ROLE OF CYCLIC NUCLEOTIDES IN THE IMMUNOMODULATING ACTION OF HISTAMINE IN MICE

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A role for histamine in the regulation of the immune response has recently been found. Histamine receptors have been shown to be present on immunocompetent cells [3, 5, 11]. The present writer demonstrated the immunoregulatory effect of histamine when injected into experimental animals; depending on the time of injection, moreover, histamine can stimulate or inhibit the immune response [2].

In the investigation described below the mechanism of the immunoregulatory action of histamine in mice was studied and the type of histamine receptor and the part played by the cyclic nucleotide system in the realization of the action of histamine were determined, for histamine has been shown to change the intracellular levels of both cAMP and cGMP [4, 9].

EXPERIMENTAL METHOD

Experiments were carried out on 50 C57BL/6 mice sensitized by a single intraperitoneal injection of 5 μg ovalbumin with 5 mg aluminum hydroxide gel. The mice were decapitated at different times after sensitization and the spleen was removed and a lymphocyte suspension obtained from it by gentle homogenization [1]. To discover the population of histamine-sensitive lymphocytes the spleen cells were fractionated by spontaneous sedimentation at 1 g [6]. Cell fractions with sedimentation rates of 1-4, 4-6, and 6-8 mm in 1 h in Hanks' solution in Ficoll with a density of 1010, which according to data in the literature correspond to B lymphocytes, T helpers, and T suppressors [8], were investigated. In the course of 2 h, $2 \cdot 10^6$ to $5 \cdot 10^6$ cells of each fraction were cultured in medium 199 with 10% isologous blood serum, $5 \cdot 10^{-5}$ M 2-mercaptoethanol, and 100 units penicillin in 1 ml in the presence of 10^{-3} M histamine and $5 \cdot 10^{-3}$ M each of mepyramine and metiamide, blockers of H_1- and H_2-histamine receptors respectively. The rate of cell proliferation was estimated from incorporation of labeled ^{3}H-thymidine, added at the beginning of culture in a concentration of 2 μCi/ml.

To study the effect of histamine on the cyclic nucleotide level in the cells they were incubated in medium 199 with 10^{-3} M histamine, with $5 \cdot 10^7$ cells in the sample. After incubation for 10 min the reaction was stopped with 5 N HCl, the cells were homogenized, and the protein was precipitated by boiling for 2 min in a water bath and centrifugation for 20 min at 1000g. The supernatant was used to determine the cAMP and cGMP concentrations by a radioimmunologic method using reagents in accordance with instructions supplied by the Radiochemical Centre, Amersham, England.

The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The fractions isolated from splenic lymphocytes of sensitized mice differed in their sensitivity to the action of exogenous histamine (Table 1). The cells of the fastest sedimented fraction changed the degree of proliferation in the presence of 1 mM histamine, whereas cells of the other fractions virtually did not react to its presence. The degree and direction of the changes in proliferation largely depended on the period of sensitization. In the early stages (1st or 2nd days of sensitization) histamine reduced the degree of proliferation of the rapidly sedimented fraction of lymphocytes, on the 3rd day of sensitization it stimulated proliferation, whereas in the later stages it had little effect on the degree of proliferation of the cells of this fraction (Table 2).

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TABLE 1. Action of Histamine on Proliferation of Lymphocytes from Mouse Spleen (4th day of sensitization, $1 \cdot 10^6$ cells per sample)

Fraction (sedimentation rate in mm/h)	Incorporation of ^{3}H-thymidine without histamine, cpm	Incorporation of ^{3}H-thymidine with 1 ml histamine, cpm
1—4	4650±750	6420±2300
4—6	3520±160	3870±580
6—8	8070±1200	2870±1060*

*P < 0.01.

TABLE 2. Effect of Histamine on Proliferation of Rapidly Sedimented (6-8 mm/h) Lymphocyte Fraction from Mouse Spleen at Different Times of Sensitization

Day of sensitization	No. of cpm of sample with 1 mM histamine / No. of cpm of sample without histamine
1	0,54±0,06
2	0,31±0,08
3	1,45±0,09
5	0,76±0,04
8	1,10±0,20
15	0,80±0,07

TABLE 3. Cyclic Nucleotide Levels in Cells of Different Lymphocyte Fractions from Mouse Spleen under the Influence of Histamine on 3rd Day of Sensitization (cpm/1·10^6 cells)

Fraction (sedimentation rate in mm/h)	Control		Histamine		Histamine + metiamide		Histamine + mepyramine	
	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
1—4	6,0±0,7	2,1±0,1	6,0±0,3	2,7±0,3	—	—	—	—
4—6	5,4±0,2	3,6±0,3	5,6±0,2	3,6±0,5	—	—	—	—
6—8	5,7±0,4	4,8±0,2	10,3±0,7*	4,2±0,6	4,6±0,5	5,4±0,2	12,6±0,8*	2,4±0,2

*P < 0.01.

The study of the cyclic nucleotide content in the lymphocytes showed changes under the influence of histamine only for the fastest sedimented fraction of splenic lymphocytes (Table 3). Under the influence of histamine significant changes were observed in the intracellular content of cAMP only, and not of cGMP. Metiamide, which blocks H_2-histamine receptors, abolished the increase in the intracellular cAMP concentration under the influence of histamine. Mepyramine had no such action.

Histamine thus affects proliferation of the rapidly sedimented splenic lymphocyte fraction from sensitized mice. The intensity and direction of the changes in the degree of proliferation depended on the time of sensitization, and were opposite in the inductive and productive phases of the immune response. The action of histamine was mediated through H_2-histamine receptors, for it could be abolished by metiamide. The action of histamine on proliferation of the rapidly sedimented splenic lymphocyte fraction from sensitized mice was connected with adenylate cyclase activation, which led to an increase in the intracellular cAMP concentration. Data in the literature show that only T suppressor lymphocytes are sensitive to the action of histamine [7, 10], whereas the fastest sedimented cells, represented in the present experiments by the histamine-sensitive fraction, also belong mainly to the T suppressor lymphocytes [8]. Since the direction and degree of change of the immune response through the action of exogenous histamine differed in different stages of sensitization, depending on the time of injection of histamine either stimulation or inhibition of the immune response is possible.

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RISE IN SERUM LEVEL OF NATURAL COLD LYMPHOCYTOTOXINS AFTER AUTOHEMOSTIMULATION

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The stimulating action of blood transfusions on the biological systems of the body is well known. Transfusions of blood or its components and also autohemostimulation raise the levels of antibacterial antibodies [7, 8], complement [2], lysozyme, properdin [1], normal antitissue autoantibodies [5], the phagocytic activity of leukocytes [3], and activity of various other factors of cellular and humoral immunity.

This paper describes data showing increased activity of natural cold isolymphocytotoxins (NCILCT) as a result of hemostimulation with autologous blood in man.

EXPERIMENTAL METHOD

Activity of NCILCT was studied in the blood serum of 16 healthy volunteers who received intramuscular injections of 5-9 ml whole autologous blood once every 2 days. A course of five injections of autologous blood was given to 11 subjects and 10 injections to five subjects.

Serum for investigation was taken before autohemostimulation and before each injection of autologous blood, i.e., after 2, 4, 6 days, and so on. At the end of the course of autohemostimulation, serum was taken once every 10 days for 1.5-2 months.

The microlymphocytotoxic test was performed by the usual method [10] (lymphocytes + serum + rabbit complement), the only difference being that the cells were incubated with sera at 14°C for 1 h and the mixture after addition of complement was incubated at 14°C for 40 min. Lymphocytes of 20 standard donors were used in the reaction in the form of a suspension enriched with B and T cells.

Lymphocytes were isolated by allowing 80 ml heparinized blood to stand for 30 min at 37°C. The supernatant, containing the leukocyte suspension, was transferred to other tubes, where iron carbonyl was added in the proportion of 10 mg to 20 ml suspension, then the mixture was incubated for 20 min at 37°C to sediment the monocytes, after which it was centrifuged in a dextran–verografin density gradient [6] for 20 min at 170g. The dextran–verografin solution was made up from eight parts standard 6% dextran and two parts standard 76% verografin. Its specific gravity was 1076-1077.

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