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ENZYMIC AND IMMUNOLOGICAL ACTIVITIES OF LYMPHOCYTES DURING CHEMICAL CARCINOGENESIS

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During chemical carcinogenesis in Wistar rats the activity of acid phosphatase and certain dehydrogenases in the blood lymphocytes was compared with the development of cellular immunity revealed by the macrophage migration inhibition test. In the early periods of carcinogenesis the changes in succinate dehydrogenase and acid phosphatase activity were shown to correspond to a high level of immunological reactivity of cellular type in 66% of the animals. During progressive growth of the tumors in the rats the enzyme balance in the lymphocytes was disturbed and, at the same time, the immunological response was depressed.

KEY WORDS: chemical carcinogenesis; lymphocytes; enzyme activity; cellular immunity.

Because of the inadequacy of immunological protection against malignant growth, the discovery of the mechanism of this phenomenon is extremely important. It follows from communications published previously [1, 2, 4, 7-10, 12] that during the development of the immune response of the organism to antigens of infectious and noninfectious nature definite changes arise in metabolism of the lymphocytes. Suggestions have been made for an approach to the elucidation of the degree of informativeness of the cytochemical changes in the cells of the immunocompetent system as a means of predicting the course of diseases and the effectiveness of treatment [3].

The object of this investigation was to study the enzyme activity of lymphocytes and to compare this activity with the manifestation of reactions of cellular immunity in the course of chemical carcinogenesis. No investigations of this type on this particular model have been described.

EXPERIMENTAL METHOD

The carcinogen DMBA was injected intramusuclarly in a dose of 3 mg per animal into Wistar rats. Before treatment with the carcinogen and for the 5 months after its injection, the dynamics of activity of three enzymes was studied in the blood lymphocytes: succinate dehydrogenase (SD; EC 1.3.99.1), α-glycerophosphate dehydrogenase (α-GPD; EC 1.1.2.1), and acid phosphatase (AP; EC 3.1.3.2). Activity of the dehydrogenases was determined by Nartsissov's method [6] and of AP by the method of Goldberg and Barka [11]. In order to characterize the activity of these enzymes after the number of stained granules had been counted in 50 lymphocytes, mean values per cell were obtained.

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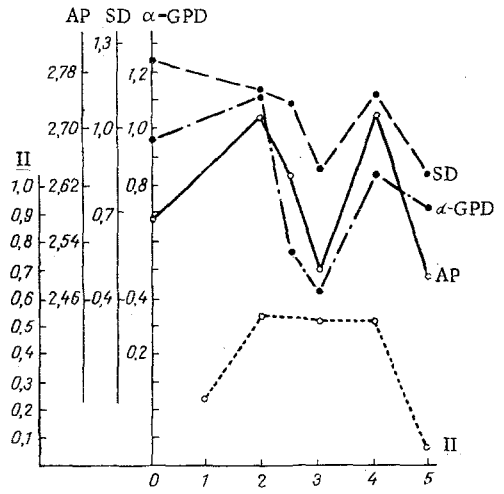

Fig. 1. Comparison of enzymic and immunological activity of lymphocytes during carcinogenesis. Abscissa: months of carcinogenesis; 0) indices before experiment; ordinate: scale II) index of inhibition of migration of macrophages from capillary tubes; log of mean values of AP, SD, and α-GPD activities.

TABLE 1. Rate of Change of Mean Values of Logarithms of Enzyme Activities in Lymphocytes during Carcinogenesis*

Enzyme	Time of carcinogenesis (in months)			
	0—2	2—3	3—4	4—5
SD	—0,012	—0,065	+0,054	—0,012
α-GPD	+0,012	—0,341	+1,106	—0,024
AP	+0,022	—0,041	+0,059	—0,042

*Rate of decrease of enzyme activity preceded by a minus sign, increase by a plus sign.

Cellular reactions of immunity at different times of carcinogenesis were studied with the aid of the method of inhibition of migration of macrophages from capillary tubes described previously [5]. The results were subjected to statistical analysis by the Minsk-22A computer. To normalize the distribution of the values for scrutiny, logarithms were taken of the indices of enzyme activity, after which the normality of the distribution was tested by the χ^2 criterion. In this way a relationship was obtained between the values of the logarithms and their direct values. Standard deviations (dispersions) were calculated. The values of enzyme activity obtained were subjected to correlation analysis. The coefficients of correlation were determined both to detect correlation between the activities of each enzyme at different times in the course of carcinogenesis and their initial values under normal conditions, and also to study correlation between the activities of individual enzymes. Piecewise-linear approximation of the resulting curves to functions of the type $\log Q = f(t)$ was carried out. The rates of change of enzyme activity in the different time intervals were calculated. The degree of significance of differences between groups was determined by the t test.

EXPERIMENTAL RESULTS

In this investigation, three enzymes characterizing different metabolic pathways in these cells (the Krebs cycle, glycolysis, and hydrolysis) were chosen for the cytochemical study of the peripheral blood lymphocytes during the development of chemically induced sarcomas. The results of the experiments to compare enzymic and immunological activity of the lymphocytes during carcinogenesis are illustrated in Fig. 1.

Analysis of these indices shows that during carcinogenesis the enzymic activity of the lymphocytes undergoes definite cyclic changes.

After 2 months of carcinogenesis, when the immunodepressive action of the carcinogen was reduced, a significant decrease was observed in SD activity and an increase in AP activity. According to data in the literature, such changes in lymphocyte metabolism are characteristic of the development of hypersensitivity of delayed type, which arises in various immune states. These results show that at this stage of carcinogenesis 66% of the animals were producing one of the mediators of cellular antitumor immunity, for the macrophage migration inhibition index was 0.55.

By the third month of carcinogenesis, a time when progressive growth of the tumors began in most rats, the level of all three enzymes was considerably reduced. This decrease in enzymic activity of the lymphocytes had already started after 2.5 months. Low dehydrogenase activity is linked with increasing severity of the pathological process. The macrophage migration inhibition index remained at its previous level, but this test

was positive in only 58% of animals. The decrease in AP activity at this stage of carcinogenesis will be noted but is difficult to explain. It may perhaps be connected with the beginning of exhaustion of the immunological response.

In fact, by 4 months the macrophage migration inhibition index remained fairly high (0.53) in only 36% of rats. Although the enzyme activity was increased again at this time, the dehydrogenase activity had not regained its original level.

Changes in the enzymic status of the lymphocytes during carcinogenesis were also revealed by correlation analysis. Before administration of the carcinogen, moderate positive correlation was found between the activity of the dehydrogenases, and the coefficient of correlation (r) was 0.40. After 2 months of carcinogenesis, correlation between them was now negative ($r = -0.43$), confirming a compensatory increase in α-GPD activity associated with a decrease in SD activity. Later association between the dehydrogenases was absent ($r = -0.13$), and as the tumors developed progressively, it was not again restored.

The disturbance of the energy balance of the lymphocytes was confirmed by determination of correlation between the activities of each of the enzymes and this index under normal conditions. The data on α-GPD activity are particularly interesting in this respect. Significant negative correlation was found for this enzyme at 4 months of carcinogenesis ($r = -0.53$), but by 5 months, when correlation had already ceased for the other two enzymes, it still continued to rise for α-GPD ($r = -0.68$).

The calculation of the rate and direction of changes in the mean values of the logarithms of enzyme activity is given in Table 1. Examination of these results shows that between the third and fourth months of carcinogenesis the rates of increase of SD (0.054) and AP (0.059) activity did not differ significantly from their decrease in the previous time interval. Meanwhile the rate of rise of α-GPD activity (1.106) was more than three times greater than the rate of its decrease between the second and third months.

To sum up these findings, it can be postulated that in the later stages of carcinogenesis there is a disturbance of the enzyme metabolism of the lymphocytes, so that the α-glycerophosphate shunt possesses the greatest activity.

The results of these experiments thus showed that during carcinogenesis complex enzymic changes, which affect the development of immunological insufficiency during growth of malignant neoplasms in a definite manner, take place in the lymphocytes.

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