ANTITOXIC AND PYROGENIC PROPERTIES OF RABBIT POLYMORPHS

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Leukocytes from peritoneal exudate of rabbits obtained after intraperitoneal injection of physiological NaCl solution secrete substances neutralizing staphylococcal toxin and possessing a pyrogenic action. The antitoxic activity of the leukocytes rose sharply after contact with pyrogenal or with vaccine obtained from Bacillus mesentericus. Extracts of the leukocytes, and fractions of the granules, mitochondria, and hyaloplasm do not possess antitoxid or pyrogenic activity.

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Our previous investigations [2] showed that after contact with bacterial pyrogens (pyrogenal, vaccine obtained from Bacillus mesentericus) the leukocytes of a peritoneal exudate of albino mice acquire the power of neutralizing staphylococcal toxin. The nature of this phenomenon is uncertain. All that can be said is that the antitoxic properties of the leukocytes appear under the same conditions as leukocytic pyrogen. Production of leukocytic pyrogen (like acquisition of antitoxic properties by leukocytes) takes place only by living cells, and in experiments in vitro it is stimulated by addition of bacterial pyrogens [1, 3, 4, 9, 11-13, 14].

In the present investigation the antitoxic and pyrogenic properties of leukocytes were compared in rabbits, because all the data on leukocytic pyrogen which were mentioned above were obtained with peritoneal exudate leukocytes of rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 216 rabbits weighing 2.5-3 kg. To obtain exudate the rabbits were injected intraperitoneally with 400 ml 0.85% NaCl solution or with the same volume of physiological saline with 20 μg pyrogenal or 10 billion cells of heat-killed B. mesentericus vaccine. A second injection of 400 ml 0.85% NaCl solution, but without pyrogenal or B. mesentericus vaccine, was given 18 h later. The rabbits was sacrificed by air embolism 3 h after the second injection. Exudate from the peritoneal cavity was collected in cooled glass flasks and heparin was added (5 units/ml). The number of cells in the exudate was 3-5 millions per ml, 80-90% of them polymorphonuclear leukocytes. The exudate was centrifuged at 1500 rpm for 10 min and the residue of leukocytes made up into a suspension in Hanks' salt solutiom, which was then mixed with staphylococcal toxin (1:1).

Leukocytes were used in concentrations of 60, 150, or 300 millions/ml. The mixtures of leukocytes with toxin were incubated on a water bath at 37° for 2 h, with periodic agitation. Neutralization of toxin by the leukocytes was judged from the results of intranasal administration to albino mice (weighing 10-12 g) of 0.025 ml of leukocyte suspension containing 0.012 ml staphylococcal toxin, and in control experiments from the results of administration of the same dose of toxin, but without leukocytes. Altogether 2320 albino mice were used in the experiments. The toxin was prepared in the department of microbiology of the Institute of Experimental Medicine. A pathogenic lecithinase-positive staphylococcus (strain 5/2) was grown for 5 days on 0.3% semisolid agar in an atmosphere containing 30% CO_2, the resulting culture was filtered, and the filtrate was used in the experiments. The pyrogenic activity of the preparations was tested by injecting them into rabbits intravenously in a dose of 1 ml/ kg. The rectal temperature was measured twice with

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an electrothermometer: before the injection to determine the initial background, and every 30 min thereafter for 3 h. In the course of the preparatory work steps were taken to prevent contamination of the preparations with bacterial pyrogens.

EXPERIMENTAL RESULTS

Antitoxic and Pyrogenic Properties of Whole Leukocytes. We began the investigations with the study of the antitoxic properties of leukocytes extracted from the peritoneal cavity of rabbits after two injections of 0.85% NaCl solution. Total suppression of activity of the toxin occurred when a leukocyte suspension containing 300 million cells/ml was used. With a decrease in concentration of the suspension by half, only part of the staphylococcal toxin was neutralized, and a concentration of 60 million cells/ ml was ineffective. The ability of the leukocytes to neutralize staphylococcal toxin increased sharply after injection of bacterial pyrogens into the rabbits. Leukocytes obtained from peritoneal exudates of rabbits 21 h after injection of pyrogen (20 μg) or B. mesentericus vaccine (10 billion bacterial cells) completely suppressed activity of the staphylococcal toxin, even in a concentration of 60 million cells/ml. The results of tests of the pyrogenic properties of whole leukocytes showed that following incubation of leukocytes in vitro for 2 h in 0.85% NaCl solution at 37°, an accumulation of pyrogenic substances took place in the liquid layer.

To determine whether the mechanism of the antitoxic and pyrogenic action of the leukocytes is associated with substances contained within the cells, we carried out a series of experiments with subcellular fractions of leukocytes.

Antitoxic and Pyrogenic Properties of Subcellular Fractions of Leukocytes. Extracts and certain subcellular components of the leukocytes were used. The extracts were prepared as described by Hirsch [10], and fractions of granules, mitochondria, and hyaloplasm were isolated by the method of Cohn and Hirsch [7]. No antitoxic or pyrogenic activity was found in extracts or fractions of granules, mitochondria, and hyaloplasm after incubation of these fractions in 0.85% NaCl solution at 37° for 2 h, either separately or when pooled mechanically.

We were thus unable to find antitoxic and pyrogenic substances within the leukocytes. It may be that these substances are present in the cell in very small amounts, are extracted from it gradually, and accumulate in the surrounding medium. All subsequent experiments were carried out with leukocytic pyrogen contained in each cell-free layer.

Antitoxic and Pyrogenic Properties of Leukocytic Pyrogen. To obtain leukocytic pyrogen a suspension of leukocyte in 0.85% NaCl solution was incubated at 37° for 2-4 h with periodic shaking. After incubation, the leukocytes were sedimented by centrifugation at 1500 rpm for 10 min [3, 5, 6]. The top cell-free layer contained leukocytic pyrogen, which, when injected intravenously into rabbits, caused elevation of the body temperature (Fig. 1). Addition of the top layer to the staphylococcal toxin and incubation of the mixture at 37° for 4 h led to neutralization of the toxin (Fig. 1). As in the experiments with whole leukocytes, this effect was dependent on the concentration of leukocytes taken for preparation of the leukocytic pyrogen. Considerable depression of activity of the toxin was observed when a top layer obtained after incubation of 60 million cells/ml was used. Of the 40 mice receiving a mixture of top layer with toxin by the intranasal route 10 (25%) died, whereas the mortality of the control mice from the same dose of toxin was 95%. With a decrease in concentration of the suspension to 300 million cells/ml no difference was found between the effects of intranasal administration of the top layer plus toxin and of toxin alone to the mice. Native liquid fraction of the peritoneal exudate likewise possessed no antitoxic activity.

Different results were obtained (under the same conditions) with leukocytes from the peritoneal cavity of rabbits after administration of pyrogenal to the animals (20 μg in 400 ml physiological saline). Contact for 4 h between the toxin and top layer of a leukocyte suspension containing 300 million cells/ ml at 37° led to neutralization of all the toxin.

The most marked pyrogenic and antitoxic properties were exhibited by a concentrate of leukocytic pyrogen prepared by lyophilization. The resulting preparation retained its properties when kept in the cold for more than 6 months (period of observation).

In the next experiments the effect of the time of incubation of the leukocytes in vitro with 0.85% NaCl solution on secretion of biologically active substances into the surrounding medium was studied. According to Collins and Wood [8], if leukocytes are incubated in 0.85% NaCl for 24 h, the top layer acquires the

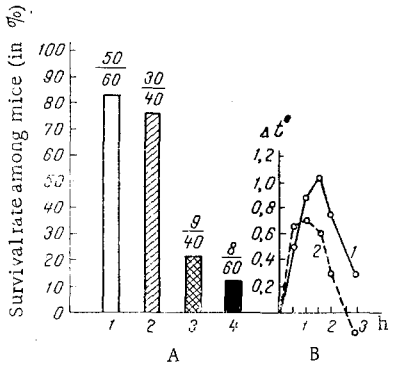

Fig. 1. Pyrogenic and antitoxic activity of leukocytic pyrogen. A) Survival rate of albino mice after intranasal injection of staphylococcal toxin, incubated with leukocytic pyrogen obtained after lyophilic drying (1), after incubation of leukocytes for 2 h (2) and 18 h (3), and control (4). Numerator gives number of surviving mice, denominator number of mice taken in experiment: B) temperature reaction of rabbits to intravenous injection of leukocytic pyrogen obtained by incubation of leukocytes at 37° for 4 (1) and 18 h (2). Mean results of 5 observations for each group.

property of inhibiting pyrogenic activity of bacterial endotoxins. Studies of the antitoxic properties of the top layer after incubation of leukocytes for short and long periods showed that the factor inhibiting activity of the staphylococcal toxin appears in the top layer at the same time as leukocytic pyrogen. As Fig. 1 shows, after incubation of leukocytes for 2 h, the top layer contained a very large amount of leukocytic pyrogen and the antitoxic activity of this layer was high. After incubation for 18 h the top layer lost its power to neutralize staphylococcal toxin and its pyrogenic activity also diminished.

The cause of disappearance of the antitoxic factor during prolonged incubation of the leukocyte suspension was examined in experiments with two preparations of the top layer: one cell-free, the other containing leukocytes. Of 40 mice receiving a mixture of toxin with a "2-h" cell-free top layer (original, and allowed to stand at 37° for 18 h), none died. The results were different when top layer incubated at 37° for 18 h along with leukocytes was tested: under these conditions the top layer did not possess antitoxic properties.

Intravital observations on leukocytes (moist preparations stained with 0.01% trypan blue solution) showed that after incubation for 2 h in physiological saline at 37° the leukocytes remained viable. Prolongation of the incubation period to 18 h caused damage to about 30% of cells.

The antitoxic factor (like leukocytic pyrogen) is thus produced only by living leukocytes, but it evidently disappears from the top layer if damaged cells appear in that layer.

The results described above show that, when exposed to the stimulant action of an inadequate medium (0.85% NaCl), leukocytes from peritoneal exudate of rabbits acquire the property of neutralizing staphylococcal toxin. The antitoxic activity of the leuckocytes rises sharply after contact with pyrogenal or B. mesentericus vaccine. The staphylococcal toxin is inactivated by an antitoxic factor produced in the leukocytes and rapidly secreted by the cell into the surrounding medium. The nature of this factor is unknown. All that can be said at present is that there is no connection between lysosomes of the leukocyte, containing phagocytin and other bactericidal substances, and the antitoxic factor. The antitoxic factor shares many common features with leukocytic pyrogen. It is difficult to say at present, however, whether the two substances are identical or whether they are two different substances formed and secreted by leukocytes in response to the action of the same stimulus.

LITERATURE CITED

1. P. N. Veselkin, Fever [in Russian], Moscow (1963), p. 116.
2. V.E. Pigarevskii and G. I. Il'in, Dokl. Akad. Nauk SSSR, 163, No. 3, 734 (1965).
3. A. V. Sorokin, Pyrogens [in Russian], Leningrad (1965), p. 49.
4. E. Atkins, Physiol. Rev., 40, 580 (1960).
5. J. L. Bennett and P. B. Beeson, J. Exp. Med., 98, 477 (1953).
6. D. L. Bornstein, C. Bredenberg, and W. B. Wood, J. Exp. Med., 117, 349 (1963).
7. Z. A. Cohn, J. G. Hirsch, and E. Wiener, In: Ciba Foundation Symposium on Lysosomes, edited by A. V. S. de Reuck and M. P. Cameron, Boston (1963), p. 126.
8. R. D. Collins and W. B. Wood, J. Exp. Med., 110, 1005 (1959).
9. H. Fessler et al., J. Exp. Med., 113, 1127 (1961).
10. J. G. Hirsch, J. Exp. Med., 103, 613 (1956).
11. H. K. Kaiser and W. B. Wood, J. Exp. Med., 115, 27 (1962).
12. M. K. King and W. B. Wood, J. Exp. Med., 107, 279 (1958).
13. R. G. Petersdorf and J. L. Bennett, Bull. Johns Hopk. Hosp., 100, 277 (1957).