EFFECT OF HUMAN TONSILLAR LYMPHOCYTE EXTRACT ON THE MACROPHAGE MIGRATION INHIBITION TEST IN GUINEA PIGS EXPOSED TO VARIOUS ANTIGENS

N. V. Medunitsyn, A. N. Mats, and O. V. Protasova

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Guinea pig peritoneal exudate cells were treated with human tonsillar lymphocyte extract in vitro or in vivo. The macrophage migration inhibition test was performed with normal cells and with cells treated with the extract. Old tuberculin, purified tuberculin, staphylococcal toxoid, and staphylococcal antiphagin were used as antigens. Lymphocyte extract without antigen did not affect macrophage mobility. Staphylococcal antiphagin without lymphocyte extract caused weak inhibition of cell migration. Preliminary treatment of the cells in vivo or in vitro with lymphocyte extract considerably increased the sensitivity of the cells to staphylococcal antiphagin. The remaining antigens did not inhibit macrophage migration whether before or after treatment of the cells with lymphocyte extract. KEY WORDS: transfer factor; increased sensitivity of delayed type; macrophage migration inhibition test; bacterial peptidoglycans.

Increased sensitivity of delayed type can be induced in man and monkeys with the aid of human lymphocyte extract. This sensitivity arises even if the recipients are kept under sterile conditions [4, 7, 8]. As a rule it is impossible to induce delayed hypersensitivity in small laboratory animals by means of human lymphocyte extract. It appears only after preliminary sensitization, i.e., after preliminary contact between recipient and antigen [9]. The activity of lymphocyte extracts is assumed to depend on the presence of a special low-molecular-weight transfer factor, capable of either inducing or potentiating hypersensitivity of delayed type.

This paper describes an attempt to modify the sensitivity of guinea pig cells by treating them in vivo or in vitro with human tonsillar lymphocyte extract.

EXPERIMENTAL METHOD

Guinea pigs were used. Intradermal tests with old tuberculin in a dilution of 1 : 10 and with undiluted antiphagin revealed no increased cutaneous sensitivity to these antigens in the intact animals.

Four preparations were used as reagents for the macrophage migration inhibition test. 1) Staphylococcal antiphagin produced by the I. I. Mechnikov Moscow Research Institute of Vaccines and Sera. This preparation was obtained by extracting a thick suspension of cultures of 10 to 12 strains of Staphylococcus aureus and coagulase-positive Staphylococcus albus with salt solution at 100°C for 1 h, followed by filtration of the bacterial suspension [3]. 2) Native staphylococcal toxoid produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology. This preparation was a filtrate of a staphylococcal broth culture, detoxicated by heating in formaldehyde, and with an activity of not less than 5 fixation units/ml. 3) Old tuberculin produced by the Gor'kii Research Institute of Epidemiology and Microbiology, with an activity of 90,000 tuberculin units/ml. 4) Dry purified tuberculin produced by the Leningrad Research Institute of Vaccines and Sera, with an activity of 50,000 tuberculin units per ampul. This preparation was diluted in 1 ml isotonic NaCl solution.

Guinea pig peritoneal exudate cells for the macrophage migration inhibition test were obtained on the third day after intraperitoneal injection of 10 ml of 2% peptone solution with glycogen (1 mg/ml) and cultured in capillary tubes in medium 199 with 5% heated guinea pig serum and with various concentrations of the test preparations. The first dilutions of the preparations were: staphylococcal antiphagin 1 : 10, staphylococcal

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TABLE 1. Effect of Intravenous Injection of Lymphocyte Extract on Migration of Guinea Pig Peritoneal Exudate Cells after Contact with Various Antigens

Antigen	Injection of lymphocyte extract	No. of animals in group	Index of macrophage migration with consecutive tenfold dilution of antigen		
			I*	II	III
Staphylococcal antiphagin	—	5	68	88	95
	+	8	47†	68†	87
Staphylococcal toxoid	—	5	106	102	112
	+	5	114	87	102
Old tuberculin	—	6	100	101	104
	+	5	101	108	102
Purified tuberculin	—	2	98	92	97
	+	3	93	98	96

* For details of first dilution of antigens see "Experimental Method."
† Effect of lymphocyte extract significant at $P = 0.05$.

TABLE 2. Migration of Guinea Pig Peritoneal Exudate Cells after Incubation in vitro with Human Tonsillar Lymphocyte Extract

Antigen	Incubation of cells with lymphocyte extract	No. of animals in group	Index of macrophage migration with consecutive tenfold dilution of antigen		
			I*	II	III
Staphyloccal antiphagin	—	7	84	103	112
	+	11	54†	85†	104
Staphyloccocal toxoid	—	4	117	114	111
	+	5	120	101	110
Old tuberculin	—	4	97	93	122
	+	5	100	95	102

* For details of first dilution of antigens see "Experimental Method."
† Effect of lymphocyte extract significant at $P < 0.05$.

toxoid 1:100, old tuberculin 1:100, and purified tuberculin 1:10. The macrophage migration index (as a percentage of the area of migration of cells in the culture without the antigen) was determined after culture for 18 h [2].

To obtain the lymphocyte extract, human tonsils were taken not later than 3 h after tonsillectomy, freed from connective tissue, minced to a single-cell suspension from which clumps and adhesive cells were removed by filtration through a Kapron sieve and column of cotton wool. The suspension was freed from red cells with the aid of ammonium chloride and the cells were washed with Hanks' solution, frozen to −30°C, and thawed at 37°C in 10 volumes of distilled water. Insoluble components were removed by centrifugation of 6000 and 20,000 g and filtration of the supernatant through a 0.22 μ Millipore membrane. The extract was then treated with 0.25 M sucrose and lyophilized.

EXPERIMENTAL RESULTS

In the experiments of series I 24 h before the peritoneal exudate was taken the guinea pigs were given an intravenous injection of a dose of lymphocyte extract equivalent to $2 \cdot 10^8$ lymphocytes, in a volume of 1 ml. The mean migration indices of the peritoneal cells obtained both from the experimental (after injection of lymphocyte extract) and from control (intact) animals, are given in Table 1. They show that preliminary injection of the lymphocyte extract increased the sensitivity of the cells to the inhibitory action of staphylococcal antiphagin but did not affect sensitivity to other antigens.

In the experiments of series II, $6 \cdot 10^7$ peritoneal exudate cells from intact guinea pigs were treated with extract obtained from $2 \cdot 10^8$ human tonsillar lymphocytes. The cells were incubated for 90 minutes at room temperature, then washed in medium 199 and cultured in capillary tubes.

The experimental results given in Table 2 show that preliminary treatment of the peritoneal cells with lymphocyte extract in vitro increased the sensitivity of the cells to the inhibitory action of staphylococcal antiphagin. Just as in the experiments in vivo, sensitivity to staphylococcal toxoid and to old tuberculin remained unchanged.

It can tentatively be suggested that the phenomenon of increased sensitivity of the macrophages to staphylococcal antiphagin discovered in these experiments was connected with transfer factor. Such a factor must be present in an extract of the tonsillar lymphocytes of patients who, as a rule, have increased sensivitity to staphylococci. Staphylococcal antiphagin, like tuberculin, can induce skin reactions of delayed type [1]. Finally, guinea pigs, through their constant contact with staphylococci, may have a certain initial degree of increased sensitivity detectable by the macrophage migration inhibition test. In the present experiments staphylococcal antiphagin, in low dilutions, could suppress migration of cells obtained from intact animals. Preliminary treatment of the cells with lymphocyte extract merely potentiated this effect. This explanation of the phenomenon is in agreement with modern views on the adjuvant action of transfer factor.

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.

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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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