P5-03 Development of a *B. melitensis* Rev.1 mutant lacking streptomycin resistance

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Abstract

Brucella melitensis is the etiological agent of ovine and caprine brucellosis. Since this species is the major cause of human brucellosis, vaccination of small ruminants is essential for its control and eradication avoiding brucellosis in humans. However, *B. melitensis* Rev.1 (the only vaccine available) is abortifacient when applied to pregnant sheep and goats, virulent to humans and resistant to streptomycin (Strp), one of the antibiotics of choice for treating human brucellosis. The goals of this work were to study the streptomycin resistance mechanisms in Rev.1 strain and to correct genetically this resistance to develop a potentially safer vaccine. In bacteria, Strp resistance is associated with mutations in the 16SrRNA, in proteins of the 30S ribosomal subunit and in enzymes modifying the 16SrRNA. Thus, we compared the corresponding genes in Rev.1 and *B. melitensis* 16M (reference strain of the biovar 1 and Strp-sensitive [Strps]). Whereas the 16SrRNA sequences were identical, we identified point mutations in Rev.1 rpsL (encoding the 30S S12 protein) and rsmG (encoding a methyltransferase acting on N7 of G527 of 16SrRNA) leading to P91L (mutation previously described) and P81R changes, respectively. Consistently with rpsL essentiality, we could not obtain Rev.1rpsL mutants. However, we demonstrate the involvement of rpsL in Rev.1 Strp resistance by introducing the 16M rpsL gene in Rev.1. The rsmG deletion in 16M (BmeΔrsmG mutant) conferred Strp resistance, while rsmG deletion in Rev.1 (Rev.1ΔrsmG) did not affect Strp resistance. Introduction of 16M rsmG in Rev.1ΔrsmG (Rev.1ΔrsmG::Tn7BmersmG -abbreviated as Rev.1StrpS-) resulted in increased Strp sensitivity. Rev.1StrpS showed an attenuated profile both in BeWo trophoblasts and THP-1 monocyte-derived-macrophages. In mice, Rev.1StrpS conferred similar protection against *B. suis* and resulted in lower residual virulence and abortifacient effects than Rev.1. The mutant was proven safe in pregnant sheep (see the results of the work presented by, P. M. Muñoz.). Protective efficacy experiments are in progress in sheep.
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