CHANGES IN THE CYTOCHEMICAL STRUCTURE OF THE OF THE BLOOD LYMPHOCYTE POPULATION UNDER THE INFLUENCE OF ANTILYMPHOCYTIC SERUM

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Activity of dihydro-orotate dehydrogenase and succinate dehydrogenase was studied cytochemically in the blood lymphocytes of rats and dogs before and at various times after injection of antilymphocytic serum and antilymphocytic globulin. Under the influence of these substances the number of lymphocytes without dehydrogenase activity in the peripheral blood stream increased, and this was accompanied by an increase in the number of cells with high activity of these enzymes. The changes in the cytochemical structure of the populations are examined from the standpoint of the depressive and, at the same time, the activating effect of the preparations.

The object of this investigation was to study the activity of succinate dehydrogenase (SDH), reflecting energy processes in the mitochondria, and of dihydro-orotate dehydrogenase (DDH), participating in the synthesis of pyrimidine bases, in the blood lymphocytes of animals at various times after injection of antilymphocytic serum (ALS) and antilymphocytic globulin (ALG).

EXPERIMENTAL METHOD

Experiments were carried out on male August rats (13 animals) weighing 150-200 g and on six male dogs weighing 20-25 kg. On alternate days for 1 week the rats received an intraperitoneal injection of 0.5 ml rabbit ALS (nine animals) and of normal rabbit serum (four animals). Daily for 14 days the dogs received ALG intramuscularly in a dose of 5 mg protein/kg body weight. The ALG (batch 66/1) was prepared at the Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR, and had a lymphoagglutinin titer of 1:560 and a lymphocytotoxin titer of 1:1280. The ALS was prepared by immunization of rabbits with lymphocytes obtained from rat lymph glands followed by adsorption with antierythrocytic antibodies. The titers of lymphoagglutinins and lymphocytotoxins were 1:560 and 1:1280, and no antibodies against serum proteins were found. The titer of hemagglutinins was below 1:2.

Before and after the injections of ALS and ALG into the animals the total leukocyte count and blood formula were determined. Activity of SDH (1.99.3.1) and DDH (1.3.3.1) in the lymphocytes was determined cytochemically by Nartsissov's method [3]. The number of granules of reaction product was counted in each lymphocyte (altogether 50 cells). By carrying out the analysis in this way the combined dehydrogenase activity in 50 cells or the mean activity per lymphocyte could be expressed and the cytochemical structure of the peripheral lymphocyte population could be studied by arranging them in corresponding classes depending on the level of cell enzyme activity.

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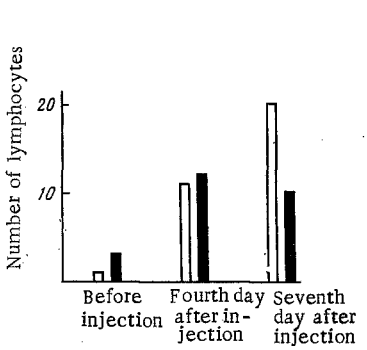

Fig. 1

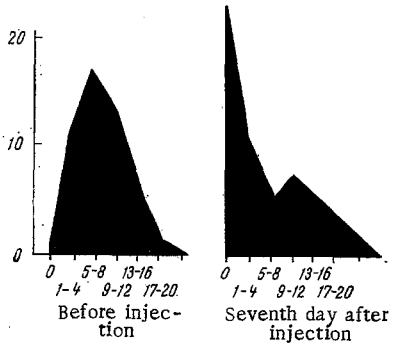

Fig. 2

Fig. 1. Changes in number of lymphocytes without and with high DDH activity at various times after injection of ALS. Unshaded columns, lymphocytes with no enzyme activity; shaded columns, lymphocytes with high enzyme activity.

Fig. 2. Frequency polygon of lymphocytes subdivided by DDH activity before and after injection of ALS. Abscissa, number of granules in lymphocytes; ordinate, number cells.

The results were subjected to statistical analysis. The significance of the increase in number of lymphocytes with no activity and with high activity of the enzyme was assessed by means of Wilcoxon's nonparametric criterion. To determine the structure of the lymphocyte population with respect to enzyme activity the parameters of distribution were calculated and their arithmetic mean, standard deviation, and coefficients of variation, asymmetry, and excess were determined. The difference between the dispersions were estimated by means of Fisher's criterion and subdivision into two subpopulations was carried out by Urbakh's method [6]. Differences between the mean populations before and after the injections were estimated by Student's criterion.

EXPERIMENTAL RESULTS

The study of DDH activity showed that at certain stages the injection of ALS into intact animals had virtually no effect on the total activity of the enzyme, i.e., the total number granules in 50 lymphocytes remained constant. A significant decrease in DDH activity was discovered only after three or four injections of the preparation (5th-7th day after the beginning of injection). However, these mean indices of enzyme activity largely masked the character of the change arising in the peripheral blood lymphocyte population after administration of ALS. Cells with no enzyme activity began to appear in the blood stream 2 h after injection of ALS; meanwhile the number of lymphocytes with high DDG activity increased. These changes were particularly clear ($P \leq 0.05$) on the 4th and 7th days after the beginning of injection of the preparation (Fig. 1). Results of this nature are evidence of an increase in the metabolic heterogeneity of the lymphocytes. Each subsequent injection of ALS increased this heterogeneity and this, in turn, was reflected in the curve of distribution of lymphocytes as a function of their degree of DDH activity, and at certain times this curve became asymmetrical in character (Fig. 2, Table 1).

These results suggest that two populations of lymphocytes exist; statistical populations showed that the mean of one subpopulation was 3.68 ($m_1 = 0.9$) and of the other 13.5 ($m_2 = 1.6$).

The dynamics of SDH activity in the peripheral blood lymphocytes very closely resembled the character of the changes in DDH activity; differences could be found only in the times of appearance of these changes.

Control experiments showed that injection of the corresponding doses of normal rabbit serum into the rats led to a sharp increase in dehydrogenase activity in the lymphocytes; no cells with low enzyme activity were found.

Intramuscular injection of ALG into dogs caused changes in the population structure of the peripheral lymphocytes by dehydrogenase activity analogous to the changes observed in rats after injection of ALS.

TABLE 1. Statistical Parameters of Population Structure of Rat Lymphocytes by Dihydro-orotate Dehydrogenase Activity before and after Administration of ALS

Time of determination	$\bar{X}$	σ	V	A	E
Before injection	7,3*	3,32†	45,5	0,550	—1,17
On 7th day after injection.	5,9	6,9	117	0,850‡	—0,2

Legend: $\bar{X}$) mean enzyme activity; σ) standard deviation; V) coefficient of variation; A) coefficient of asymmetry; E) coefficient of excess.

*Difference between means by Student's criterion ($P < 0,01$).
†Difference between dispersions by Fisher's criterion 4.3, $P < 0,01$.
‡Distribution before injection of ALS symmetrical, after injection asymmetrical ($P < 0,01$ for 50 cells).

The number of cells with high and with reduced enzyme activity in the blood stream also increased, and this was reflected in the character of the distribution curve, which became sharply asymmetrical as the total quantity of the preparation injected increased. A general decrease in DDH activity in the dogs appeared after 10-12 injections (by the 10th-11th day after the beginning of injection of ALG).

The character of the cytochemical changes in the peripheral lymphocytes depends not only on the duration of injection (or, correspondingly, on the total dose) of ALS and ALG. As preliminary tests showed, the character of the changes was closely connected with the initial and concomitant state of the animal. In particular, injection of ALG into dogs after skin grafting was accompanied by an increase in the number of only those lymphocytes with low DDH activity, and this was combined with a decrease in the total activity of the enzyme. Similar changes were found in rats with nephrotoxic nephritis after injection of ALS.

The dynamics of dehydrogenase activity after injection of ALS and ALG clearly shows that besides quantitative changes in the blood lymphocytes there are also qualitative changes, the nature of which (the appearance of cells with sharply reduced enzyme activity) corresponds to known views regarding the depressive influence of these preparations. In turn, the marked decrease in DDH (decreased synthesis of pyrimidines) and SDH (decrease in the energy supply) activity which occurred in part of the peripheral lymphocyte population must be regarded not only as a criterion, but also as one cause of the immunodepressive action of ALS and ALG.

Meanwhile, as the investigations showed, the changes arising in the lymphocyte population structure cannot be completely explained by the depressive effect of the preparations. In particular, the increase in heterogeneity of the lymphocytes with respect to dehydrogenase activity after injection of ALS and ALG, observed also in certain allergic diseases [5] and in the period of antibody synthesis [4], is evidently one of the signs reflecting the state of functional activity of the lymphoid tissue. The appearance of a state of this type after administration of ALS and ALG is also evidence of an increase in the number of lymphocytes with high dehydrogenase activity.

The increase in dehydrogenase activity in the peripheral blood lymphocytes is a feature constantly accompanied by a state of increased activity both of the cells themselves (in particular, in blast transformation [1]), and in the lymphoid tissue in the period of antibody synthesis [1, 2]. However, in these states no increase in the number of cells with low enzyme activity is observed, or this increase is transitory in character [4]. After injection of ALS and ALG the increase in the number of circulating lymphocytes with high dehydrogenase activity may be connected with replenishment of the peripheral blood stream with new cells from the lymph glands [7]. However, the possibility of sterile activation of the cells arising under the influence of these preparations, and also of their differential action on lymphocytes of different origin [8], cannot be ruled out.

The investigations thus showed that after injection of ALS and ALG in the period of stabilization of quantitative shifts, changes whose character indicates the presence of a simultaneous depressive and activating influence of the preparations can be detected cytochemically in the lymphocyte population structure.

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