F. TULARENSIS INFECTION-INDUCED CELL CYCLE BLOCK AND INDUCTION OF APOPTOSIS

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Generally, macrophages initiate the innate immune response by recognizing pathogens, phagocytosing them and secreting inflammatory mediators. However, intracellular bacterial pathogens have developed plenty of strategies to influence processes in eucaryotic host cells to establish appropriate conditions for their own growth and proliferation.

Francisella tularensis is a highly virulent intracellular pathogen responsible for tularemia. This bacterium is capable of infecting many mamalian species and various types of cells, but little is known about the mechanisms of survival and interactions with host cells.

Initially, the murine macrophage-like cell line J774 was infected by *F. tularensis* LVS using a multiplicity of infection MOI=100. We examined a number of infected J774 cells and cytopathogenicity (fig.1).

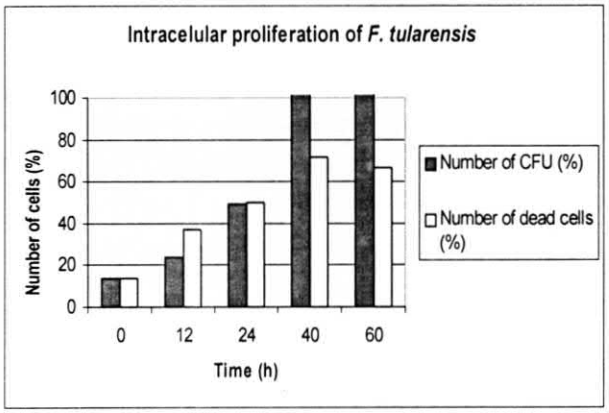

Figure 1: Intracellular proliferation of F. tularensis in J774 cells. Infection was accomplished by protocol used in the Proteome center. Numbers of CFU and dead cells were counted from total quantity of cells in the given time periods.

We investigated the influence of infection by *F. tularensis* on cell cycle status by determination of DNA content of J774 cells. (fig. 2, 3).

Our idea was to investigate any possible connection between susceptibility of J774 cells to *F. tularensis* infection and the cell cycle.

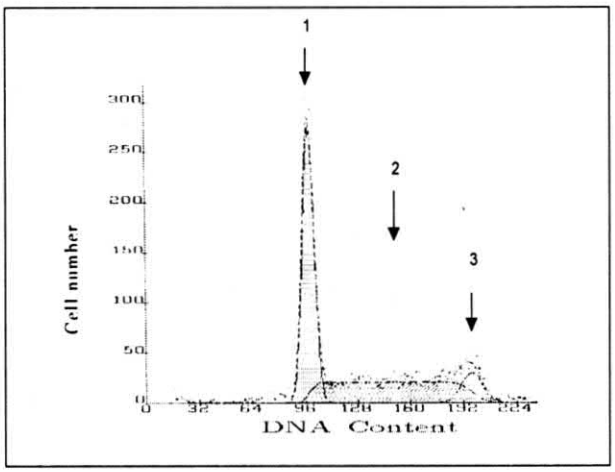

Fig.2 Demonstration of the acquired results by flow cytometry. 1 points at G1-peak, 2 points at S-phase and 3 points at G2/M-peak.

We detected cell cycle block in G1-phase, approximately 6–12 h after infection. We obtained the same results by using heat-dead *F. tularensis*. When zymosan was used to simulate bacterial infection, cells were also blocked in G1-phase, but cells needed a longer time period to return to the normal stage. Unfortunately we gained similar results by determination of the cell cycle in the control cells.

To summarize these observations, the apparent cell cycle block may be caused by presence of bacterial agent, but this effect is not specific for *F. tularensis* and the effect is minimal, since 70–80% of the cells are naturally situated in G1 phase 6–12 h after passage.

One of the most important factors that influences bacterial pathogenicity is the creation of signals that influence successive events in the infected cell and in surrounding, uninfected cells, after primary interaction with the pathogen. Therefore we have investigated whether primary interaction of the host cell and *F. tularensis* induces apoptosis or necrosis in J774 cell cultures.

To determine if there is a role of apoptosis in infection-induced cell death, we monitored the presence of surface-exposed phosphatidylserine,

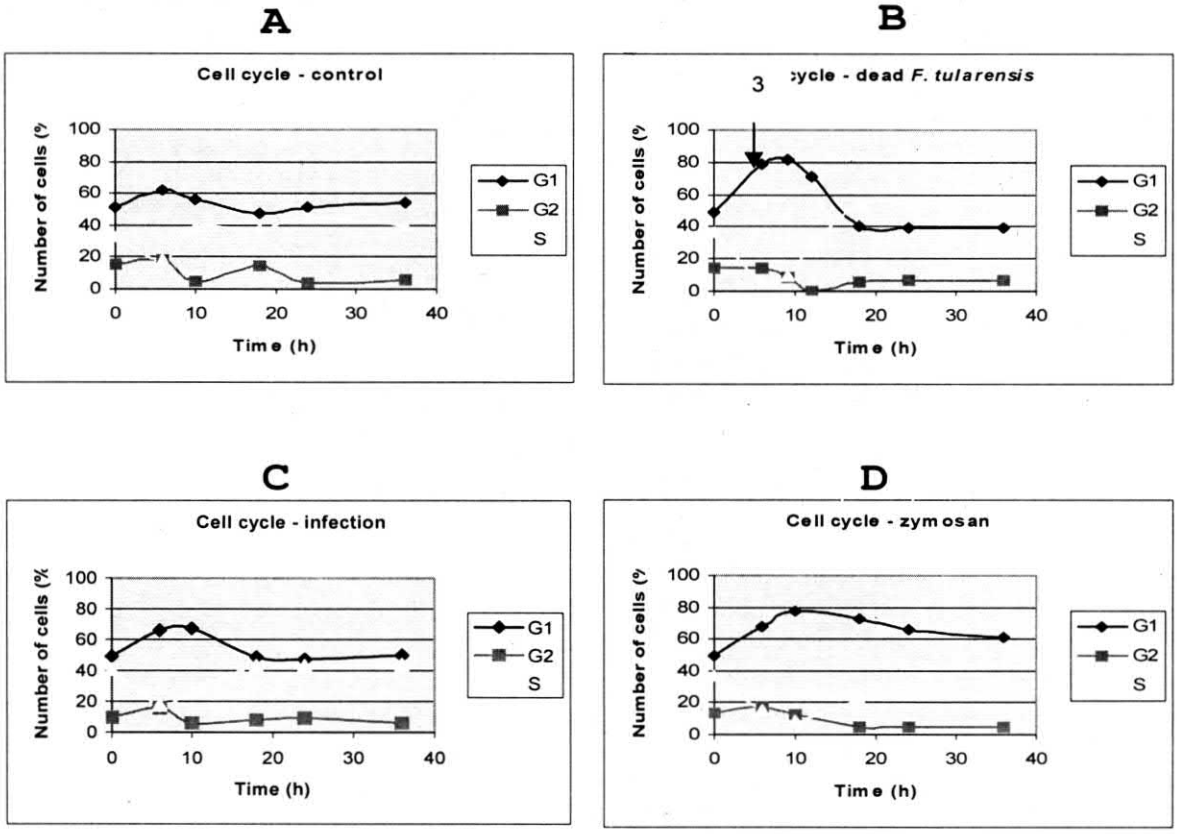

Fig.3: Graphs show the changes in the single phases of the cell cycle at the given time periods. (A) Control cells. (B) To the culture medium with J 774 cells was added heat-killed F.tularensis. (C) In this case was added F.tularensis LVS (MOI=100). (D) In this case was added zymosan with aim to simulate bacterial infection.

the appearance of terminal deoxynucleotidyl-transferase-mediated a template independent addition of FITC-dUTP for labeling DNA breaks (TUNEL)-positive infected cells and the release of nucleosomes using a photometric enzyme--immunoassay.

Surface exposure of phosphatidyl serine was examined by labeling with fluorescein isothiocyanate-conjugated annexin V and was analyzed by flow cytometry (fig.4).

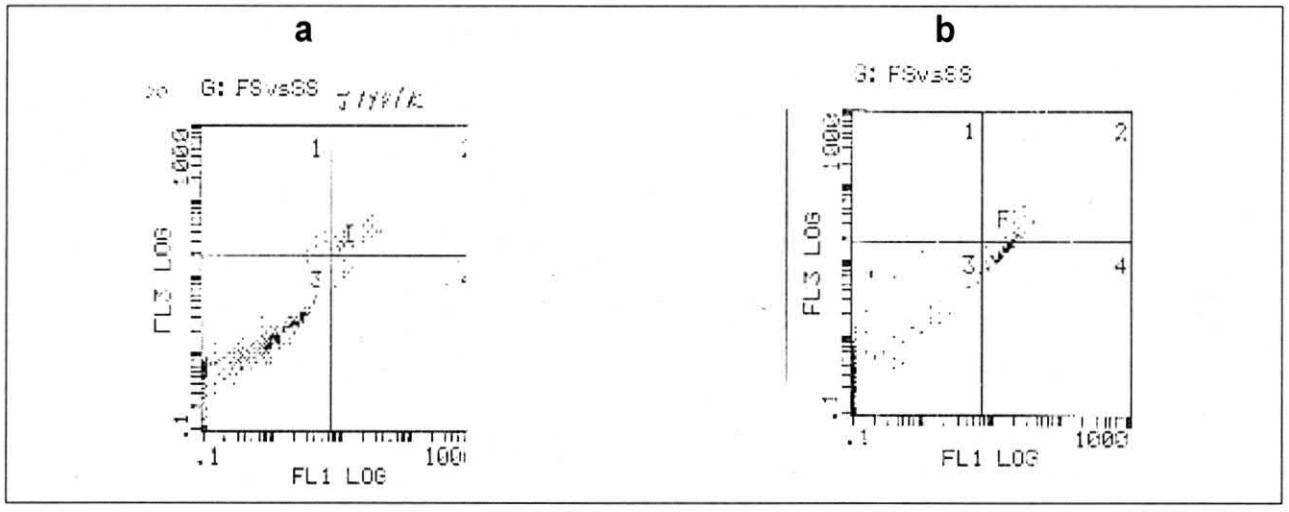

Fig. 4: Flow cytometric analysis of Annexin V staining. (a) Control cells, 18 h culturing. X-axis represent Annexin V staining and Y-axis represent PI staining. (b) F. tularensis-infected J 774 cells after 18 h culturing (MOI=100).

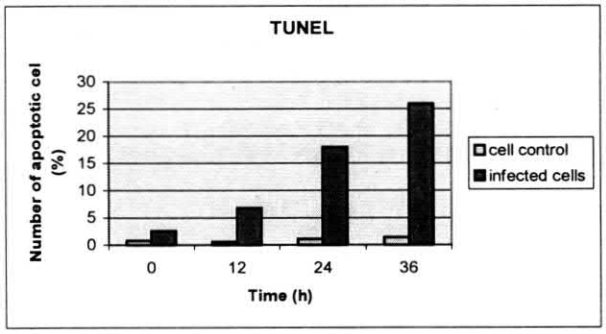

Fig.5: Determination of apoptosis by means of TUNEL in J774 cells after infection by F. tularensis (MOI=100).

One of the later steps in apoptosis is DNA fragmentation, a process which results from the activation of endonucleases during the apoptotic program. We used the APO-DIRECT™ assay, a single-step method for labeling DNA breaks with FITC-dUTP followed by flow cytometry analysis (fig.5).

Cell death was also determined by a photometric enzyme-immunoassay for the in vitro determination of cytoplasmic histone-asociated-DNA-fragments (Cell death detection ELISA PLUS). This method is useful not only to measure apoptotic cell death, but also to determine necrosis (fig.6). From these measurements it is clear that *F. tularensis* kills J774 cells through apoptosis from 10 hours and later after infection by this pathogen. At late time points we can detect mass annihilation of the murine macrophages through necrosis.

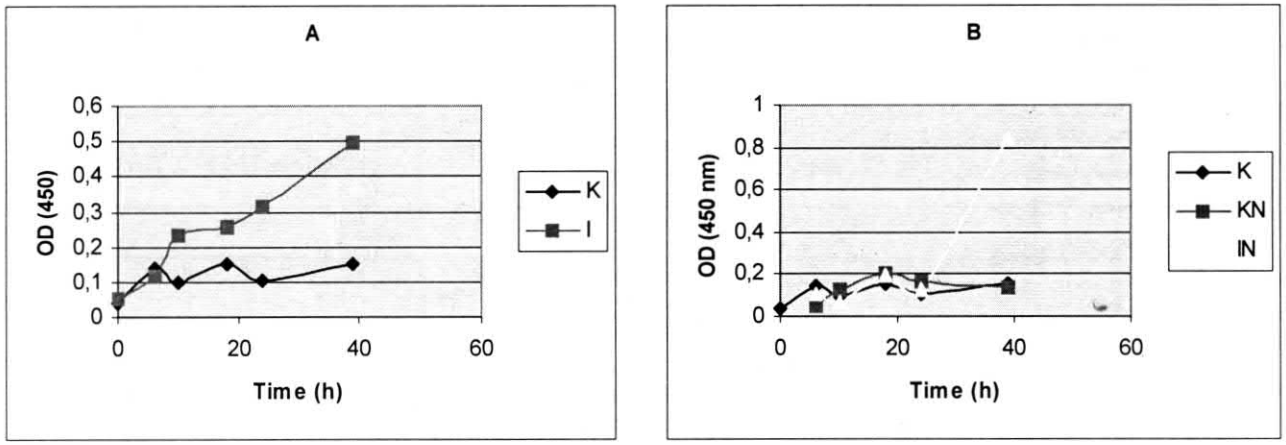

Fig. 6 (A) Induction of apoptosis in J 774 cells after infection by F. tularensis (MOI=100), K = cells control, I = infected cells. (B) Determination of necrosis in J 774 cells after infection by F. tularensis, K = cell control, KN = determination of necrosis in cell control, IN = determination necrosis in infected cells.

Conclusion

Cell cycle block may be caused by presence of *F. tularensis*, but the effect is minimal and not specific.

Infection of J774 cells by F. tularensis induces cell death through apoptosis during early time periods.

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