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PLoS one

Brucellosis Vaccines: Assessment of *Brucella melitensis* Lipopolysaccharide Rough Mutants Defective in Core and O-Polysaccharide Synthesis and Export

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Abstract

Background: The brucellae are facultative intracellular bacteria that cause brucellosis, one of the major neglected zoonoses. In endemic areas, vaccination is the only effective way to control this disease. *Brucella melitensis* Rev 1 is a vaccine effective against the brucellosis of sheep and goat caused by *B. melitensis*, the commonest source of human infection. However, Rev 1 carries a smooth lipopolysaccharide with an O-polysaccharide that elicits antibodies interfering in serodiagnosis, a major problem in eradication campaigns. Because of this, rough *Brucella* mutants lacking the O-polysaccharide have been proposed as vaccines.

Methodology/Principal Findings: To examine the possibilities of rough vaccines, we screened *B. melitensis* for lipopolysaccharide genes and obtained mutants representing all main rough phenotypes with regard to core oligosaccharide and O-polysaccharide synthesis and export. Using the mouse model, mutants were classified into four attenuation patterns according to their multiplication and persistence in spleens at different doses. In macrophages, mutants belonging to three of these attenuation patterns reached the *Brucella* characteristic intracellular niche and multiplied intracellularly, suggesting that they could be suitable vaccine candidates. Virulence patterns, intracellular behavior and lipopolysaccharide defects roughly correlated with the degree of protection afforded by the mutants upon intraperitoneal vaccination of mice. However, when vaccination was applied by the subcutaneous route, only two mutants matched the protection obtained with Rev 1 albeit at doses one thousand fold higher than this reference vaccine. These mutants, which were blocked in O-polysaccharide export and accumulated internal O-polysaccharides, stimulated weak anti-smooth lipopolysaccharide antibodies.

Conclusions/Significance: The results demonstrate that no rough mutant is equal to Rev 1 in laboratory models and question the notion that rough vaccines are suitable for the control of brucellosis in endemic areas.

Citation: González D, Grilló M-J, De Miguel M-J, Ali T, Arce-Gorvel V, et al. (2008) Brucellosis Vaccines: Assessment of *Brucella melitensis* Lipopolysaccharide Rough Mutants Defective in Core and O-Polysaccharide Synthesis and Export. PLoS ONE 3(7): e2760. doi:10.1371/journal.pone.0002760

Editor: Kirsten Nielsen, University of Minnesota, United States of America

Received April 23, 2008; **Accepted** June 24, 2008; **Published** July 23, 2008

Funding: This work was funded by the European Commission (Research Contract QLK2-CT-2002-00918) and the Ministerio de Ciencia y Tecnología of Spain (Proyecto AGL2004-01162/GAN).

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Brucellosis is a group of closely related zoonotic bacterial diseases caused by the members of the genus *Brucella*, a group of gram-negative bacteria that behave as facultative intracellular parasites. There are several *Brucella* species, and they infect a wide range of mammals in which they are a main cause of abortions and infertility. In addition, they are readily transmitted to human beings where they produce a grave and debilitating disease that requires a long and costly antibiotic therapy and that often leaves permanent sequelae [1]. Because of its high incidence in developing countries, economic consequences, and difficult eradication, the World Health Organization considers brucellosis as one of the seven neglected zoonoses, a group of diseases that contribute to the perpetuation of poverty [2].

Ruminants are highly susceptible to brucellosis. Cattle are most often infected by *B. abortus* whereas sheep and goats are the preferred hosts of *B. melitensis*, the *Brucella* species most virulent for