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A capsule-deficient mutant of *Francisella tularensis* live vaccine strain is significantly more attenuated than LVS yet induces comparable protection in mice against *F. tularensis* challenge

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Aim: To develop a live attenuated vaccine against *Francisella tularensis* that is safer than LVS and comparably potent.

Methods: A putative capsule-deficient antibiotic marker-free mutant (LVS Δ capB) of *F. tularensis* Live Vaccine Strain (LVS) was constructed by allelic exchange. LVS Δ capB was evaluated for serum sensitivity, virulence in human macrophage-like THP-1 cells, and virulence and protective immunity in BALB/c mice after intranasal (i.n.) and intradermal (i.d.) immunization. BALB/c mice were immunized with LVS Δ capB at doses ranging from 10³ to 10⁷ CFU i.n. and 10⁶ to 10⁸ CFU i.d. Four weeks later, the mice were challenged i.n. with 10⁴ CFU LVS (>5 x LD₅₀), and at 5 days post-challenge, the bacterial burden in the lung, liver and spleen was assayed. Sham-immunized mice and mice immunized with LVS at doses ranging from 3 x 10² to 10⁴ CFU i.n. and 10⁵ to 10⁷ CFU i.d. served as controls.

Results:

- (I) LVS Δ capB, which retains the O-Antigen, was serum resistant.
- (II) In a competition experiment, LVS Δ capB was outgrown by parental LVS in THP-1 cells.
- (III) LVS Δ capB was significantly attenuated in mice and caused no weight loss, obvious signs of illness, or deaths at any dose tested; hence the LD₅₀ i.n. was > 10⁷ CFU vs. 1.8 x 10⁸ CFU for LVS and the LD₅₀ i.d. was > 10⁸ CFU vs. 3.2 x 10⁷ CFU for LVS.
- (IV) Mice immunized with LVS Δ capB i.n. or i.d. and then challenged 4 weeks later with a lethal dose of LVS i.n. were 100% protected from illness and death.

In mice immunized with LVS Δ capB, the bacterial burden in the lung was 3 – 5 logs lower than in sham-immunized animals, and the bacterial burden in the spleen and liver was 3 – 4 logs lower than in sham-immunized animals, comparable to that in mice immunized with LVS. This indicated that both local replication in the lung and systemic dissemination of *F. tularensis* was strongly inhibited by immunization with LVS Δ capB.

Conclusions and discussion:

- (I) LVS Δ capB is serum resistant but significantly attenuated in both human macrophages and mice, providing a safer vaccine candidate than LVS.
- (II) Immunization with LVS Δ capB by the i.d. or i.n. route induces strong protective immunity, comparable to that induced by LVS at maximum tolerated doses, against lethal i.n. challenge with *F. tularensis*.

Future studies will examine the capacity of LVS Δ capB to protect against aerosol challenge with *F. tularensis* subsp. *tularensis* Schu S4.