Iranian Journal of Microbiology
Volume 5 Number 1 (March 2013) 19-23

Immune reactivity of *Brucella melitensis*–vaccinated rabbit serum with recombinant Omp31 and DnaK proteins

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Received: October 2012, **Accepted:** December 2012.

ABSTRACT

Background and objectives: *Brucella melitensis* infection is still a major health problem for human and cattle in developing countries and the Middle East.

Materials and Methods: In this study, in order to screen immunogenic candidate antigens for the development of a *Brucella* subunit vaccine, a cytoplasmic protein (DnaK) and an outer membrane protein (Omp31) of *B. melitensis* were cloned, expressed in *E.coli* BL21 and then purified using Ni-NTA agarose. Immunized serum was prepared from a rabbit inoculated with attenuated *B. melitensis.*

Results and Conclusion: It was proved that immunized serum contains antibodies against recombinant Omp31 (rOmp31) and DnaK (rDnaK) by Western blot and ELISA assays. The results may suggest the importance of these proteins as subunit vaccines against *B. melitensis* as well as targets for immunotherapy.

Keywords: *Brucella*, Cloning, Immune Reactivity, ELISA, Protein Expression, Purification

INTRODUCTION

Brucella spp. are intracellular pathogens which were originally defined as facultative intracellular bacteria that preferentially infect macrophages (1, 2). Human infections with *B. melitensis* are endemic in many developing countries (3), and the incidence of brucellosis in livestock is of great economic concern due to reduced productivity, increased numbers of abortions and weak offspring, and is a major impediment to trade and export of livestock. Human brucellosis is a severe debilitating disease that requires prolonged treatment with several antibiotics, and also involves considerable medical expense, as well as loss of working hours (4). *B. melitensis Rev.1*, an attenuated smooth strain used to control *B. melitensis* infection gives heterologous protection against other *Brucella* spp. and is currently considered as the best vaccine for the prophylaxis of caprine brucellosis (5). However, major problems like the ability of this strain to cause infection in humans (6) and the development of resistance to streptomycin used to treat brucellosis, have made the health officials to prohibit its use for human vaccination (7). Therefore, a subunit vaccine that is protective against *B. melitensis* is desirable. There is an increasing interest in the study of immunogenicity and protective effects of *Brucella* outer membrane proteins (OMPs) and cytoplasmic proteins (8-10). For the first time Omp31 was cloned from *Brucella melitensis* 16M, and its predicted

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