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A novel recombinant multi-epitope protein against *Brucella melitensis* infection

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ABSTRACT

Live, attenuated *Brucella* vaccines are considered effective but can induce abortions in pregnant animals and are potentially infectious to humans. There is a strong need to improve the immunoprotective effects and safety of vaccines against *Brucella*. Currently, subunit vaccines have been demonstrated to be safe and efficacious alternatives in both humans and animals. In this study, we employed bioinformatics tools to predict B and T cell epitopes to aid development of a novel recombinant multi-epitope antigen for brucellosis vaccination. To evaluate the protective capacity of the recombinant antigen, the antigen's efficacy was studied in a mouse model of brucellosis. Our results indicated that BALB/c mice immunized with this recombinant multi-epitope antigen showed mixed Th1-Th2 immune responses with high levels of specific IgG and exhibited high degrees of IFN-γ and IL-6 and significantly higher CD3, CD4, and CD8 frequencies compared to the control group. The recombinant antigen and vaccine strain M5-90 also provided protection against *Brucella melitensis* 16 M infection. Using bioinformatics tools to develop candidate vaccines is a promising strategy for the development of *Brucella* vaccines.

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1. Introduction

As a neglected zoonosis, high brucellosis incidence is reemerging worldwide, especially in developing countries [1]. Brucellosis is caused by *Brucella* spp., which are Gram-negative, facultative, intracellular pathogens. *Brucella* spp. (*Brucella abortus*, *Brucella canis*, *Brucella melitensis*, and *Brucella suis*) may lead to abortions in livestock or wildlife and cause a group of illnesses in humans [2,3]. Protection against *Brucella* infection was validated in a series of animal models, including mice [4], rabbits [5], ruminants [6], and primates [7]. *Brucella*-infected hosts exhibit mixed Th1 and Th2 responses, with a number of studies demonstrating the importance of the Th1 response, particularly the roles of CD4 and CD8 T cells, against *Brucella* [4]. Antibodies and cytokines play key protective roles against *Brucella* infection. IgM, IgG3, interferon-gamma (IFN- γ), and interleukins (IL- 6 and IL-10) display strong bactericidal activity, while both the humoral and cellular immune responses provide intracellular killing mechanisms [4]. The three distinct pathological phases observed in infected hosts include the incubation phase, the acute phase, and the chronic phase. The chronic phase involves severe organ damage and pyrexia, hematological, osteoarticular, and hepatobiliary damage, and includes non-specific clinical symptoms in humans, such as weakness, fever, sweating, and arthralgia [8]. Due to the significant health problems and important economic losses associated with *Brucella* infection, vaccination of susceptible animals is considered a crucial means of controlling brucellosis.

Currently, several available vaccines are used to protect domestic livestock. *B. melitensis* Rev.1 is the vaccine used worldwide to protect goats and sheep against *B. melitensis* infection. *B. melitensis* M5-90, derived from the virulent *B. melitensis* M28 strain, is primarily administered to goats and sheep to prevent brucellosis in China [9]. Additionally, *B. abortus* RB51 [10], strain 19(S19) [10], and strain 82(S82) [11] are the three primary vaccines recommended for protecting cattle against brucellosis caused by *B. abortus*. Although these vaccines are effective in reducing brucellosis prevalence in natural hosts, a series of disadvantages exist [1,9]. Currently used, live, attenuated *Brucella* vaccines are considered effective in both stimulating the immune system and replicating in the host [12]; however, these attenuated vaccines can induce abortions in pregnant animals and are potentially infectious to humans [13]. There is a great need for new strategies or improved vaccines against

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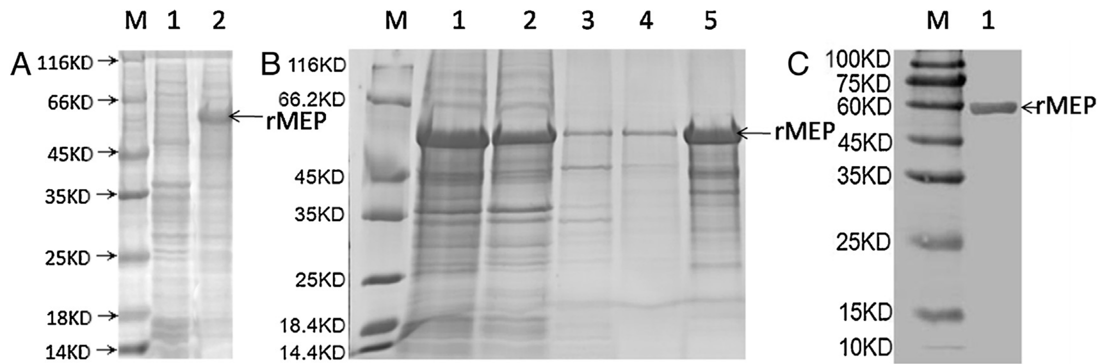

Fig. 1. SDS-PAGE and western blot analysis of rMEP. (A) Expression analysis of rMEP. M: marker; lane 1: uninduced; lane 2: after 0.2 M IPTG induction. (B) Purification analysis of rMEP. M: marker; lane 1: supernatant of lysis; lane 2: flow through; lane 3: elution with 10 mM imidazole; lane 4: elution with 20 mM imidazole; lane 5: elution with 50 mM imidazole. (C) Western blot analysis of rMEP with anti-His-tag monoclonal antibody. M: marker; lane1: purified rMEP.

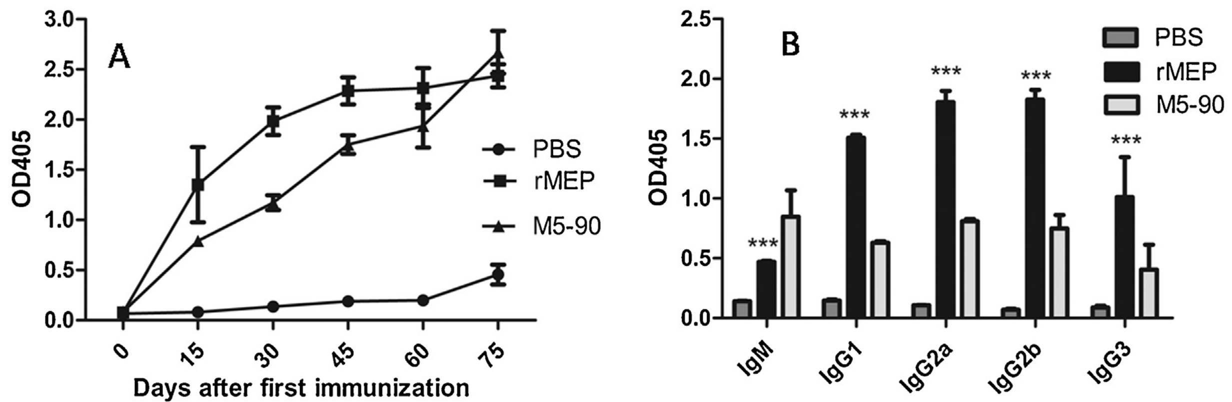

Fig. 2. Post-immunization response of rMEP- and M5-90-specific antibody. (A) Kinetics of the humoral response elicited after inoculation with PBS or immunization with rMEP or M5-90. IgG antibody levels were evaluated by ELISA. OD_{405} values are represented as the mean ± SD. (B) Profiles of antibody isotypes as measured by ELISA. Comparison of the anti-rMEP isotype level to that of PBS-inoculated and M5-90-immunized mice, respectively (***$p < 0.001$).

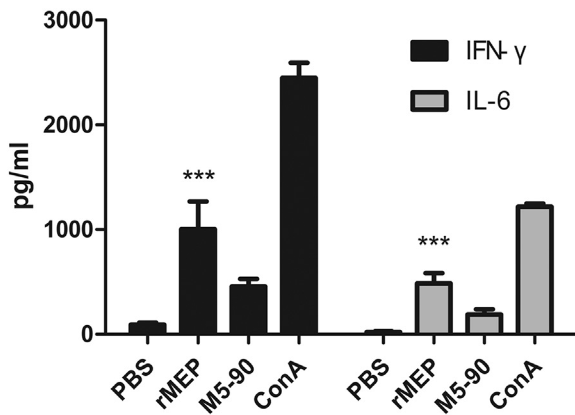

Fig. 3. Quantitative determination of IFN-γ or IL-6 production in splenocytes. Splenocytes from PBS-inoculated or rMEP- or M5-90-immunized mice were obtained 2 weeks after the last immunization, followed by *in vitro* incubation with 1 μg/mL rMEP for 48 h. Cytokine concentrations in culture supernatants were measured by ELISA. The data are represented as the mean ± SD. Comparisons are between PBS-inoculated, rMEP-immunized, and M5-90-immunized groups, respectively (***$p < 0.001$).

Brucella. In order to ameliorate immunoprotective effects and safety, subunit vaccines that depend upon antigen identification have been developed. Currently, numerous protein antigens, particularly those associated with outer membrane proteins (OMPs), induce immunoreactivity and protective immune responses in mouse models [14,15]. These immunodominant examples include OMP16 [16], OMP25 [17], OMP31 [18], OMP2b [19], and periplasmic protein BP26 [20]. Therefore, to develop efficient subunit vaccines, screening and assessment of new protective antigens is crucial.

A promising and standard approach for screening specific and immunogenic epitopes is bioinformatics analysis, with numerous studies having employed this method to predict antigenic protein epitopes [21–23]. Methods of immunoinformatics analysis used for predicting B cell epitopes are based on the properties of each amino acid, such as hydrophilicity [24] and surface accessibility [25], or structural characteristics, including β turns [26] and flexibility [27]. The development of these bioinformatics methods provide more cost-effective approaches, enabling the creation of *Brucella* vaccines and determination of diagnostic antigens associated with brucellosis.

In this study, we combined bioinformatics analysis using such tools as ABCPred [28], COBEPro [29], and BepiPred [30] to predict B cell epitopes and T cell epitope prediction using IEDB (http://www.iedb.org/), to design a novel recombinant multi-epitope antigen for vaccination against brucellosis.

2. Materials and methods

2.1. Bacterial strains and animals

B. melitensis M5-90 (vaccine strain) and *B. melitensis* 16 M (virulent strain) were gifted by the Plague and Brucellosis Prevention and Control Base, Chinese Centers for Disease Control and Prevention, Baicheng City, Jilin Province, China. Specific-pathogen-free,

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