THE USE OF ANTILEUKOCYTIC SERUM TO STUDY THE ROLE OF ENDOGENOUS PYROGENS IN THE MECHANISM OF THE FEBRILE REACTION IN IMMUNOPATHOLOGICAL PROCESSES

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UDC 612.57-06:615.365.018.53]-06:612.017.1

Antileukocytic serum (ALS) injected into rabbits has a marked effect on the blood leukocyte count and raises the body temperature. Addition of ALS to whole rabbit blood causes endogenous pyrogens to accumulate in the plasma. The results confirm a possible dependence of the febrile reaction in immunopathological processes on the formation of endogenous pyrogens by interaction between specific antibodies and leukocytes.

Many immunopathological diseases are accompanied by a febrile reaction. Endogenous pyrogens formed by interaction between the corresponding antibodies and the blood polymorphs are considered to play an important role in the mechanism of its development [1, 2, 5-7, 12, 13, 20]. Such a mechanism of development of fever is particularly likely in the case of immune and autoimmune diseases of the blood. However, no evidence on this problem is available.

The investigation described below, using antileukocytic serum (ALS), was undertaken to study whether endogenous pyrogens are formed by lecukocytes during their interaction with specific antibodies.

EXPERIMENTAL METHOD

Experiments were carried out on 65 rabbits weighing 2.5-3.5 kg and on 50 guinea pigs weighing 250-350 g. The polymorphs were obtained from peritoneal exudate of rabbits [2, 3, 8], the suspension was freed from contamination with erythrocytes by the brief action of distilled water (not more than 1 min), and guinea pigs were immunized with the cell suspension together with Freund's complete adjuvant, mixed in equal volumes [2, 10, 15-17, 19, 21]. The mixtures were injected subcutaneously 3 times at intervals of 10-12 days in doses of between 50 and 100 million cells per injection. Blood was taken from the guinea pigs by cardiac puncture 10-12 days after the 3rd injection, the serum was heated to 56° C for 30 min, cultured for sterility, and kept at −20° C. The titer of ALS was 1:128 by the leukoagglutination test [11]. Normal serum (NS) was obtained from healthy guinea pigs. The formation of endogenous pyrogen by the leukocytes of the exudate was studied by the method described elsewhere [2, 3, 8] by incubating a suspension of leukocytes with a concentration of 50 million cells per ml in 0.85% NaCl solution at 37° C for 2 h with the addition of ALS or NS in dilutions of 1:5 and 1:20. In two series of experiments fresh guinea pig NS was added as complement in a proportion of 1:10. After incubation the cells were removed by centrifugation, and the pyrogenic activity of the cell-free supernatant was tested by injection intravenously into rabbits in a dose of 1 ml/kg. Interaction between ALS and blood leukocytes was studied by adding the ALS and NS to fresh heparinized rabbit blood in the ratio of 1:20 and subsequently incubating the mixture at 37° C for 2 h. After centrifugation at 2500 rpm and at 4° C for 25 min, the resulting plasma was tested for pyrogenic activity by intravenous in-

Department of General Pathology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR, P. N. Veselkin.) Translated from Byulleten' Éksperimental'noi Biologiya i Meditsiny, Vol. 76, No. 10, pp. 77-80, October, 1973. Original article submitted October 17, 1972.

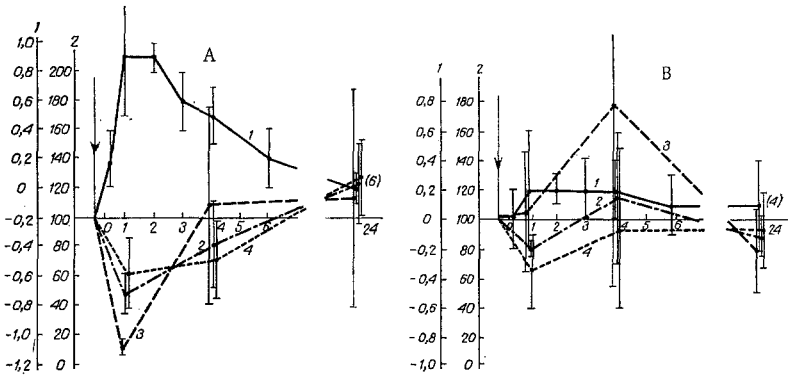

Fig. 1. Changes in body temperature and in blood leukocyte count after intravenous injection of ALS and NS into rabbits: A) injection of ALS in dose of 3 ml/kg; B) injection of NS in the same dose; 1) body temperature; 2) total leukocyte count; 3) neutrophil count; 4) lymphocyte count. Arrow indicates time of injection. Vertical lines show confidence limits. Number of animals given in parentheses. Abscissa: time (in h); ordinate: 1) change in body temperature (in deg); 2) change in number of cells (in per cent of initial level).

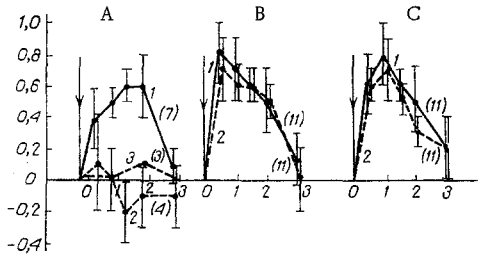

Fig. 2. Pyrogenic activity of supernatant after incubation of ALS and NS with blood leukocytes and exudate. A) Incubation of heparinized blood with ALS and NS and injection of plasma into rabbits in dose of 10 ml/kg; B) incubation of exudate leukocytes with ALS and NS; C) the same but with the addition of complement; 1) injection of supernatant after incubation of leukocytes with ALS; 2) injection of supernatant after incubation of leukocytes with NS; 3) change in body temperature after injection of ALS only in the same dose. Ordinate, change in body temperature of animals (in deg). Remainder of legend as in Fig. 1.

jection into rabbits in a dose of 10 ml/kg. The rabbit's temperature was measured in the rectum by a resistance thermometer two or three times at intervals of 30 min to establish its initial level, and thereafter at the same interval for 3-6 h after administration of the preparation. The greatest care was taken to ensure that the pyrogens were free from bacterial contamination (the vessels were sterilized at 170° C for 2 h, all solutions were tested for absence of pyrogens, and so on). Statistical analysis of the experimental results was carried out by the aid of Student's criterion.

EXPERIMENTAL RESULTS

Intravenous injection of ALS into rabbits in a dose of 3 ml/kg caused a marked fall in the total number of leukocytes and severe granulocytopenia, which in some rabbits reached the level practically of agranulocytosis. It will be noted that at the time of the maximal decrease in the number of neutrophils the rabbits' body temperature was considerably raised (Fig. 1A). The changes in the number of circulating neutrophils and the increase in body temperature followed a similar time course and had the character of a cyclic reaction. After injection of NS into rabbits neutrophilia was observed, but the body temperature did not rise. In both cases the lymphocyte count fell by the same degree (Fig. 1B). These changes in the control evidently reflect the general reaction of the organism to injection of foreign protein[4].

The phase of leukopenia was presumably attributable to agglutination of the leukocytes and their removal from the circulation while elevation of the body temperature depended on the formation of endogenous pyrogens by the leukocytes as a result of their interaction with antibodies. This hypothesis is confirmed by the results of the next experiments.

Addition of ALS to whole heparinized rabbit's blood followed by incubation for 2 h at 37° C led to the accumulation of endogenous pyrogens in the plasma, as was conclusively proved by the injection of this plasma into recipient rabbits in a dose of 10 ml/kg (Fig. 2a). Guinea pig NS had no such action. ALS in the dose

used for incubation with blood (1:20), when injected intravenously into rabbits, did not affect the body temperature (Fig. 2A). According to the available evidence, the formation of endogenous pyrogen by polymorphs during their interaction with various stimuli, for example, during phagocytosis of bacteria, is biphasic in character: in the first phase there is activation of the leukocytes (possibly the synthesis of a propyrogen), while in the second phase the active protein leukocytic pyrogen is formed and liberated from the cell [14, 18]. As these experiments show, the addition of ALS to intact blood cells triggers the complete cycle of formation of endogenous pyrogen and its accumulation in the plasma. The next step was to study the effect of ALS on pyrogen formation by exudate leukocytes which had passed through the activation phase during inflammation. It will be clear from Fig. 2B that incubation of exudate leukocytes with the addition of ALS did not lead to an increase in the pyrogenic activity of the supernatant. The addition of complement to the system likewise did not result in the stimulation of pyrogen formation (Fig. 2C). The exudate leukocytes were probably already highly activated as a result of inflammation so that in the experiments in vitro no further stimulant effect of ALS was obtained on the formation of endogenous pyrogen.

The results of these experiments thus show that ALS has a marked effect on parameters such as the number of polymorphs in the blood and the body temperature, the changes in which coincide in time. The brevity of the reaction depends on the small dose and the single injection of ALS. The discovery of endogenous pyrogens in the plasma during incubation of whole blood with ALS indicates that they are formed by interaction between specific antibodies and leukocytes and it confirms the possibility that the pyrexia after intravenous injection of ALS into rabbits may depend on this process. The use of ALS into rabbits may depend on this process. The use of ALS offers prospects for the analysis of the immunological and biochemical mechanism of formation of endogenous pyrogens by the leukocytes in immunological reactions.

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