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Review

Host response to *Brucella* infection: review and future perspective

Mohamed G Elfaki[1,2], Alwaleed Abdullah Alaidan[1], Abdullah Abdulrahman Al-Hokail[2,3]

[1] *Department of Infection and Immunity, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia*
[2] *Alfaisal University College of Medicine, Riyadh, Saudi Arabia*
[3] *Department of Medicine, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia*

Abstract

Brucellosis is a zoonotic and contagious infectious disease caused by infection with *Brucella* species. The infecting brucellae are capable of causing a devastating multi-organ disease in humans with serious health complications. The pathogenesis of *Brucella* infection is influenced largely by host factors, *Brucella* species/strain, and the ability of invading brucellae to survive and replicate within mononuclear phagocytic cells, preferentially macrophages (Mϕ). Consequently, the course of human infection may appear as an acute fatal or progress into chronic debilitating infection with periodical episodes that leads to bacteremia and death. The existence of brucellae inside Mϕ represents one of the strategies used by *Brucella* to evade the host immune response and is responsible for treatment failure in certain human populations treated with anti-*Brucella* drugs. Moreover, the persistence of brucellae inside Mϕ complicates the diagnosis and may affect the host cell signaling pathways with consequent alterations in both innate and adaptive immune responses. Therefore, there is an urgent need to pursue the development of novel drugs and/or vaccine targets against human brucellosis using high throughput technologies in genomics, proteomics, and immunology.

Key words: brucellosis; pathogenesis; immunity, Saudi Arabia.

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Introduction

Brucellosis is a zoonotic and contagious infectious disease caused by bacteria of the genus *Brucella*. There are 10 species of *Brucella* that have been classified on the basis of primary host specificity, biochemical characteristics, and antigenic component [1]. Among the known species of *Brucella*, *B. melitensis*, *B. abortus*, and *B. suis* are the most pathogenic to humans [2]. However, in Saudi Arabia, *B. suis* does not exist due to the lack of its natural animal reservoir, the swine species, leaving *B. melitensis* and *B. abortus* as the two most common brucellae that cause human infection in this region [3-5]. The bacterium is a small, Gram-negative, non-motile coccobacillus adapted to intracellular living within host mononuclear phagocytic cells [1,6]. The bacterium infects primarily ruminant animals and is transmitted to humans through contact with infected animals, including handling of infected carcasses, consumption of unpasteurized milk or milk products, or, in rare cases, through inhalation of aerosols from infected specimens [7,8]. Thus, humans are accidental hosts of brucellosis. Transmission is also possible by inhalation of fomites, as brucellae have the ability to survive in dusty environments and free-range rearing conditions for several months without losing infectivity [9]. In rare cases, hospital-acquired infection may occur [10] due to the volatile nature of brucellae and a low dose requirement to cause infection. Thus, the spread of brucellae increases public panic and anxiety worldwide.

Most of the infected animals in Saudi Arabia are inhabitants of rural areas where intense human interaction with animals coupled with the nomadic lifestyle of the inhabitants favor the transmission of brucellae [11]. The natural animal reservoirs for brucellae in this region include camels, cattle, sheep, and goats. The disease is economically important in farm animals and will be a major public health problem worldwide unless stringent control measures are undertaken in the livestock industry [12]. The prevalence rate of brucellosis has increased recently in traditionally endemic regions such as the Mediterranean basin countries, the Arabian Peninsula, South America, and Southeast Asia [13]. The disease annually infects 500,000 humans worldwide, a number that may be higher in depressed regions of the world. In Saudi Arabia, however, the incidence rate of

brucellosis has risen (70 in every 100,000), and the disease prevalence is considered among the top five communicable diseases as reported by the Saudi Ministry of Health (MOH) (http://www.moh.gov.sa).

Pathogenesis and clinical presentation

Brucella has the propensity to localize inside Mϕ [6,14] of the liver, spleen, bone marrow, uterus, heart, and brain with protean clinical manifestations [15]. The disease is characterized by undulant fever, arthritis, spondylitis, endocarditis, osteomyelitis, and hepatosplenic abscesses or brain abscesses in rare events [16,17]. In animals, however, brucellosis is characterized by spontaneous abortion at late gestation, orchitis, undulant fever, and infertility, leading to severe economic losses of farm animals [7].

The adaptation of *Brucella* to live inside Mϕ [18] is managed by its ability to block receptors for innate immunity [19], inhibit phagolysosome fusion, inhibit apoptosis, and downregulate antigen presentation [20], which collectively leads to their escape from effector immune responses [21]. However, the propensity of *Brucella* to localize inside reticuloendothelial cells of parenchymatous organs, especially the liver and the spleen [22], is responsible for the consequential formation of granuloma in these organs. In conditions of immune challenges, the hidden brucellae will be released from their harbored sites with consequent relapse of infection and the appearance of chronic fatigue. Episodes of the systemic release of these brucellae may result in the development of bacteremia with consequent expression of undulant fever, splenomegaly, hepatomegaly, endocarditis, meningitis, arthritis, or osteomyelitis [16, 17] in most chronic cases. Based on these broad and serious clinical sequelae where multi-organ systems are implicated [15], the diagnosis and control of brucellosis pose a serious challenge to healthcare professionals.

Cell response to *Brucella* infection

The intracellular nature of *Brucella* organisms makes it difficult for these bacteria to be completely eliminated by the host cellular responses [20] or be eradicated by antimicrobial drugs [23, 24]. Thus, brucellae evade the host's cellular response with subsequent tissue retention, and relapse of infection ensues [21]. The mechanistic events involved in the cascade of this process are not fully understood and require further study. However, previous studies showed that several regulatory molecules secreted by Mϕ may contribute to the intracellular survival of brucellae in murine Mϕ. Macrophage-derived cytokines such as interleukin 1 (IL-1), IL-12, and tumor necrosis factor alpha (TNF-α) contribute to the control of early *Brucella* spp. infection via the IFN-γ pathway [25, 26]. Indeed, IFN-γ has been reported to reduce the intracellular growth of *Brucella* by upregulating both Mϕ effector function and the secretion of TNF-α and iron molecules [27]. Other regulatory molecules produced by Mϕ such as reactive oxygen intermediates and nitric oxide have been reported to control the growth of *B. abortus* [28]. In essence, the persistence of brucellae in Mϕ is broadly attributed to failure of phagolysosomal fusion [29, 30], inhibition of the oxidative burst [31, 32], or to the synthesis of Cu-Zn superoxide dismutase [33], which inactivates oxygen radicals and promotes the intracellular survival of brucellae inside the Mϕ niches. A recent study by Barrionuevo *et al.* [34] demonstrated that *B. abortus* utilizes its lipoproteins to inhibit the monocytes/macrophages activation mediated by IFN-γ and to subvert host immunological responses. Consequently, the marker for Mϕ activation (FcγR1, CD64) was downregulated and no FcγR1-mediated phagocytosis ensued.

In addition to the role of Mϕ in immunity, T cell subsets play a major role in the resolution of infection due to *Brucella* spp. $CD4^+$ T cells secrete potent cytokines such as IFN-γ and IL-10 that are known, respectively, to enhance protection or exacerbate infection due to brucellae [35-37]. The precise role of T cells in protection against *Brucella* infection has been unequivocally shown in studies using adoptive transfer [38] and gene knockout experiments [39]. In the murine model of infection [38], $CD4^+$ T lymphocytes have been demonstrated to exert their protective effect by the production of IFN-γ, which activates rodent Mϕ to halt the replication of intracellular brucellae [37]. The effector molecules in killing brucellae by IFN-γ-activated Mϕ were related to the production of reactive oxygen intermediates but not to nitric oxide [28]. Regulation of IFN-γ production in this process is mediated by the cytokines TNF-α and IL-12 [40], which were both secreted by activated Mϕ. In contrast, the counter-regulator of IFN-γ production, IL-10, has been demonstrated to exacerbate infection due to *B. abortus* [41]. Based on these studies, both $CD4^+$ and $CD8^+$ T cells were implicated in protection against brucellosis in the murine model of *Brucella* infection. While $CD4^+$ T cells exert their effect via cytokine secretion, cytotoxic $CD8^+$ T cells eliminate infection by lysing autologous Mϕ infected with *Brucella* spp. Recent studies by Cha

et al. [42] corroborated the above findings by demonstrating increased production of IFN-γ in mice inoculated with *B. abortus* outer membrane proteins compared to animals inoculated with whole live bacteria.

In humans, little is known about the immunological control of *B. melitensis* infection. Previous studies by Zaitseva *et al*. [43] demonstrated the expression of Th1 cytokines *in vitro* by $CD4^+$ and $CD8^+$ T cells stimulated with heat-inactivated *B. abortus*. There have been no *in vivo* studies to corroborate these findings, due to restrictions imposed on the study of human subjects as well as ethical conduction rules in the study of human models. However, a recent study by our group [44] demonstrated an increased production and expression of transforming growth factor beta 1 (TGF-β1) in sera and peripheral mononuclear blood cells (PMBCs) of patients with brucellosis. The increased TGF-β1 production in these patients correlated well with depressed T cell proliferation and IFN-γ production. On the basis of the above studies, it is obvious that *Brucella* infection causes secondary immunosuppression. However, the extent of immunosuppression in humans is difficult to evaluate due to patient dropout, ignorance of most patients about the importance of subsequent medical exams or tests, and the hidden medical history of patients suspected with chronic debilitating infections. Thus, brucellae constitute a major challenge to physicians and a threat to infected humans by evading the host immune response. The cellular cascade and mechanistic events involved in the generation of human infection are not fully understood.

Humoral response to *Brucella* infection

Antibodies of the IgG, IgM, or IgA classes may play a role in protection against brucellosis. Anti-*Brucella* antibodies are proteins that cause agglutination, complement fixation, and precipitation when reacted with their homologous antigens [45] derived from brucellae. Most of the reactive antibodies were elicited by *Brucella* lipopolysaccharide (LPS) rather than by cytoplasmic proteins. Indeed, IgM antibodies against *Brucella* LPS were the first to appear following infection and rise gradually during the course of acute infection. In contrast, IgG anti-*Brucella* antibodies appeared later after the onset of infection and were likely provoked by *Brucella* cytoplasmic proteins. On this basis, most of the available serological tests were based on LPS to differentiate between infected and uninfected hosts. For cytoplasmic proteins, there is no single antigenic determinant that differentiates between infected humans and naturally infected animal hosts. Thus, the humoral response of humans to *Brucella* infection is different from that exhibited in animals, the natural reservoirs for brucellosis. A recent study that used protein microarrays [46] supported this notion and highlighted the importance of certain antigens as potential markers for each host response and disease pathogenesis. However, the differential power of these antigens in the differentiation of brucellae from their closely related bacteria was not reported by these investigators [46].

It is well known that anti-*Brucella* antibodies have the potential to cross-react with antibodies raised against heterologous *Brucella* strains or against some enteric bacteria. Cross-reactions in serum agglutination have been observed in infections caused by some enteric bacteria [47]. This cross-reactivity hampered the interpretation of many of serological tests used in the diagnosis of brucellosis [48]. The situation is worsened in farm animals vaccinated with the live-attenuated Rev1 vaccine, which makes it difficult to differentiate between vaccinated and infected animals [49]. In humans, the use of serological assays had complicated the clinical differentiation between acute, chronic, or chronic persistent forms. Thus, identification of key antigenic determinants among strains of *Brucella* spp. that do not cross-react with other bacteria is crucial for appropriate diagnosis. However, efforts to develop recombinant antigens for *Brucella* have been initiated with the goal to use them as subunit vaccines or purified antigens for definitive diagnosis [50]. Recently, unpublished proteomic data presented by Elfaki *et al.* at the 115th General Meeting of the American Society for Microbiology (May 30-June 2, 2015), holds promise as diagnostic markers for the differentiation of *Brucella* species and strains.

Future research perspectives

Since the host response to *Brucella* is largely mediated by immune cells, elucidation of common proteomes elicited by peripheral blood mononuclear cells of infected patients as well as proteomes expressed by infecting brucellae are imperative to define both drug and vaccine targets against brucellae. A recent study in our laboratory showed a resolution of ≥ 600 spots per *Brucella* strain with a minimum of 88% homogeneity (Elfaki *et al.*, unpublished data). Marked quantitative and qualitative changes were observed in *Brucella* protein patterns measured by

principal component analysis (PCA) and hierarchical cluster analysis. Furthermore, most of the differential peptides studied thus far were involved in cell metabolism, cell proliferation, and immune regulation (Elfaki *et al.*, unpublished data). The goal of our current study is to elucidate markers implicated in microbial pathogenesis, disease progression, and immune regulation of *Brucella* infection. However, further research is needed to dissect the cellular pathways during *Brucella* infection. Of major interest, studies on the role of microRNAs in $CD4^+$ or $CD8^+$ T cell responses mounted against infection with brucellae. Such information is vital to define the regulatory role of microRNAs in phagocytosis or antigen presentation by either Mϕ or dendritic cells infected with brucellae. Other points of interest that complement our current knowledge include the role of brucellae in the induction of inflammasomes, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), toll-like receptors (TLR), and T regulatory cells. The outcome of the aforementioned prospective is to define conditions that elicit infection, inflammatory response, bacterial colonization and clearance, disease progression, and protection by immune effector cells. Unfortunately, most of the available data on immunity to brucellosis were collected from the murine model of infection. However, the response of murine species to *Brucella* infection is completely incompatible with responses encountered in the natural hosts of *Brucella*, such as ruminant animals, or even with that seen in accidental hosts such as humans. All of these factors led to controversial interpretations when comparing the animal response to that encountered in humans. Therefore, there is an urgent need to develop an animal model compatible with human brucellosis. In our judgment, unless the above research issues are well defined, no vaccine is possible against human brucellosis under these adversaries.

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Corresponding author

Prof. Mohamed G. Elfaki,
Department of Infection and Immunity, King Faisal Specialist Hospital and Research Centre,
P. O. Box 3354, MBC 03, Riyadh 11211, Saudi Arabia.
Phone: +966-546-271-771
Email: elfaki@kfshrc.edu.sa

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