EFFECT OF BIOLOGICALLY ACTIVE SUBSTANCES OF LEUKOCYTIC ORIGIN ON RESISTANCE OF ALBINO MICE TO STAPHYLOCOCCAL TOXIN

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Studies of the pyrogenic and antitoxic activity of fractions of native rabbit leukocytic pyrogen showed that both these activities are present in the low-molecular-weight fraction and that by their resistance to heat they can be divided into two parts: thermolabile (leukocytic pyrogen proper) and thermostable (antitoxic factor).

When leukocytes from aseptic peritoneal exudate of rabbits are incubated in 0.85% NaCl solution substances with pyrogenic activity [3, 5, 6, 8, 13] and with a protective action against toxic staphylococcal pulmonary edema in albino mice [4] accumulate. It is not clear whether leukocytic pyrogen possesses antitoxic activity or whether two different substances are formed and secreted by leukocytes in response to the action of the same stimulus.

To investigate this problem the pyrogenic and antitoxic activity of different fractions of native rabbit leukocytic pyrogen was investigated.

EXPERIMENTAL METHOD

A preparation of native leukocytic pyrogen was obtained from leukocytes of sterile peritoneal exudate of rabbits by the method of Bennett and Bisson as modified by Sorokin and Efremov [6]. The leukocytic pyrogen was fractionated by the method of Hadley et al. [12] and also with butanol and methanol as described by Rafter et al. [16].

Analytical fractionation of native and heated leukocytic pyrogen was carried out on Sephadex G-75, in a column measuring 1.2 × 100 cm at the rate of 10-12 ml/h. Proteins were determined by the method of Lowry et al. [14], total lipids after Folch et al. [10], and DNA after Burton [9].

Staphylococcal toxin was prepared by growing a pathogenic lecithinase-positive staphylococcus (strain 14-21) for 5 days on 0.3% semiliquid agar with 30% carbon dioxide. The resulting culture was filtered and the filtrate used in the experiments.

To determine its direct antitoxic action the leukocytic pyrogen was mixed with staphylococcal toxin (1:1), and the mixture was incubated at 37°C for 2, 4, and 18 h. After the end of incubation serial dilutions of toxin (from 1:16 to 1:2048) were prepared and the α-hemolysins titrated [7].

The protective activity of the leukocytic preparations was estimated from the survival rate of albino mice (weight 12-14 g) after intranasal administration of staphylococcal toxin in a dose of 1.6-1.7 LD_{50} in a

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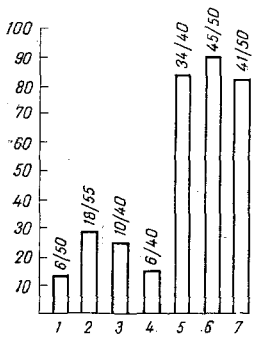

Fig. 1

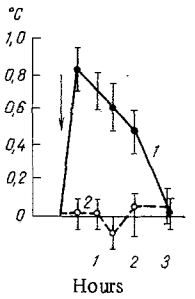

Fig. 2

Fig. 1. Survival rate after intranasal injection of staphylococcal toxin (1.7 LD_{50}/0.03 ml) into control mice (1) and mice receiving preliminary (24 h beforehand) injections of pyrogen-free physiological saline (2), solution of horse serum albumins (3), high-molecular-weight (4) and low-molecular-weight (5) fractions of leukocytic pyrogen, and native (6) and heated (7) preparation of leukocytic pyrogen.

Fig. 2. Temperature response of rabbits to intravenous injection of native (1) leukocytic pyrogen and of the same pyrogen heated to 90°C for 30 min (2) (mean results of five observations for each group). Abscissa, time (in h) after injection of pyrogen; ordinate, change in body temperature (in deg).

volume of 0.03 ml. The substances for testing were injected intranasally under superficial ether anesthesia in a dose of 0.04 ml 24 h before administration of the staphylococcal toxin.

Pyrogenic activity was determined after intravenous injection of the leukocytic preparations into the rabbits in a volume of 1 ml/kg [6]. At all stages of the work steps were taken to prevent contamination of the preparations by bacterial pyrogens.

Statistical analysis of the results was carried out with the aid of Student's criterion.

EXPERIMENTAL RESULTS

At all periods of incubation of the mixture of staphylococcal toxin with leukocytic pyrogen (1:1) the titer of α-hemolysins remained the same as in the original toxin (1:256). However, despite the absence of any direct neutralization effect, the preliminary (24 h beforehand) intranasal injection of 0.4 ml leukocytic pyrogen protected the mice against staphylococcal toxin in a dose of 1.7 LD_{50}/0.03 ml (Fig. 1).

It was not clear whether this protective effect was the result of a nonspecific stressor response or whether it depended on some particular properties of the leukocytic pyrogen. It will be clear from Fig. 1 that the high-molecular-weight protein fraction of the leukocytic pyrogen, sterile pyrogen-free physiological saline, aqueous solution of horse serum albumins with the same protein concentration as the preparation of native pyrogen, and aqueous solution of the low-molecular-weight protein fraction of F_{2b} histone of calf thymus with a protein concentration of 30 to 3000 μg/ml had no protective action against staphylococcal toxin. Different results were obtained with a preparation of native leukocytic pyrogen and its low-molecular-weight fraction precipitated by the method of Hadley et al. [12]. Both preparations protected mice against staphylococcal toxin in a dose of 1.7 LD_{50}/0.03 ml.

The experiments were repeated using the fractions of leukocytic pyrogen obtained by the method of Rafter et al. [16]. High-molecular-weight proteins sedimented during dialysis and precipitated with meth-

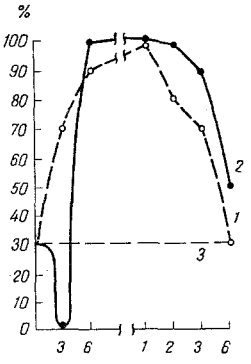

Fig. 3. Effect of preliminary intranasal injection of pyrogenal (1 μg/0.04 ml) on leukocytic pyrogen (0.04 ml) on survival rate of mice receiving standard time dose of staphylococcal toxin at various times after prophylactic injection of pyrogens: 1) leukocytic pyrogen; 2) pyrogenal; 3) control. Abscissa, time of injection of toxin after preliminary treatment with pyrogens; ordinate, survival rate of mice (in %).

anol had no protective action against staphylococcal toxin. A protective effect was observed in tests of the supernatant (low-molecular-weight fraction) in which the whole of the pyrogenic activity of the preparation of native leukocytic pyrogen was concentrated. Of 40 mice each receiving 0.04 ml of the supernatant, none died from the effects of staphylococcal toxin. Meanwhile 13 of 40 control mice receiving the same dose of toxin died (32.5%).

To abolish its pyrogenic activity the preparation of native pyrogen was heated to 90°C for 30 min. The resulting residue was further sedimented by spinning at 3000 rpm for 20 min. The supernatant was used as the apyrogenic part of the native preparation. Heating led to loss of the pyrogenic activity but reduced the protective action of the preparation only a little (Fig. 2).

The chemical nature of the thermostable substances contained in leukocytic pyrogen and increasing the level of resistance of the mice to staphylococcal toxin has not been determined. All that is known is that after heating at 90°C for 30 min followed by spinning at 3000 rpm for 20 min the protein content in the leukocytic pyrogen fell from 230 to 95-97 μg/ml. This took place on account of disappearance of the peak of high-molecular-weight "protein" fraction. Besides protein components, the heated preparation also contained carbohydrates. Negative results were obtained in tests for lipids and DNA.

A study of the dynamics of resistance showed that intranasal injection of leukocytic pyrogen led to a rapid increase in resistance without the negative phase characteristic of the protective action of bacterial preparations (Fig. 3).

It is clear from these results that leukocytic pyrogen has a protective action and raises the level of resistance of mice to the effect of intranasal injection of staphylococcal toxin in a dose of 1.7 LD_{50}/0.03 ml. This action develops rapidly without a negative phase; this evidently explains the fact observed previously that the preparation is active not only when given prophylactically but also when administered at the same time as the staphylococcal toxin [2].

A study of the pyrogenic and antitoxic activity of the various fractions of leukocytic pyrogen showed that both activities are localized in the low-molecular-weight fraction and consist of two parts: thermolabile (leukocytic pyrogen proper) and thermostable (antitoxic factor). With respect to these features and to its ability to increase the resistance of the animal the antitoxic factor is similar to the low-molecular-weight components of macrophages known collectively as monocytin [11, 15].

The discovery of pyrogenic and antitoxic activity in stimulated leukocytes points to close interaction between the immediate mechanisms of formation of the febrile reaction and nonspecific resistance and confirms the view that fever is a nonspecific, adaptive response of the body formed in the course of evolution [1].

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