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Review

Brucellosis vaccines based on the open reading frames from genomic island 3 of *Brucella abortus*

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ABSTRACT

Brucella abortus is the etiological agent of brucellosis, a zoonotic disease affecting cattle and humans. This disease has been partially controlled in cattle by immunization with live attenuated *B. abortus* S19 and RB51 strains. However, use of these vaccine strains has been associated with safety issues in animals and humans. New vaccines have since emerged in the prevention of brucellosis, particularly DNA vaccines, which have shown effectiveness and a good safety profile. Their protection efficacy in mice is associated with the induction of Th1 type and cytotoxic T cell mediated immune response against structural antigens and virulence factors expressed during *B. abortus* infection. Some antigenic candidate for vaccine design against brucellosis (mainly DNA vaccines) have been obtained from genomic island 3 (GI-3) of *B. abortus*, which encodes several open reading frames (ORFs) involved in the intracellular survival and virulence of this pathogen. The immunogenicity and protection conferred by these DNA vaccines in a murine model is reviewed in this article, suggesting that some of them could be safe and effective vaccine candidates against to prevent *B. abortus* infection.

Contents

1. Introduction

Brucella abortus is a facultative intracellular pathogen that causes brucellosis, an endemic zoonosis affecting bovines and humans in several regions of the world. It is a Gram-negative bacterium characterized as a small, microaerophilic, non-spore-forming, slow growing, coccobacillus [1]. This pathogen is one of the most virulent species of *Brucella* genus infecting humans [2]; infection usually occurs through ingestion of contaminated food or through direct contact with infected animals [3]. After initial contact with the host, *Brucella* adheres to and penetrates the

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mucous membrane and travels through the lymphatic system and bloodstream and finally reaching the bone marrow, lymph nodes, liver and spleen. In some case, the bacterium can be found in bones, joints, male reproductive organs, placenta and the fetuses of pregnant females [4]. In these tissues, *Brucella* survives and replicates within various cell types, including but not limited to dendritic and epithelial cells, placental trophoblasts, and macrophages [5]. The ability of this pathogen to adapt to intracellular environments is essential for establishment of infection. The infection in human exhibit an acute phase, associated with undulant fever, fatigue and headaches, followed by a chronic phase, which can manifest into arthritis, orchitis, hepatitis, endocarditis or neurobrucellosis [6]. Due to these complications, brucellosis is considered a crippling disease, annually affecting more than half a million people in the world [3]. In bovines, brucellosis causes abortion in pregnant females, stillborn or weak calves, retention of placentas and reduced milk yield. Besides, *B. abortus* can be found in the male reproductive organs causing infertility [7]. All these complications produce major economic losses and public health problems necessitating the containment and treatment of the disease in humans and bovines [8].

Prevention of brucellosis primarily depends on immunization; which plays a key role in containing the disease in animals before it spreads to humans. In order to develop safe and effective vaccines against bovine and human brucellosis, significant efforts must be made to identify the genes that encode *B. abortus* virulence factors. Using these antigenic factors as vaccine candidates, the host immune responses can be directed towards these virulence factors. To this end of identifying antigenic targets for the development of vaccines against brucellosis, this study will discuss the immunogenicity and protection conferred in murine model by vaccines that were developed based on the open reading frames (ORFs) from genomic island 3 (GI-3) of *B. abortus*. The results suggest that some these constructs discussed in this reports could be used as safe and effective vaccines against *B. abortus*.

2. Bovine brucellosis vaccines

Prevention of bovine brucellosis primarily done by using licensed live attenuated *B. abortus* S19 and RB51 vaccine strains. *B. abortus* S19 is a smooth attenuated strain, effective against brucellosis in cattle [9]; however, it can induce abortions in immunized females and is infective in humans. Additionally, the presence of lipopolysaccharide (LPS) in this strain makes it difficult to differentiate between immunized versus naturally infected animals because of the anti-LPS antibody cross-reactivity [10]. Although S19 strain is used in several countries because of its protective efficacy in cattle, the aforementioned diagnostic and safety issues allowed for the introduction of *B. abortus* RB51 rough strain [11]. This vaccine strain has similar protective efficacy but is less abortive in cattle than the *B. abortus* S19 strain [11]. Furthermore, since *B. abortus* RB51 is a mutant lacking the O-antigen of LPS, it does not interfere with the diagnosis between immunized versus infected bovines. However, RB51 strain is infectious to humans and resistant to rifampicin; one of the major antibiotics used to treat human brucellosis [9,12]. These vaccines have been shown to induce protective immunity in mice, associated with strong $CD4^+$ Th1 cell-mediated immune response with production of IFN-γ (but not IL-4), and specific $CD8^+$ cytotoxic T cells [13]. In cattle, immunization with *B. abortus* RB51 and S19 vaccines induces an immune response characterized by proliferation of $CD4^+$ and $CD8^+$ T-cells; IFN-γ and IL-17A production by $CD4^+$ T-cells and; $CD4^+$ and $CD8^+$ memory cells [14].

Safety limitations of *B. abortus* S19 and RB51 strains-based vaccines warranted the search for safer and more effective experimental vaccines. Some of these newer vaccine strains are based on attenuated strains, protein subunits or DNA fragments. Their protections levels are defined by the difference between the mean of $\log_{10}$ CFU/spleen values of the negative control group (non immunized group) and the mean of $\log_{10}$ CFU/spleen values of the immunized group (1, 2 and 3). These vaccines have shown a high variability in the protection levels depending on the type of vaccine, route of administration, doses or the challenge time used in each experiment [15]. Among them, main attenuated *B. abortus* 2308 strains described are those mutated for the gene encoding for phosphoglycerate kinase (Δ*pgk*), phosphoglucomutase (Δ*pgm*), zinc uptake system (Δ*znuA*) and glycosyltransferase *wbkA* (Δ*wbkA*) [16–19]. Besides these vaccines, promising results have been achieved using live attenuated S19 Δ*vjbR*::Kan in encapsulated or non-encapsulated alginate microspheres. This vaccine based on *vjb*R, a regulatory system of quorum sensing (QS) controlling the expression of *Brucella* virulence factors genes, is safer than S19 vaccine and it induces protection level of immunity in mice (Table 1) [20]. Although attenuated strains are usually more immunogenic vaccines, there is a risk that mutations can be reversed, thereby promoting infection and miscarriage in immunized animals [21]. These safety issues were absent in those immunized with subunit vaccines such as those containing periplasmic binding protein P39, periplasmic peptidyl cis–trans isomerase SurA, as well as diverse outer membrane proteins (Omps). These vaccines have shown to provide protection in mice (Table 2) [22–30]. The search for safe and effectiveness vaccine candidates has been extended to DNA vaccines as well; a strategy that continues to raise considerable

Table 1
Live attenuated vaccines based on genes deleted from *Brucella abortus*.

Target	Mice model	Route	Vaccine doses (CFU) × mice	Strain challenge/Doses (CFU) × mice	Challenge time	Protection levels[a]	Ref.
Δ*pgk*	BALB/c C57BL/6 129/Sv	i.p.	1×10^5	*B. abortus* 2308/1 x 10^6	2 wks	0.96* 1.36* 3.28*	[16]
Δ*pgm*	BALB/c	i.p.	1×10^7	*B. abortus* 2308/5 × 10^5	2 wks 4 wks	2.25* 1.93*	[17]
Δ*znuA*	BALB/c	i.p.	1×10^8 2 (1×10^8)	*B. abortus* 2308/5 × 10^4	4 wks	1.78* 1.67*	[18]
Δ*znuA* Δ*purE*	BALB/c	i.p.	2 (1×10^8)	*B. abortus* 2308/5 × 10^4	4 wks	0.79*	[18]
ΔwbkA	BALB/c	i.p.	1×10^6	*B. abortus* 2308/1 × 10^6	2 wks 4 wks	2.22* 1.76*	[19]
S19 Δ*vjbR*::Kan	BALB/c	i.p.	Encapsulated (1×10^5) Non-encapsulated (1×10^5)	*B. abortus* 2308/1 × 10^5	1 wk	3.86* 3.06*	[20]

Significant values (*); Intraperitoneal (i.p.) route; weeks (wks).
[a] Protection levels are measured by units of protection, represented by the difference between the mean of $\log_{10}$ CFU/spleen values of the negative control group and the mean of $\log_{10}$ CFU/spleen values of the immunized group.