ANTIMICROBIAL CATIONIC PROTEINS OF NEUTROPHILIC GRANULOCYTES IN EXPERIMENTAL Q-RICKETTSIOSIS

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Interest in the study of phagocytes as the effector stage of immunity has increased considerably in recent times. Ideas have crystallized on the cationic proteins (CP) of neutrophilic granulocytes (NG) as one of the leading molecular mechanisms maintaining the defensive reactions of the body [2, 4, 5, 12]. It has been suggested that there are three interconnected mechanisms of the antimicrobial action of the granular CP of neutrophils: preparation of bacteria for phagocytosis, a direct antimicrobial action, and stimulation of phagocytosis and of the antimicrobial activity of macrophages [2, 12]. The cytochemical method of estimation of CP, which has been called the lysosomal-cationic test (LCr), has been found to be an effective method of studying the functional activity of NG and their ability to inactivate microorganisms [3].

Information on the functional state of NG in the case of infection by *Coxiella burneti* is scanty [10, 11] and insufficient, and it was therefore decided to determine the dynamics of changes in the LCT findings in animals infected and immunized with *C. burneti,* and to evaluate the effect of preliminary immunization on the antimicrobial potential of NG.

EXPERIMENTAL METHOD

Experiments were carried out on male guinea pigs weighing 250 ± 20 g. The animals as a whole were divided into six groups, 10-12 in each group. Experimental guinea pigs of groups 1 and 5 were given subcutaneous injections of corpuscular antigen, prepared from *C. burneti* phase 1, strain "Yellow-necked field mouse – Luga" in a concentration of 50 μg per animal. Experimental animals of group 2 were given an injection of a living culture of *C. burneti* of this same strain, subcutaneously, and those in groups 3 and 4 – intraperitoneally. The culture of *C. burneti* was titrated in albino mice weighing 12 g. The infective doses were expressed as ID_{50}, calculated by the method of Reed and Muench, as described previously [8]. Animals of the five groups were immunized beforehand with *C. burneti* antigen, those of group 6 with prowazeki antigen (produced at the N. F. Gamaleya Institute) in a dose of 4 complement-fixing units per animal, and they were infected on the 28th day with *C. burneti.* The dose of infection was $2 \cdot 10$ ID_{50} (D1), and in group 4 this was increased to $2 \cdot 10$ ID_{50} (D2). The presence of rickettsias in blood films was determined by the method of Zdrodovskii [1] and Coons.

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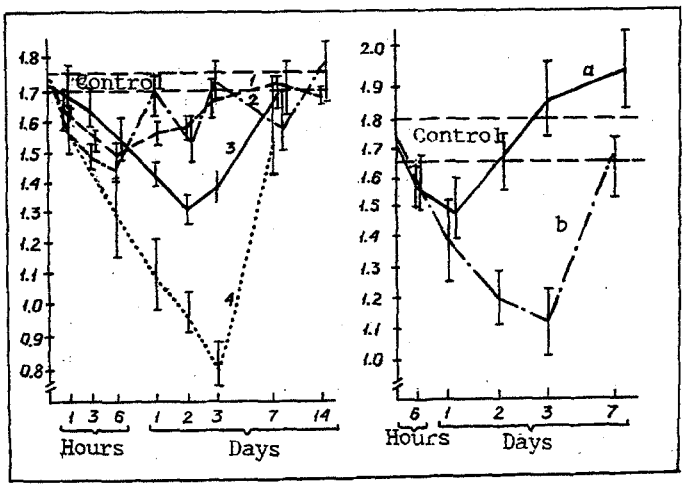

Fig. 1

Fig. 2

Fig. 1. Dynamics of change in antimicrobial potential of NG depending on mode of administration and dose of *C. burneti*. Abscissa, time after injection of *C. burneti*; ordinate, parameters of LCT (MCC): 1) subcutaneous injection of antigen from *C. burneti*, 2) subcutaneous injection of living culture of *C. burneti*, 3) intraperitoneal infection in a dose of D1, 4) intraperitoneal infection in a dose of D2.

Fig. 2. Change in antimicrobial potential of NG in animals immunized beforehand with autologous and heterologous antigen. Abscissa, days after infection; ordinate, parameters of LCT (MCC): a) animals immunized with antigen obtained from *C. burneti*; b) animals immunized with antigen obtained from *C. prowazeki*.

To assess the functional state of NG, films of the peripheral blood were made before infection and during the course of the vaccinal and infectious processes, after 1, 3, and 6 h and on the 1st, 2nd, 3rd, 7th, and 14th days, and used for cytochemical determination of CP with the aid of LCT [6]. Unfixed blood films were immersed in buffered alcoholic solution of Fast Green (pH 8.15) for fixation and staining, sprinkled with water, and transferred to azure solution, washed with water, dried, and examined under the microscope with magnification of 900-1000. To assess the results of cytochemical analysis, a semiquantitative method based on determination of the mean cytochemical coefficient (MICC) was used. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Experiments with subcutaneous injection of antigen (group 1) showed that 1 h after immunization the content of CP was lower than initially (1.77 ± 0.07) in the peripheral blood neutrophils (Fig. 1), and minimal values were reached after 6 h (1.50 ± 0.06, $p < 0.01$). Starting with the 2nd day, the LCT values showed an increase, and by the 3rd day their initial values were restored. Later, throughout the period of observation, the CP level remained within normal limits. There was no rise of body temperature in the experimental animals of this group.

After subcutaneous injection of the living agent (group 2) the fall in the CP level was found to be wavelike and decremental in character (Fig. 1) with the maximal decrease of its values likewise 6 h after infection (1.45 ± 0.03, $p < 0.01$). Rickettsias were found in the blood of three animals of this group on the 2nd-7th days after infection, and during the same period the body temperature rose to 39.8°C.

After intraperitoneal infection with *C. burneti* a significant (1.59 ± 0.09, $p < 0.01$) fall of the CP level did not begin until 6 h after injection of the agents, and continued for 2-3 days, to reach minimal values (1.32 ± 0.04). The CP concentration in peripheral blood neutrophils was not restored until the 7th day. The body temperature of all the animals was raised as early as 2 days after infection and on the 3rd day it reached 40.5°C, returning to normal at the end of the week. The presence of coxiellas in the animals' blood could not be detected after the 7th day of the disease.

An increase in the dose of material injected intraperitoneally (group 4) led to the greatest decrease (by half) in the CP content (0.82 ± 0.09), and to slower recovery of the antimicrobial potential of NG. Two guinea pigs, which had the lowest LCT parameters (1.01 and 0.80) 24 h after infection, died on the 8th and 9th days of the disease. At autopsy, hundreds of *C. burneti* cells were found in each field of vision in their spleens.

In experiments with intraperitoneal infection of previously immunized animals (group 5) the response of the body was found to develop much more rapidly (Fig. 2). On the 1st day after infection minimal values for the CP content were found in the blood neutrophils (1.47 ± 0.04, $p < 0.01$), but by the 2nd day this parameter had returned to its initial value (1.70 ± 0.03). Starting with the 3rd day, the LCT parameters were higher than their initial level, and remained so until the 7th day after infection (1.95 ± 0.03, $p < 0.01$). On infection of guinea pigs immunized beforehand with prowazeki antigen the time course of the CP content was virtually identical with that in immunized animals (Fig. 2).

Significant changes in the antimicrobial potential of the blood NG were thus found in the course of both vaccinal and infectious processes in experimental Q-rickettsiosis, the severity and character of which were largely determined by the dose on mode of administration of *C. burneti.* Subcutaneous injection of the antigen caused a decrease in the LCT values, and in this case this was evidence of intensive secretion of CP by neutrophils into the blood plasma, followed by gradual restoration of the antimicrobial potential of NG on account of cells of the bone-marrow reserve. Subcutaneous injection of the living agent led to a fluctuating change in the level of CP in the neutrophils, evidently associated with the periodic entry of coxiellas into the blood, as described by Khavkin [9]. In the course of development of the infectious process the antimicrobial potential of NG fell sharply on the 2nd-3rd day, at a time when rickettsiemia was beginning, with the appearance of interleukin-1 [13]. The LCT parameters returned to normal by the 6th-7th day, at a time when, in experiments on mice, antibodies were found to be appearing, and the DTH reaction was beginning to develop [7, 14]. It must be emphasized that the presence of immunity to C. burneti reduced almost by half the decrease in antimicrobial potential, and subsequently a higher protective potential was formed.

The results suggest that NG participate directly in the development of the infectious process in Q-rickettsiosis, secreting antimicrobial CP into the blood stream. Intensive disintegration of NG in infected tissues also leads to a high CP concentration outside the granulocytes. Their utilization by macrophages may determine development of the resorptive resistance effect [4].

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