3. SUMMARY

Modulation of *Francisella tularensis* LVS *in vitro* infection by interferon-γ and lipopolysaccharide

Background: *Francisella tularensis,* highly virulent bacteria responsible for tularaemia, is known to replicate within the host macrophages. However, the immunopathogenesis of this infection is still incompletely understood. We focused on mutual interactions between intracellular parasitic bacteria *F. tularensis* and murine macrophage-like cell line J774.2.

Aims: To examine the capability of recombinant murine lymphokine **interferon-γ** (INF-γ) and/or **lipopolysaccharide** (LPS) derived from *E. coli* to stimulate *in vitro* antimicrobial activity of macrophage-like J774.2 cell line against the live vaccine strain (LVS) of *Francisella tularensis* through their ability to produce proinflammatory cytokines and chemokines and to inhibit the growth of bacteria.

Methods: Proliferation of microbes in *in vitro* culture was acquired by technique of population analysis. Recombinant murine INF-γ, in concentrations 100 I.U./ml or 1000 I.U./ml of medium, and/or bacterial LPS derived from *E. coli*, serotype O55:B5 in concentrations 10 ng/ ml of medium or 50 ng/ml of medium, was used for stimulations immediately after infection or 3 hrs before infection. ELISA method was used for evaluation of cytokines and chemokines in culture supernatants. Following cytokines and chemokines, interleukin 12p40 subunit (IL-12 p40), interleukin 18 (IL-18), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), monocyte chemotactic protein 5 (MCP-5), RANTES (Regulated upon Activation, Normal T Expressed, and presumably Secreted), MIP-1γ (Macrophage Inflammatory Protein 1 gamma) and MIP-2 (Macrophage Inflammatory Protein 2) were tested. Viability of cells was calculated using Trypan blue exclusion test (0,5% TB in saline) and concentration of NO_2^- was determined by Griess reagent. Results expressed as mean ± S.D. were derived from three independent experiments.

Results: We demonstrated that *F. tularensis* LVS infection up-regulates IL-12p40 production by stimulated macrophages and down-regulates TNF-α production. *F. tularensis* LVS infection was not capable to affect the production of IL-18, IL-6, MCP-5, RANTES, MIP-1 and MIP-2 by stimulated macrophages. Both stimulation of J774.2 cells either by INF-γ alone or especially in combination with LPS before infection *F. tularensis* LVS revealed protective effects. Higher concentrations of INF-γ with LPS were needed to inhibit ongoing *F. tularensis* LVS infection. The generation time of *F. tularensis* in J774.2 cells was calculated to be 3.5 h and the stability of culture is guarantied for the first 12 hours after infection.

Conclusions: *F. tularensis* LVS infection modulates the cytokine synthesis by J774.2 macrophage-like cell line. On the other hand, stimulation of J774.2 cell line by combination of INF-γ with LPS inhibits the growth of *F. tularensis* LVS.