FORMATION OF ENDOGENOUS PYROGEN BY BONE MARROW CELLS

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Bone marrow cells of rabbits can produce endogenous pyrogen after stimulation with bacterial lipopolysaccharide. The optimal conditions for liberation of the pyrogen are incubation of the cells in medium No. 199 with the addition of 15% homologous serum. Participation of bone marrow cells in the formation of endogenous pyrogen and in the mechanism of fever also may occur, it is suggested, in the intact organism.

KEY WORDS: fever; pyrogens; leukocytes; bone marrow.

The febrile reaction to injection of bacterial endotoxins and lipopolysaccharides is mediated through the formation of pyrogens in the body [1, 2, 4, 10]. The main source of endogenous pyrogens under these circumstances is the granulocytes and monocytes of the blood [5-7]. However, after injection of endotoxin into rabbits with a sharply reduced circulating blood leukocyte count even to the extent of agranulocytosis, fever nevertheless still develops [8, 9]. It can tentatively be suggested that in these cases bone marrow cells may be the source of the endogenous pyrogens. There is no information in the literature on the ability of bone marrow cells to produce endogenous pyrogen and the investigation described below was accordingly carried out to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 42 chinchilla rabbits of both sexes weighing 2.5-3.5 kg. Bone marrow was obtained from the femora and tibiae, minced, and passed through Kapron gauze. After centrifugation at 1500 rpm for 10 min at 4°C a suspension of bone marrow cells with a concentration of $50 \cdot 10^6$ cells/ml was prepared: a) in 0.85% NaCl solution with 15% homologous serum, b) in medium No. 199, and c) in medium No. 199 with 15% serum. To stimulate the cells, the bacterial lipopolysaccharide pyrogenal was added to the suspension before incubation in a dose of 0.5 minimal pyrogenic dose (MPD) to $300 \cdot 10^6$ cells. Incubation was carried out at 37°C for 20 h with periodic shaking during the first 4 h. The cells were then removed by centrifugation at 2000 rpm for 20 min at 4°C. The supernatant containing the endogenous pyrogen was injected intravenously into rabbits in a dose of 2 ml/kg. To rule out the possibility of pyrogenicity due to the pyrogenal added for stimulation, subthreshold doses of this substance were used and the tests of endogenous pyrogen were performed on tolerant rabbits. Tolerance was produced by intravenous injection of pyrogenal in a dose of 50 MPD 24-48 h before the experiment. The rabbit's body temperature was measured at intervals of 30 min to establish the initial level, and then at the same intervals for 2 h after injection of the substances. In the course of the work conditions preventing the possibility of contamination with bacterial pyrogens were observed: sterilization of the glassware at 170°C for 2 h, testing all solutions for absence of pyrogenicity, and so on. The experimental results were subjected to statistical analysis by the use of Student's criterion.

EXPERIMENTAL RESULTS AND DISCUSSION

The control experiments showed that bone marrow cells, after incubation in medium No. 199 with the addition of 15% homologous serum, do not liberate pyrogen spontaneously, in the absence of stimulation. The addition of pyrogenal to the cells led to pyrogen formation. Intravenous injection of bone-marrow pyrogen induced a brief febrile reaction, typical of endogenous pyrogens, with the highest peak recorded after 30 min and terminating after 2 h (Fig. 1A).

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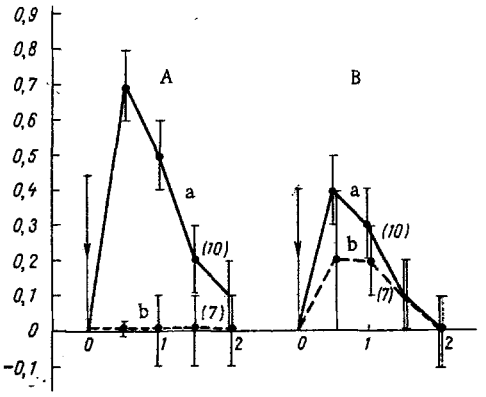

Fig. 1. Pyrogenic activity of supernatant after incubation of bone marrow cells. Dose injected 2 ml/kg. A) Incubation in medium No. 199 with 15% serum: a) with pyrogenal, b) without pyrogenal; B) incubation with pyrogenal: a) in 0.85% NaCl solution with 15% serum, b) in medium No. 199 without serum. Abscissa, time, in h; ordinate, rise of body temperature (in °C). Arrow indicates time of injection. Vertical lines represent confidence limits; number of animals in parentheses.

The optimal conditions for pyrogen production were found to be incubation of the cells in medium No. 199 with the addition of 15% homologous serum. Much less pyrogen was liberated during incubation of bone marrow cells in 0.85% NaCl solution with 15% serum or in medium No. 199 without the addition of serum (Fig. 1B).

Bone marrow cells, like the leukocytes of the blood, can thus produce endogenous pyrogen in the presence of additional stimulation. Compared with circulating blood cells, which liberate adequate amounts of pyrogen during incubation in 0.85% NaCl solution with the addition of 15% serum, bone marrow cells require a more enriched incubation medium in order to produce endogenous pyrogen. Discovery of the fact of endogenous pyrogen formation by bone marrow cells suggests that they participate in the formation of endogenous pyrogen and in the mechanism of fever in the intact organism also. In particular, these results shed light on the cause of development of fever in agranulocytosis, when the functional state of the circulating leukocytes is considerably weakened [3, 8, 9]. In such cases the reaction is probably mediated through the formation of endogenous pyrogen not by the leukocytes of the blood, but by the reserve of bone marrow cells.

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