Vaccine 33 (2015) 3659–3666

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

Immune response triggered by *Brucella abortus* following infection or vaccination

Elaine M.S. Dorneles[a], Andréa Teixeira-Carvalho[b], Márcio S.S. Araújo[b], Nammalwar Sriranganathan[c], Andrey P. Lage[a,*]

[a] *Laboratório de Bacteriologia Aplicada, Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, 31270-901, Belo Horizonte, Minas Gerais, Brazil*
[b] *Laboratório de Biomarcadores de Diagnóstico e Monitoração, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Avenida Augusto de Lima 1715, 30190-002, Belo Horizonte, Minas Gerais, Brazil*
[c] *Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, 1410 Prices Fork Rd, Blacksburg, VA 24061, USA*

ARTICLE INFO

Article history:
Received 13 February 2015
Received in revised form 16 May 2015
Accepted 21 May 2015
Available online 3 June 2015

Keywords:
Bovine brucellosis
Brucellosis vaccines
Immune response
RB51
S19

ABSTRACT

Brucella abortus live vaccines have been used successfully to control bovine brucellosis worldwide for decades. However, due to some limitations of these live vaccines, efforts are being made for the development of new safer and more effective vaccines that could also be used in other susceptible species. In this context, understanding the protective immune responses triggered by *B. abortus* is critical for the development of new vaccines. Such understandings will enhance our knowledge of the host/pathogen interactions and enable to develop methods to evaluate potential vaccines and innovative treatments for animals or humans. At present, almost all the knowledge regarding *B. abortus* specific immunological responses comes from studies in mice. Active participation of macrophages, dendritic cells, IFN-γ producing $CD4^+$ T-cells and cytotoxic $CD8^+$ T-cells are vital to overcome the infection. In this review, we discuss the characteristics of the immune responses triggered by vaccination *versus* infection by *B. abortus*, in different hosts.

1. Introduction

Brucellosis is one of the major zoonosis in public and animal health worldwide. Infection by *Brucella* spp. leads to important economic losses and affects numerous livestock, wildlife and humans [1–3]. In cattle, infection is predominantly due to *B. abortus* and usually causes placentitis followed by abortion in pregnant cows, epididymitis and orchitis in bulls [1].

Vaccination is one of the most effective measures to reduce the prevalence of bovine brucellosis and has largely contributed to the success of many control programs [4]. S19 and RB51 are the two *B. abortus* vaccines more broadly used in the control of brucellosis in cattle, being effective in the prevention of abortion and infection, besides offering long lasting protection [5–8]. However, due to some issues presented by these current vaccines, such as be pathogenic for humans, cause abortion in pregnant cows and, for S19, induce antibodies that interfere with the serological tests employed in the diagnosis, great effort have been made to find a better and safer brucellosis vaccine. Characterization of the immune profile associated with resistance to *B. abortus* infection is critical, since the advances in genomics, proteomics and recombinant DNA technology have allowed the exploration of new vaccines, more effective and safer [9–11].

However, at the present, most of our understanding about protective immune response against *B. abortus* infection/vaccination comes from studies using mouse model. In contrast, there is a limited amount of information concerning the immune mechanism by which the *B. abortus* vaccines confer protection in cattle. Therefore, in this review, we opt to broaden the discussion on the host/*B. abortus* interaction, including vaccination and infection in the natural host, cattle, or in animal models of infection, in order to understand which immunological mechanisms and events are stimulated by this pathogen.

2. Innate immune response

In case of brucellosis as well as in other diseases, the innate immune system will act as the first line of host defense,

* Corresponding author at: Universidade Federal de Minas Gerais. Av. Antônio Carlos, 6627, 31270-901, Belo Horizonte, MG, Brazil. Tel.: +55 31 3409 2081; fax: +55 31 34 09 20 80.
E-mail address: alage@vet.ufmg.br (A.P. Lage).

http://dx.doi.org/10.1016/j.vaccine.2015.05.057
0264-410X/

responsible for preventing replication, reducing the initial number and killing of the microorganism, besides creating conditions for the generation of an effective adaptive immune response [12]. This first line of defense include phagocytosis of pathogens by cells such as neutrophils, macrophages and dendritic cells (DC), death by natural killer (NK)-cells, secretion of cytokines and chemokines, recognition of molecules typical of a microbe [pathogen-associated molecular patterns (PAMPs)] by pattern-recognition receptors (PRRs), and activation of the complement system [12].

2.1. Cells

Macrophages, DCs, along with NK-cells and neutrophils are the first cells to respond against infection [13]. Natural killer-cells are activated by *B. abortus* or their antigenic fractions [14] and are thought to be important in the activation of B-cells and consequently to antibody production [15]. However, even though NK-cells may be activated following infection, they seem to be not crucial in controlling brucellosis in mice, since its depletion *in vivo* does not affect the course of infection [14]. Likewise, it has been shown that human NK-cells did not express IFN-γ mRNA or secrete IFN-γ protein in response to *B. abortus* [16] and have a significantly depressed cytotoxicity in patients with acute infection, suggesting that NK-cells are also not critical to immune response against *B. abortus* in humans [17].

Neutrophils are the most numerous and important short-lived phagocytes in innate immune response, but in case of *B. abortus* infection, after phagocytosis the neutrophils are not stimulated to induce an effective level of degranulation [18–20]. Studies have demonstrated that neutrophils appear not to play a significant role in the clearance of *B. abortus* from infected mice [18]. On the contrary, later in the infection of mice, during the chronic phase (after 15 days post-infection), *B. abortus* is killed more efficiently in the absence of neutrophils than in their presence [21]. It was suggested that neutrophils limit and regulate the activation of adaptive immune response against intracellular *B. abortus* infection, mainly throughout decreasing T lymphocytes activation [21]. In addition, a response consistent with an activation profile, increase in the expression of CD35, CD11b and IL-8 and, decrease of CD62, has been observed in human neutrophils and associated with pathogenesis of brucellosis, contributing to localized tissue injury and inflammation (Fig. 1) [22]. Also, human neutrophils have been implicated in potential mechanisms of tissue damage during liver brucellosis, since hepatic cell apoptosis was significantly enhanced by stimulation with supernatants from *Brucella*-infected neutrophils [23]. Therefore, activation of neutrophils seems not to be associated with protective immunity against *B. abortus*, but rather, it appears to be related to tissue damage and down regulation of adaptive immune response.

In contrast to secondary involvement of neutrophils and NK-cells, macrophages play a central role in *B. abortus* infection. In the earlier stages of infection in mice, before the development of an adaptive immune response by the host, macrophages allow the replication and survival of the microorganism, whereas in the later stages they are the main cells responsible for the elimination of *B. abortus* [18,24–26]. *B. abortus* enter in mouse macrophages, remodel their phagosomes and avoid the fusion of late endosomes and lysosomes, forming special phagosomes called *Brucella*-containing vacuoles (BCVs) at endoplasmic reticulum [27]. After internalized by macrophages, BCVs interact with endoplasmic reticulum and establish a replicative niche by mainly up-regulation of the virB type IV secretion system [28]. The mechanisms used by *B. abortus* to remodel the phagosome and successfully establish a replicative compartment are promising targets to develop an attenuated mutant that could be explored as potential vaccine. Moreover, in these early stages of infection in mice, *B. abortus* induce the expression of low levels of proinflammatory cytokines and high levels of anti-inflammatory cytokines [18,29–32]. All together, these mechanisms allow survival of *Brucella* in phagocyte cells. Once inside the mouse macrophages, *B. abortus* replicates extensively without inducing toxic effects to the cell and spreads throughout the host (lymph nodes, spleen, liver and bone marrow) *via* lymphatic and hematogenous [18,33]. In later stages, after the establishment of antimicrobial mechanisms by adaptive immunity, activated macrophages are the primary source of *B. abortus* elimination in the infected mice [24,26,34]. The bactericidal activity of activated mouse macrophages are mainly due to reactive nitrogen intermediates (RNIs) and reactive oxygen intermediates (ROIs), which are induced by gamma interferon (IFN-γ) and tumor necrosis factor (TNF-α) and increased in the presence of iron (Fig. 1) [25,35]. However, a small population of bacteria may still survive inside the macrophages, leading to recurrence of the disease and chronic infection.

Dendritic cells form a key link between innate and adaptive immune systems. *B. abortus* down-modulates DC maturation in mice by interfering with the toll-like receptor 2 (TLR2) signaling pathway [36]. It has been shown that cattle DCs are resistant to *B. abortus* infection, in spite of exhibiting some signs of maturational and activation impairment and lack of up-regulation of co-stimulatory molecules and IL-12p40 after infection [37]. Mice and human DCs are susceptible to *B. abortus* infection [36,38]. These differences in susceptibility of DCs among hosts may be related to the differences observed in the progression of the disease, since cattle is more able to control the infection, showing less clinical signs compared to humans and some mice strains. However, rough *B. abortus* strains are able to induce higher phenotypic and functional maturation of human and murine DC cells, characterized by IL-12 and TNF-α secretion, and naive CD4 T-lymphocytes stimulation, compared to smooth strains [38–40]. Higher exposition of outer membrane proteins (Omp) in rough strains, compared to smooth strains, has been indicated as responsible for the stronger DC maturation in infection by rough strains [39]. The maturation and activation of DCs along with cytokine secretion after *B. abortus* infection seem to be dependent of caspase-2 and TLR6 [38,41]. Caspase-2 plays different roles during rough and smooth strains infections, being critical to mouse DC maturation and cytokine production in rough strains infection, whereas in infection by smooth strains it promotes the cell death, favoring bacterial dissemination [38]. TLR6 is required by mouse DC to induce TNF-α and IL-12 [41]. Furthermore, rough *B. abortus* RB51-infected murine DCs show up-regulated expression of MHC class II and costimulatory molecules CD40, CD80 and CD86, suggesting that RB51 vaccine strain is capable of inducing significant innate immune response [38–40,42].

2.2. Cytokines, chemokines and PRRs/PAMPs

During bacterial infection the antimicrobial activity of macrophages is modulated by sequential production of cytokines, some of these secreted by the macrophages themselves (TNF-α and IL-12), and others produced by neighboring cells (IFN-γ). Tumor necrosis factor-α is one of the first cytokines released following *B. abortus* infection of macrophages, and its production results from direct interaction between *Brucella* and macrophages [31]. Experimental evidence shows that human macrophages activated by TNF-α inhibit the replication of *Brucella* spp. *in vitro* [31] and that TNF-α and IL-12 are directly involved in resistance to brucellosis in mice [43–45]. Furthermore, TNF-α release is increased by the phagocytosis of opsonized bacteria, indicating that the Fcγ receptor regulates the expression of TNF-α in a positive manner [31]. Nevertheless, it was observed that *B. abortus* actively prevents the release of TNF-α by human and mouse macrophages during infection, indicating that this is the basic mechanism of