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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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TABLE 1. Effect of Autohemostimulation on Activity of Natural Cold Isolymphocytotoxins in Healthy Subjects

Surname	Cytotoxic activity of sera, % of dead cells			
	with B lymphocytes		with T lymphocytes	
	before injection of autologous blood	after injection	before injection of autologous blood	after injection
S.	20 (30)	75 (30)	75 (35)	100 (45)
Sh-v	20 (25)	50 (25)	50 (25)	75 (30)
K-na	20 (25)	50 (30)	20 (30)	50 (30)
P.	20 (30)	50 (30)	50 (30)	50 (30)
O-i	20 (30)	50 (30)	20 (50)	50 (50)
K-v	20 (30)	75 (30)	20 (10)	50 (15)
Sh-va	20 (25)	75 (40)	20 (30)	20 (30)
M-a	20 (40)	75 (40)	20 (20)	75 (20)
I.	20 (15)	50 (15)	20 (30)	75 (30)
M-o	50	75	50	75
V.	50	75	50	75
D.	50	75	50	75
O-k	50	75	50	75
K-o	50	75	75	80
T.	20	20	50	50
L.	20 (10)	20 (10)	50 (15)	50 (15)

Legend. Frequency of reaction of serum (in %) given in parentheses.

The ring on the boundary between the two phases, consisting of a mixture of T and B lymphocytes, was washed off with Hanks' solution and resuspended in the same solution, the lymphocyte concentration being adjusted to 2000-4000 cells/mm^3. Next, papainized sheep's red blood cells (SRBC) were added to the suspension at the rate of 2 ml of a 20% suspension of SRBC to 1 ml lymphocyte suspension. The mixture of cells was kept for 15 min at 18-25°C, centrifuged for 5 min at 170g, and placed for 30-40 min in a refrigerator at 6-8°C. After the end of this time the residue was resuspended, layered above dextran–verografin solution, and centrifuged for 20 min at 170g to remove rosettes formed by T lymphocytes with papainized SRBC. The cell suspension remaining at the partition boundary between the two phases, enriched with B lymphocytes, was washed off with Hanks' solution and a suspension containing 1000-3000 cells/mm^3 was prepared.

The SRBC were papainized with a 0.25% solution of papain (from Merck, West Germany) L-cysteine hydrochloride for 10 min at 37°C. The ratio of SRBC to enzyme solution was 2:1.

A lymphocyte fraction enriched with T cells was obtained from the residue of lymphocytes which formed rosettes with papainized SRBC by lysis of the latter with 10-20 volumes of distilled water for 30-40 sec. The T lymphocytes were then washed off with Hanks' solution and a suspension containing 1000-3000 cells/mm^3 was prepared.

The concentration of T lymphocytes in the enriched fraction was determined by the method of spontaneous rosette formation with native SRBC [9]. The concentration of B cells was determined by two methods: by the allogeneic rosette formation test with Rh-positive erythrocytes loaded with incomplete Rh-antibodies [4] and the heterologous rosette formation test with SRBC, loaded with rabbit antiserum [9]. A suspension of T and B cells with a purity of 80-90 and 70-80% respectively was used in the lymphocytotoxic test.

EXPERIMENTAL RESULTS

In the overwhelming majority of subjects tested on the 6th-10th day after the beginning of autohemostimulation a definite increase was found in NCILCT activity relative to both B and T lymphocytes (Table 1). Only in two subjects was NCILCT activity unchanged. One of them exhibited a paradoxical reaction to injection of autologous blood, consisting of a brief fall in anti-T NCILCT compared with the initial level. During the 1.5-2 months after the end of the course of autohemostimulation, NCILCT activity of all subjects gradually declined to its initial level.

The frequency of reaction of NCILCT (the percentage of positively reacting specimens of lymphocytes relative to the total number tested) in the overwhelming majority of cases was the same both before and after autohemostimulation. The exception was the individual cases

in which the frequency of reaction after autohemostimulation was 5-15% above the initial level. These differences can evidently be explained on the grounds that additional scatter or activation of NCILCT which were present in the recipients before hemostimulation, but which were not revealed because of their low activity, takes place in the course of hemostimulation.

These results not only confirm the existing view on the stimulating action of injections of autologous blood, but they can also be used for applied purposes: to raise NCILCT activity in diagnostic sera.

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EFFECT OF RABBIT ANTISERUM TO MOUSE BRAIN ON BONE MARROW CELLS FORMING GRANULOCYTE-MACROPHAGE COLONIES IN AGAR CULTURE *in vitro* AND *in vivo*

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It was shown previously [8, 9, 12, 13] that rabbit antiserum against mouse brain (RASMB) possesses activity against mouse pluripotent hematopoietic stem cells (CFU-S), for treatment of bone marrow with this antiserum considerably reduces their ability to form colonies in the spleen of lethally irradiated mice. It has been suggested that activity of RASMB against hematopoietic stem cells is due to the presence of an antigenic marker for CFU-S. However, specificity of RASMB for CFU-S has not been finally proved. The writers have postulated [4, 15] that RASMB inactivates not the pluripotent hematopoietic stem cells, but a different cell population contained in bone marrow and playing a subsidiary role relative to hematopoietic stem cells in splenic colony formation. This hypothesis is based on the following experimental data: Injection of intact syngeneic thymocytes into recipient mice together with RASMB-treated bone marrow considerably reduces the effect of the antiserum [4, 15].

Interaction between thymus-dependent lymphocytes and hematopoietic stem cells has been described [5, 6].

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