ENDOGENOUS PYROGEN FORMATION BY MONONUCLEAR PHAGOCYTES

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Cells of the mononuclear phagocyte system play an important role in resistance [3, 4] for they produce various biologically active substances or monokines [10, 15], which include endogenous pyrogens (EP), which trigger and maintain the febrile reaction in the intact organism [2, 7, 11]. Interest in EP has increased because this particular monokine is regarded as interleukin I and characterized by polyfunctional properties [12, 15].

As a continuation of earlier studies [1, 8], in the present investigation particular features and individual aspects of the mechanism of EP formation by human and rabbit mononuclear phagocytes were studied under comparable conditions.

EXPERIMENTAL METHOD

The general principles of the work with EP were described previously [1, 8]. To isolate monocytes from human blood, 1000 ml of blood from donors was used, whereas to obtain rabbit monocytes and alveolar macrophages, 30 chinchilla rabbits of both sexes weighing 2.5-3.0 kg were used. The pyrogenic activity of the preparations was tested on 110 rabbits. Monocytes were isolated by fractionating cells from heparinized blood by the method in [9] and alveolar macrophages were isolated by flushing out cells from the bronchial tree of a rabbit's lungs [13]. In control experiments leukocytes were incubated in medium 199 with the addition of 15% heated homologous serum in a concentration of 5×10^6 cells/ml at 37°C for 18 h. For the purpose of stimulation mononuclear phagocytes were incubated just as in the control experiments, in the same medium and in the same concentrations with the addition of heat-killed *Staphylococcus epidermidis* cells (in the ratio of 30 bacterial cells to 1 phagocyte), of homologous granulocytes from a blood film (3:1), or their products obtained by destruction of granulocytes by a single freezing and thawing. In some experiments, besides bacteria, actinomycin D (from Reanal, Hungary), an inhibitor of protein synthesis, was added to the incubation medium in a dose of 10 μg/ml, together with cytochalasin B (5 μg/ml, from Sigma, USA), which inhibits uptake of various particles by phagocytes. The viability of the leukocytes was determined by staining the cells with a 0.25% solution of trypan blue. Films were stained with May–Gruenwald solution and the phagocytic activity of the leukocytes was estimated by counting and calculating the phagocytic number and index. At the end of incubation the cells were removed by centrifugation and the supernatants were tested for pyrogenic activity, by injecting them intravenously into rabbits in a dose of 1 ml/kg body weight. The animals' body temperature was measured rectally with an electrothermometer twice or three times before administration of the substances and during the 4 h after injection. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

On incubation of all types of mononuclear phagocytes chosen for testing, namely human and rabbit blood monocytes and rabbit alveolar macrophages, in medium 199 with the addition of 15% homologous serum at 37°C for 18 h without any additional stimulation, EP formation was not observed (Figs. 1 and 2). After isolation and incubation the proportion of viable leukocytes was 80-85%.

Incubation of human blood monocytes for 18 h with staphylococci (phagocytic number 72 ± 10, phagocytic index 8 ± 1.5) was accompanied by pyrogen formation. Optimal doses of EP equivalent to 5 ± 10^6 cells/kg body weight evoked a brief monophasic pyrexial response with mean

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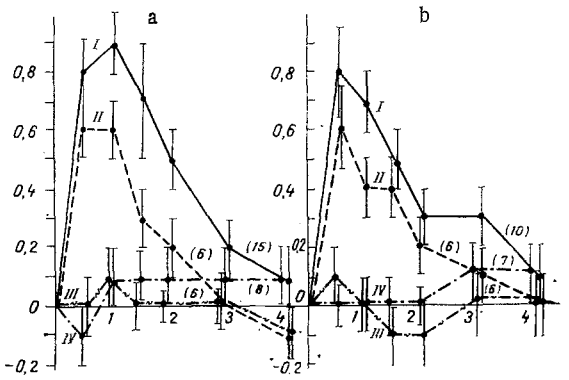

Fig. 1

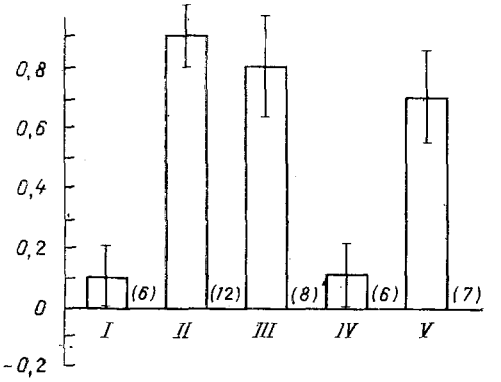

Fig. 2

Fig. 1. Temperature responses of rabbits to injection of incubated human (a) and rabbit (b) monocytes: abscissa, time (in h); ordinate, body temperature (in deg C). 0) Time of injection of samples. Vertical lines indicate confidence limits (number of animals in parentheses). I) Stimulation with bacteria in a dose of 5×10^6 (a) and 10×10^6 (b) cells/kg body weight; II) stimulation with bacteria in a dose equivalent to 1×10^6 (a) and 5×10^6 (b) cells/kg body weight; III) stimulation by products of disintegrated homologous granulocytes in a dose equivalent to 5×10^6 (a) and 10×10^6 (b) cells/kg body weight; IV) without additional stimulation, in same dose as in a, and b, respectively.

Fig. 2. Temperature reactions of rabbits to injection of incubated rabbit alveolar macrophages. I) Without additional stimulation, II) stimulation with bacteria, III) stimulation with bacteria and cytochalasin B, IV) simultaneous stimulation with bacteria and actinomycin D, V) under the influence of bacteria and addition of actinomycin D 4 h after beginning of incubation of leukocytes. Columns indicate mean maximal rise of body temperature after injection of sample. Remainder of legend as to Fig. 1.

rise of temperature by 0.9°C ($P < 0.01$) 30-45 min after injection. Injection of threshold doses of pyrogen equivalent to a dose of 1×10^6 cells/kg body weight caused the development of significant ($P < 0.05$), although weaker, responses (Fig. 1a). Incubation of rabbit blood monocytes under similar conditions with bacteria (phagocytic number and index 73 ± 8.0 and 10.0 ± 1.8, respectively) also led to EP formation, inducing significant ($P < 0.01$) temperature responses when injected into rabbits in optimal doses equivalent to 10×10^6 cells/kg body weight. Injection of threshold doses of homologous monocytic pyrogen into the animals in a dose equivalent to 5×10^6 cells/kg body weight was accompanied by a rise of body temperature on average by 0.6°C ($P < 0.05$) (Fig. 1b).

To investigate the possible mechanisms of activation of mononuclear phagocytes during aseptic inflammation, homologous granulocytes and also products obtained from their destruction, were used for stimulation under conditions of aseptic inflammation. Incubation of monocytes under these conditions (the proportion of viable phagocytes was 75-80%) did not lead to EP formation: Injection of samples into rabbits in a dose equivalent to 5×10^6-10×10^6 cells/kg body weight was not accompanied by the development of febrile reactions (Fig. 1).

Investigation of the biochemical mechanisms of activation of mononuclear phagocytes was carried out on rabbit alveolar macrophages. On incubation of the leukocytes in medium 199 with homologous serum for 18 h with the addition of bacteria (phagocytic number 77 ± 6.0, phagocytic index 12 ± 2.0) revealed marked EP formation (Fig. 2). Addition of cytochalasin B in a dose of 5 μg/ml, inhibiting the phagocytic activity of alveolar macrophages (phagocytic number 5.0 ± 2.0, phagocytic index 0.6 ± 0.3; $P < 0.05$), to the incubation medium simultaneously with the microorganisms did not lead to inhibition of pyrogen formation — the dose of EP equivalent to 10×10^6 cells/kg body weight, evoked significant ($P < 0.05$) temperature reactions when injected into rabbits (Fig. 2).

In a special series of experiments, when actinomycin D, which blocks protein synthesis, was added simultaneously with bacteria to the incubation medium in a dose of 10 μg/ml, complete inhibition of EP production by alveolar macrophages was observed. Actinomycin D, if added to the incubation medium 4 h after the beginning of stimulation of the macrophages by staphylococci, i.e., when activation of the leukocytes had already taken place, lost its in-

hibitory activity; however, samples incubated for 18 h in a dose equivalent to 10×10^6 cells/kg body weight, if injected into rabbits, evoked marked febrile ractions (Fig. 2).

Mononuclear phagocytes of different types, not containing preformed EP, thus form it when activated by certain stimuli [1, 5, 10, 12] (in the present investigation, by heat-killed bacteria). To study the neglected question of the possible mechanisms of EP production by leukocytes under conditions of aseptic inflammation, experiments were first conducted with stimulation of monocytes by homologous intact and destroyed granulocytes. Under these conditions the monocytes did not secrete pyrogen. In inflammation, various complex mechanisms, including interaction between leukocytes and the vascular endothelium, and the subsequent stage of emigration of cells into the inflammatory focus, probably take part in activation of phagocytes and triggering of EP formation. Inhibitor analysis using actinomycin D and cytochalasin B yielded fresh evidence of the important role of initial interaction between stimulus and leukocyte membrane in the mechanism of activation of mononuclear phagocytes and of the biphasic nature of the process of EP formation [5, 6, 10, 14].

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