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Attenuated IglH and DsbA deletion mutants created in *Francisella tularensis ssp. Holarctica* demonstrating different level of protection have distinct intracellular fate

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Francisella tularensis, the causative agent of severe disease tularemia, is a highly infectious intracellular pathogen. Nevertheless, there is no licensed vaccine available up today. To move a step closer to the vaccine we carried out a comparative proteomic analysis of different *F. tularensis* strains and we discovered potential candidate proteins involved in *F.tularensis* virulence mechanisms, in particular IglH, a product of the *Francisella* Pathogenicity Island and DsbA, a homolog of the disulfide oxidoreductase DsbA family. Subsequently, an importance of these proteins was studied for *F.tularensis* viability in host cells and virulence in mice, respectively. We performed non-polar deletion of genes in the clinical isolate of *Francisella tularensis ssp. holarctica* denoted FSC200. The iglH and the dsbA mutants were then characterized employing *in vitro* and *in vivo* methods and moreover, their possible immunostimulatory capacity was studied. We showed that both proteins are important for intracellular growth within macrophages. Further, both mutants have defects in their intracellular fate showing impaired ability to escape from the phagosome. Additionally, the BALB/c mice infected with the dsbA mutant or the iglH mutant survived the infection without any signs of disease. Importantly, the difference between these two mutants was found in the protective effect against another infection with *F.tularensis*. Since all mice vaccinated with the dsbA mutant in any dose given survived the challenge with the FSC200 strain, the pretreatment with the iglH mutant led to protection only at the dose higher than 3×10^7 CFU/mouse. The ongoing work is aimed at elucidation of the mechanisms causing protective effects described, in particular colocalization of studied mutants with early and late endosomal markers, and finally with lylososomal marker cathepsin D is being investigated.

Keywords: Francisella tularensis, intracellular fate, attenuation, protective effect