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Immunogenic and protective antigens of *Brucella* as vaccine candidates

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ABSTRACT

Brucella is an intracellular pathogen that causes abortion in domestic animals and undulant fever in humans. Due to the lack of a human vaccine against brucellosis, animal vaccines play an important role in the management of animal and human brucellosis for decades. Strain 19, RB51 and Rev1 are the approved *Brucella* spp. vaccine strains that are most commonly used to protect livestock against infection and abortion. However, due to some disadvantages of these vaccines, numerous studies have been conducted for the development of effective vaccines that could also be used in other susceptible animals. In this review, we compare different aspects of immunogenic antigens that have been a candidate for the brucellosis vaccine.

1. Introduction

Brucellosis remains an important health problem in the Iran and other parts of the world. Brucellosis is a zoonosis affecting approximately 500,000 people annually around the world [1]. To date, ten *Brucella* species have been characterized including *Brucella melitensis, Brucella abortus, Brucella suis, Brucella canis, Brucella neotomae, Brucella ovis, Brucella microti, Brucella inopinata, Brucella ceti,* and *Brucella pinnipedialis,* all of which except for *B. neotomae* and *B. ovis* are human pathogens [2,3]. The most frequent symptoms of human brucellosis are fever, chills or shaking rigors, malaise, generalized aches, pain all over the body, joint and low back pain, headaches, anorexia, easy tiredness, and general weakness. The disease remains endemic in many regions of the world including Africa, Latin America, Middle East, Asia, and the Mediterranean basin [4,5]. The World Health Organization(WHO) consider brucellosis as one of the seven neglected zoonoses, a group of diseases that contribute to the perpetuation of poverty [6]. Some developed countries have eradicated brucellosis in domestic animals by sustained S19, RB51, 45/20 vaccine, SR82 vaccine strains and Rev1 vaccination of young animals in combination with test and slaughter [7], whereas in most developing countries, brucellosis causes a significant economic loss due to abortion, and infertility in pregnant livestock [8,9]. Nowadays, the current vaccines including S19, RB51, and Rev1 widely are successfully used to prevent livestock brucellosis worldwide. In the last decades, due to some drawbacks shown by current vaccines, numerous studies have been performed to design and develop safer and more protective *Brucella* vaccines for animals and human [4]. Various antigenic fractions have been extracted from *Brucella* and examined as vaccine candidates in the mouse model, and some of these have revealed protective efficacy. The new vaccines include subunit vaccines, DNA vaccines, recombinant proteins, outer membrane vesicles and vector vaccines [10]. Since there is no safe human vaccine, livestock vaccination is also an effective method to prevent human infections. The purpose of this review is to provide an overview of the properties of *Brucella* immunogenic antigens and its role in humoral and cellular immunity.

2. Cell wall structure of *Brucella*

The first step for design an effective and safe brucellosis vaccine is identification of antigenic determinants and new understanding of the molecular interaction of *Brucella* spp. and the host [11]. Similar to other Gram-negative bacteria, the *Brucella* cell wall consists of inner and outer membranes (OM) (Fig. 1). In addition, a peptidoglycan layer strongly associated with the outer membrane consisting of at least 75 proteins with various outer membrane proteins (Omps) is reported [12]. The Lipopolysaccharide (LPS) molecule constitutes a major component of *Brucella* outer membrane. The LPS is composed of three domains including lipidA, core, and O antigen [13]. The structure of lipid A in *Brucella* shows several differences to other Gram-negative enterobacteria which are important for evading the host immune system during the early stages of infection [14]. Based on the presence of diaminoglucose and glucosamine, there are two groups of core lipid A molecule. The fatty acid chains contain long saturated molecules

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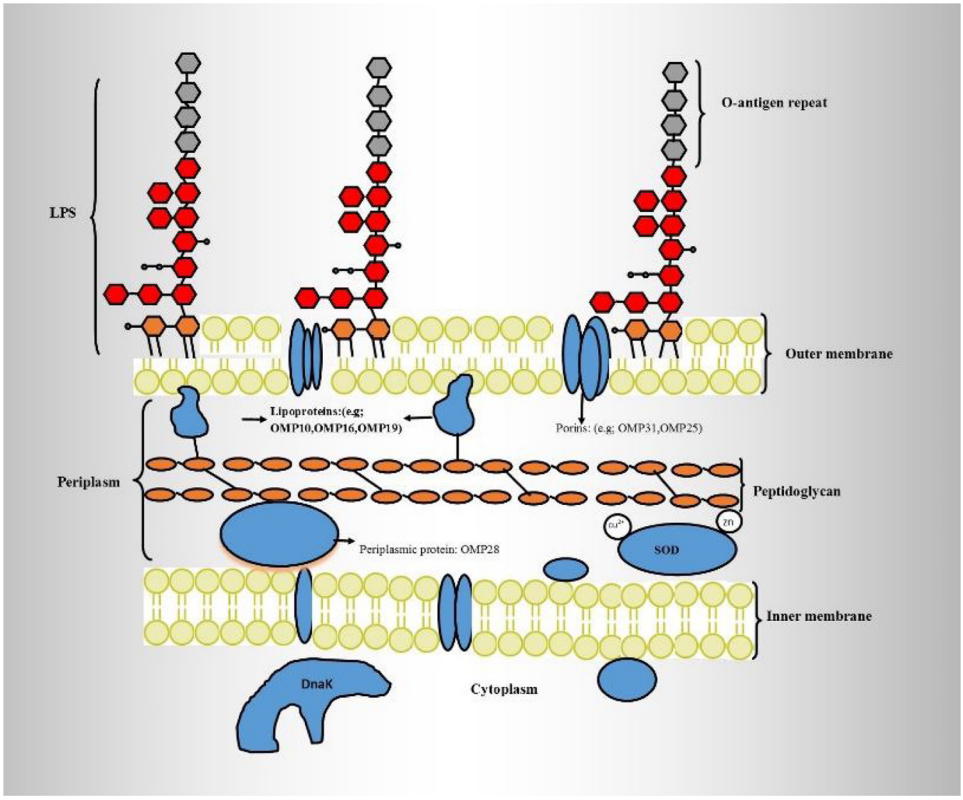

Fig. 1. Immunogenic antigens of *Brucella*: Lipopolysaccharide (LPS), Outer membrane proteins (OMPs), Heat shock proteins (e.g; DnaK), Periplasmic proteins (e.g; OMP28, SOD).

(C16:0 to C18:0) and a very-long-chain molecule, 27-hydroxy-octacosanoate (27-OH-C28:0) [15]. Also, *Brucella* lacks neutral sugars, ethanolamine, and phosphate [13]. The structure of the core region is also different from the enterobacterial core. In *Brucella*, the major components of the core region include glucose, mannose, quinovosamine (2-amino-2,6-dideoxy D-glucose), small amounts of other sugars and nonsubstituted KDO that possess different from the enterobacterial core [15]. Another major property is the absence of the heptose region [16]. Besides, *Brucella* spp. produces proteins (e.g. Vi antigen), which make a capsule around the LPS and limit its contact with TLR4 receptors [17,18]. Smooth strains of *Brucella* generally contain LPS that is modified with an O-antigen and invade the host cells more efficiently than rough strains which lack the O-antigen [19]. Thus, the LPS O chains plays a key role in virulence. Also, the LPS O chains avoid the immune response activation by inhibition of cellular apoptosis [20]. Generally, LPS possesses unusual immunological properties such as low toxicity which is considered as a main virulence factor of *Brucella* [17]. The outer membrane also contains a set of proteins involved in physiologic mechanisms and pathogenesis. The *Brucella* outer membrane proteins as immunogenic antigens have been proposed in *Brucella* vaccine candidate [21,22]. In the structure of OMPs, the NH 2-terminal signal peptides contain a tetrapeptide, showing a high degree of similarity to the consensus sequence required for the modification and procession of bacterial lipoprotein precursors: the lipobox Leu-(Ala or Ser)-(Gly or Ala)-Cys at the 23 to 11 positions [23,24]. The first lipoprotein described for *Brucella* spp. is the equivalent to the peptidoglycan-linked Braun lipoprotein (murein lipoprotein). This polypeptide contains fatty acids, both ester and amide linked. It is partially exposed on the surface of smooth *B. abortus* and *B. melitensis* [23]. The usage and development of specific protein antigens appears to be a possible strategy for decreasing cross-reaction with other microorganisms in the diagnosis and prevention of brucellosis [25].

3. Immune response against *Brucella*

Generally, the host immune response against intracellular pathogens such as *Brucella* is divided into innate and adaptive immunity [26]. The innate immune system includes anatomical barriers, complement system and cellular populations, such as phagocytes, and innate lymphocyte subsets (natural killer and $\gamma\delta$ T cells). The adaptive immunity, also known as the acquired immunity is composed of T lymphocytes, and B lymphocytes [27,28].

3.1. B cells- mediated immunity

The role of humoral arm in protection against *Brucella* is less defined than that of the cellular arm [11]. The main role of B cells includes antibody production and cytokine secretion. Under certain circumstances, B cells act as antigen presenting cells for CD4+ helper T cells or CD8 + T cells. Anti-*Brucella* antibodies are proteins that cause agglutination, complement fixation, and precipitation when reacting with their homologous antigens derived from *Brucella* [29]. Many protection studies suggest the significance of humoral immunity in animal models of brucellosis. For example, passive transfer of the sera containing anti-LPS antibodies to mice could protect against challenges with virulent *B. abortus* [30,31]. Most of the reactive antibodies were induced by *Brucella* LPS rather than through cytoplasmic proteins [29]. However, other than LPS, protein antigens (e.g. OMPs and stress-response proteins GroEL, DnaK and HtrA) are also identified by the humoral response [32]. Goenka et al. mentioned that both BALB/c and C57BL/6 B cell deficient mice clear *Brucella* more rapidly than the wild type (WT) mice through an antibody independent mechanis [33]. Furthermore, B cell deficient BALB/c and C57BL/6 mice produced significantly less TGF-β and IL-10, respectively three weeks post infection, suggesting that B cells enhance susceptibility to *Brucella* infection through both IL-10 and TGF-β dependent mechanisms [33,34]. However, it is generally

accepted that the cellular response is also required for complete protection [11,35].

3.2. T cells- mediated immunity

Although both innate and adaptive immune responses are involved in response to *Brucella* infection, a cell-mediated immune response particularly macrophages and T-cells have been demonstrated to be critical for protection against *Brucella* and other intracellular bacterial pathogens [28,36,37]. Helper T-cell mediated protection is primarily associated with a Th1 T-cell response and persistence (i.e. chronic brucellosis) with a Th2 response [11]. CD4+ cells play a central role in coordinating and intensifying the adaptive immune response by separating themselves into functional subsets, such as the Th1 type, a subset that is associated with production of interferon-γ (IFNγ); IL-2; and tumor necrosis factor-α (TNF-α) [20]. CD8+ cells are considered to be important for protective immunity because of their ability to lyse or kill the infected cells, thereby releasing *Brucella* from intracellular hiding and exposing to extracellular bactericidal mechanisms [11]. Cytokines released by CD4+ and CD8+ cells may activate macrophages and dendritic cells, thereby increasing their bactericidal activity against *Brucella* [38]. Moreover, previous studies have suggested that mutations in genes encoding the cytokines IFN-γ, IL-6, TNF-α, and IL-10 contribute to increased susceptibility to human brucellosis [38,39]. Antigen-presenting cells also present co-stimulatory molecules on their surface that are important for stimulation/activation of T-cells [10]. In addition to the differences in immunization regimens, various factors such as protein length and sequence, and the route of vaccination may greatly influence the types of T cell responses induced [40].

3.3. Brucella evasion of immune response

Brucella can efficiently colonize mononuclear phagocytes and replicate to high numbers in the liver and spleen. Once the bacteria are phagocytosed by the macrophages, dendritic cells, and other antigen presenting cells (APCs), approximately 10% of the bacteria resist digestion within these cells [41,42]; however, failure to lyse the *Brucella* within phagosomes leads to formation of *Brucella*-containing vacuoles and thus replication in phagosomes [43]. Although, *Brucella* lack well-known bacterial virulence factors such as cytolysins, capsules, exotoxins, secreted proteases, fimbriae, phage-encoded toxins, and virulence plasmids [32,44], many *Brucella* virulent factors, such as LPS, type IV secretion system, and the BvrR/BvrS two-component system are critical in the intracellular survival and replication. In this regard, live or killed *Brucella* or its outer membrane fragments containing significant quantities of lipoproteins, ornithine containing lipids, Br-LPS, and related polysaccharides can induce weakly innate immunity [41,45]. In addition, *Brucella* can evade from the host immune response through internalization in a lipid-raft containing vacuole that acquires EEA-1 and Rab5 antigens [19], and modifications of virulence factors such as lipopolysaccharide (LPS) and flagellin [38]. The flagellin protein in *Brucella* spp. cannot induce TLR5 receptors, and is another way for the bacteria to evade the immune system during early infection [17,46]. As noted above, *B. abortus* and *B. melitensis* that have smooth LPS are able to survive intracellularly [47]. Also, *Brucella* species inhibit the apoptosis of the host cells to benefit their own intracellular survival and replication [48]. Both attenuated and virulent strains of *B. abortus* and attenuated *B. melitensis* are eliminated by PMN, while virulent *B. melitensis* is more resistant to intracellular death, a feature which probably sttributes its higher virulence to both man and animals [20,45].

4. Vaccination with animal vaccines

Vaccination is an important inexpensive tool used in the control, management, and elimination of brucellosis [49]. Nevertheless, vaccination alone is not sufficient for controlling and preventing brucellosis. It should be associated with test and slaughter policies, pasteurization dairy products, surveillance and hygiene procedures [49–51] In addition, due to the lack of human brucellosis vaccine, the most effective strategy to control human brucellosis is to control brucellosis in animal populations. During the last decades, the development of an effective vaccine for brucellosis control/eradication has been a challenge for researchers [42,49]. According to vaccinology studies, a safe and protective vaccine against brucellosis should: (a) have a strong induction Th1 response; (b) not interference with the serological tests; (c) not cause disease or persistent infection in vaccinated animals nor be pathogenic for humans; (d) not cause abortion in pregnant animals (e) not lead to seroconversion on revaccination; (f) be stable and not revert virulence in vivo nor in vitro; and (g) be inexpensive, easy to produce and to administer [49]. Although there is not still an ideal vaccine for protection against brucellosis, vaccination with available vaccine strains (eg; S19, Rev1, Rb51) remains the most effective method for the prevention and control of brucellosis in domestic livestock, being a critical component of most brucellosis control and eradication programs throughout the world [50]. Immunization with *B. abortus* S19 and RB51 for cattle and *B. melitensis* Rev1 for sheep and goats remains the most effective vaccines for more than 60 years (Table1). There are no vaccines against brucellosis in camels, water buffaloes, yacks, swine and other domestic or semi-domestic livestock [7]. However, classic vaccines can lead to abortion, interfere with serological tests and be infective for the human. For solving these drawbacks, there have been a large number of attempts to develop better vaccines. (DIVA, DNA, rOMP, OMV vaccines)

5. Vaccine candidate antigens of *Brucella*

5.1. LPS

Brucella LPS has been considered the most important antigen during the immune response in brucellosis. The LPS O-polysaccharide severely impacts the outcome of infection with *B. abortus, B. melitensis* and *B. suis* [19]. LPS elicits long-lasting serological response in both vaccinated and infected animals. The lipopolysaccharide (LPS) of smooth *Brucella* species is by far the strongest antigen when compared to other antigenic molecules. *Brucella* strains found in human infections are generally characterized by a smooth LPS phenotype [19,52]. *Brucella* LPS has been shown to diminish anti-bacterial responses by inhibiting complement and anti-bacterial-peptide attacks and by preventing the synthesis of immune mediators [17]. The studies of Ferguson et al. demonstrated that infection with a *Brucella* attenuated containing *bacA* mutation, bac a is a cytoplasmic protein that is involved in the lipid A biosynthesis, can result in increased host pathology without an increase in bacterial load, crucial considerations for vaccine candidates [53,54]. Brucella LPS interferes with the MHCII-dependent antigen processing machinery of macrophages by clustering with MHCII molecules, probably resulting in a downregulated T cell activation [55]. The current diagnostic tests such as standard agglutination test, rose bangle test, and complement fixation tests are mainly based on the detection of antibodies directed against the lipopolysaccharide (LPS) portion of the cell membrane. Therefore, it is difficult to differentiate between vaccinated and infected animals using LPS-based serological tests [56]. In addition, tests based on anti-LPS antibodies give false positives because of cross-reactivity with other Gram-negative bacteria like *Yersinia enterocolitica* O:9, *Salmonella* species, and *Escherichia coli* [16]. Therefore, this and other drawbacks of anti-LPS antibodies have led to an increased interest in the detection of antibodies to alternative antigens, mainly outer membrane proteins and cytoplasmic proteins.

5.2. Outer membrane proteins

In the early 1980$_s$, OMPs were initially characterized as protective

Table 1
The current available brucellosis vaccines.

Vaccines	Date of developing	Strain of *Brucella*	Humoral immunity	Cellular immunity	Abortion in pregnant animals	Pathogenic For Human	Interfere to serologic tests	More details
S19	1923	*B. abortus* strain 19	+	+	+	+	+	This vaccine carries no resistance to any of the antibiotics used to treat human brucellosis.
RB51	1982	*B. abortus* strain RB51	+ (12 week after vaccination)	+	+	+	–	Resistant to rifampicin
					–			
45/20	1922	Heat-killed *B. abortus* biovar1 strain45/20	+	+	–	–	+	Used in some European countries reversion to smooth strain coadminstrated with adjutant
			–	–			–	
SR82	1974	*B. abortus* biovar 6	+	+	+	Low pathogenicity in humans	Weak responses on agglutination tests	This strain is still massively used in the Russian Federation, Azerbaijan, Tajikistan and other countries in the region
					–			
Rev1	1957	WT *B. melitensis 6056*	+	+	+	+	+	Resistant to streptomycin

antigens. Outer membrane proteins (OMPs) are located on the bacterial surface and are in direct interaction with immune system cells (Fig. 1) [23,56]. In addition, expression of these proteins is often regulated by environmental signals; these proteins of bacteria play important roles in direct interaction with host cells. Furthermore, these proteins are involved in resistance to bactericidal cationic peptides and polycations, permeability to hydrophobic agents, resistance to divalent cation chelators, and poor activation of bactericidal mechanisms by lipopolysaccharide [57]. In *Brucella*, the outer membrane protein genes are located on the chromosome I [58]. The *Brucella* OMPs are divided based on their molecular mass into three major groups: group 1 is a 94 kDa Omp, group 2 antigens of approximately 41–43 kDa (eg. Omp2a, omp2b), and group 3 antigens of 30 kDa (omp25, omp31). These OMPs are also present in the other non-human species, but with a quantitative difference [59]. In all *Brucella* strains, the group 2 proteins by far are the most abundant. The group 3 proteins were assumed to be analogs of OMP A, but the sequence data have not confirmed this. All these OMPs are associated with virulence [58]. The use of highly specific recombinant antigens, particularly the highly immunogenic OMPs, in an enzyme-linked immunosorbent assay (ELISA) format offers high sensitivity and specificity in addition to a field-usable format [21,60]. A range of bacterial OMPs has been associated with the immunogenicity of *Brucella* spp. including OMP10, OMP16, OMP19, OMP25, OMP28, and OMP31 (Table 2). The benefit of specific protein antigens appears to be a possible strategy for minimal or no cross-reaction with other microorganisms in the diagnosis of brucellosis [61,62]. Besides, combinations of antigens can induce better protection than univalent vaccines, especially when different antigens are expressed at different phases of the pathogen's life cycle [62]. However, some OMPs, such as the 18-kDa OMP, can induce appropriate immunity but may not play a crucial role in host-acquired protective immune mechanisms to brucellosis [63].

5.2.1. Omp10

The sequencing analysis suggests that Omp10, Omp16, and Omp19 are lipoproteins [63,64]. Omp10 and Omp19 share antigenic determinants with the bacteria of the family Rhizobiaceae. Overall, bacterial lipoproteins possess various structures and functionality, ranging from bacterial physiology to pathogenesis mechanisms. There are about 80 genes encoding probable proteins in the *B. abortus* genome. In a study by Tibor et al. using a murine model, it was revealed that deletion of the *omp10* gene from *B. abortus* 544 significantly reduced bacterial virulence [57]. Another study by Simborio and colleagues has demonstrated that immunogenicity affects combined rOmps by ELISA, utilizing both standard tube agglutination test (TAT)-positive and negative serum samples from Korean native cattle. Thus, combined recombinant *B. abortus* outer membrane proteins (rOmps) and individual rOmps can be used in the serodiagnosis and vaccine production [65]. Unlike in vitro investigation, some studies indicated that these recombinant OMPs did not induced antibody responses in the cattle naturally infected by *B. abortus* [23,61].

5.2.2. OMP16

OMP16 (16 kDa) shows a significant similarity to the peptidoglycan-associated lipoproteins (PALs) of many Gram-negative bacteria [23,57]. Omp16 of *Brucella* spp. is a lipoprotein and is expressed in all biovars of *B. abortus*, *B. melitensis*, *B. suis* [66,67]. Karina and coworkers have previously reported that *B. abortus* Omp16 confers protection against a challenge with virulent *B. abortus* when administered i.p. with systemic adjuvants (IFA or aluminum hydroxide) or orally with a mucosal adjuvant (cholera toxin) [24,68]. Some studies suggest that Omp16 acts as an adjuvant by activating the CD8α+ subset of dendritic cells both in vivo and in vitro in C57BL/6 mice [34]. According to Pasquevich et al, unlipidated OMP16, as a new bacterial PAMPs, is able to activate the dendritic cells and monocytes, induces a Th1 immune response, and is a very promising self-adjuvanting vaccine against systemic, as well as

Table 2
Vaccine candidate antigens in *Brucella*.

Antigens	Species	Subcellular location	Induction of Antibody	Cellular immunity	More details Ref: Uniprot KB
LPS	*Brucella. spp*	Outer membrane	+ + +	+ + +	–
OMP10	*Brucella (abortus, melitensis, suis)*	Outer membrane	+	+	Elicits an immune response in *B.melitensis*-infected sheep but not in *B.abortus*-infected cattle.
OMP16	*Brucella (abortus, melitensis, suis)*	Outer membrane	+	+	Part of the Tol-Pal system, which plays a role in outer membrane invagination during cell division and is important for maintaining outer membrane integrity.
OMP19	*Brucella (abortus, melitensis, suis)*	Outer membrane	+	+	Elicits an immune response in humans, mice, sheep and goats infected with *B.melitensis* or *B.abortus*, but not in *B.abortus*-infected cattle
OMP25	*Brucella.* Spp.	Outer membrane	+ +	+ +	25 kDa outer-membrane immunogenic protein
OMP28 (BP26)	*Brucella (melitensis, suis)*	Periplasmic space	+	+	26 kDa periplasmic immunogenic protein
OMP31	*Brucella* spp. except of *B. abortus*	Outer membrane	+ +	+ +	Major outer membrane protein associated with peptidoglycans May function as a porin.
HSPs (e.g;DnaK)	*B. melitensis, B. ovis B. suis, B. abortus*	Cytoplasm	+	+	Acts as a chaperone.
SOD	*Brucella* spp.	Periplasmic space	+	+	Destroys radicals which are normally produced within the cells and which are toxic to biological systems.

orally acquired brucellosis [24]. In general, vaccination with Omp16 induces a strong Th1, humoral response, and mucosal protection against multiple *Brucella* spp [24,69].

5.2.3. OMP19

B. abortus unlipidated 19 kDa outer membrane protein (UOmp19) is a promising candidate for a subunit vaccine against brucellosis [70]. The UOmp19 is associated with virulence and is expressed broadly within the *Brucella* genus [68]. Fiorentino et al. have demonstrated that expression of OMP19 is important for the induction of a protective response by the vaccine strain *B. abortus* S19 since the abrogation of its gene in this strain leads to the loss of its protective ability in heifers [71]. Another study showed that plant-expressed U-Omp19 can induce significant protection when administered to BALB/c mice by the oral route as purified proteins and within the crude leaf material of transgenic tobacco plants. The protection level gained was equivalent to those elicited by the oral administration of S19 or RB51 [72]. In previous studies, U-Omp19 elicited a T helper 1 response and oral protection with cholera toxin (CT) as a mucosal adjuvant against *B. abortus* infection. Furthermore, intera gastric (i.p) immunization of mice with rOmp19 has been suggested to induce a Th1 and Th17 response while intraperitoneal (i.p.) vaccination only induced a Th1 response [68,70,72].

5.2.4. OMP25

Molecular analyses indicate that the Omp25 gene is highly conserved among the *Brucella* spp. [73,74]. Sequencing comparative analysis has shown that the *Brucella* Omp25 protein displays about 40% identity with several hypothetical OMP from other members of the Rhizobiaceae group of bacteria [22]. Also, it exhibits some sequence homology and antigenic relationship with *Escherichia coli* OmpA and cadF from *Campylobacter jejuni* [21]. In previous studies, it has been demonstrated that Omp25 was involved in the pathogenesis process, and deletion of gene Omp25 resulted in a decrease in the bacterial virulence in animal models [75]. Outer membrane protein 25 from *B. suis* has been reported to inhibit the production of TNF-α by human macrophages. Furthermore, *B. suis*-infected macrophages secreted significantly lower amounts of TNF-α than did macrophages infected with a ΔOMP25 *B. suis* mutant [21,48]. Several reports have shown that administration of Omp25 DNA vaccine or recombinant Omp25 is protective against the virulent *B. melitensis* or *B. abortus* challenge in mice which makes Omp25 a viable vaccine target [74,76]. Overall, immunization with Omp25 inconsistently results in TNF-α production which is contradictory to evidence that Omp25 of *B. suis* inhibits TNF-α production in human macrophages and allows secretion of periplasmic proteins in acidic medium [34,77–79]. Omp25 is also known to bind the outer membrane to the peptidoglycan layer, which suggests a role in the membrane stability, persistence, TNF-α inhibition, and adaptation to the acidic environment [78,80]. On the other hand, Omp25 has also been reported to be involved in the virulence of *B. melitensis*, *B. abortus*, and *B. ovis* [75]. *Brucella* species lacking Omp25 have been shown to be attenuated in mice as well as cattle [80]. The study carried out by Commander et al. indicated that vaccination of mice with DNA omp25 resulted in the significant production of IFN-γ and TNF-α, but not IL-4 or IL-10, indicating that a memory Th1 immune response was induced [76].

5.2.5. OMP31

OMP31, which is 34% homologous with *Brucella* OMP25 was reported to be an important immunogenic major outer membrane protein and antigen, which is present in all *Brucella* species, with the exception of *B. abortus* [81]. Among *Brucella* outer membrane proteins, members of the *Brucella* OMP25/OMP31 family show strong antigenicity and can be used to diagnose brucellosis and candidate vaccine [82]. Some of the studies suggested that a high percentage of *B. canis*-infected dogs developed detectable levels of specific antibodies against recombinant

Omp31 (rOmp31) from *B. melitensis* [83]. As a result, recombinant Omp31 could be a useful candidate for the development of a subunit vaccine against *B. canis*. Besides, the nucleotide sequence of this protein is quite conserved in the genus, and the *B. canis* Omp31 sequence displays only one nucleotide substitution in comparison with *B. melitensis* Omp31 [84,85]. Omp31 plays an important role in cellular and humoral immune protective responses against Brucella infection [48,86]. Also, in 2013, Snyder et al. mentioned that OMP31 can not only play a role in protective humoral immunity but also induce specific cellular immunity. The recombinant 31 kDa outer membrane protein (Omp31) in aluminum hydroxide or incomplete Freud adjuvant (IFA) induced significant levels of protection against *B. melitensis* challenge in the mouse model [4,87].

5.2.6. Protein BP26

This protein, also known as Omp28, is located in the periplasmic space of *Brucella* and has been identified as an important diagnostic antigen in brucellosis [88]. BP26 is highly conserved among *B. abortus, B. suis, B. ovis, B. canis, B. neotomae*, and *B. melitensis* and was sensitive and specific for the diagnosis of *Brucella* infection in animals by ELISA [88,89]. Protein BP26 can be used not only in diagnosis of brucellosis but also as a protective antigen for control and prevention of brucellosis. Jeong Ju Lim et al. have revealed that rOmp28 of *B. abortus* is a good vaccine candidate for protection against *B. abortus* infection [90]. In general, BP26 is capable of inducing both humoral and cellular responses and is protective as a subunit or DNA vaccine [34,91,92]. In 2004, Cloeckaert and coworkers showed that the lack of gene bp26 in strain Rev1 still protects against *B. melitensis* in sheep or *B. ovis* in rams. Besides, in an evaluation of humoral and cellular immune response to the periplasmic protein BP26 and the outer membrane protein OMP31 in M5-90 vaccinated Chinese merino and Kazak sheep, Wang et al. showed that antibody reactivity was strong to the native membrane protein extract (NMP) but weak to both rBP26 and rOMP31 in these animals [92]. Also, IFN-γ response induced by reactive peptides was overall relatively weak. In contrast, Clapp et al. have shown that DNA vaccination of bison with pcDNA3.1-bp26 and pcDNA3.1-TF result in developing enhanced antibody, proliferative T cell, and interferon-gamma (IFN-γ) responses [93]. Generally, the results of previous studies contribute to designing and developing a more effective vaccine for use in animals or humans.

5.3. Cu/Zn superoxide dismutase(SOD)

This periplasmic protein (18.5–20 kDa) is expressed in all of *Brucella* spp. and causes a good protection against *Brucella* infection in the murine model [94]. In *Brucella*, like other aerobic bacteria, these metaloenzymes catalyze the dismutation of superoxide (O2) to hydrogen peroxide (H2O2), which can then be further detoxified through the action of catalases and peroxidases [95]. Tatum et al. showed the deletion of Cu-Zn sod from *B. abortus* caused decreased survival during the initial phase of infection and reduced spleen weights in mice [96]. In contrast, three peptides were evaluated for determination of immunity to Cu-Zn superoxide dismutase from *B. abortus* in mice; however, only one was able to induce significant protection against *B. abortus* 2308 [97]. Furthermore, Stevens et al. showed that following vaccination of cattle and mice with S19 or RB51, no immune responses were induced in superoxide dismutase and synthetic peptides of superoxide dismutase [98]. However, immunization with SOD has been found to have more efficient protection against brucellosis when administered in the form of either recombinant peptides, DNA vaccines, whole recombinant proteins, overexpression mutants, or through co-immunization with other fractions [34]. Studies have shown that a nasal vaccination with SOD-DNA produces both IgG2a and IgA antibodies as well as CD8+ splenocytes and systemic protection against *B. abortus* in mice [99]. SOD DNA vaccinated calves showed significant IgG1 and IgG2a titers and IFN-γ+ peripheral blood monocyte (PBMC) but no detectable TNF-α or IL-4 on stimulation with rSOD or crude *Brucella* proteins [100].

5.4. Heat shock proteins (HSPs)

Bacterial heat shock proteins (HSPs) not only contribute to intracellular survival against harsh environmental conditions, but also are immunogenic, and they are immunodominant targets of both the humoral and cellular immune responses [101,102]. The expression of GroEL, GroES and HtrA, as well-known members of HSP family, may be effective in resistance to bactericidal mechanisms of macrophages. More recently, a study was performed by Hop et al., showing that GroEL in combination with OMP25, OMP31 can have a positive impact on cell-mediated immunity [103]. However, some studies have demonstrated that *Brucella* GroEL could not be a protective antigen against brucellosis in mice [102,104]. The molecular chaperone DnaK, the member of hsp70 family, acts together with DnaJ in a variety of cellular mechanisms [105,106]. Expression of DnaK in *B. suis* was induced by heat-shock conditions, and acidic condition inside the phagosomes for resisting intracellular killing [107]. Pervious investigations have indicated that the induction of DnaK is an essential event in resistance to antimicrobial defense mechanisms of the macrophage in the host [106–108]. On the other hand, vaccination with rDnaK induced a cytotoxic T lymphocyte and Th1 type immune response [105,109]. Besides, Ghasemi et al. demonstrated that immunized serum contains antibodies against recombinant DnaK (rDnaK) by Western blot and ELISA techniques [110]. Thus, these periplasmic proteins can be used as *Brucella* vaccine.

6. Conclusion

As noted above, for effectively control of brucellosis, an ideal vaccine against brucellosis must induce a strong Th1 immune response, including both CD4+ and CD8+ cells. Selecting the ideal antigens is the main step in vaccine design. Depending on the desired response, the antigenic proteins should contain specific epitopes to B-cell receptors and can be recognized by the T-cell receptor in a complex with MHC molecules. For this purpose, different research groups have examined various subunit fractions from *Brucella* as recombinant proteins vaccines in mouse and animal models. Although during recent decades numerous studies were performed related to immunogenic and protective *Brucella* antigens, However, the most immunogenic antigens cannot be still, considered as effective vaccine against brucellosis. To design and develop an effective vaccine against *Brucella*, there is a need to further understand the mechanisms of *Brucella* pathogenesis, the bacterial cell wall, and the optimal methods for antigen purification, selection of animal model, as well as effective adjuvants. In addition, the host genetic differences and variety of immune response among animals and humans should be considered. Hence, in the past decade the mechanisms of *Brucella* pathogenesis, and host protective immunity against *Brucella* infections have widely been investigated using systems biology, and bioinformatics approaches for design effective brucellosis vaccines. For instance, bioinformatic technologies were used to identify the regions within these protective antigens most probably to elicit a protective immune response. In this regard, determination of the epitope of a protein is a very complex process and antigenic epitopes may depend on protein folding, epitope length, targetingthe N-terminus or C-terminus, continuous and discontinuous epitopes as well as on primary structure. These high throughput technologies are able to aid in facilitating vaccine development and predicting basic molecular mechanisms of host–pathogen interactions. Therefore, more studies recommended to be conducted in order to identify new details on *Brucella* pathogenesis at the molecular level, which will create new strategies for controlling and preventing brucellosis.

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