Expression and Localization of Green Fluorescent Protein in *Brucella abortus* strain RB51.

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(ABSTRACT)

Brucella abortus is a facultative intracellular bacterial pathogen, which causes abortion in cattle and undulant fever in human. *B. abortus* strain RB51 (Strain RB51) is the official vaccine for bovine brucellosis in the USA. *B. abortus* strain RB51 can be used as a vector for the over-expression of its own (homologous) as well as heterologous protective antigens. The immune system can detect these heterologous antigens and produce a response. Expressing a protein in different bacterial compartments has been shown to affect its accessibility to the immune system and the way the antigen is processed by antigen presenting cells. In order to determine if the immune response is affected by the localization of the antigen, green fluorescent protein (GFP) was expressed at three different locations in *B. abortus* strain RB51, outer-membrane (OM), periplasmic space (PS) and in the cytoplasmic region (CR) of *B. abortus* strain RB51. This localization was obtained by transforming strain RB51 with plasmids pBBg18sGFP and pBBgSsGFP, in which the 18 kDa *Brucella* lipoprotein and the *Brucella* Cu/Zn SOD protein signal sequences were added to the GFP sequence to cause OM and PS expression respectively. No signal sequences were added to the plasmid pBBgGFP for CR only expression. Expression and localization of GFP in the different compartments in recombinant *B. abortus* strain RB51 were confirmed by electron microscopy and antibody absorption experiments. Groups of 5 female BALB/c mice each were injected and boosted with three recombinant strains and appropriate controls. Mice were bled and their anti-GFP antibody production was assessed. None of the immunized mice produced specific antibodies against GFP, probably due to the low expression of the heterologous antigen observed in this study by strain RB51 observedin this study. It will be necessary to produce new recombinants which are able to express higher amounts of GFP to answer if localization of heterologous antigen within the recombinant RB51 affects the level of a specific immune response