LOCAL *IN VIVO* HOST RESPONSES TO INFECTION OF MICE WITH *FRANCISELLA TULARENSIS* LVS

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Francisella tularensis is the causative agent of the disease tularemia. Inhalation of infected dust leads to the respiratory form of the disease, while transmission by arthropods or direct contact with an infected animal causes the ulceroglandular form of the disease. Lethality in mouse model is much more dependent on the route of infection than on the genetic background of mice. LD50 after i.d. infection with *F. tularensis* LVS at both – either susceptible (BALB/c) or resistant (C57Bl/10) mouse strain is higher than $1x10^7$ microbes per mouse [1]. On the other hand, LD50 after aerogenic infection is $3x10^1$ microbe/mouse of strain BALB/c and $1x10^2$ microbe/mouse of strain C57Bl/10 [3].

1. Intradermal infection

The presence of F. tularensis microbes in different organs of infected mice was followed for 15 days after intradermal inoculation of sub lethal dose of app. 0.1 LD50 (5×10^5 microbes per mouse). Bacterial numbers increased during first 3 days in the skin, axillary lymph nodes and spleen, then CFU started to decline in all these organs and 15 days after infection were bacteria found only in the spleen [2,3].

Phenotype of cells isolated from the skin [3]. As few as 3.8% of cells carrying Common Leukocyte Antigen (CD45) was detected in the skin cell suspension of nonifected mice. Majority of CD45+ cells was created by T-cells, mostly γ/δ TCR-positive cells (70% of CD3+ cells) that are known as DETC – dendritic epidermal T cells. These cells express an oligoclonal repertoire that enables them to respond to a variety of antigens without the need for diverse T-cell receptor, possibly by recognition of stress-induced self-antigens or of conserved foreign antigens. Proportion of CD45 positive cells increased after infection and reached 14% on day 6. Proportion of T-cells rised at 24 hours after inoculation of *F. tularensis* and this enhancement was caused by influx of CD4 cells. Very early after infection the increase in percentage of B-cells was observed, from 0.3 % CD19+ cells in control mice to 1.9 % CD19+ cells of all measured skin cells 24 hours after infection. Only minimal numbers of monocytes, macrophages and dendritic cells were detected in skin suspension of control mice and this proportion persists up to day 6 after i.d. infection when the F4/80-/MHC class II- cells (monocytes, immature macrophages) and F4/80+/MHC class II+ (mature macrophages, immature dendritic cells) increased and reached 4% of all events. During next 3 days all immature cells (7% of all events) switched to F4/80-/MHC class II+ phenotype, which is typical for dendritic cells called in the skin Langerhans cells. Langerhans cells are able to prime nad've CD4+ T-cells and are responsible for antigen processing and presentation in the skin.

Determination of expression and level of cytokines in the skin. It is known that initial survival of *F. tularensis* is clearly depend on the production of cytokines IFN-γ and TNF-α. At the early stage of murine tularemia IL-12 and TNF-α. are expressed by infected mononuclear phagocytes, and these cytokines are believed to trigger the production of IFN-γ by NK-cells. Although it is quite difficult to determine production of cytokines in the skin, the results from immunohistochemistry study [2], RT-PCR [3] and ELISA [3] were obtained corresponding to numerous other works: the production of IFN-γ and TNF-α was detected at the site of infection. But, in comparison with production of TNF-α in the spleen after s.c. or i.v. infection, it is necessary to say that amount of TNF-α at the site of infection is much lower. Presence of IL-12 was also detected in the skin after infection by immunohistochemistry and ELISA.

2. Aerogenic infection

Several reports were published regarding aerogenic infection by *Francisella* in the past – in the 60´s and 70´s. The scientists were mostly interested in possible protection against inhaled microbes of virulent strains. On this account the majority of reports described numbers of CFU in the lungs and lymphoid organs, level of antibodies or histological study of lung tissue after infection. The authors used virulent strains: either Type A (*F. tularensis tularensis*) or Type B (*F. tularensis palearctica*) in these years [4,5].

Number of CFU in the lungs after aerogenic infection with *F. tularensis* SCHU strain (Typ A) and *F. tularensis* 130 strain (Typ B) grew a few

logarithms a day up to the death on day 5 at all infected animals [6]. The growth of bacterial number in the lungs after aerogenic infection with vaccine strains (LVS and EG-15) showed similar trend as the virulent strains in the beginning of infection but after 6 days decreasing number of CFU was observed [3,5]. *F. tularensis* LVS was also still detected 4 weeks after aerogenic infection of BALB/c (susceptible) but not of C57Bl/10 (resistant) mouse strain. Granulomas were located by haematoxilin-eosin and their presence and intensity corresponded with detection of *F. tularensis* in lung tissue.

Phenotype of cells isolated from bronchoalveolar lavage and lung tissue from the C3H/HeN mouse strain [3]. The number of about 2% CD45 positive cells found in the bronchoalveolar lavage grew after infection and reached 25% 10 days after aerogenic infection. The majority of these cells expressed F4/80+/MHC class II+ cell phenotype. The situation was different for lung tissue cells. CD45 positive cells composed more that 70% of isolated cells and the proportion was almost constant in the course of infection. Lymphocytes constituted most of leukocytes (80%) while monocytes and macrophages represented only 20% of measured cells. In control mice half of them were of F4/80+/MHC class II- phenotype and the rest was F4/80+/MHC class II+. The number of mature macrophages (F4/80+/MHC class II+) was broadly enhanced in the course of infection and also mild growth of dendritic cells was observed. Unlike the situation in the skin, the decay of T cells (CD3) was registered 24 hours after infection in the lungs. Both CD4 and CD8 participated in the reduction of T-lymphocytes. B-cells, which composed 16% of CD45 positive cells in control mice, increased 24 hours after infection and reached 25% of CD45+ cells. Presence of neutrophils in lung tissue was detected by histochemistry very early after infection (after 2 hours for resistant mouse strain and 4 hours for susceptible strain) however already after 12 hours neutrophils were not found.

Determination of cytokines produced by cells of bronchoalveolar lavage (TNF-α, IL-1β) and by cells isolated from the lung tissue (IFN-γ, IL-4) was provided using ELISA method [4]. The enhancement of TNF-α production by cells from susceptible mouse strain BALB/c was detected 24 hours after aerogenic infection. Detected basal level of TNF-α production in control mice C57Bl/10 was lowered in the course of infection. The high expression of TNF-α-receptor type II early after infection and binding of cytokine to this receptor is a possible explanation. Resistant mice started to produce other inflammatory cytokine IL-1 on day 3 after infection while in susceptible BALB/c mice this cytokine was not detected. Among cytokines produced by lymphocytes Th1 cytokine IFN-γ was produced by resistant strain in the course of infection and reached 3 100 pg/ml on day 10. Interleukin 4, as cytokine causing switch of the immune response to Th2 branch, was also measured. It seems that production of this cytokine influences ability to eliminate bacteria but the mechanism is unknown. Resistant mice C57Bl/10 produced 350 pg/ml of IL-4 only at 24 hours after infection. Similar results were obtained by Macela [5] after s.c. and i.p. infection when only resistant strain produced IL-4 after tularemia infection.

Production of antibodies. The role of specific antibodies is less well understood. Nevertheless, in murine model of disease, it has been shown that passive transfer of immune serum causes a decrease in mortality rate [5,6]. The level of total specific antibodies in the bronchoalveolar lavage fluid (BALF) and their isotypes was determined by ELISA method [3]. Increased level of specific antibodies was detected only at 3 days after infection in BALF obtained from BALB/10 mice. As prevailing isotype IgG1 was identified. Seven days after infection in both strains – susceptible and resistant –enhancement of specific antibodies was observed and specific antibodies persist for weeks in BALF and serum [3,5]. IgM and IgA isotypes created main proportion of specific antibodies in C57Bl/10 mice and IgM, IgG2a and IgG2b in BALB/c mice at later intervals in the course of infection.

Summary

Phenotype of cells and mechanisms participated in the innate immune response caused by infection with *Francisella tularensis* LVS in the skin and lungs are different. While the majority of immunocompetent cells in the skin create γ/δ T-cells, mostly monocytes and macrophages are present on the alveolar surface of the lungs at the control mice. Macrophages are also detected in interstitium of the control lungs although lymphocytes create majority of CD45 positive cells in the suspension isolated from the lung tissue. Proportion of T-lymphocytes increases in the course of infection in the skin and this enhancement is caused by elevation of CD4 positive cells at early stage of infection followed by expansion of γ/δ T-cells on day 3. In contrast to the skin, among lung T-lymphocytes proportion of CD8 and γ/δ T-cells decreases 24 hours after aerogenic infection. It seems that influx of B-cells into site of infection and adjacent lymph organs is independent on the genetic background and the route of infection. The late influx of macrophages and their

switching to dendritic cell phenotype is observed in the skin while lung macrophages, carrying F4/80 marker together with MHC class II molecules, are present even in control mice. Their number is increased early after infection but conversion to dendritic cells is much weaker than in the skin. The large production of IFN-γ and IL-12, Th1 cytokines, and low amount of proinflammatory cytokine TNF-α is present in the skin whereas considerable production of TNF-α and weak production of IFN-γ follow aerogenic infection in the lungs. The mechanisms of defence vary at the site of infection and are more effective in the skin.

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