Review

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Research progress on the role of immune cells in *Brucella* infection

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Abstract: Brucellosis is one of the most prevalent zoonoses in the world. Incidence of the disease has increased significantly in recent years and has seriously affected the health of human beings and the development of animal husbandry. The pathogenesis of brucellosis remains unclear. Current studies suggest that this disease may be related to changes in natural killer cells, dendritic cells, and macrophages in immune cell subsets. Brucellosis may be also related to T helper (Th) 1 cell/Th2 cell imbalance in the CD4⁺ T cell subset, immunoregulation of regulatory T cells and Th17 cells, and the mechanism of action of CD8⁺ T cell. This paper aims to review the research progress on these inherent immune cells, the CD4⁺ T cell subset, and CD8⁺ T cells in *Brucella* infection.

Keywords: brucellosis, CD4⁺ T cell, CD8⁺ T cell, inherent immune cell

1 Introduction

Brucellosis is a zoonosis caused by *Brucella* [1]. *Brucella* is a genus of Gram-negative intracellular bacteria. The prevalent *Brucella* species in China mainly include three kinds, namely, *B. abortus*, *B. ovis*, and *B. suis*. Among these species, *B. ovis* features the strongest pathogenicity to humans [2-4]. People mainly become infected by *Brucella* by touching infected animals or ingesting contaminated meat and dairy products [5]. *Brucella* infection commonly manifests as fever, which is easily misdiagnosed as malaria, typhoid, rheumatic fever, osteoarthritis, and other diseases [6]. The pathogenesis of *Brucella* infection in humans remains unclear. In the presence of additional complications, diagnosis using epidemiological and clinical symptoms poses difficulties. Once it has entered into the chronic phase, curing the disease becomes impossible. Therefore, the pathogenesis of brucellosis is considered a research hot spot. *Brucella* infection is considered to be related to inherent immunity, adaptive immunity, and immunomodulatory mechanisms [7].

Papadopoulos et al. [8] have observed from in vitro implant bone model that *Brucella* can infect and replicate in bone marrow-derived dendritic cells (DCs) through granulocyte-macrophage colony-stimulating factor. Gao et al. [9] have discovered that when *Brucella* invades the host, the function of natural killer (NK) cells is inhibited, which can cause abortion. When type 1 T helper (Th1) cell is downregulated, the in vivo Th1 cell/Th2 cell imbalance can also cause the animal host to abort [10,11]. Th17 cells provide the host organism with additional defense against a number of extracellular and intracellular microbial infections and can defend against invasion of *Brucella* through cooperation with Th1 cells [12]. The antibody response to *Brucella* proteins, mediated by interleukin 17 (IL-17) secreted by Th17 cells, plays a key role in brucellosis vaccine [13]. Regulatory T cells (Tregs) inhibit the immune function of effector T cells, promoting *Brucella* invasion [14]. Therefore, the occurrence of brucellosis may be related to the imbalance in Treg/Th17 cells. Clapp et al. [15] have observed from nasal mucosa infection that when *Brucella* invades organisms through the nasal mucosa, CD8⁺ T cells play an important role in immune protection. Based on these research reports, we summarize the pathogenesis of inherent immune cells, as well as the role of Th1 cell/Th2 cell in CD4⁺ T cell subsets, Treg and Th17 cells, and CD8⁺ T cells in brucellosis.

2 Inherent immune cells and *Brucella*

2.1 NK cells and *Brucella*

NK cells belong to a group of important inherent immune cells in organisms. They play an important role in the frontline defense system and participate in immunoregulation, as well as antitumor, anti-autoimmune disease and antiviral activities [16]. NK cells can directly dissolve Gram-negative bacteria and kill the target cells infected by microbes, thus playing an immunoregulatory role in *B. abortus* brucellosis [9]. The main cytokines that scavenge *Brucella* comprise tumor necrosis factor- α (TNF- α), IL-6, IL-12, and interferon γ (IFN- γ). Among these cytokines, IFN- γ can activate the phagocytosis of macrophages, directly killing *Brucella*. Under stimulation by macrophages and the TNF- α secreted by NK cells, the process of phagocytosis for killing *Brucella* reaches the highest [9]. Jiménez et al. [17] have shown that in the case of lack of T cells and B cells, activation of NK cells serves as the critical factor for survival of *Brucella*-infected mouse. NK cells are activated in the host with brucellosis to participate in the control of propagation and diffusion of *Brucella* within the organism. To sum up, NK cells can be involved in scavenging *Brucella* by secreting TNF- α and other cytokines to confer immune protection on organisms.

2.2 DCs, macrophages, and *Brucella*

DCs include unique antigen-presenting cells with the strongest organism function and can activate resting T cells. DCs can efficiently take up, process, and present antigens. Immature DCs exhibit strong mobility, whereas the mature ones can effectively activate naïve T cells. DCs act as the central link in starting, regulating, and maintaining the immune response. When *Brucella* invades organisms, cyclic β -1,2-glucan in the bacterium seriously infects the DCs, causing the immune balance of CD4⁺ T cells to tend toward Th2 cell subsets. This condition can aid the invasion of pathogens [18]. Macrophages can identify and kill *Brucella*. However, the *Brucella* species that are not killed by macrophages continually reproduce in phagocytic cells. This condition damages macrophages and causes release of *Brucella*, which enters the body's circulation to cause bacteremia or septicemia. Ayarçı et al. [19] have observed that when hosts are infected by *Brucella*, the level of high mobility group box 1 (HMGB1) in the serum increases, leading to septicemia and severe damages to the host. HMGB1 stimulates mononuclear cells and macrophages in the body to produce proinflammatory cytokines [20], including TNF- α , IL-1 β , IL-6, and macrophage inflammatory protein-1 β , to increase the expression and release of various inflammatory molecules, resulting in aggravation and worsening of inflammation by brucellosis.

3 CD4⁺ T cells and *Brucella*

3.1 Th1 cells/Th2 cells and *Brucella*

CD4⁺ T cells play an important role in the immune process of resisting *Brucella* invasion. CD4⁺ T cells can activate the bactericidal functions of macrophages through secretion of IFN- γ [21]. Dorneles et al. [22] have noted that after vaccinating cattle with *Brucella*, CD4⁺ Th1 cells, induced by IFN- γ , participate in the initiation and development of control of *Brucella*, whereas CD8⁺ T cells, B cells, and Th2 cells exert no significant effects [23]. IFN- γ secreted from Th1 cells plays an important role in the control of brucellosis. IFN- γ can avoid utilizing the immune response induced by Th1, providing for development of a highly efficient vaccine to prevent brucellosis [24,25]. IFN- γ secreted from Th1 cells and IL-4 secreted from Th2 cells are mutually antagonistic. IFN- γ secreted from Th1 destroys the macrophages damaged by *Brucella* to provide immune protection to the organism [26]. Th1 cell/Th2 cell balance is very important in maintaining the normal immune response of organisms. Changes in Th1 cell/Th2 cell balance can cause various immune diseases, including autoimmune

diseases, allergies, tumors, and infections [27]. Clausse et al. [28] used *Brucella* lumazine synthase and outer membrane protein 31 to stimulate the immune response of Th1 cells, Th2 cells, and mixed cells to generate IFN- γ under adjuvant induction. Results indicated that the chimeric protein of *Brucella* can activate macrophages, induce the immune response mediated by Th1 cells, and confer protection to the body. Goel and Bhatnagar [29] evaluated the potential immunogenicity and protective effects of *Brucella* outer membrane protein 25 in BALB/c mice and the toxic effects of brucellosis. These researchers showed increased immunoglobulin G (IgG)1/IgG2b ratio, as well as increased level of IL-4 in the mouse serum and increased immune response of Th2 cells. This condition caused an imbalance in the Th1 cell/Th2 cell ratio. Therefore, the increase in Th2 cells in the host may inhibit the immune response by Th1 cells. Imbalance in the Th1 cell/Th2 cell ratio results in the occurrence of brucellosis.

3.2 Treg and Th17 cells and *Brucella*

Treg is a kind of CD4⁺ CD25⁺ Foxp3⁺ T cell subset with negatively regulatory function. This cell can play an immunosuppressive function through the release of IL-10, transforming growth factor β , and cytokines. IL-17 is the characteristic cytokine secreted by Th17 cells; it can resist invasion by pathogenic microbes, playing an important protective role in maintaining the immune homeostasis of organisms. IL-17 secreted by Th17 cells plays a core role in protective immunization against brucellosis [30]. Treg/Th17 balance plays an important role in controlling inflammation and pathogenesis through immune escape [31]. In cases of tumors, inflammatory responses, or autoimmune diseases, Treg/Th17 cell balance is destroyed, leading to a series of diseases. Wang et al. [32] have observed that in the body of aborted mice infected by *Brucella*, IL-10 secreted by Treg leads to functional imbalance between macrophages and the initial proinflammatory/anti-inflammatory cytokines, promoting the growth of and persistent infection by *Brucella*. Studies have shown that when the body of a sheep was immunized with *Brucella* M5-90 outer membrane protein, Treg exerted an immunosuppressive function. However, when Treg was downregulated, the immune response of IFN- γ , CD4⁺ T cell, and CD8⁺ T cell improved [32]. Experts speculated that brucellosis may be related to Treg/Th17 cell imbalance. However, in relation to the occurrence and development of the disease, the kind of immune mechanism induced by Treg/Th17 cell imbalance remains unclear. Thus, further studies should be conducted.

4 CD8⁺ T cell and *Brucella*

Mature T lymphocytes can be divided into CD4⁺ T cells and CD8⁺ T cells, which play an important role in cellular immunity. Activated CD4⁺ T cells differentiate into Th cells, which can enhance elimination of *Brucella*. Activated CD8⁺ T cells differentiate into cytotoxic T lymphocytes (CTL), which can dissolve macrophages infected by *Brucella* through perforin release to kill the organism intracellularly. Barrionuevo et al. [33] have observed from their study on the mechanism of *B. abortus* infection in organisms that invasion of *Brucella* can activate CTL, leading to an immune response in cells. When the immune function of the major histocompatibility complex class I is inhibited, *B. abortus* escapes the attack of CTL and undergoes immune escape, which further promotes chronic infection by brucellosis. Durward-Dioia et al. [34] have assumed that CD8⁺ T cell failure inhibits the occurrence of IFN- γ and the adaptive immune response, playing an important immune inhibition role in the chronic phase of brucellosis infection. Yin et al. [35] have discovered from a study on *Brucella* epitope vaccine that in BALB/c mice immune with recombinant protein, mixed Th1 cells–Th2 cell immune response mainly occurred. The ratio of CD4⁺ T cells/CD8⁺ T cells and the expressions of IgG and IFN- γ also increased. Attenuated brucellosis live vaccine M5–90 Δ wboa [36] is a potential vaccine for prophylaxis of the disease. This vaccine can activate CD4⁺ T cells and CD8⁺ T cells, can cause high expression of IFN- γ in the body, and can provide desirable immune defense function. Therefore, if studies center on researching and developing a targeted vaccine that can activate CD4⁺ T cells in the early phase and CD8⁺ T cells in the late phase of brucellosis, the incidence of the disease may be significantly decreased.

5 Summary and prospects

Brucellosis may be related to the immune regulatory function of inherent immune cells and CD8⁺ T cells, as well as imbalances in Th1 cells/Th2 cells and Treg/Th17 cells in CD4⁺ T subsets. However, the specific mechanism of the interaction of Treg/Th17 cells in brucellosis remains unclear and must be further investigated. With a wide range of epidemic areas and absence of typical clinical manifestations at the early stage, brucellosis is easily misdiagnosed and not easily eradicated upon entering into the chronic period. Therefore, prevention and control measures at the early stage of the disease bear importance. To prevent the occurrence of brucellosis, studies have focused on the development of vaccines. At present, the developed brucellosis vaccines mainly refer to attenuated live vaccines, including the following: S19, S2, M5, and RB51; inactivated vaccines, including 45/20, H38, 19-B, and 104M; mutant vaccines, including RB51, VTRM1 (rough mutant *B. melitensis* 16M), and VTRSI (rough mutant *B. suis*); and subunit vaccines, such as BLS-L7/L12. These vaccines enhance the corresponding immune response and perform protective roles. The scientific community is currently concentrated on studies of the immune mechanism of brucellosis to explore or to develop high-potency protective *Brucella* vaccines. Outbreak or prevalence of brucellosis in pastoral areas can be prevented upon development of a high-potency vaccine for brucellosis or discovery of a new method for regulating Th1 cell/Th2 cell balance and Treg/Th17 cell balance for brucellosis treatment.

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