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Review Article

Vaccines and Vaccine Candidates against Brucellosis

Noormohamad Mansoori[1], Mohammad Reza Pourmand[*1]

[1]Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran

*Corresponding author: Mohammad Reza Pourmand, Department of Pathobiology, School of Public Health, and Biotechnology Research Center,Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +98 21 88954910, E-mail: mpourmand@tums.ac.ir

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Abstract

Brucella is a facultative intracellular pathogen, and brucellosis is commonest zoonotic disease worldwide. *Brucella* species, isolated from domestic animals, are important pathogen for humans. Annually, more than 500,000 new cases of brucellosis are reported, and this figure is an underestimate due to extended under-reporting cases in several endemic countries. *Brucella* has a variety of virulence mechanisms that prevent detection and activation of innate immunity, but protection against intracellular pathogen is represented by cell-mediated immunity. As yet, much research has been performed to develop a safe *Brucella* vaccine to control the disease in human and animals. Despite the availability of several live attenuated vaccine for animals, currently, no effective human vaccine is available. Moreover, due to the potential use of *Brucella* in bioterrorism or biowarfare, development of an effective vaccine against brucellosis for human use is necessary. In this paper, we aimed to review and discuss the efforts of researchers to develop vaccines against Brucellosis.

Keywords:Brucella, Vaccine, Brucellosis, Zoonoses

1.Background

Brucella is a facultative intracellular pathogen causeing severe febrile illness in human, known as brucellosis (1). Brucellosis is the commonest zoonotic is the commonest zoonotic disease worldwide. annually more than 500,000 new cases of brucellosis are reported, and this figure is an underestimate due to extended under-reporting cases in several endemic countries (2). In many countries, Brucellosis is a serious public health problem, especially those around the Middle East, Mediterranean Sea, and South America as it is endemic in this areas (2,3).

Domestic and wild animals are the primary hosts for *Brucella*. Four species of *Brucella*, isolated from domestic animals, are important pathogens for humans. *Brucella* strains may either expressing smooth lipopolysaccharide (S-LPS) or rough lipopolysaccharide (R-LPS). This species are different in their pathogenicity and their host preference and include: *Brucella melitensis* (goatsandsheep), *B. abortus* (cattle), *B. suis* (swine), and *B. canis* (dogs), which cause abortion in ewes and goats, resulting in huge economic losses (4,5). Common routes of human infection are the ingestion of unpasteurized dairy products such as cheese or milk, contact with infected animals and inhalation of aerosols (6).

The disease has the tendency to affect several organs; then to cause chronic diseases such as arthritis, spondylitis, encephalitis, meningitis, orchitis, prostatitis, and endocarditis; and to persist for prolonged periods in the reticuloendothelial system of infected hosts (2,7). It has been reported that despite early diagnosis and treatment, chronic disease develops in 10-30% of the cases, and approximately 2% of untreated patients die from brucellosis (8,9). In the zoonotic hosts, *Brucella spp*. infect the reproductive tract, and can cause infertility or abortion (10,11).

Although brucellosis in most developed countries has been controlled in domestic animals, but it remains as a public and animal health problem in the developing countries. In order to prevent brucellosis, it is crucial that intervention strategies in animals and humans be improving. Over the last decades, most promising strategies have been conducted to control and eradicate the disease by developing safer and more effective vaccines for animals, but there is no licensed vaccine against human brucellosis yet (2,12).

Human vaccine would be applied to protect laboratory personnel, farmers, veterinarians, and general population living in brucellosis endemic areas (13). Moreover, *Brucella* bacteria can be used as a biological weapon due to their highly infectious nature and the potential use of the agents as a weapon for biowarfare or bioterrorism (14,15).

In this paper, we aimed to review and discuss the efforts of researchers to develop vaccines against Brucellosis.

2. Context

2.1. Immune system response

2.1.1.Innate immune system

Human immune system interaction with the *Brucella* is critical for the development of chronic disease or clearance of infection. Upon arrival, *Brucella* has a four-week latency period before becoming symptomatic (16,17). Detection of the bacteria inside of the body is mediated by the innate immune system with pattern recognition receptors, including the nucleotide binding and oligomerization domain-like receptors (NLRs), the toll-like receptors (TLR1, TLR2, TLR4, TLR5 and TLR6), and alternative complement pathway (18).

On the other hand, *Brucella* has a variety of virulence mechanisms that prevent detection and activation of innate immunity such as producing poorly recognizable Lipid A and flagellin, which lack the TLR5 agonist domain and molecules, which suppress innate immune signalling (19,20).

2.1.2.Cell-mediated immunity

Protection against intracellular pathogens represented by *Brucella*, depends on cell-mediated immunity involving activated macrophages, dendritic cells and, T-lymphocytes ($CD4^+$, $CD8^+$ and γδ T cells), whereas humoral immunity has a minor role in the control of infection (16,21).

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Activated macrophage and dendritic cells present *Brucella* immunogenic antigens to T-lymphocytes and induce differentiation of T-helper 1 (Th1). Then Th1 produces cytokines, and this mechanism has an essential role in clearance of infection (16,22). After entering the host, *Brucella* is taken up by macrophage and dendritic cells. *Brucella* can survive and replicate in this immune cells and evad adaptive immune system (21,23). Macrophages are key elements in the cellular immune response against intracellular bacteria like *Brucella* (17). Infected macrophages produce critical cytokines such as TNF-α, enhancing the bactericidal activity of phagocytes, and IL-12, driving the Th1 immune response. IL-12 induces the production of IFN-γ from $CD4^+$, $CD8^+$, and γδ T lymphocytes, resulting in the Th1 immune response and bactericidal activity of macrophages, which in turn lead to the prevention of the intracellular survival of *Brucella* (17,24). In addition, cytotoxic activity of the $CD8^+$ and $\gamma\delta$ T cells are significant for killing the infected macrophages (25). In the mouse model, IgG2a isotype antibody, opsonises the bacteria and facilitates effective phagocytosis (14,16).

Despite the mechanisms mentioned above, *Brucella* produces various virulence factors that modify those mechanisms, then it can survive and replicate for many years in hosts reticuloendothelial system, afterwards it can produce chronic and persistent infection (26).

2.2. Prevention against brucellosis

As mentioned, brucellosis is transmitted through contact with infected animals or dairy products, so the disease control programmes in countries with a high prevalence mainly have been focused on vaccination of animals with killed and live attenuated strains (14).

2.2.1. Killed vaccines

Over the years, a wide variety of killed vaccines such as *B. abortus* strain 45/20 and *B. melitensis* H38 have been developed to protect animals against brucellosis, but they have had limited success because they induce persistent antibody titters that can interfere with common serological tests; in addition, protection after challenge by this strains are insufficient (27).

2.2.2. Live attenuated vaccines

Live attenuated vaccines carry several advantages over killed vaccines. They are less expensive, as live vaccines are administered, the organism is allowed to replicate within the host and to permanently induce cellular immunity (27,28).

B. abortus strain19 (S19)

B. abortus S19 is live smooth weakened vaccine used to control of bovine brucellosis. This strain was isolated in the early twentieth century and naturally attenuated when an infectious culture of *B. abortus* was left at room temperature for a year (29). After vaccination, the animal is protected against brucellosis for many years, which can be drawn-out by revaccination (4,29). Despite of being attenuated, it is serologically indistinguishable from infectious strains due to its smooth nature. It induces strong antibody response against the LPS O-side chain (30).However, *B. abortu*s S19 is not completely avirulent, significant reduction in milk production and low rate of abortion in cows have been reported with this vaccine (31,32). Side effects associated with using live attenuated vaccines, prevent their widespread use in humans. In 1952, a derivative vaccine of S19, *B. abortus* VA 19, was used in the former USSR (Union of Soviet Socialist Republics) as a live vaccine for human but unfortunately some of those people were diseased with vaccine strain because vaccine was found to be insufficiently attenuated (33,34).

Some studies focused on the development of live attenuated *Brucella* vaccines for human by deletion of important genes required for survival. A *vjbR*, quorum sensing-related transcriptional regulator, knockout was generated in the S19 vaccine and investigated for its potential as a vaccine on mice model. To enhance vaccination efficacy, the live S19 Δ*vjbR* was encapsulated in alginate microspheres containing the parasite *Fasciola hepatica* nonimmunogenic eggshell precursor protein. Vaccine candidate was able to elicit an anti-*Brucella*-specific IgG response and to protect mice in challenge with virulent *B. abortus* strain 2308 (35).

However, *B. abortus* S19 is not entirely avirulent in humans, cases have been reported that in which veterinarians were infected with the vaccine strain (4).

B. abortus RB51

B. abortus RB51 is R-LPS mutant that is spontaneously attenuated and obtained by subculturing the virulent strain of *B. abortus* 2308 on medium containing penicillin and rifampicin (36). *B. abortus* RB51 is very stable and in some countries introduced instead of *B. abortus* S19 as a vaccine for cattle. RB51strain has low virulence and does not interfere with diagnostic serology tests but can induce very low level of abortion (32,37).

RB51 carries an IS771 insertion disrupting the *wbo*A gene, a gene encoding a glycosyl transferase that is responsible for O-side chain synthesis. It is thought that this strain has several unknown mutations (38). Vaccine strain RB51 can infect humans, but it is less virulent than S19 strain(39). On the other hand, it is resistant to rifampicin which is used in the groups of brucellosis patients who cannot be treated with routine drugs; for example, children, pregnant women, endocarditis and neurobrucellosis cases; therefore, it is considered unsuitable as human vaccine (32,34).

B. melitensis Rev.1

B. melitensis Rev.1 is live smooth attenuated vaccine used for immunization of sheep and goats. This strain was derived from a virulent strain, it is resistant to 2.5 $\mu g.mL^{-1}$ streptomycin and susceptible to 5 IU penicillin G. Having S-LPS phenotype, *B. melitensis* Rev.1 raises antibody response in serological tests, so that is difficult to distinguish between vaccinated and infected animals (37). *B. Melitensis* Rev.1 retains some virulence, leading to abortions in pregnant animals. In some cases, it has been reported that *B. melitensis* Rev.1 was excreted into the milk of animals, which enhance concerns about the vaccine strain infect other animal and humans (37,40). It has also been reported that veterinarians vaccinating sheep, were infected with this organism (33).

A number of genetically attenuated mutants have been developed, but their suitability for human use has not been evaluated (28,41). A genetically defined, attenuated *pur*EK mutant of *B. melitensis* strain 16M was developed, and it was found that it protects mice against disseminated infection of spleens and livers caused by virulent strain of *B. Melitensis* (42).

2.3. Subunit vaccines against Brucellosis

The live attenuated strains are good choice for vaccination due to induction high level of protection and being less expensive, but they produce some unpleasant side effects such as abortion in pregnant animals and infection in humans (4,29,36). Currently, there is no immunization strategy for human; thus, the development of an effective subunit vaccine is necessary.

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There have been many studies showing the protective effect of subunit vaccines, formulated either as DNA, purified proteins, and antigenic fractions[e.g. LPS, ribosomal L7/L12 protein, P39, 31 kDa outer membrane protein (Omp31) and Outer membrane vesicles (OMVs), as mentioned below], which are extracted from *Brucella* and tested as vaccine candidates on the animal models. Some of mentioned antigens tend to poorly stimulate immune system and require to coadministration with an adjuvant (43).

Brucella LPS has been shown to be 268-fold less pyrogenic than *Escherichia coli*, but it has been shown that adjuvant protein could enhance expression of costimulatory molecules on murine B cells and humoral responses to polysaccharide antigens as well(44).

Subcutaneous immunization of mice by *Brucella* LPS in conjugation with *Helicobacter pylori*'s recombinant CagA protein, significantly increased immune system response when challenged with *B. abortus* strain 544 intraperitoneally (44). Ribosomal vaccines have been proposed against different disease. In one model, immunization of mice with recombinant *B. abortus* ribosomal L7/L12 protein has been provide reduction of *Brucella* in spleen and to elicite some levels of protection (45). The 39-kDa protein (P39) is one of the most immunodominant proteins detected in *Brucella* infections; P39 elicits production of IFN- γ from mononuclear cell (45).

The *Brucella spp*. major outer membrane proteins (OMPs) were identified and characterised as immunogenic and protective antigens. Mice immunized with recombinant Omp31 (rOmp31) or rOmp31 plus R-LPS, provide the best protection level against *Brucella ovis* (46).

OMVs are bilayer membrane vesicles haveing the outer membrane and periplasmic components, released during the growth of *Brucella* by a mechanism involving cell wall turnover (47). Proteins present in OMVs from *B. melitensis* are SOD, co-chaperonin GroES, Omp19, Omp25, Omp31, bp26, and Omp16. A group of researchers purified OMVs from both *B. melitensis* strain 16M (smooth strain) and VTRM1 (rough strain) and used them for mice immunization. OMVs from a rough *B. melitensis* VTRM1 induced significantly higher expression of IL-12, TNF-α and IFN-γ genes. Mice immunized intramuscularly with rough OMVs shown protection against challenge with virulent *B. melitensis* strain 16M. It is possible that the absence of the O-side chain inpurified OMVs from rough strain, could allow to higher exposure of bacterial surface molecules, such as OMPs, to immune receptors (48).

In addition to fractions mentioned above, number of vaccine candidates such as; Omp25 (49), rOmp28 (50), rOmp31 (51), rOmp16 and rOmp19 (52), recombinant S-adenosyl-lhomocysteine hydrolase (53), rDnaK and rSurA (54), SodCprotein (55), *sod*C gene(56), Lumazine synthase (57), Bp26(58),Heat shock protein (59), recombinant superoxide dismutase (rSOD) proteins (60) were identified and examined by different research teams.

3. Conclusion

Brucellosis is a highly contagious zoonotic disease that is an important economic and sanitary problem affecting millions of people worldwide. *Brucella* was classified as biosafety Level 3 agent and the Centers for Disease Control and Prevention designated it as Class B bioterrorist threat agent because of being potential abiological weapon (61,62).

The best effective alternative approach to control animal brucellosis is the use of vaccination programs (63). S19, Rev.1and RB51 vaccines have been used successfully in eradication and control programs against animal brucellosis in many countries (64). Side effects associated with live attenuated strains of *Brucella*, prevent widespread use of this type of vaccines in human. Therefore, they are considered unsuitable as human vaccine.

Subunit vaccines are good choice for vaccination due to induction good level of protection in animal model. In compare to other subunit vaccines, OMPs and OMVs are characterised as immunogenic and protective antigens and therefore considered suitable candidates as human vaccine. OMVs have considerable advantages; they are multicomplex antigens that strongly activate the host innate and acquired immune response pathways and are less expensive in terms of purification. However, further research's are required to fully evaluate the benefits and risks of Subunit vaccines.

At present, there is no licensed vaccine for prevention of human brucellosis, and current animal vaccines are both virulent in humans and lack clinical efficiency (37).

Vaccination of human beings could be thought as a different attitude towards the prevention of naturally acquired disease and as a defence strategy against bioterrorism or biowarfare as well (65). Subunit vaccines could avoid the drawbacks of live weakened vaccines because of being avirulent, , nonviable, and we can select and provide good protective immunodominant antigens, different from those used for immunodiagnosis

Conflict of Interests

Authors declare they have no conflict of interests.

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Authors' Contribution

All authors contribute in writing different parts of this manuscript.

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