MECHANISMS OF FORMATION OF ENDOGENOUS PYROGEN BY LEUKOCYTES

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The development of fever under the influence of various pathological stimuli takes place through the formation of endogenous pyrogens in the body by "professional phagocytes" [1, 2, 4, 6, 9]. The writers showed previously that a key stage in this process is activation of leukocytes [2, 3].

Data are given below on the kinetics of formation of endogenous pyrogen by rabbit leukocytes and on the possible role of activated leukocytes in self-regulation of its formation. Information on this matter in the literature is scanty and contradictory [6-8, 10].

EXPERIMENTAL METHOD

The general conditions of sterility during the work and methods of isolation of leukocytes from the blood and exudate of rabbits were described previously [2, 4]. The buffy coat obtained from blood and a suspension of peritoneal exudate leukocytes contained 35-40 and 90-95% of neutrophilic granulocytes and 2-3% of monocytes, respectively. The viability of the cells was determined by staining with a 0.25% solution of trypan blue [11]. To study the kinetics of formation of leukocytic pyrogen (LP) blood leukocytes were suspended in 0.15 M NaCl with the addition of 15% rabbit plasma in a concentration of $25\text{-}30 \cdot 10^6$ cells/ml and incubated for 2, 4, and 18 h at 37°C. The cells were stimulated with the bacterial lipopolysaccharide, pyrogenal, in a dose of 0.5 MPD (minimal pyrogenic dose) per $120 \cdot 10^6$ leukocytes, and the pyrogenic activity of the samples was tested on tolerant rabbits [2]. Exudate leukocytes were suspended in 0.15 M NaCl in a concentration of $(30\text{-}35) \cdot 10^6$ cells/ml and incubated for 1 h at 37°C, after which the cells were separated, washed, and reincubated for 1 h under the same conditions. To determine the intracellular LP content, the pyrogenic activity of the soluble fraction of blood and exudate leukocytes, disintegrated by freezing and thawing, was titrated [7]. Pyrogenic activity was tested on rabbits of both sexes weighing 2.8-3.0 kg, by intravenous injection of the samples in different doses. The cell-free supernatant layer containing products of cells activated during inflammation, including LP, obtained after incubation of the leukocytes in 0.15 M NaCl, was fractionated under sterile conditions by the method described previously [5] on a Sephadex G-75 (Pharmacia, Sweden) column. Fractions eluted from the column, in a final concentration of 3.75 μg/ml, or pyrogenal were added to the blood leukocyte suspension in a concentration of $30 \cdot 10^6$ cells/ml in 15% serum and incubated for 18 h at 37°C. The supernatant obtained after incubation of $120 \cdot 10^6$ leukocytes was injected into rabbits weighing 2.8-3.0 kg. The animals' rectal temperatures were measured with an electrothermometer for 4 h after injection of the samples at intervals of 30 min. The experimental results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Kinetics of Pyrogen Formation by Blood Leukocytes. No pyrogen was present in inactivated leukocytes isolated from blood. A low level of pyrogenic activity was found in leukocytes incubated for 2 h with pyrogenal. Injection of supernatant obtained after destruction of $120 \cdot 10^6$ leukocytes into a rabbit caused the animal's body temperature to rise on average by 0.5°C (Fig. 1A). However, liberation of LP was not yet observed at this period of incubation (Fig. 1B). During incubation of leukocytes for 4 h endogenous pyrogen accumulated in the medium. Supernatant obtained after incubation of $120 \cdot 10^6$ leukocytes with pyrogenal for 4 h possessed pyrogenic activity (Fig. 1B). The intracellular content of pyrogen after incubation

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TABLE 1. Incubation of Blood Leukocytes with Products of Activated Exudate Leukocytes Fractionated on Sephadex G-75 Column

Mean maximal rise of body temp. of rabbits in response to intravenous injections of supernatant after incubation for 18 h, °C				
of blood leukocytes in 15% serum				
with addition of pyrogen	without addition	with addition of fraction eluted as principal protein peak	with addition of pyrogenic protein	of pyrogenic fraction in 15% serum
0.1 ± 0.1 (9)	0.2 ± 0.1 (10)	0.2 ± 0.1 (10)	0.6 ± 0.1 (10)	0.5 ± 0.1 (14)

Legend. Number of observations given in parentheses.

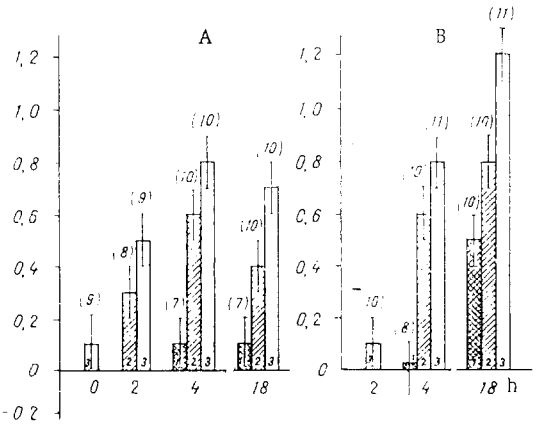

Fig. 1. Kinetics of pyrogen formation by blood leukocytes. Abscissa, incubation time (in h); ordinate, change in rabbits' body temperatures (in °C). Vertical lines represent confidence limits. Number of observations shown in parentheses. A) Intracellular pyrogen content after incubation of suspension of blood leukocytes with pyrogenal for different periods; B) content of extracellular pyrogen after incubation of suspension of blood leukocytes with pyrogenal for different periods. Columns indicate mean maximal rise of body temperature after injection of supernatant obtained by disintegration (A) or incubation (B) of $20 \cdot 10^6$ (1), $60 \cdot 10^6$ leukocytes (3).

for 4 h corresponded to the content of extracellular LP secreted during this incubation period by an equal number of leukocytes (Fig. 1A). Supernatant after incubation of simulated leukocytes for 18 h possessed even greater pyrogenic activity. A temperature reaction developed even after injection of supernatant equivalent to $20 \cdot 10^6$ cells (Fig. 1B). However, the intracellular pyrogen content in such leukocytes was actually a little lower than after incubation of these cells for 4 h (Fig. 1A).

Kinetics of Pyrogen Formation by Exudate Leukocytes. Soluble fractions of unincubated exudate leukocytes possess pyrogenic activity. Injection of supernatant after destruction of $50 \cdot 10^6$ such cells into a rabbit raised the animal's body temperature by 0.8°C (Fig. 2A). The same reaction was observed in rabbits in response to injection of cell-free incubation medium containing LP, liberated by $15 \cdot 10^6$ leukocytes in the course of 1 h of incubation. No pyrogenic activity was found in supernatant corresponding to $5 \cdot 10^6$ incubated leukocytes (Fig. 2B). The intracellular pyrogen content at the end of this incubation period was approximately the same as that in unincubated leukocytes (Fig. 2A). The extracellular pyrogen content during the second incubation period was greater than during the first period. The rabbits developed only a weak temperature reaction to injection of supernatant obtained after reincubation of $15 \cdot 10^6$ cells (Fig. 2B). The intracellular LP content in leukocytes incubated twice also was considerably less than before incubation and after incubation for 1 h (Fig. 2A).

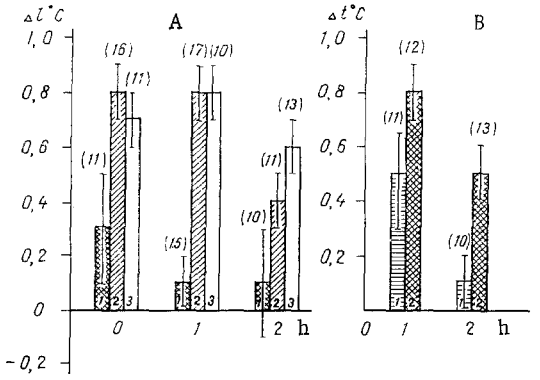

Fig. 2. Kinetics of pyrogen formation by exudate leukocytes. A) Intracellular pyrogen content after incubation of exudate leukocytes for different periods in 0.15 M NaCl. B) Content of extracellular pyrogen after incubation of exudate leukocytes for different periods in 0.15 M NaCl. Columns indicate mean maximal rise of body temperature after injection of supernatant obtained by destruction (A) of $15 \cdot 10^6$ (1), $50 \cdot 10^6$ (2), and $100 \cdot 10^6$ leukocytes (3) or after incubation (B) of $5 \cdot 10^6$ (1) and $15 \cdot 10^6$ leukocytes (2). Remainder of legend as to Fig. 1.

Incubation of Blood Leukocytes with Products of Exudate Leukocytes Activated during Inflammation. On fractionation of a freeze-dried preparation containing products of activated exudate cells on Sephadex two fractions were obtained: a fraction eluted from the column with the principal protein peak and not containing pyrogenic activity, and a pyrogenic fraction of lower molecular weight. Since the writers' previous experiments showed that these two fractions differ in their effect on hematopoiesis in intact rats [5], it was decided to study their possible stimulating action on pyrogen formation by blood leukocytes. During incubation of blood leukocytes with the nonpyrogenic fraction of the preparation for 18 h no pyrogen was produced (Table 1). The temperature reaction of the rabbits to injection of supernatant after incubation of leukocytes with the pyrogenic fraction of the preparation was approximately the same as the change in the animals' body temperatures in response to injection of this fraction incubated under the same conditions, but in the absence of leukocytes (Table 1). Consequently, the pyrogenic fraction of the preparation likewise did not stimulate pyrogen production by normal leukocytes.

In control tests the cells were incubated with pyrogenal for 18 h. The resulting cell-free incubation fluid possessed high pyrogenic activity (Table 1). Consequently, during isolation and incubation the leukocytes did not lose their ability to form LP in response to appropriate stimulation. After incubation of the leukocytes both in 15% serum alone and in serum with the addition of the test fractions and pyrogenal, 90% of the cells remained viable.

Blood leukocytes thus do not contain preformed LP. Weak pyrogenic activity was found after incubation of the cells for 2 h with pyrogenal, but no liberation of pyrogen was observed during this period of incubation. Formation and secretion of pyrogen by blood leukocytes took place intensively 4 h after the beginning of incubation with pyrogenal and gradually diminished until 18 h. Secretion of LP is evidently preceded by its accumulation in the cell. Since exudate leukocytes secreted much more LP in the course of incubation for 1 h than they contained before incubation, it can be concluded that these cells not only liberate but also form an active pyrogen, in agreement with data in the literature [10]. After intensive liberation of LP during incubation for 1 h its intracellular content remains high. The decrease in the extracellular concentration of pyrogen during the second period of incubation was accompanied by a decrease in its intracellular concentration. Data obtained on leukocytes from blood and exudate suggest that after the LP concentration in the cell reaches a certain level, the processes of formation of active pyrogen and its secretion are interconnected: The LP formed is secreted from the cell; the reduction in the intracellular concentration of pyrogen is accompanied by a decrease in its concentration in the medium.

Products of leukocytes activated by inflammation were found to have no stimulating effect on the process of pyrogen formation by blood leukocytes. Development of the febral reaction

during inflammation evidently takes place with the involvement of a complex system of factors and cell-tissue reactions.

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GASTRIC SECRETION AND EXCRETION UNDER MINERALOCORTICOID DEFICIENCY

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Besides its basic digestive function the stomach also participates in the regulation of the acid–base balance and water metabolism of the body.

The object of this investigation was to study gastric secretion in dogs with mineralocorticoid deficiency resulting from bilateral adrenalectomy and receiving replacement injections of hydrocortisone (1.2-1.7 mg/kg body weight), maintaining the blood 11-hydroxycorticosteroid concentration at the control level. As a result of complete removal of the adrenals a deficiency of gluco- and mineralocorticoids only develops, for compensatory shifts of sympathetic nervous activity and of gonadal function are observed [6-9].

EXPERIMENTAL METHOD

Experiments were carried out on 14 mongrel male dogs with Pavlov gastric pouches. Gastric secretion was stimulated by subcutaneous injection of histamine and by feeding with meat (samples of juice were collected after 15 min and hourly, respectively). The volume of gastric juice (expressed per kg body weight), its acidity, and its content of ammonia [2] and also of the exogenous dye neutral red [1] were determined. After a control series of experiments on eight dogs, one-stage bilateral adrenalectomy was performed. The subsequent experiments began on the 8th-9th days and ended on the 16th-17th days after the operation. Experiments were carried out at these same times on six dogs undergoing mock operations.

EXPERIMENTAL RESULTS

The results showed that all changes in gastric secretion were due to a primary mineralocorticoid deficiency, for the corresponding parameters in dogs undergoing the mock operation did not differ from the controls (Tables 1 and 2).

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