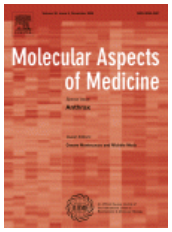

To protect or adversely affect? The dichotomous role of the NLRP1 inflammasome in human disease

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Article

ASC-Mediated Inflammation and Pyroptosis Attenuates *Brucella abortus* Pathogenesis Following the Recognition of gDNA

Juselyn D. Tupik [1], Sheryl L. Coutermarsh-Ott [1], Angela H. Benton [1], Kellie A. King [1], Hanna D. Kiryluk [1], Clayton C. Caswell [1] and Irving C. Allen [1,2,*]

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Abstract: *Brucella abortus* is a zoonotic pathogen that causes brucellosis. Because of *Brucella's* unique LPS layer and intracellular localization predominately within macrophages, it can often evade immune detection. However, pattern recognition receptors are capable of sensing *Brucella* pathogen-associated molecular patterns (PAMPS). For example, NOD-like receptors (NLRs) can form a multi-protein inflammasome complex to attenuate *Brucella* pathogenesis. The inflammasome activates IL-1β and IL-18 to drive immune cell recruitment. Alternatively, inflammasome activation also initiates inflammatory cell death, termed pyroptosis, which augments bacteria clearance. In this report, we assess canonical and non-canonical inflammasome activation following *B. abortus* infection. We conducted in vivo studies using *Asc*$^{-/-}$mice and observed decreased mouse survival, immune cell recruitment, and increased bacteria load. We also conducted studies with *Caspase-11*$^{-/-}$mice and did not observe any significant impact on *B. abortus* pathogenesis. Through mechanistic studies using *Asc*$^{-/-}$macrophages, our data suggests that the protective role of ASC may result from the induction of pyroptosis through a gasdermin D-dependent mechanism in macrophages. Additionally, we show that the recognition of *Brucella* is facilitated by sensing the PAMP gDNA rather than the less immunogenic LPS. Together, these results refine our understanding of the role that inflammasome activation and pyroptosis plays during brucellosis.

Keywords: brucellosis; canonical inflammasome; non-canonical inflammasome; NLR; pyroptosis; ASC; caspase-11; caspase-1; IL-1β; gDNA

1. Introduction

Brucellosis is a zoonotic bacterial disease that exhibits pathogenesis consistent with inflammation. Transmitted through *Brucella* spp. primarily from agricultural animals to humans in unpasteurized dairy products, brucellosis symptoms in humans often include inflammatory or influenza-like characteristics such as arthritis, undulant fevers, and neurological manifestations [1,2]. Because there is currently no human vaccine for brucellosis and effective antibiotic regiments for the disease require long treatment durations, these symptoms often persist throughout the infected individual's lifetime due to the well-adapted ability of *Brucella* to evade immune recognition [3]. Unlike the classical lipopolysaccharide (LPS) layer of Gram-negative bacteria such as *Escherichia coli* that contain a glucosamine backbone with short acyl groups, *Brucella* spp. contain a modified lipid A layer that consists of a diaminoglucose backbone with long branching acyl groups [2]. This deviation from

35. Englen, M.D.; Valdez, Y.E.; Lehnert, N.M.; Lehnert, B.E. Granulocyte/macrophage colony-stimulating factor is expressed and secreted in cultures of murine L929 cells. *J. Immunol. Methods* **1995**, *184*, 281–283. [CrossRef]
36. Sharma, A.; Puhar, A. Gentamicin Protection Assay to Determine the Number of Intracellular Bacteria during Infection of Human TC7 Intestinal Epithelial Cells by Shigella flexneri. *Bio-Protocol* **2019**, *9*. [CrossRef]

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Review

Detecting DNA: An Overview of DNA Recognition by Inflammasomes and Protection against Bacterial Respiratory Infections

Juselyn D. Tupik [1], Justin W. Markov Madanick [1], Hannah M. Ivester [1] and Irving C. Allen [1,2,*]

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Abstract: The innate immune system plays a key role in modulating host immune defense during bacterial disease. Upon sensing pathogen-associated molecular patterns (PAMPs), the multi-protein complex known as the inflammasome serves a protective role against bacteria burden through facilitating pathogen clearance and bacteria lysis. This can occur through two mechanisms: (1) the cleavage of pro-inflammatory cytokines IL-1β/IL-18 and (2) the initiation of inflammatory cell death termed pyroptosis. In recent literature, AIM2-like Receptor (ALR) and Nod-like Receptor (NLR) inflammasome activation has been implicated in host protection following recognition of bacterial DNA. Here, we review current literature synthesizing mechanisms of DNA recognition by inflammasomes during bacterial respiratory disease. This process can occur through direct sensing of DNA or indirectly by sensing pathogen-associated intracellular changes. Additionally, DNA recognition may be assisted through inflammasome–inflammasome interactions, specifically non-canonical inflammasome activation of NLRP3, and crosstalk with the interferon-inducible DNA sensors Stimulator of Interferon Genes (STING) and Z-DNA Binding Protein-1 (ZBP1). Ultimately, bacterial DNA sensing by inflammasomes is highly protective during respiratory disease, emphasizing the importance of inflammasome involvement in the respiratory tract.

Keywords: inflammasome; NLRP3; AIM2; IFI16; DNA; STING; ZBP1; Type I IFN; bacterial respiratory infection; pyroptosis

Citation: Tupik, J.D.; Markov Madanick, J.W.; Ivester, H.M.; Allen, I.C. Detecting DNA: An Overview of DNA Recognition by Inflammasomes and Protection against Bacterial Respiratory Infections. *Cells* **2022**, *11*, 1681. https://doi.org/10.3390/cells11101681

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1. Introduction

1.1. Inflammasome Sensing of Bacteria

The innate immune system serves as one of the primary mechanisms of host–pathogen defense. One key effector in this system is a multi-protein complex termed the inflammasome (Figure 1) [1]. The canonical inflammasome typically consists of three components: (1) a Pattern Recognition Receptor (PRR) that senses pathogen components, (2) the adaptor protein Apoptosis-associated Speck-like protein containing a CARD (ASC), and (3) activated caspase-1 that cleaves and activates cytokines and other proteins. Inflammasomes first assemble in response to PRR sensing of conserved genetic motifs known as pathogen or damage-associated molecular patterns (PAMPS/DAMPS) [1]. Inflammasomes sense LPS, bacterial surface proteins, and nucleic acids, which facilitates activation and host defense [2]. Inflammasome-forming PRRs consist of the intracellular NOD-like receptors (NLRs) and AIM2-like receptors (ALRs) (Figure 1).

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