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Identification of a new immunogenic candidate conferring protection against Brucella melitensis infection in Mice

Amir Ghasemi [a], Amir-Hassan Zarnani [b,c,**], Abolfazl Ghoodjani [d], Simin Rezania [e], Mohammad Hossein Salari [a], Mahmood Jeddi-Tehrani [f,*]

[a] Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[b] Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
[c] Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran
[d] Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
[e] Institute for biophysics, Medical University of Graz, Austria
[f] Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

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abstract

Identification of bacterial proteins that contribute to the replication and survival of the engulfed bacteria within phagolysosome is critical in the pathogenesis of intracellular bacteria. Heat shock proteins (HSPs) are molecular chaperones that prevent unwanted protein aggregation and protect the bacteria against cell stress. In order to study the potential of HspA for development of a Brucella subunit vaccine, immunogenicity and protective efficacy of recombinant HspA (rHspA) from Brucella melitensis was evaluated in BALB/c mice. The hspA gene was cloned in pDEST42 and the resulting recombinant protein was used as subunit vaccine. rHspA elicited mixed TH1/TH2 immune responses with higher titers of specific IgG1 than IgG2a. In lymphocyte transformation assay, splenocytes of immunized mice exhibited a strong recall proliferative response with high amounts of IFN- , IL-12, IL-10 and IL-6 and very low levels of IL-5 and IL-4 production. The protective effect of rHspA was evaluated by administering rHspA to mice that resulted in a significant reduction in bacterial load and high degree of protection against B. melitensis challenge compared to control mice ($p < 0.001$). These results suggest that rHspA may be a useful candidate for the development of subunit vaccine against brucellosis.

1. Introduction

Brucella species are Gram negative, facultative intracellular pathogens that cause abortion in domestic animals (sheep, cattle, and goats) and a severe illness in humans (Corbel, 1997). Brucellosis is mostly exhibited as an endemic disease, especially in developing countries and continues to impose significant health problems and important economic losses (Ko and Splitter, 2003). Brucella melitensis is the most common and pathogenic species that infects humans worldwide and the least species specific, infecting livestock (Young, 1995). For the time being, the live attenuated B. melitensis, Rev.1 strain, is the most widely used vaccine for controlling brucellosis in sheep and goat (Garin-Bastuji et al., 1998; Scharp et al., 1999). The Rev.1 vaccine was firstly obtained by using streptomycin as the selective agent to develop the attenuated streptomycin-resistant Rev.1 strain from streptomycin-dependent bacteria selected from a virulent strain of B. melitensis in the 1950s (Elberg and Faunce, 1957). However, it is less than ideal because Rev.1 elicits a long-lasting serological response against the O polysaccharide making it difficult to differentiate vaccinated animals from those naturally infected (Baldi et al., 1996; Zygmunt et al., 1994). Moreover, due to occasional problems caused by Rev.1, its use is prohibited in countries free of B. melitensis (Jimenez de Bagues et al., 1994). These problems include occasional induction of abortion when adminis- tered during pregnancy, pathogenicity for humans (Moriyon et al., 2004) and resistance to streptomycin which is one of the preferred antibiotics for treatment of brucellosis (Delpino et al., 2007). In order to avoid these drawbacks, alternative vaccination approaches like subunit vaccines are highly demanded and identification of Brucella-associated antigens is the subject of extensive research in many countries. In this regard, different components of B. melitensis have been proposed as candidates for subunit vaccines (Al-Mariri,

* Corresponding author at: Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran PO. Box: 19615-1177.
** Corresponding author at: Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran PO. Box: 19615-1177.
E-mail addresses: zarnani@avicenna.ac.ir (A.-H. Zarnani), Mahjed@avicenna.ac.ir (M. Jeddi-Tehrani).

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