AN *IN VITRO* MODEL OF *FRANCISELLA TULARENSIS* LVS INFECTION

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Francisella tularensis live vaccine strain (LVS) is a facultative intracellular bacterium, which causes lethal disease in several mouse strains (Fortier et al. 1991). A huge body of information dealing with the mechanisms of *Francisella tularensis* infection of various mouse cell lines is available (Macela 1980, Fortier et al. 1992). In this study we used *in vitro* infection of murine macrophage–like cell line J774 for the standardization of another *in vitro* model, which could be more universal for the studies of bacteria – host pathogen interactions, more precise assignment of early stages of primary infection of host cells, and characterization of intracellular compartment associated with ingested bacteria, respectively. A subsequent goal of our study was identification of the expression of genes encoding for selected cytokines under the influence of infection and the study of final fate of primary infected cells.

A population analysis was chosen for studying of *in vitro* model of *F. tularensis* LVS infection. Conditions of infection were selected to ensure the multiplication of infection 1:100 (cell:bacteria). J774 cells (ECACC, No: 85011428) were cultured for 48 h in a complete culture medium without gentamicin. *Francisella tularensis* LVS strain (ATCC 29684, American Type Culture Collection, Manassas, Va.) was cultured on Mc'Leod agar plates for 24 h at 37°C in atmosphere containing 5% CO_2. J774 cells were exposed to LVS strain for 2 h at 37°C in culture medium without gentamicin. Infected cells were than cultivated in culture medium supplemented with gentamicin for 1 h at 37 °C in atmosphere containing 5% CO_2 and then washed three times with culture medium without gentamicin. The number of J774 cells was calculated and cell viability was assessed using Trypan blue exclusion test.

Final multiplication of *F. tularensis* infection ranged from 1:123 to 1:239. Our results indicate no significant variations in the number of J774 cells per ml during the post–infection periods (figure 1.). The most profound decrease in the viability was seen at twelve hours after infection (figure 2.), but this decrease did not reach statistical significance.

Proliferation curve of *F. tularensis* in J774 cell culture (figure 3 – the result of one representative experiment is displayed) shows geometric dependence after first 6 h post-infection. First six hours after initiation of infection represent probably the lag phase. This result was subsequently successfully repeated in several independent experiments and all data are in a good concordance with result presented here.

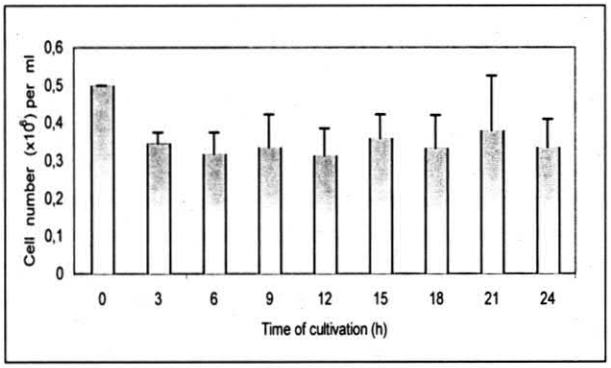


Fig. 1: The number of cells in infected cultures (n=4) during 24 hours of post-infection period

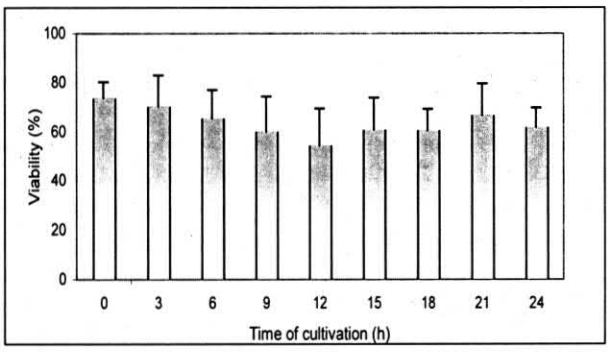


Fig. 2: Viability of cell line J774 (n=4) infected by Francisella tularensis LVS strain

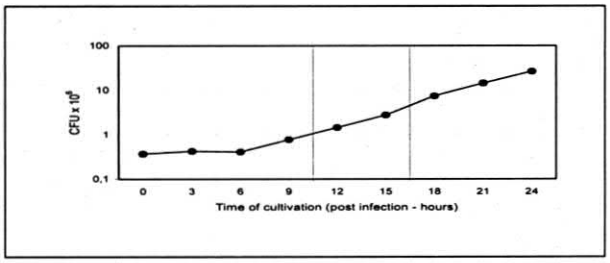


Fig. 3: Proliferation of Francisella tularensis LVS strain in J774 cell culture during 24 hours post-infection

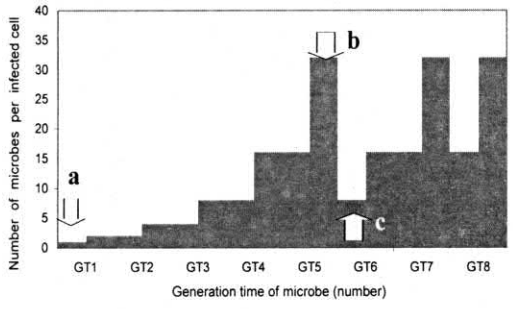
Generation time for bacterium *Francisella tularensis* LVS in J774 cells was calculated from experimental data obtained from intervals started at 3 and ended at 24 hours post–infection (four independent experiments). Generation time for bacterium was calculated to be 3.5 h. Generation time in J774 cells is comparable to the generation time of *F. tularensis* LVS strain in inflammatory peritoneal cells (table 1) published by Anne Fortier and coworkers (1992), but comparison of results has to be interpreted with caution. Our results were drowning from completely *in vitro* system using established macrophages cell line. Other authors used either *in vivo* infected cells or *ex vivo* cultivated peritoneal exudates cells for infection. Only the same population-based approach was used in all above-mentioned studies. Furthermore, the generation time of bacterium *Francisella tularensis* LVS in J774 cell line is quite dissimilar to the generation time of bacterium calculated for multiplication of bacteria in resident macrophages (Fortier et al. 1992). Our results are not in accordance with those obtained from the *ex vivo* system based on adherent peritoneal cells (4.6 h) and in so–called „immune" peritoneal cells (4.6 h) also (Macela 1980). A. Macela used single cell analysis; it means the calculation of fluorescent labelled microbes in individual cells for the evaluation of generation time of *Francisella tularensis* LVS and strain 130 in rodent (murine) macrophages.

Table 1

Comparison of generation time of calculated for *Francisella tularensis* LVS in phagocytic cells

Type of cell culture	Technique	Generation time (h)	Author
J774	Population analysis	3.5	M. Èerná (2002)
Adherent peritoneal cells	Single cell analysis	4.6	A. Macela (1980)
"Immune" peritoneal cells	Single cell analysis	4.6	A. Macela (1980)
Resident peritoneal cells	Population analysis	6.0	A. Fortier (1992)
Inflammatory peritoneal cells	Population analysis	3.0	A. Fortier (1992)

In summary, our results were used for the delineation of a model for the evaluation of strictly primary interaction of intracellular bacterial pathogen with the phagocytes that are one of the components of the host immunoregulatory system. The simple scheme of this interaction is presented on figure 4.



a: originally infected with one microbe per cell
b: host cell death
c: other cells infected with unpredictable number of microbes

Fig. 4: The single cell model of intracellular replication of Francisella tularensis LVS strain

In our system, the stability of cell culture is sufficient for the first 12 hours after infection. Than, the infected host cells are ruptured and bacteria are spreading to cultivating medium and are for disposal for subsequent phagocytosis by other phagocytic cells in culture. These "secondary" infected cells can be modified by autocryne/paracrine manner so that the mechanism of handling with intra–cellularly located microbes can be dissimilar to that used by primary infected cells (at time zero). Characterization of possible cell–cell interaction in *in vitro* system, including the analysis of cytokines produced in the course of primary infection of murine macrophage–like cell line J774, infected by *F. tularensis* LVS strain, will be the next goal of our study.

References

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