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DYNAMICS OF NON-SPECIFIC ANTIBACTERIAL ACTIVITY OF THE PERITONEAL CELLS OF MICE INDUCED WITH *COXIELLA BURNETII* ANTIGEN

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Summary. - It was found that primary immunization with *Coxiella burnetii* antigen increased mouse resistance to *Salmonella typhimurium* infection as evidenced by acceleration of bacterial elimination from the peritoneal cavity and a decrease in lethality of experimental animals. The existence of two rises of bactericidal activity of mouse peritoneal cells was ascertained: the „early" on days 1 and 2, and the "late" on day 14 after *C. burnetii* administration. The first rise was accompanied by some increase in the number of peritoneal cells as well as by some change of their qualitative representation. The second increase of antibacterial activity was detected during the pronounced cellular and humoral immune responses to *C. burnetii.*

Key words: Coxiella burnetii; Salmonella typhimurium; specific and nonspecific resistance; peritoneal cells

Introduction

The increasing bactericidal action of macrophages is one of the most effective criteria for evaluation of their participation in the formation of cellular immunity and their activation by cytokines including those that control the functions of macrophages (Freidlin, 1984).

In this connection it was interesting to trace the dynamics of the antibacterial activity of mouse peritoneal cells immunized by *Coxiella burtnetii* antigen and to compare the level of non-specific defence against *Salmonella typhimurium* with the level of acquired specific anti-*Coxiella* immunity.

Materials and Methods

Outbred and C3HA mice weighing 20-25 g were used as experimental model. A corpuscular phase I *Coxiella burnetii* antigen of *"Apodemus flavicollis*-Luga" strain was used for their immuni-

zation (3rd chick embryo passage). The antigen was standardized by the method of enzymoimmunoassay (Tokarevich *et al.*, 1989) and diluted in phosphate-buffered saline (PBS) pH 7.4. The animals were immunized intraperitoneally (i.p.) with 100 μg of antigen suspended in 0.5 ml of the PBS. Control animals were injected 0.5 ml of PBS.

Virulent cultures of S. typhimurium were opsonized by suspending in PBS containing 10 per cent of anti-*Salmonella* antibody. After 30 min of incubation at 37 °C the *Salmonella* were injected i.p. into mice. Immediately and 2 hr later each mouse was injected i.p. with 3 ml of PBS containing 10 units of heparin per ml. The mice were immediately killed by cervical dislocation and the fluid from the peritoneal cavity was removed. The BSA-water was added to lyse the phagocytes. Three serial 1:10 dilutions were made at 0 °C and were plated on bismuth sulphite agar. The colonies were counted after 24 to 48 hr of incubation at 37 °C. The fraction of *Salmonella* killed *in vivo* was calculated as follows: 1 g CFU in peritoneal fluid immediately after injection minus 1 g CFU in peritoneal fluid after 2 hr. The antibacterial activity of the peritoneal cells from mice which had been previously immunized with *C. burnetii* antigen was counted at different periods and expressed as follows; the amount of the killed *S. typhimurium* within 2 hr by macrophages from animals minus the amount of the killed *S. typhimurium* within 2 hr by macrophages from control animals.

The influence of preliminary *C. burnetii* immunization on the survival of the mice infected with *S. typhimurium* was followed after intraperitoneal test-strain injection. Infecting dose was 10^5 microbial cells per animal. The dynamics of the total number and relative quantity of peritoneal exudate was studied at the mice after intraperitoneal injection of antigen (these were animals of the test group) and of PBS (the control group of the animals). The initial level of indices was determined in intact animals. The relative quantity of peritoneal exudate cells was detected in the smears coloured according to Romanovsky-Giemza.

To control the dynamics of the specific response onto injection of *C. burnetii* antigen we used complement fixation test (CFT), indirect immunofluorescence reaction (IIFR), leukocyte adherence inhibition (LAI) and delayed hypersensitivity test (DHT). Lymphocyte sensibilization with the help of LAI test was detected according to the method (Halliday, 1972) in our own modification (Tokarevich, 1985). DHT was conducted according to the method (Kazár, 1982) with the antigen obtained by extraction with trichloroacetic acid of *C. burnetii* corpuscular antigen at phase I. The obtained data were analysed statistically with Student's criterion (under $P \leq 0.05$).

Results and Discussion

Specific response dynamics was typical for *C. burnetii* antigen immunization. Antibodies to phase II antigen were discovered 6 days after immunization, the

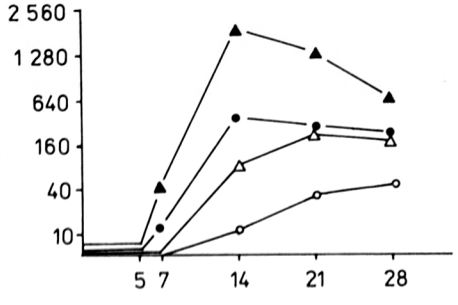

Fig. 1
Detection of antibodies in mice immunized by *C. burnetii* antigen
Antibodies to *C. burnetii:*
phase II indirect immunofluorescence ▲
phase I test △
phase II complement fixation test ●
phase I ○
Vertical bars - confidence intervals.
Abscissa - days after immunization, ordinate - mean antibody titres.

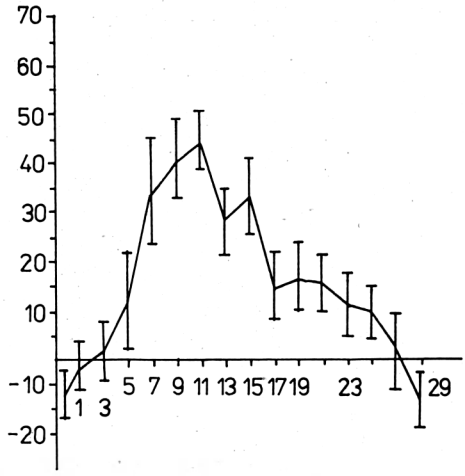

Fig. 2
Leukocyte adherence inhibition test of mice immunized by *C. burnetii*
Vertical bars - confidence intervals.
Abscissa - days after immunization, ordinate - the level of sensibilization

IIFR titres being lower than the CFT titres (Fig. 1). By the 14 days the serum antibody titres reached maximum levels. Thereafter their levels began to decrease gradually but still remained rather high till the end of the observation period. Antibodies to *C. burnetii* phase I antigens were discovered later, their level was lower than the level of the antibodies to phase II antigen. Positive DHT was not found till the 7th day after immunization and lasted for the whole testing period.

Lymphocyte sensibilization according to LAI test was revealed from the 4–5 day, maximum sensibilization level appeared after the 1st week post- immunization (Fig. 2).

Microscopic examination of the peritoneal exudate demonstrated that injection of *C. burnetii* antigen caused short increase of the total amount of peritoneal cells that reached their maximum 6 hr after injection of preparation (Fig. 3-I).

There were some significant changes in the relative quantity of cells of the peritoneal exudate in experimental animals: for example, by 6 hr after immunization a great increase of neutrophiles was found. It appeared that the granulocytes made 30 per cent from the total cell count during a moderate increase in the amount of lymphocytes. The increase of the number of these cells in control animals during the same period was insignificant (Fig. 3-II). On the 2nd day after immunization the amount of neutrophiles and lymphocytes decreased in test animals, however, the level of neutrophiles (0.48 mln/ml) remained rather high in comparison with the control mice where the relative quantity of the cells returned to normal and did not change practically in future. The relative quantity of the cells in peritoneum at test animals was restored by

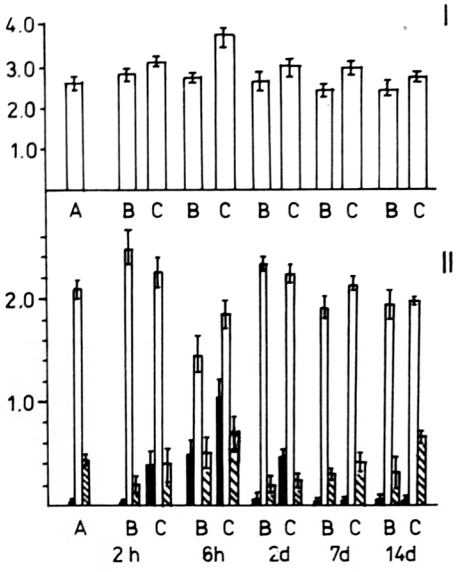

Fig. 3
Influence of *C. burnetii* antigen immunization on the relative composition of peritoneal exudate.
I. Total amount of cells per 1 ml of peritoneal exudate.
II. Dynamics of changes in the relative composition of peritoneal exudate cells.
A - intact animals
B - mice inoculated with PBS
C - mice inoculated with *C. burnetii* antigen
Abscissa - time (hours, days) after immunization, ordinate - number of cells (x 10^6); black columns - number of neutrophiles, empty columns - number of macrophages and monocytes, shaded columns - number of lymphocytes.

the 7th day, however, on the 14th day after immunization there was a second increase of the number of lymphocytes unlike to the control group.

After inoculation of intact mice with *S. typhimurium* a moderate clearance of these bacteria from the peritoneum could be observed for 2 hr. Thus, previous immunization of mice with *C. burnetii* antigen at least at distinct periods caused marked increase of clearance activity (Fig. 4). One can distinguish two peaks of this activity: an early one - from 6 to 48 hr and a late one - on the 14th day after immunization. This 2nd activity increase lasted for a rather long period between 7th till the 21st day post-immunization.

The obtained data prompted us to examine the influence of preliminary immunization with *C. burnetii* antigen on survival of the mice infected with *S. typhimurium*. It was established that 87.6 per cent ±6.1 of non-immunized mice (control group) infected with *S. typhimurium* succumbed (observation period - 6 days). The mice which had been immunized preliminary with *C. burnetii* 48 hr and 14 days before, succumbed to *Salmonella* infection more seldom than controls (29.0 %±16.9 and 17.0 %±14.7, respectively). There were also some differences in the dynamics of death of animals in comparison with the control group (Fig. 5).

In accordance with the results of others (Damrow *et al.*, 1981; Kazár *et al.*, 1984; Kelly, 1987) we have shown that *C. burnetii* immunization resulted in marked increase of the host resistance to heterogenous microorganisms. Our experiments established that changing activity in peritoneum has a cyclic character. It must not be excluded that to a certain extent at least the first peak of this activity is connected with the increase of the number of peritoneal cells and with the quality changes in their relative quantities. For example, 2–6 hr after *C. burnetii* immunization there was a total increase of their number; contemporarily greatly increased the amount of neutrophiles which have antimicrobial activity against *Salmonella* as well (Khavkin *et al.*, 1977). However, the peak of antibacterial activity is registered on the background of normalization of the total amount of peritoneal cells and some decrease of the amount of neutrophiles (in comparison with maximum levels). Decisive significance in the increase of clearance of *Salmonella* from peritoneal cavity belongs to the activated macrophages (Dunn *et al.*, 1985).

As known, *C. burnetii* antigens are capable to activate macrophages (Kelly, 1977; Freidlin, 1984). Besides, it was established that during the first hours after *C. burnetii* antigen immunization of mice the interferon system activity also increased (Brezina *et al.*, 1968; Kramskaya *et al.*, 1989). Activating effect of

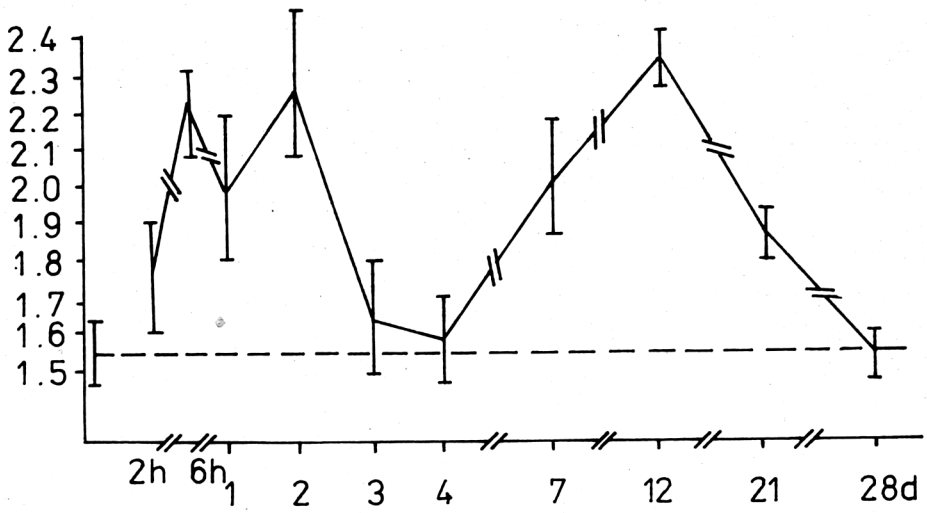

Fig. 4

Antibacterial activity of peritoneal cells of mice immunized by *C. burnetii* antigen

———— experiment

– – – – control

Vertical bars – confidence intervals.

Abscissa – time (hours, days) after immunization, ordinate – amount of killed *S. typhimurium* (logarithm difference).

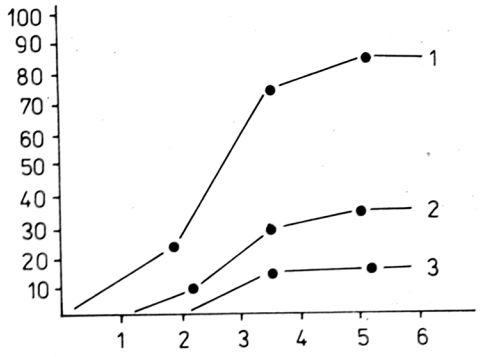

Fig. 5
Lethality dynamics of mice infected by *S. typhimurium:* immunized with by *C. burnetii* antigen (experiment) and non-immunized (control)
1 - intact animals (control)
2 - animals immunized 2 days before infection
3 - the same 14 days before to infection
Abscissa - time (days) after infection, ordinate - amount of succumbed animals (%).

interferons onto macrophages was registered by many authors (Freidlin *et al.*, 1990). Activating effect of the interferons onto natural killers was also described. At the same time it was established that 6 hr after *C. burnetii* immunization - during the first peak of clearance activity - there was a great increase of natural cytotoxicity of mice splenocytes (Macela *et al.*, 1985; Tokarevich *et al.*, 1990), which did not only perform extensive regulatory functions through the system of numerous mediators but had a direct antibacterial effect (Lomakin, 1989).

The 2nd peak of the antibacterial activity was registered from the 7th day after immunization at the period when antibodies to *C. burnetii* appeared in the blood and positive LAI test and DHT could be detected. Maximum of this activity is registered on the 14th day and is accompanied by increasing the number of lymphocytes in the peritoneal exudate of mice and high indices of cellular and humoral immunity.

One can suppose that during the second increase of antibacterial activity a noticeable role is played by lymphokines which at the same time affect leukokocyte adherence (Halliday, 1979). One may suppose that monitoring of γ-interferon can be used for analysis of post vaccinal immunity (Kazár, 1988).

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