

Analysis of vaccine potential of live recombinant and inactivated wild strains of *Corynebacterium pseudotuberculosis*

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Caseous lymphadenitis (CLA) is a worldwide chronic disease that affects sheep and goats, whose etiological agent is *Corynebacterium pseudotuberculosis*. Despite all the economic losses caused by CLA, there is not much information about molecular mechanisms in the pathogenesis of this bacterium, and immune prophylaxis against the infection is not able to reduce the incidence of illness. Recently, our research group obtained 34 alkaline phosphatase-positive recombinant strains of *C. pseudotuberculosis*, through random mutagenesis using TnFuZ, of which we identified 21 different loci. The aim of the present study was to employ these 21 previously obtained mutants in mouse vaccination trials in order to search for new alternative vaccines against CLA. Analyses of immunoglobulins and cytokines were carried out to identify the immune response pattern after immunization. Among the 21 strains tested, the live attenuated strain CP13 showed the best protection level, 75% on average, in immunized mice. Moreover, this strain was able to elicit a significant level of IgG and IgG2a, characterizing a Th1 immune response responsible for eliminating intracellular pathogens, such as *C. pseudotuberculosis*. Interaction assays with murine J774 cells, *in vitro*, revealed that CP13 had diminished intracellular viability. This may be due to the lack of the protein coded by the transposon interrupted gene, an iron transport system binding (secreted) protein. These results showed the vaccine potential of this mutant against CLA. In another aspect, in this study, four inactivated wild strains of *C. pseudotuberculosis* were also tested in infection trials in BALB/c mice and subjected to the same immunological analysis. The formalin-inactivated strain T1, consisting of somatic and secreted antigens of *C. pseudotuberculosis*, showed the best protection level, above 70% on average, in immunized mice, and also induced significant levels of the immunoglobulins assayed. The results obtained here point out CP13 and formalin-inactivated T1 strains as potential live and inactivated vaccines, respectively, against CLA selected in a murine model.

Key words: *Corynebacterium pseudotuberculosis*; Vaccine; Caseous lymphadenitis; Goat; Sheep