

Analyses of phagocytic ability of rat macrophages for *Francisella tularensis*

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Aims: The Fisher 344 rat, in comparison to the mouse, may represent an infection model which is more representative of human *F. tularensis* infections. In this study, we sought to examine the phagocytic activity and inflammatory cytokine production of bone marrow derived macrophages (BMDM) from F344 rats exposed to different *F. tularensis* strains.

Methods: F344 rat BMDM were infected with various MOIs of LVS, *F. tularensis* subsp. *holarctica* (Type B) or *F. novicida*. At specified intervals, cells were lysed and bacteria enumerated by serial dilution plating. Culture supernatants were analyzed for the production of nitric oxide and TNF- α . Virulence of the different *Francisella* species also was ascertained by intra-tracheal pulmonary challenges.

Results: Using inert fluorescent microbeads (100 beads:1 cell), it was determined that the basal phagocytic uptake of rat BMDM was greater than that of mouse BMDM (87.1% vs. 33%). There was minimal uptake/replication of LVS in rat macrophages, and marginal replication (1-2 log₁₀) of *F. novicida*, in contrast to the 3-4 log₁₀ increase of Type B at 72 hr. Levels of nitric oxide (LVS 25 μ M, *F. novicida* 50 μ M, Type B < 10 μ M) and TNF- α (LVS 930 pg/ml, *F. novicida* 2040 pg/ml, Type B 72 pg/ml) production were inversely correlated with intramacrophage replication. Finally, *in vivo* pulmonary challenges revealed that both LVS (LD₅₀ > 10⁷ CFU) and *F. novicida* (LD₅₀ > 10⁵ CFU) were highly attenuated for virulence in rats as in humans.

Conclusions: LVS and *F. novicida* exhibit minimal replication in rat macrophages. Moreover, rats were greatly resistant to pulmonary challenge with LVS and *F. novicida*, correlating with *in vitro* production of nitric oxide and TNF- α . Collectively, the *in vitro* phagocytic analyses and rat model of pulmonary tularemia may serve to extend our current understanding of the pathogenesis of *Francisella tularensis*. This project has been funded in whole or in part with Federal funds from the National Institute of Allergies and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN266200500040C.