On the other hand, an analogous phenomenon could not be obtained with staphylococcal toxoid or with the antigen most commonly used for such purposes – tuberculin. Antiphagin and antitoxin differ in their method of preparation. The decisive factor is evidently the vigorous extraction of antiphagin from bacterial cells and the high peptidoglycan concentration in its composition. Staphylococcal peptidoglycan inhibits migration of macrophages of both sensitized and normal guinea pigs [5]. This action of staphylococcal peptidoglycan and of other analogous bacterial glycoproteins is considered to be nonspecific [5, 6]. From this point of view, lymphocyte extract increases only the nonspecific sensitivity of cells to staphylococcal antiphagin.

Human tonsillar lymphocyte extract thus contains a factor which potentiates the macrophage migration inhibition reaction under the influence of staphylococcal antiphagin. The specificity of action of this factor and its relationship to transfer factor are problems that still await solution.

LITERATURE CITED

1. F. G. Berngof, Staphylococcal Antiphagin and Treatment of Suppurative Diseases of the Skin [in Russian], Moscow (1952).
2. O. V. Protasova, I. M. Pozina, O. S. Sergeeva, et al., Lab. Delo, No. 11, 667 (1966).
3. Handbook on the Use of Bacterial and Virus Preparations [in Russian], Moscow (1975), p. 165.
4. J. W. Eichberg, R. S. Steele, S. S. Kalter, et al., Cell. Immunol., 26, 114 (1976).
5. A. Grov, Acta Path. Microbiol. Scand., Sect. B, 84, 315 (1976).
6. B. Heymer, B. Bültmann, W. Schachenmayer, et al., J. Immunol., 111, 1743 (1973).
7. R. W. Steele, J. W. Eichberg, R. L. Heberling, et al., Cell. Immunol., 22, 110 (1976).
8. C. B. Sue, M. A. South, H. W. Jordan, et al., Exp. Haematol., 3, 327 (1975).
9. T. M. Welch, R. Triglia, L. E. Spitler, et al., Clin. Immunol. Immunopath., 5, 407 (1976).

MECHANISMS OF LEUKOCYTE ACTIVATION DURING THE FORMATION OF LEUKOCYTIC PYROGEN

E. G. Rybakina and A. V. Sorokin

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Actinomycin D and cyclohexamide, inhibitors of protein synthesis, inhibit the formation of endogenous pyrogen by the blood granulocytes induced by bacterial lipopolysaccharide and specific antigranulocytic serum but do not affect the secretion of pyrogen by exudate leukocytes. This shows that the inhibitors inhibit the activation phase but not the process of liberation of the pyrogen. KEY WORDS: fever; pyrogen; leukocytes; actinomycin D; cyclohexamide.

In the modern view the onset and maintenance of the febrile response in various pathological states are mediated through the formation of an endogenous pyrogen by the leukocytes [2, 5, 13]. Great importance is therefore attached to the study of the mechanisms of its formation. Intact blood granulocytes have been shown not to contain pyrogen in the preformed state [6, 9], but they form it in response to inflammation, interaction with bacterial endotoxins, or interferonogens, during phagocytosis, and also in certain immunologic reactions [4, 6, 7, 10, 11]. The formation of pyrogen by leukocytes takes place in two phases. In the first phase of activation mechanisms regulating the formation of leukocytic pyrogen are triggered, after which pyrogen is produced and secreted into the surrounding medium. One approach to the study of the activation phase of the leukocytes is inhibition of protein synthesis in them by inhibitors such as actinomycin D, cyclohexamide, and puroamycin. Data in the literature on this problem are contradictory [6-8, 12].

The object of the present investigation was to study the effect of inhibitors of protein synthesis (actinomycin D and cyclohexamide) on the activation phase of the leukocytes in three models: stimulation by bacterial

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.)

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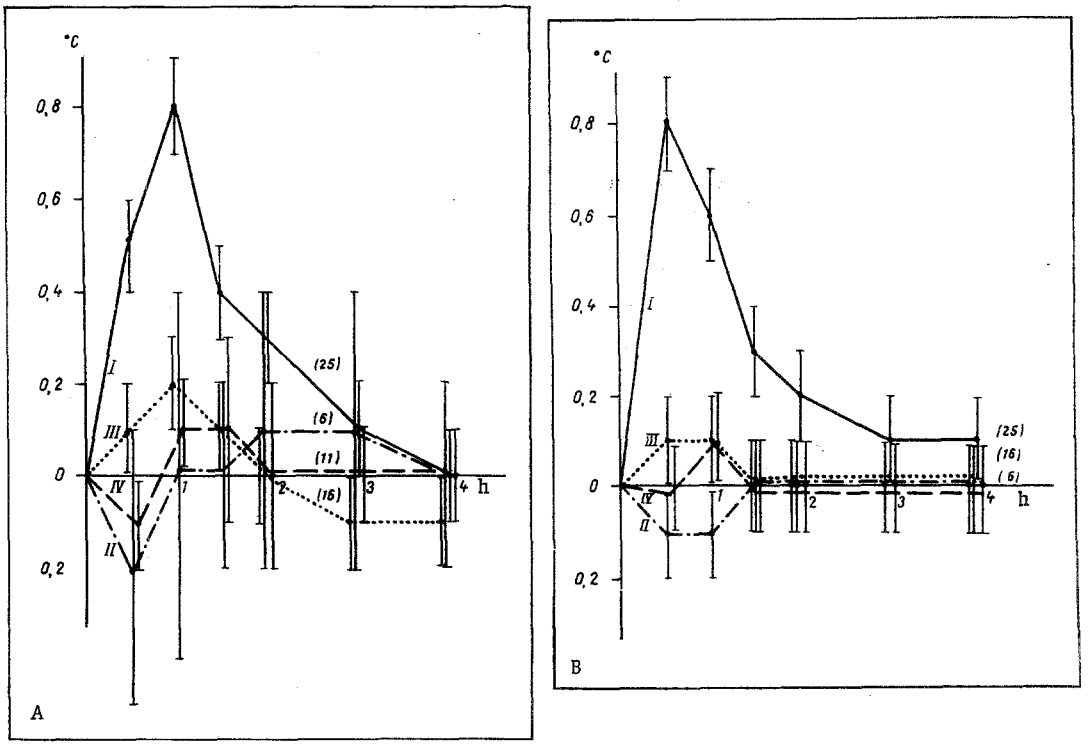

Fig. 1. Pyrogen formation by blood leukocytes in response to stimulation by pyrogenal and effect of actinomycin D and cyclohexamide on this process. A) Injection of plasma after incubation of whole blood with pyrogenal; B) injection of supernatant after incubation of leukocyte suspension with pyrogenal. I) Incubation of leukocytes with pyrogenal; II) the same, without pyrogenal; III) incubation of leukocytes with pyrogenal and with addition of actinomycin D; IV) the same, but with addition of cyclohexamide. Vertical lines represent confidence limits. Number of animals shown in parentheses. Abscissa, time in h; ordinate, change in body temperature, in °C.

lipopolysaccharide, stimulation by antigranulocytic serum (AGS), and aseptic inflammation. This is the first attempt to study biochemical mechanisms of pyrogen formation by granulocytes during their interaction with specific antiserum, and it is a continuation of the writers' previous investigations [4].

EXPERIMENTAL METHOD

A general condition of the work was strict observance of measures preventing contamination with bacterial pyrogens [3]. Heparinized blood was obtained by cardiac puncture from rabbits of both sexes. Leukocytes were isolated as the buffy coat [7]. Between 200 and 250 million leukocytes containing 35-40% of granulocytes were obtained from 100 ml blood. Leukocytes were isolated from the peritoneal exudate of rabbits 18 h after receiving an intraperitoneal injection of 400-500 ml of a sterile 0.2% solution of glycogen in 0.85% NaCl solution [3, 9]. From each rabbit 1.5-2 billion leukocytes were obtained, of which 90-95% were granulocytes. Guinea pig AGS was obtained by the method described previously, namely triple immunization of guinea pigs with leukocytes from rabbit exudate [4]. The AGS titer determined by the leukocyte agglutination test was 1:256.

Blood leukocytes were suspended in 15% rabbit serum in 0.85% NaCl in a concentration of 25-30 million cells/ml and incubated for 1, 2, 4, and 18 h at 37°C. The bacterial lipopolysaccharide pyrogenal, in a concentration of 0.15 MPD/ml of suspension (MPD = minimal pyrogenic dose) and AGS in a dilution of 1:20, i.e., in a small dose, were used to activate the leukocytes. Small doses of AGM, just as of other cytotoxic sera, are known to stimulate the functional activity of the cells against which they were formed, by contrast with large doses, which have an inhibitory action [1, 4]. In control tests instead of AGS an equal volume of normal guinea pig serum (NS) was added. Exudate leukocytes were suspended in 0.85% NaCl, for in some experiments in 15% rabbit serum, in a concentration of 30-35 million cells/ml. Whole blood was incubated for 30 min at 4 and 18 h with pyrogenal in a concentration of 0.01 MPD/ml blood and AGS in a dilution of 1:20. At the end of incubation the cells were removed by centrifugation and the supernatant was tested for pyrogenic activity by intra-

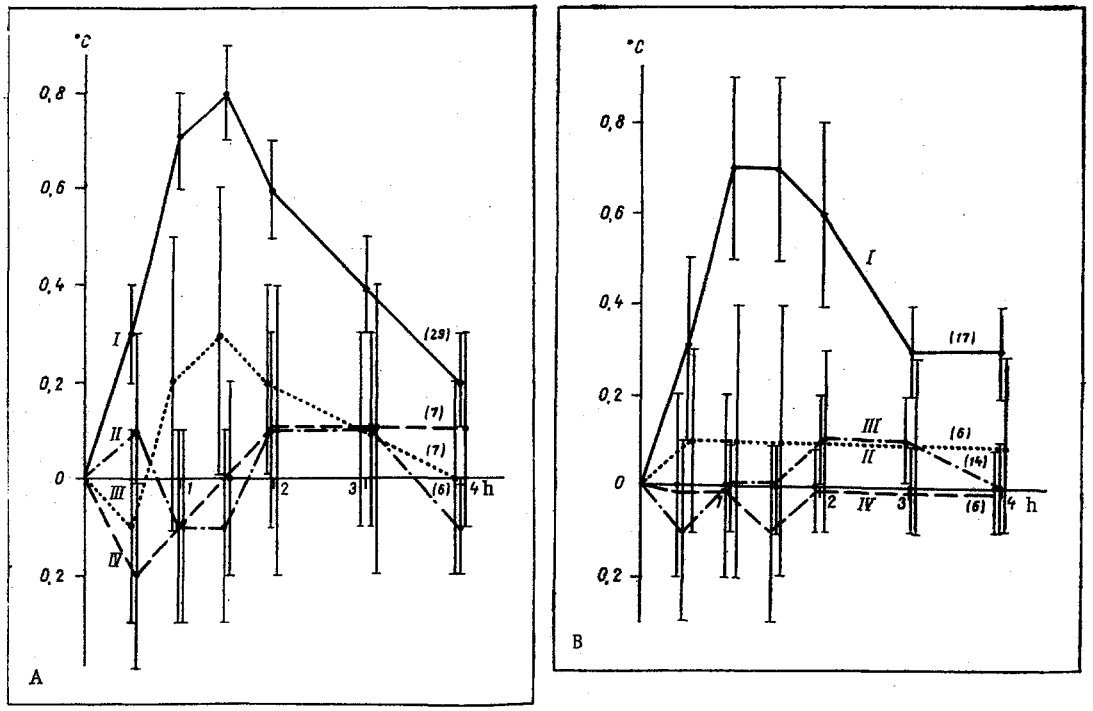

Fig. 2. Pyrogen formation by blood leukocytes in response to interaction with AGS. A) Injection of plasma after incubation of whole blood with AGS; B) injection of supernatant after incubation of leukocyte suspension with AGS. I) Incubation of leukocytes with AGS; II) incubation of leukocytes with NS; III) incubation of leukocytes with AGS on addition of actinomycin D; IV) the same, with addition of cyclohexamide. Remainder of legend as in Fig. 1.

venous injection into rabbits in the following volumes: 1.5 ml/kg body weight in the case of incubation of exuleukocytes isolated from blood, 1 ml/kg per animal in the case of incubation of exudate leukocytes, and 10 ml/kg of plasma obtained after incubation of whole blood. Actinomycin D (Reanal) and cyclohexamide (Serva), in final concentrations of 10 μg/ml, were used as inhibitors of protein synthesis. The body temperature of the rabbits was measured in the rectum by means of an electrothermometer twice or three times before administration at intervals of 30 min and during the 4 h after administration of the preparation at the same intervals. The pyrogenic activity of samples in which pyrogenal was used as the activator were tested on tolerant rabbits, into which pyrogenal was injected in doses of 50 MPD 2 days before their pyrogenicity was tested. The experimental results were subjected to statistical analysis by means of Student's t-test.

EXPERIMENTAL RESULTS

1. Pyrogen Formation by Blood Leukocytes Stimulated by Pyrogenal. During incubation of whole blood for 4 h no pyrogen was formed. The addition of pyrogenal led to significant ($P < 0.01$) accumulation of endogenous pyrogenal in the plasma after incubation for 4 h (Fig. 1A). Intravenous injection of plasma evoked a monophasic febrile reaction with maximal rise of the body temperature of the rabbits 0.5-1 h after injection, and terminating after 3-4 h. Actinomycin D and cyclohexamide, if added to whole blood simultaneously with pyrogenal, significantly ($P < 0.01$) inhibited pyrogenal production by the blood leukocytes after incubation for 4 h (Fig. 1A).

The addition of pyrogenal to a suspension of leukocytes isolated from blood also led to the accumulation of pyrogen in the incubation medium, after incubation for 4 h, with a febrile reaction of the same character after intravenous injection of the supernatant into rabbits. If actinomycin D or cyclohexamide was added to the leukocyte suspension simultaneously with pyrogenal, no pyrogen was formed (Fig. 1B).

2. Pyrogen Formation by Blood Leukocytes in Response to Interaction with Specific AGS. AGS added to whole blood induced the accumulation of pyrogen in the plasma ($P < 0.01$) after incubation for 4 h (Fig. 2A). NS had no such action.

Actinomycin D, added to whole blood simultaneously with AGS, depressed pyrogen production by the blood leukocytes after incubation for 4 h ($P < 0.01$). Cyclohexamide, under the same conditions, suppressed pyrogen production completely (Fig. 2A).

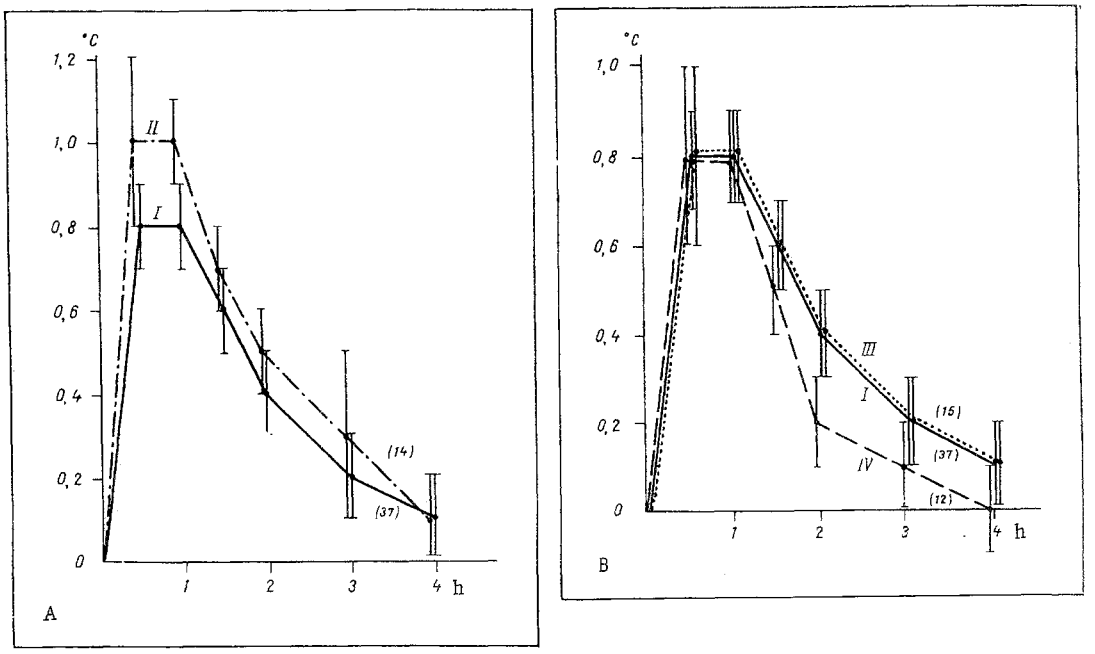

Fig. 3. Secretion of pyrogen by exudate leukocytes in response to addition of pyrogenal and inhibitors of protein synthesis. A) Incubation of leukocytes with pyrogenal; B) incubation of leukocytes with actinomycin D and cyclohexamide. 1) Addition of supernatant after incubation of leukocytes in 0.85% NaCl solution; 2) injection of incubated material after stimulation of leukocytes by pyrogenal; 3) injection of supernatant after incubation of leukocytes with actinomycin D; 4) the same, with addition of cyclohexamide. Remainder of legend as in Fig. 1.

Leukocytes isolated from blood also produced pyrogen in response to the addition of AGS to the incubation medium. These leukocytes stimulated by AGS lost their ability to produce pyrogen if actinomycin D or cyclohexamide was added to the incubation medium simultaneously with the stimulator (Fig. 2B).

The study of the dynamics of pyrogen formation by the blood leukocytes showed that endogenous pyrogen, in amounts detectable by the methods used, began to appear in the plasma after incubation of whole blood leukocytes for 30 min both with AGS (Δt°C 0.9 ± 0.2) and with pyrogenal (Δt°C 0.7 ± 0.1). After incubation for 4 h there was no increase in the pyrogenic activity of the plasma (in both cases Δt°C was 0.8 ± 0.1). Incubation for 18 h increased the quantity of pyrogen in the plasma compared with after incubation for 4 h, in response to stimulation both by AGS (Δt°C 1.0 ± 0.1) and by pyrogenal (Δt°C 1.1 ± 0.2).

Leukocytes isolated from blood did not produce pyrogen after incubation with pyrogenal for 1 and 2 h, unlike leukocytes of whole blood (Δt°C 0.1 ± 0.1). Endogenous pyrogen began to appear in the incubation medium after 4 h (Δt°C 0.8 ± 0.1). Lengthening the incubation time to 18 h led to an increase in the quantity of pyrogen (Δt°C 1.2 ± 0.1). Cyclohexamide, if added to the incubation medium 1 and 2 h after the beginning of incubation with pyrogenal, i.e., during the period before any liberation of pyrogen by leukocytes took place, like cyclohexamide added to the medium simultaneously with pyrogenal, blocked production of pyrogen by the blood cells (Δt°C 0.2 ± 0).

3. Pyrogen Formation by Exudate Leukocytes. By contrast with the blood leukocytes, exudate leukocytes when incubated with 0.85% NaCl were able to liberate pyrogen without any additional activator [3, 9]. These leukocytes, from the standpoint of triggering of pyrogen formation, were already activated in response to inflammation. Comparison of the ability of the blood and exudate leukocyte to produce pyrogen and secrete it from the cell thus enables the phases of activation and liberation of the pyrogen to be analyzed to some extent separately. In these experiments incubation of exudate leukocytes in 0.85% NaCl and also in 15% rabbit serum led to accumulation of pyrogen. The addition of pyrogenal increased pyrogenic activity somewhat (Fig. 3A). Actinomycin D and cyclohexamide, unlike their effect on blood leukocytes, did not inhibit pyrogen formation by exudate leukocyte (Fig. 3B).

The two inhibitors of protein synthesis used, namely actinomycin D (an inhibitor of transcription), and cyclohexamide (an inhibitor of translation), thus inhibit pyrogen formation by blood cells stimulated by AGS

and pyrogenal if added simultaneously with the stimulator. However, they cannot block liberation of pyrogen by exudate cells which have already been activated. This is evidence that inhibitors of protein synthesis inhibit the activation process, during which the new cell proteins are evidently synthesized, but they do not inhibit the process of liberation of pyrogen.

LITERATURE CITED

1. O. A. Bogomolets, in: Anticytotoxic Serum in Internal Medicine [in Russian], Kiev (1967), p. 15.
2. A. V. Sorokin, Pyrogens [in Russian], Leningrad (1965).
3. A. V. Sorokin and O. M. Efremov, in: Proceedings of the Third Scientific Conference of Pathophysiologists of the North Caucasus [in Russian], Rostov-on-Don (1969), p. 238.
4. A. V. Sorokin, O. M. Efremov, and O. A. Él'kina, Byull. Éksp. Biol. Med., No. 10, 77 (1973).
5. E. Atkins and P. T. Bodel, in: Pyrogens and Fever, Edinburgh (1971), p. 81.
6. P. T. Bodel, Yale J. Biol. Med., 43, 145 (1970).
7. C. G. Cox and G. W. Rafter, Biochem. Med., 5, 227 (1971).
8. M. K. Fleetwood, G. W. Gander, and F. Goodale, Proc. Soc. Exp. Biol. (New York), 149, 336 (1975).
9. H. K. Kaiser and W. B. Wood, J. Exp. Med., 115, 27 (1962).
10. D. M. Moore, S. F. Cheuk, J. D. Morton, et al., J. Exp. Med., 131, 179 (1970).
11. E. S. Shell and E. Atkins, Am. J. Physiol., 212, 1103 (1967).
12. I. Taborsky, Acta Virol., 16, 376 (1972).
13. W. B. Wood, Lancet, 2, 53 (1958).

COMMON ANTIGENS OF STABLE L-FORMS OF GROUP A STREPTOCOCCUS AND HUMAN MYOCARDIAL MUSCLE FIBERS

L. V. Beletskaya, Yu. V. Vul'fovich, É. V. Gnezditskaya, S. A. Goncharova, G. A. Ugryumova, and M. Ya. Korn

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Common antigenic features of stable streptococcal L-forms and of the cytoplasmic membrane of muscle fibers of the human myocardium were demonstrated by an immunofluorescence method. The common antigen is a component of the surface membrane of the muscle cell, adjacent to the sarcolemmal sheath, and of the membranes of the transverse tubules of the macroplasmic reticulum, which pass through the sarcomeres of the muscle fiber in the zone of the L-disks. The reaction was completely prevented by exhaustion of the antiserum against antigen of L-forms by means of a human myocardial tissue homogenate or a suspension of cultures of L-forms grown on a meat or casein medium. Exhaustion with a tissue homogenate of other organs (liver) or with concentrated nutrient medium had virtually no effect on the intensity of the reaction. In the authors' view, the presence of common antigens in cultures of stable L-forms of group A streptococcus and the myocardium may be one cause of the long persistence of L-forms in the human and animal body. KEY WORDS: stable streptococcal L-forms; myocardial muscle fibers; common antigens.

One of the conditions for long persistence of bacteria in the human or animal body is mimicry due to common antigenic components of the microorganism and tissues of the host. It is well known, for example, that hyaluronic acid in the capsule of microorganisms, including the streptococcus, is identical with the hyaluronic acid of the connective tissue of man and animals [11]. Similarity has been demonstrated between the polysaccharide of group A streptococci, which is a component of the cell wall, and the antigens of certain mam-

Laboratory of Streptococcal Infections and Laboratory of Mycoplasmas, Department of General Microbiology, and Laboratory of Opticophysical Methods of Investigation, N. F. Gamaleya Institute of Epidmiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Aacdemician of the Academy of Medical Sciences of the USSR V. D. Timakov.)

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