PHENOTYPIC PROFILE OF PHAGOCYTIC CELLS J774.2 INFECTED WITH *FRANCISELLA TULARENSIS* LVS MICROBES

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The overall aim of this study was to analyze the phenotypic changes of the host cells that can occur during the primary interaction of *Francisella tularensis* microbes with professional phagocytes. The cell line J774.2, derived from female BALB/c mouse (ECACC, No. 85011428), was used as a model of host cell, which can represent first line of defense in host tissues infected with intracellular bacterial pathogens. *Francisella tularensis* live vaccine strain (*Francisella tularensis* LVS, ATCC 29684, American type culture collection, Manassas, Va, USA) was used for infection *in vitro*. Detection and identification of the changes induced by infection was done by flow cytometry. For all selected markers the commercially available specific monoclonal antibodies (Immunotech, BD Pharmingen or Serotech) including negative isotypic controls were used. All of them were directly conjugated with fluorescein isothiocyanate (FITC) or phycoerythrine (PE) fluorochromes. After standard staining procedure the flow cytometric analysis using flow cytometer Coulter^{R} Epics^{R} XL (Coulter, Fullerton, USA) equipped with software version Epics XL Flow Cytometry Work Station – System II^{TM} ver. 3.0 was performed under standard operating procedure. Only identification of intracellular marker MOMA-2 required two additional steps during staining procedure – fixation and permeabilization. The flow cytometric phenotyping of J774.2 cells was monitored before and during 24 hours of infection *in vitro* with *Francisella tularensis* LVS, each 6 hrs and than after 48 hrs.

The markers selected for this study are listed in table 1. All CD markers listed in table 1 have surface epitopes on macrophage membrane, except the marker MOMA-2, which has an intracellular localization.

For the stability of the *in vitro* system, the viability of J774.2 cells was monitored before and during 24 hours of infection *in vitro* with *Francisella tularensis* LVS, each 3 hrs and than after 48 hrs. Viability was monitored in FS (forward scatter) vs. SS (side scatter) dot-plot histogram, two samples and 10 000 cells in each sample were analyzed by flow cytometry in all time intervals. The results

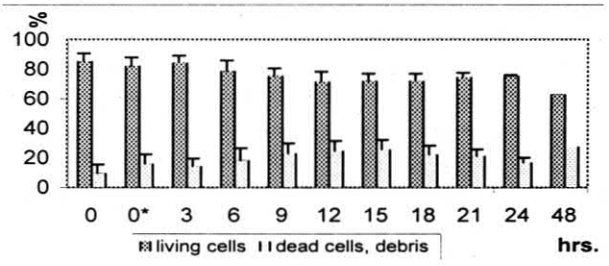
Table 1

List of markers

Marker	Short characterization of molecule
MHC II class	major histocompatibility complex antigens class II
CD11a	leukocyte integrin adhesion molecule
CD11b	leukocyte integrin adhesion molecule
CD14	myelomonocytic receptor for LPS-LBP
CD16/32	Fcγ low afinity receptor for IgG
CD21/35	complement receptor (C3b)
CD45	leukocyte common antigen
CD54	ICAM-1, adhesion molecule
CD71	transferrin receptor
CD80	costimulation ligand for T-lymphocytes
CD86	costimulation ligand for T-lymphocytes
F4/80	MoAb specifically recognized murine macrophages
MOMA-2	murine intracellular macrophage-associated antigen

Table 2

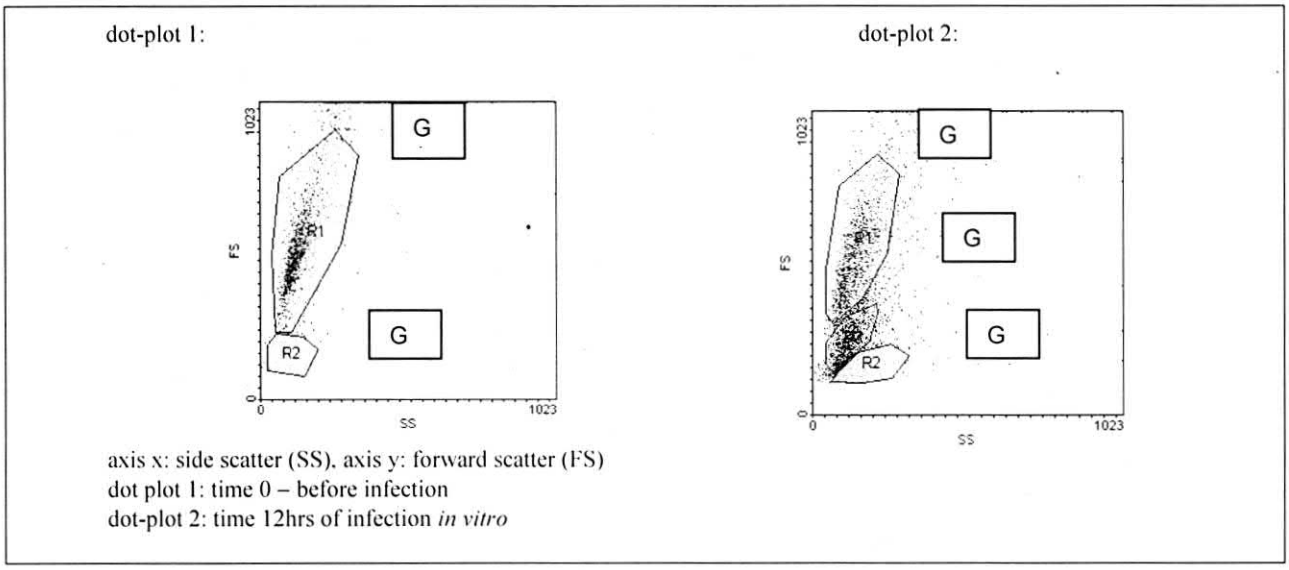
Viability of J774.2 cells during 48 hours of infection with *Francisella tularensis* LVS



axis x: time of *in vitro* infection (hrs)
axis y: percentage of G1 (living cells) and G2 (dead cells, debris)

showed that the cycle of primary infection is finished of about 12 hrs after infection (table 2).

During the course of infection a new subpopulation of cells have appeared in FSxSS scattergram in the middle of upper G1-subpopulation and lower G2-subpopulation seen before infection (figure 1 – dot-plot 2). The existence of third G3-subpopulation has been observed regularly since 12 hrs of infection and later, in some experiments the sign of the existence of third subpopulation was detected as soon as 3 hrs after infection. The frequency of surface markers inside the major subpopulation (gated as G1) was rather stable during the whole cultivation period (48 hrs of infection – table 3).



axis x: side scatter (SS), axis y: forward scatter (FS)
dot plot 1: time 0 – before infection
dot-plot 2: time 12hrs of infection *in vitro*

Figure 1

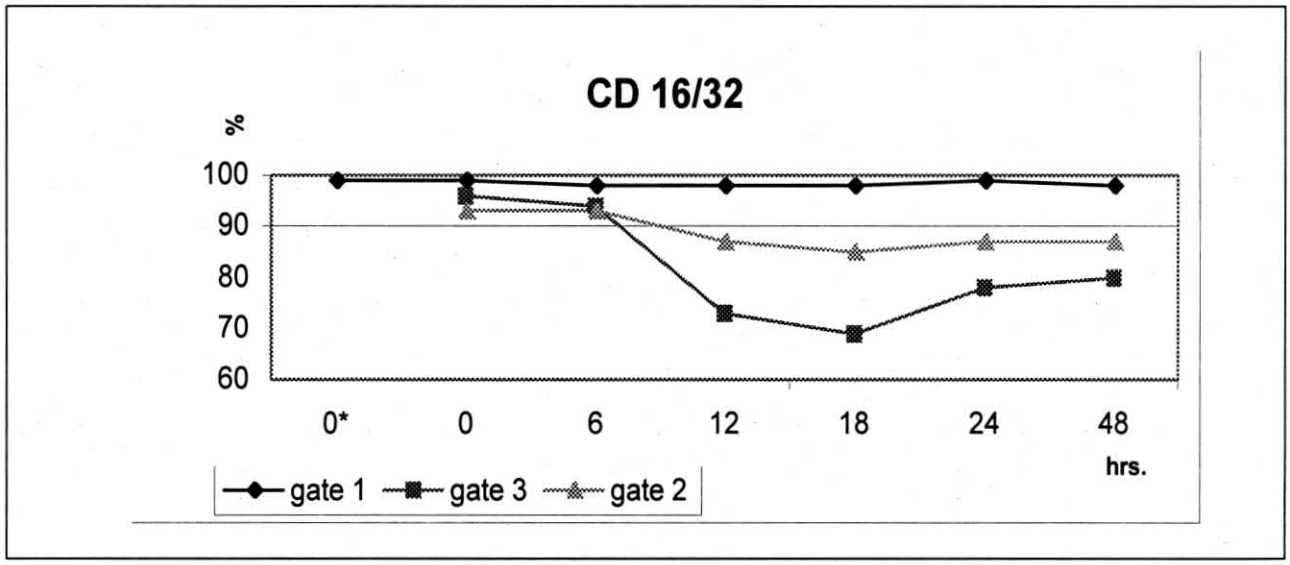
Table 3

Phenotype of J774.2 cell line during infection *in vitro* with *Francisella tularensis* LVS

Time (hrs)	0*	0	6	12	18	24	48
MHC II	1	1	2	2	2	2	2
CD11a	99	98	97	95	96	97	93
CD11b	99	99	99	98	99	99	98
CD14	32	17	34	39	34	40	12
CD16/32	99	99	98	98	98	99	98
CD21/35	97	96	88	90	91	90	92
CD45	99	98	99	97	99	99	99
CD54	98	97	97	95	95	96	97
CD 71	99	96	97	96	96	96	83
CD80	7	8	18	14	11	12	7
CD86	99	98	94	93	95	97	97
F4/80	100	100	99	99	100	100	100
MOMA-2	99	98	99	99	99	99	99

0* * cells J774.2 before infection
0 - time "zero" according Standard infection protocol (cells are de facto already 3 hrs in contact with *Francisella tularensis* microbes)

It seems likely that subpopulation G1 represents the cells primary un-infected (at time zero). On the other side, the phenotype of the cells of subpopulation G3 underwent significant changes and can represent the primary infected cells. The example of one of trends in the phenotype changes induced by infection (the changes in expression of CD16/32) shows figure 2. In this case, the cells of G2 and G3 gates down regulated the expression of CD16/32 after 6 hrs of infection. Similarly, the expression of adhesion CD11a, CD11b and CD54 molecules decreased substantially. The fall of frequency of these surface markers reached 30%. The down-regulation of expression of adhesive molecules could suggest the need of translocation of infected cells to the compartments where the processing and presentation of immune signals will be terminated. The decrease in the same extent was measured also for receptor for transferrin CD71 expression. One from the explanations of this change could be the different need of the infected cell for the delivery of Fe ions. This fact could have special importance for the intracellular multiplication of *Francisella tularensis* microbes, which are fully dependent on the delivery of Fe ions from extra- and intracellular space to the cell compartments where the microbes are multiplied. One can understand to this phenomena as a one of the steps participating in the protective mechanisms of the cell. Deep decrease in surface membrane expression of Fcγ low affinity receptor CD16/32 could be in the same way explained as another step of protection of once infected cells against invasion of another microbes through antibody-Fc receptor mediated entrance. Another explanation for all down-regulated changes could be associated with the induction of apoptosis of infected cells caused by infection. This can be true especially for the down regulation of integrine adhesion molecules expression.



axis x: time of infections (hours)
axis y: % of surface expression (relative values)
gate 1: upper cell subpopulation G1 in FSxSS scattergram
gate 2: lower cell subpopulation G2 in FSxSS scattergram
gate 3: middle cell subpopulation G3 in FSxSS scattergram

Figure 2: Surface membrane expression of CD16/32 marker in three different cell subpopulations in the course of infection in vitro

MHC class II molecules expression displays the second type of changes. The expression of MHC II antigens was substantially up regulated in the course of infection. One can speculate that the over-expression of MHC II molecules could be associated with more prominent antigen-presenting function of infected cells. Similarly to the constant expression of markers in G1 subpopulation, the phenotype of the lowest subpopulation G2 was stable especially in the first half of the course of infection. Changes in phenotype of G2 cells were seen in later intervals, but never so deep as in the G3 subpopulation. All attempts to explain the changes in macrophage phenotype that are induced by infection with *Francisella tularensis* LVS microbes are rather speculative and need further experiments to be clarified and properly interpreted.

In conclusion, we were able to detect some changes in the phenotype of one of the subpopulations of cells inside *in vitro* cultures infected with

Francisella tularensis LVS microbes. New tasks from results of our study oriented to follow of murine macrophage cells J774.2 phenotype can be specified as follows:

1) to define the new subpopulation of cells appearing during the infection;
2) to investigate the significance of these changes during early phase of infection for induction of early stages of the immune response and for consequent inter-cellular communication in the frame of immune system;
3) to interpret the results of changes in macrophage phenotype that are induced by infection with intracellular bacterial pathogen *Francisella tularensis*.

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