EFFECT OF LEUKOCYTIC PYROGEN ON THE PHAGOCYTIC PROPERTIES OF MACROPHAGES IN TISSUE CULTURE

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The effect of rabbit leukocytic pyrogen (LP) on the phagocytic activity of peritoneal macrophages from albino mice toward shigellas was investigated. The effect was found to depend on dose: LP in a large dose inhibited phagocytosis whereas small doses of LP were not very effective; the addition of LP to macrophages in average doses, after administration of kanamycin, stimulated both the phase of ingestion and the phase of digestion of the shigellas. Stimulation of phagocytosis by LP was accompanied by an increase in the activity of the lysosomal enzyme acid phosphatase in the macrophages; changes in the RNA content were not significant.

KEY WORDS: pyrogens; phagocytosis, macrophages; acid phosphatase; RNA.

According to existing data, bacterial pyrogens can increase nonspecific resistance to bacterial and virus infections and toxicoinfections [8, 14, 15]. This effect is due to the stimulating action of pyrogens on various systems of the body, including metabolism and the functional activity of leukocytes and macrophages [16]. The ability of bacterial pyrogens to stimulate the formation of endogenous pyrogens by leukocytes has also been established [11, 13]. It has been shown that pyrogen obtained from leukocytes possesses a stimulating action on leukopoiesis [9, 10] and increases the nonspecific resistance of animals to staphylococcal and influenzal toxicoinfection [7, 8]. However, the mechanism of this action of leukocytic pyrogen (LP) has not been studied.

The object of this investigation was to study the effect of LP on the phagocytic properties of macrophages in culture.

EXPERIMENTAL METHOD

A standard lyophilized sample of rabbit LP, prepared in the Department of General Pathology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR [11, 12], was used. The activity of the sample was expressed in minimal pyrogenic doses (MPD) per kilogram body weight (the minimal quantity of the substance which, when injected intravenously into rabbits, caused the body temperature to rise by 0.6°C or more). The batches of LP used contained 350 μg protein/ml, determined by Lowry's method. To a culture of peritoneal macrophages from albino mice, with a seeding concentration of 1 million cells/ml, a freshly isolated strain of Shigella sonnei (No. 32837), obtained from the Bacteriological Laboratory, S. P. Botkin Hospital, was added in a dose of 10 million cells/ml (10 : 1). The cover slip was washed to remove unattached cells and extracellular microorganisms 1 h after infection. The phagocytic activity of the macrophages against shigellas was assessed by bacteriological analysis of cell lysates [2]. Determination of the dynamics of the change in the number of viable intracellular bacteria by seeding lysates of macrophages on Endo's medium enabled a phase of ingestion (in the early stages, namely the first hour after infection) and a phase of digestion of the shigellas by the cells (the next 2-4 h after infection) to be distinguished.

For a closer analysis of the final stage of phagocytosis, namely intracellular digestion of shigellas, cytochemical tests were carried out, including determination of the activity of the lysosomal enzyme acid phosphatase and the content of macrophagal RNA. Acid phosphatase is one of the hydrolytic enzymes that participate in the digestion of microorganisms [3]. RNA and ribonucleoproteins are utilized by macrophages while

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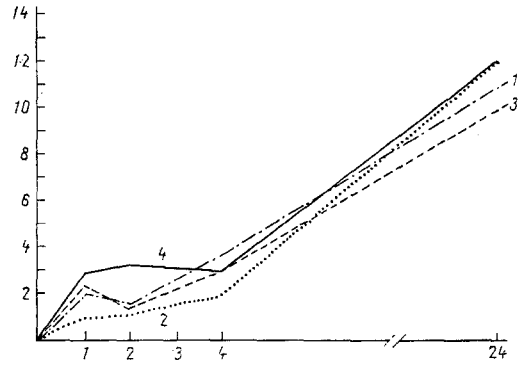

Fig. 1

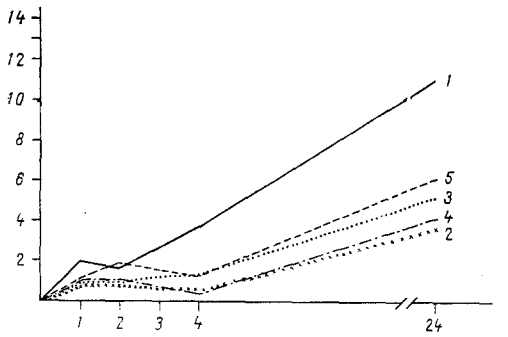

Fig. 2

Fig. 1. Effect of LP on phagocytosis of shigellas in culture of macrophages. 1) Bacterial control; 2,3,4) LP in doses of 50, 25, and 12.5 μg/ml, respectively. Here and in Fig. 2: abscissa, time (in h); ordinate, number of shigellas in 1 ml lysate (in thousands).

Fig. 2. Effect of combination of LP and kanamycin on phagocytosis of shigellas in culture of macrophages. 1) Bacterial control; 2) kanamycin 1.75 μg/ml; 3,4,5) kanamycin 1.75 μg/ml with 50, 25, and 12.5 μg/ml LP, respectively.

they perform their phagocytic function, possibly to form enzymes [5]. Evidence that RNA participates in phagocytosis was given by the results of experiments with actinomycin D. This inhibitor, which acts on RNA synthesis, inhibited phagocytosis [6].

In the present experiments acid phosphatase activity was determined by the azo-coupling method [4], and RNA by Brachet's method [1]. Stained films were studied cytophotometrically [1].

LP was tested in three doses which contained 50, 25, and 12.5 μg protein/ml, respectively. The sample was added to the cells in culture 24 h before infection. Testing LP on a model of infection involving the use of a cell culture infected with shigellas necessitated the addition of substances with antibacterial action to the cultured system. Of the known antibacterial agents kanamycin was used, for it is widely used in the treatment of dysentery. The dose of the antibiotic (1.7 μg/ml) was based on the therapeutic dose. The antibiotic was added to the cell culture simultaneously with the shigellas. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Appreciable changes were found in the phagocytic activity of the macrophages in culture as a result of the action of LP, depending on its dose (Fig. 1). In a dose of 50 μg/ml, LP inhibited the phagocytic properties of the cells, reducing the number of intracellular shigellas in the ingestion phase compared with the control (1 h of observation; $P < 0.001$) and smoothing the phase of digestion of the microorganism (1-3 h; $P < 0.01$). On the addition of small doses of the antibiotics (12.5 μg/ml) the ingestive power of the macrophages increased ($P < 0.05$) but digestive power was almost completely absent. After the use of LP in average doses (25 μg/ml) the phase of ingestion and the phase of digestion of shigellas were clearly marked ($P < 0.01$).

As a result of the action of LP in average doses (25 μg/ml) the phase of ingestion and the phase of digestion of shigellas were clearly distinguishable ($P < 0.01$).

Hence, only in the experiments with an average dose of LP (25 μg/ml) were favorable conditions created for manifestation of both the ingestive and the digestive functions of the macrophages in culture. To detect the stimulating properties of the LP on the model of a cell culture infected with shigellas, its action in conjunction with kanamycin had to be studied.

When LP was given in conjunction with kanamycin the same dose-effect dependence was found (Fig. 2). Optimal conditions for the cells were found in the experiments with LP in a dose of 25 μg/ml and kanamycin, in the presence of which the phase of ingestion was enhanced and the phase of digestion of the shigellas intensified ($P < 0.001$). Since LP had a stimulating action on the phagocytic properties of the macrophages in average doses, subsequent cytochemical investigations were carried out with the use of these doses of LP only.

TABLE 1. Changes in Acid Phosphatase Activity and RNA Content in Infected Macrophages During Treatment with LP

Index	Time of investigation, h	Without preparation (I)	P_{I-C}	LP 25 μg/ml (II)	P_{II-I}	LP 25 μg/ml + kanamycin (III)	P_{III-I}
Acid phosphatase	1	27,6±1,4	0,05	31,7±0,9	0,05	27,9±1,2	0,05
	2	29,2±0,9	0,05	32,2±1,2	0,05	26,6±0,8	0,05
	4	28,4±1,1	0,05	22,2±0,9	0,1	21,6±1,3	0,2
	Control (uninfected cells — C)	16,92±1,0					
RNA	1	5,2±1,1	0,05	4,9±0,9	0,4	4,4±0,3	0,7
	2	4,8±0,7	0,05	5,7±0,8	0,05	6,2±1,1	0,8
	4	7,7±0,4	0,1	7,1±1,4	0,7	8,1±1,3	0,7
	Control (uninfected cells — C)	9,3±0,8					

The results of the cytophotometric investigation of the samples are generalized in Table 1.

Clearly LP led to an increase in acid phosphatase activity in the early periods of observation. Maximal activity of the enzyme was detected 2 h after infection of the cells; activity then fell, although it did not reach the level of activity in the control. The dynamics of activity of the lysosomal enzyme was similar when LP was given together with kanamycin. In this case, however, a certain tendency was found for enzyme activity to fall.

As regards RNA, its content in the cell cytoplasm did not change significantly under the influence of LP. A statistically significant increase in the content of macrophagal RNA 2 h after infection pointed to the more rapid recovery of the content of ribonucleoproteins utilized in the course of phagocytosis, compared with the control. However, when LP was used against the background of kanamycin, this effect was not discovered.

The LP preparation containing substances secreted by leukocytes activated during inflammation, when used in conjunction with kanamycin, thus had a stimulating effect on the phagocytic activity of macrophages in culture against shigellas and lysosomal enzymes participating in the digestion of microorganisms.

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