that has been shown to activate caspase 3 using several T4SS substrates, including VipD, the phospholipase A2 that destabilized the outer mitochondria membrane to free cytochrome c and subsequently triggered caspase 3 [17][18][19]. *E. chaffeensis* translocated factor 1 (Etf1), *E. chaffeensis* translocated factor 2 (Etf-2), and *E. chaffeensis* translocated factor 3 (Etf-3) are a small number of T4SS effector proteins secreted abundantly during *Ehrlichia* infection [20]. Etf-3 is shown to induce ferritinophagy in the host cell, which leads to an increase in labile cellular iron in the host cell, which can activate the inflammasome [20]. However, recent studies have identified the ankyrin domain's role in stabilizing active caspase 1 [21]. Therefore, it might be possible that the T4SS effectors function as a critical inflammasome adaptor that fine tunes the activation of different caspases during *Ehrlichia* infection. Despite lacking the genes necessary for the synthesis of cholesterol in their cell walls, *Ehrlichia* hijacks the host cells and relies on the phospholipids in their cell wall for survival and infection [20][22]. These exported phospholipids and cholesterol may act as *Ehrlichia* PAMPS that elicit inflammasome activation.

Other *Ehrlichia* PAMPS that access cytosol and could trigger the activation of non-canonical inflammasome pathways are tandem repeat proteins (TRPs) [23]. TRPs are immunomodulin proteins, type I secretion effectors (T1SS), that are present at the ehrlichial surface or secreted in extracellular space [23]. TRP32, TRP47, TPR75, and TRP120 are known TRPs found in *Ehrlichia* [23]. These TRPs interact with diverse host proteins and modulate several cellular signaling events [23]. TRP32 can also control apoptotic function during *Ehrlichia* infection by interacting with GLCCI1 and TP53I11, and through its interaction with CD14, TRP32 influences MAPK, TLR, and IKK/NFκB signaling [23]. Similarly, TRP47 interacts with adenylate cyclase-associated protein 1 (CAP1) to alter its mitochondrial shuttling to promote apoptosis [23][24]. TRP47 also translocates to the nucleus using MYND-binding proteins to interact with genes that regulate actin cytoskeleton organization and immune response [25][26]. *Ehrlichia* also secretes TRP75, a predicted lipoprotein, which has been shown to interact with MMP9 (Matrix metalloproteinase-9), which has a role in cytokine-mediated signaling [23]. TRP75 can alter cellular metabolism by binding with protein kinase AMP-activated catalytic subunit alpha 1 (PRKAA1), which is the catalytic subunit of the AMP-activated protein kinase (AMPK) that promotes autophagy during energy stress [23]. Furthermore, the *E. chaffeensis* TRP120 effector protein exploits the host SUMOylation pathways for their intracellular survival [27]. In addition, TRP120 function as a nucleomodulin, which binds to GC-rich regions of host DNA to regulate multiple

cellular functions to promote *Ehrlichia* infection positively [28][29]. Canonical Notch signaling is also triggered by *E. chaffeensis* TRP120 to decrease TLR2/4 expression and increase survival within the cells [30].

In addition to *Ehrlichia* PAMPs, several potential danger-associated molecular patterns (DAMPs) generated during infection with virulent *Ehrlichia* are likely triggering activation of inflammasome [22]. Infection of macrophages with virulent IOE resulted in inhibition of autophagy induction and flux via MyD88 signaling, which result in faulty mitophagy and build-up of damaged mitochondria and ROS [2]. Consequently, the mtDNA or mtROS are likely to function as DAMPS activating NLRP3 inflammasome [2].

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