IMMUNOLOGIC ACTIVITY OF HUMAN LYMPH CELLS IN THE BLAST TRANSFORMATION REACTION

D. K. Bashirova, O. S. Kochnev,
F. A. Davletkil'deev, and M. V. Lagutina

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The immunologic activity of lymph cells obtained in the course of therapeutic drainage of the thoracic duct was studied in 14 patients with subhepatic jaundice of varied genesis. The blast transformation of lymphocytes reaction showed that lymphocytes from lymph are capable of blast formation when exposed to the action of phytohemagglutinin, antilymphocytic γ-globulin, and certain bacterial antigens. The highest intensity of the reaction was found in cultures of lymph lymphocytes taken for these purposes on the 2nd-3rd days after the beginning of lymph drainage. In the course of lymphorrhea the ability of stimulated lymph cells to undergo blast formation is reduced. In the period of lymph drainage, no blast formation takes place in stimulated cultures of patients' blood cells. The majority of antigen-sensitive lymphocytes is thus in a reserve state in the lymphoid tissue of the body.

KEY WORDS: blast transformation of lymphocytes; lymph drainage.

Many papers devoted to the study of the immunologic activity of human blood cells under normal conditions and in various diseases have recently been published [1-4, 6, 9, 11]. However, few such communications have dealt with the cells of human lymph [7, 8].

The object of this investigation was to study the immunologic activity of cells from thoracic duct lymph in patients with subhepatic jaundice of varied genesis.

EXPERIMENTAL METHOD

Therapeutic drainage of the thoracic duct with the aim of preoperative preparation and removal of between 400 and 1000 ml of lymph daily for 1-5 days were carried out on 14 patients. Meanwhile the patients received intensive treatment aimed at restoring the loss of fluid, electrolytes, proteins, etc. Among the patients studied, one had suffered from typhoid fever and two from bronchopulmonary diseases in the past.

The immunologic activity of the lymph cells, and also of blood cells, was investigated in the blast transformation of lymphocytes (BTL) reaction, by the method described by the writers previously [7]. Heparinized lymph from the patients (200 units heparin, from Richter, Hungary, to 10 ml lymph) obtained on the 1st-5th days after the beginning of drainage of the thoracic duct was used for the experiments.

Several antigens were used to stimulate the lymphocytes: dried purified tuberculin (DPT) in a dose of 50 μg/ml culture medium, combined typhoid antigen (CTA) in a dose of 15 μg/ml, dysenterin in a dose of 0.25 skin dose/ml, therapeutic dysentery vaccine (TDV), bacterial allergens (staphylococcal, streptococcal, Proteus, Escherichia coli), each in a dose of 0.1 mg in 1 ml, diluted 1:10. Lymph cells also were stimulated with phytohemagglutinin (PHA) in a dose of 20 μg/ml and antilymphocytic γ-globulin (ALG) in a dose of 0.36 mg protein/ml. Cultures of lymphocytes also were studied without antigenic stimulation.

The results of investigation of blast transformation of lymphocytes are given as mean values calculated as percentages.

EXPERIMENTAL RESULTS

The study of the cellular composition of the lymph showed that the number of lymphocytes varied depending on the time of investigation (Table 1).

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TABLE 1. Number of Lymphocytes in 1 μl Lymph during Drainage of Thoracic Duct

Time of investigation (days after drainage)	Number of lymphocytes	
	scatter	mean
1	2400—11 600	8150±1262
2	1400—8400	2020±263
3	1200—6600	2148±811
4	800—3400	1766±387
5	400—4000	1800±873

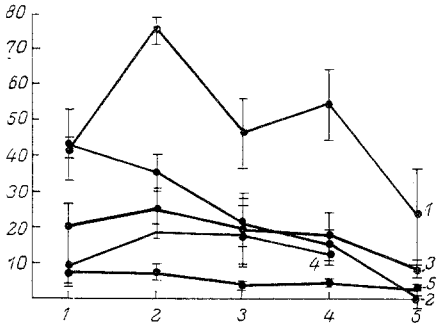

Fig. 1. Kinetics of BTL in lymph from human thoracic duct. 1) PHA; 2) DPT; 3) CTA; 4) dysenterin; 5) control (without antigenic stimulation). Abscissa, times of investigation (in days); ordinate, mean number of blast cells (in %).

As the period of lymph drainage lengthened the scatter of the lymphocyte count in the lymph increased, and on the 2nd day the mean lymphocyte count was significantly reduced ($P < 0.001$). Later (3rd-5th days) the lymphocyte count became stabilized, evidently on account of treatment. The total blood leukocyte count varied from 3900 to 38,400 cells/μl, the lymphocyte count ranged between 1 and 10%, and the mean index of the blood lymphocytes (7.14 ± 1.17) was significantly lower than initially. At all times of study BTL of the lymph in response to PHA and ALG was significant ($P < 0.05$-0.001) and the values of the reaction were 5 to 10 times greater than those of cells without antigenic stimulation (from 2.8 ± 1.8 to 8.21 ± 4.79). The kinetics of BTL was most interesting in cultures of lymph lymphocytes with PHA (Fig. 1). The mean number of blast cells was maximal on the 2nd day of investigation (75.96 ± 3.54), when it was significantly higher than on the 1st ($P < 0.001$) and 3rd days ($P < 0.05$); the BTL level at that time also was higher than the BTL level in the blood of healthy donors (47.0 ± 2.5) established by the writers previously [2]. Starting from the 3rd day the intensity of the reaction of the lymph cells during treatment with PHA fell, and on the 5th day it reached a minimum (24.0 ± 14.1). In the period of lymph drainage the number of blast cells in cultures of patients' blood cells stimulated by PHA was very low (0-10%).

The kinetics of BTL of the lymph under the influence of the various antigens is illustrated in Fig. 1. The stimulating action of DPT on lymph lymphocytes was significant on the 2nd-4th days of the investigation. At these times the mean number of blast cells (from 16.0 ± 3.65 to 36.0 ± 5.74) was 3-5 times greater than their number in the absence of stimulation. The mean BTL index fell sharply on the 5th day (1.25 ± 0.31).

A significant stimulating action of CTA, dysenterin, and TDV was found for cultures of lymph lymphocytes taken on the 2nd day of drainage of the thoracic duct. This action of Proteus, streptococcal, and staphylococcal allergens was established on the 3rd day. On the 4th-5th day the mean values of the BTL index for cultures with the above-mentioned bacterial antigens was appreciably reduced. No stimulating action of E. coli allergen on lymph cells could be found. Incidentally, blast cells were not found in cultures of blood cells following stimulation by bacterial antigens.

In patients with subhepatic jaundice blast transformation could thus be detected in cultures of lymph lymphocytes when treated both with nonspecific (PHA and ALG) and bacterial antigens. Blast transformation did not take place in the blood cell cultures because of the small number of lymphocytes in the peripheral blood. The significant stimulating effect of PHA and ALG on the 2nd day and of bacterial antigens on the 2nd

and 3rd days in cultures of lymph lymphocytes may be associated with mobilization of reserves from the circulating lymphocytes and also from cells of lymphoid tissue.

Lymph drainage thus acts as a stimulus for the mobilization of antigen-sensitive lymphocytes from lymphoid tissue. It is evidently these cells which, in ordinary situations, exist in the body in a "resting" state and enter the circulation only when maximal strain is placed on the immune system [11]. It should also be noted that this reaction is of short duration and may be followed by weakening of the immune response; for that reason, when therapeutic drainage of the thoracic duct is carried out, immunologic control is essential.

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EFFECT OF α_1-PROTEASE INHIBITOR (α_1-ANTITRYPSIN) ON THE INTENSITY OF TRANSFORMATION OF PHYTOHEMAGGLUTININ-STIMULATED LYMPHOCYTES

F. S. Baranova, A. A. Berman, and Yu. M. Zaretskaya

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α_1-Antitrypsin (α_1-AT) reduces the intensity of transformation of human peripheral blood lymphocytes stimulated by phytohemagglutinin. The degree of inhibition is determined by the antiprotease activity of the α_1-AT. Maximal inhibition of transformation was shown to be 50%. Participation of α_1-AT in the control of activity of lymphoid tissue cells is postulated.

KEY WORDS: antiproteases; lymphocyte transformation.

Natural and synthetic inhibitors of proteinases have been shown to modify the development of several immunologic phenomena [5, 6, 9]. A special place among them is occupied by blood serum proteins with antiprotease activity, in connection with their possible role in the regulation of the biological activity of lymphoid tissue cells in vivo [1, 4].

The object of this investigation was to study the effect of α_1-antitrypsin (α_1-AT) on the intensity of transformation of human peripheral blood lymphocytes stimulated by phytohemagglutinin (PHA).

EXPERIMENTAL METHOD

α_1-AT was isolated from citrated donor's plasma by Liener's method [7] without modification. The method included salting out with $(NH_4)_2SO_4$ at 50-70% saturation, chromatography on DEAE-Sephadex A-50 and concanavalin (Con) A-Sepharose. The antiprotease activity of the serum proteins and individual fractions was determined from inhibition of hydrolysis of N-benzoyl-L-arginine ethyl ester (BAEE) by trypsin [1] and was

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Laboratory of Immunogenetics, Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR, Moscow.

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