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ORIGINAL ARTICLE

Preparation and Evaluation of a New Lipopolysaccharide-based Conjugate as a Vaccine Candidate for Brucellosis

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Abstract
Objectives: Development of an efficacious vaccine against brucellosis has been a challenge for scientists for many years. At present, there is no licensed vaccine against human brucellosis. To overcome this problem, currently, antigenic determinants of *Brucella* cell wall such as Lipopolysaccharide (LPS) are considered as potential candidates to develop subunit vaccines.
Methods: In this study, *Brucella abortus* LPS was used for conjugation to *Neisseria meningitidis* serogroup B outer membrane vesicle (OMV) as carrier protein using carbodiimide and adipic acid–mediated coupling and linking, respectively. Groups of eight BALB/c mice were injected subcutaneously with 10 μg LPS alone, combined LPS + OMV and conjugated LPS–OMV on 0 days, 14 days, 28 days and 42 days. Anti-LPS IgG was measured in serum.
Results: The yield of LPS to OMV in LPS–OMV conjugate was 46.55%, on the basis of carbohydrate content. The ratio for LPS to OMV was 4.07. The LPS–OMV conjugate was the most immunogenic compound that stimulated following the first injection with increased IgG titer of ~5-fold and ~1.3-fold higher than that produced against LPS and LPS in noncovalent complex to OMV (LPS + OMV), respectively. The highest anti-LPS IgG titer was detected 2 weeks after the third injection (Day 42) of LPS–OMV conjugate. The conjugated compound elicited

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higher titers of IgG than LPS + OMV, that showed a 100–120-fold rise of anti-LPS IgG in mice.
Conclusion: These results indicate that our conjugated LPS–OMV can be used as a brucellosis vaccine, but further investigation is required.

1. Introduction

Brucellosis is one of the common bacterial zoonoses caused by organisms belonging to genus *Brucella*, a Gram-negative, non-spore-forming, facultative intracellular bacterium. Development of an efficacious vaccine against brucellosis has been a challenge for scientists for many years [1]. At present, there is no licensed vaccine against human brucellosis. To overcome this problem, currently, antigenic determinants of *Brucella* cell wall such as lipopolysaccharide (LPS) are considered as potential candidates for the development of subunit vaccines. Also, *Brucella abortus* LPS is considered as one of the most important antigens from the point of view of the primary targets of the innate immunity. The LPS of smooth strains of *Brucella* spp. comprise lipid A, fatty acids, a core region, and a polysaccharide O-side chain. The lipid A moiety alone is sufficient to activate the innate immune response; adaptive (antibody) responses are generated to the O antigen polysaccharide later in the course of infection [2].

Naturally occurring strains lacking LPS show reduced survival, therefore, LPS is considered to be a major virulence factor. Previous studies have clearly established that Smooth lipopolysaccharide is necessary for efficient intracellular survival and virulence of *Brucella melitensis*, *B. abortus and Brucella suis. B. abortus* S-LPS is 100 times less potent than that of *Escherichia coli* and *Salmonella* in inducing tumor necrosis factor γ produced by macrophages, as well as oxidative metabolism and lysozyme release by human neutrophils. This feature of S-LPS has been proposed to contribute to the survival of *B. abortus* within phagocytic cells. In addition, *Brucella* S-LPS is not susceptible to the actions of polycationic molecules, suggesting that smooth *Brucella* can resist the cationic bactericidal peptides of the phagocytes. S-LPS has also been found to confer antiphagocytic properties upon *Brucella* and is unable to activate the alternative pathway of the complement cascade [1–3].

It has been documented that polysaccharide (from bacterial capsule or LPS)–protein conjugates are usually immunogens in mice, rabbits and humans [4,5]. Many studies have shown that these conjugated vaccines can elicit humoral and cellular immunity against many human pathogens including *Neisseria meningitidis*, *Vibrio cholerae*, *Haemophilus influenzae*, *Shigella sonnei*, as well as *Brucella* spp. [4–7]. Covalent linkage of the polysaccharide, or fractions thereof, to immunogenic carriers (i.e., proteins) creates glycoconjugates that are T-dependent antigens and prime for boosting either with the glycoconjugate or the LPS. In contrast, polysaccharide or LPS–protein conjugate has been proven to be effective in several cases, and well-defined glycoconjugate vaccines have also been explored with a view to elicit discriminating immune responses [2,7,8]. Jacques et al showed the efficacy of *Brucella* O-polysaccharide–BSA conjugate in protection against *B. melitensis* H38 [9]. Other studies have been carried out to design subunit vaccines using other components and conjugated compounds such as porins and smooth lipopolysaccharide, recombinant ribosomal proteins and anti-OPS specific monoclonal antibodies [10–12].

The outer membrane vesicle (OMV) of *N. meningitidis* is among the newly studied components of microbial origin, which could be applied as a novel carrier. In addition, the potency of OMV as a carrier (conjugated to a hapten) is now proven. Overall, previous studies have shown that the predominant outer membrane proteins (PorA, PorB and RmpM) from *N. meningitidis* present in the Meningococci B Cuban vaccine had different capacities to prime the immune responses [1,13–16].

In the present study, we designed a subunit brucellosis vaccine composed of *B. abortus* S99 LPS with *N. meningitidis* serogroup B OMV as a covalent conjugate and then evaluated specific antibody response against the LPS of *B. abortus* S99.

2. Materials and methods

2.1. Bacterial strain, culture and fermentation conditions

B. abortus S99 was obtained from the Collection of Standard Bacteria, Pasteur Institute of Iran. This strain of *B. abortus* (biovar1) is smooth and CO_2 independent. It was cultured in slant *Brucella* agar medium (Merck, Germany) at 37 ± 1°C for 72 hours and then cultured in a 5-L flask containing 2 L *Brucella* broth (Merck) under aeration by a sparger at 37 ± 1°C for 72 hours to achieve the seed culture. Seed culture inoculated to the 60-L industrial fermenter (Novapaljas, contact-flow B.V, Netherlands) with a 40-L working volume [2]. The temperature and pH were adjusted to 37 ± 1°C and 6.4, respectively. Finally, after 60 hours, the fermentation process stopped by adding 10% phenol to the fermentation culture and biomass of *B. abortus* S99 harvested by centrifugation (1,19).