SENSITIVITY OF RNA-SYNTHESIZING SYSTEM OF THE LYMPHOCYTES OF PATIENTS WITH MALIGNANT NEOPLASMS TO PHYTOHEMAGGLUTININ AND DEXAMETHASONE

I. A. Ioannesyants, V. V. Adler,
Z. I. Él'kina, S. I. Artamonova,
Z. G. Kadagidze, and V. S. Shapot*

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Biosynthesis of RNA catalyzed by DNA-dependent RNA polymerase was demonstrated in a reconstructed system containing isolated lymphocyte nuclei, Mg^{2+} or Mn^{2+} salts, and ammonium sulfate, in the presence of four nucleoside triphosphates. Both Mg^{2+}- and Mn^{2+}-dependent forms of this enzyme were found in the nuclei of normal lymphocytes and of lymphocytes from patients with melanoma, lung carcinoma, and sarcoma. The activity of both forms of RNA polymerase in the nuclei of the patients' lymphocytes was higher than in normal analogs. The sensitivity of DNA-dependent RNA polymerase to dexamethasone and phytohemagglutinin was less marked in the nuclei from patients with lung carcinoma, melanoma, and sarcoma than in normal lymphocytes.

KEY WORDS: *phytohemagglutinin; dexamethasone; lymphocytes; oncologic patients.*

DNA-dependent RNA polymerase in human lymphocyte nuclei has already been studied [9, 10, 13]. Just as in other eukaryote cells, three functional forms of RNA polymerase catalyzing the specific transcription of different classes of RNA have been identified in healthy human peripheral blood lymphocytes [13]. During blast transformation of the lymphocytes under the influence of phytohemagglutinin (PHA) the dynamics of activation of individual forms of this enzyme has been traced [9, 13]. Although many investigations of the activity of DNA-dependent RNA polymerase in normal lymphocytes have been published, there are as yet no data in the literature concerning the determination of the activity of this enzyme in the lymphocytes of patients with melanoma, lung carcinoma, and sarcoma. In these malignant neoplasms the lymphocytes are virtually indistinguishable morphologically from their normal analogs, but they exhibit certain functional disturbances, notably a decrease in their sensitivity to the mitogenic action of PHA and in their ability to form rosettes with heterologous erythrocytes [5, 11].

Since one of the initial stages of blast transformation induced by PHA is induction of RNA synthesis, it was decided to compare the behavior of the DNA-dependent RNA polymerase of lymphocytes from blood donors with that from lymphocytes from patients with melanoma, sarcoma, and lung carcinoma toward glucocorticoids and, in particular, toward dexamethasone.

EXPERIMENTAL METHOD

Lymphocytes were isolated from blood by methods described previously [3]. The purity of the cell suspensions was verified in each experiment microscopically. The experimental suspensions consisted of 65-70% of small lymphocytes and they were cultivated in medium No. 99 with 20% autologous plasma at 37°C. The nuclei were isolated from the cells in medium containing 0.32 M sucrose with 0.02 M $MgCl_2$, after which the cells were disintegrated in a

*Corresponding Member, Academy of Medical Sciences of the USSR.

Laboratory of Biochemistry of Tumors, All-Union Oncologic Center of the USSR, Moscow.
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TABLE 1. Activity of DNA-Dependent RNA Polymerase in Nuclei of Lymphocytes from Donors and Patients with Melanoma, Sarcoma, and Lung Carcinoma

Experimental conditions	Indices depending on ions	Activity on DNA-depending RNA-polymerase, counts/min/μg DNA			
		donors (n = 32)	lung carcinoma (n = 18)	melanoma (n = 20)	sarcoma (n = 20)
Lymphocytes	Mg^{2+} Mn^{2+}	4 340±1120 11 270±3020	23 480±5242 36 640±5498	25 970±1890 20 480±8938	29 000±5920 80 100±6000
Lymphocytes + PHA	Mg^{2+} Mn^{2+} K_s —Mg^{2+} K_s —Mn^{2+}	9 540±520 35 700±1126 2,2 3,2	52 500±862 45 000±2968 2,2 1,2	30 000±1023 32 000±786 1,2 1,6	30 500±1750 89 000±645 1,0 1,1
Lymphocytes + PHA + dexamethasone	Mg^{2+} Mn^{2+} K_i —Mg^{2+} K_i —Mn^{2+}	8 234±673 12 340±897 1,2 2,8	44 300±1020 32 400±4343 1,2 1,3	29 200±900 27 000±534 1,0 1,2	24 300±845 73 300±326 1,2 1,2

Legend. n) Number of subjects (at least two samples were taken from each subject in each determination); K_S) ratio between activity of DNA-dependent RNA polymerase in lymphocytes treated and not treated with PHA; K_i) ratio between activity of DNA-dependent RNA polymerase in nuclei of PHA-stimulated lymphocytes treated and not treated with dexamethasone.

Potter-Elvehjem homogenizer. The purity of the nuclei was verified in the phase-contrast microscope and DNA was determined by Burton's method [7].

Activity of DNA-dependent RNA polymerase was determined by measuring the incorporation of labeled precursor into RNA in a reconstituted system containing all four nucleoside triphosphates, one of which (UTP-^{3}H) was labeled, and also a preparation of the nuclei and the necessary additives. The experimental samples (0.25 ml) contained (in millimoles): Tris-HCl, pH 7.4, 50; $MgCl_2$ 10 or $MnSO_4$ 2.5; $(NH_4)_2SO_4$ 160; UTP, CTP, GTP, and ATP 0.4; UTP-^{3}H 0.04; nuclear suspension 50-100 μg DNA. The samples were incubated for 15 min, then placed on ice and treated with 5% TCA solution in 0.01 M sodium pyrophosphate. The residues were adsorbed on Millipore filters, which were washed with sodium pyrophosphate to remove TCA and then dried, after which the radioactivity was measured with a Mark II scintillation counter in toluene scintillator; PHA and dexamethasone were used in final concentrations of 2 μg/ml and 60 μg/ml, respectively.

EXPERIMENTAL RESULTS

Activity of the enzyme was studied in the nuclei of normal lymphocytes and of lymphocytes from patients with melanoma, lung carcinoma, and sarcoma, in the presence of Mg^{2+} and of Mn^{2+}. By setting up the experiments in this way an idea could be obtained of the activity of individual forms of the enzyme, differing in location and function: Mg^{2+}-dependent, located in the nucleoli and responsible for rRNA biosynthesis, and Mn^{2+}-dependent, located in the nucleoplasm and catalyzing principally the synthesis of DNA-like RNA [13].

As Table 1 shows, the nuclei of normal lymphocytes and the lymphocytes of the oncologic patients contained both functional forms of RNA polymerase, catalyzing the incorporation of UTP-^{3}H in the presence of both Mg^{2+} and Mn^{2+} ions. The marked difference in the activity of the two RNA polymerases in the nuclei of the lymphocytes of healthy subjects and patients will be noted. For instance, the level of activity of Mg^{2+}- and Mn^{2+}-dependent RNA polymerases in the lymphocyte nuclei was higher than in the donors, by six and two times, respectively, for melanoma, by five and four times for lung carcinoma, and by seven and eight times for sarcoma.

Normal lymphocytes and lymphocytes of oncologic patients are known to react differently to PHA. Whereas donor's lymphocytes undergo blast transformation under the influence of this mitogen, accompanied by a variety of biochemical changes (intensified RNA synthesis, increased activity of DNA-dependent RNA polymerase), the indices of blast transformation of cultures of lymphocytes from patients with lung carcinoma, sarcoma, and melanoma are dis-

tinctly lowered [2, 8, 12, 16]. Accordingly, it was interesting to discover whether DNA-dependent RNA polymerase in the lymphocytes of these patients is activated by PHA. The results indicate that after exposure of normal lymphocytes to PHA for 20 h a distinct increase in the activity of DNA-dependent RNA polymerase was observed, whereas the mitogenic action of PHA in the lymphocytes of patients with lung carcinoma, sarcoma, and melanoma, under analogous conditions, was reduced to half of normal for the Mg^{2+} form and to one-third of normal for the Mn^{2+} form (Table 1).

Dexamethasone inhibited Mg^{2+}-dependent RNA polymerase of both donors and oncologic patients, when stimulated by PHA, about equally, whereas the sensitivity of the Mn^{2+}-dependent form in the patients' lymphocytes was reduced by half (Table 1).

The dependence of the sensitivity of DNA-dependent RNA polymerase to PHA and dexamethasone on the course of the tumor process also was investigated in the patients with melanoma. Tests were carried out on 25 patients with melanoblastoma, admitted to hospital with different degrees of spread of the disease. The patients were conventionally divided (in accordance with the usual TNM classification) into three groups: 1) patients with a primary tumor only: T_1-3, N-0-1a; 2) patients who had already developed regional metastases: N_1b, N_2b, N_3, M_0; 3) patients with generalized tumor growth: M-1. The investigation was carried out before and during treatment and also in the late follow-up period. The decrease in the sensitivity of the RNA-synthesizing system to dexamethasone was found to run parallel with dissemination of the process: K_i for Mn^{2+}-dependent RNA polymerase of the patients with stage I was 2.2, with stage II 1.1, and with stage III 0.9. Consequently, in the lymphocytes of the patients with lung cancer, melanoma, and sarcoma, characterized by an increased initial level of activity of DNA-dependent RNA polymerase, a decrease in the sensitivity of the RNA-synthesizing system to the inducing action of PHA and to the inhibitory action of dexamethasone was observed. Similar results were obtained with lymphocytes of patients with chronic lymphatic leukemia, on which PHA had virtually no stimulating action and in which the activity of DNA-dependent RNA polymerase was increased [1].

Since PHA selectively transforms T_1 and T_2 cells but not B cells, and since only the population of T_1 lymphocytes is sensitive to the steroid [4], the decrease in sensitivity of DNA-dependent RNA polymerase observed in the lymphocytes of the oncologic patients to these substances was probably connected with a change in the relative numbers of T and B lymphocytes in their peripheral blood during the disease. During growth of the tumor it is possible that T_1 cells sensitive to the lytic action of the steroid are predominantly affected, and this later gives rise to profound disturbances of immunity. In this connection the many clinical observations indicating the simultaneous development of Itsenko–Cushing syndrome in patients with tumors in various situations are extremely interesting [14, 15]. It is now generally accepted that the clinical picture of Itsenko–Cushing syndrome is due to excessive production of adrenocortical glucocorticoid hormones, whereas the other endocrine glands are most frequently in a state of hypofunction [6].

Experimental data and the results of the present investigation thus suggest that determination of changes in the sensitivity of the RNA-synthesizing system of the lymphoid tissue to glucocorticoids can be used for the quantitative assessment of the heterogeneity of the T cell population and that, in conjunction with the blast-transformation reaction, this analysis can be used with advantage to determine the immunological status of the patient.

LITERATURE CITED

1. M. N. Blinov, A. D. Vladimirova, and I. S. Luganova, Tsitologiya, 16, 1388 (1974).
2. S. V. Kozhanova, A. K. Kosaev, and Yu. Sh. Kaliev, in: The Immunology of Tumors [in Russian], Kiev (1975), p. 130.
3. I. S. Luganova and I. F. Seits, Lab. Delo, Nw. 9, 541 (1967).
4. R. V. Petrov et al., Usp. Sovrem. Biol., 77, No. 1, 90 (1974).
5. N. N. Trapeznikov, V. I. Kupin, R. M. Radzikhovskaya, et al., Dokl. Akad. Nauk SSSR, 222, 468 (1975).
6. N. M. Turkevich and E. M. Samundzhan, Hormones and Carcinogenesis [in Russian], Kiev (1975), p. 34.
7. K. Burton, Biochem. J., 62, 315 (1956).
8. W. Catalana, W. Sample, and P. Chretien, Cancer (Philadelphia), 31, 65 (1973).
9. A. Cooke and J. Kay, Exp. Cell Res., 79, 179 (1973).
10. S. Handmaker and J. Graef, Biochim. Biophys. Acta, 199, 95 (1970).

11. T. Kanaya, Sapporo Med. J., 43, 48 (1974).
12. A. Nagel, W. L. Piessens, and T. Stilmant, Eur. J. Cancer, 1, 41 (1971).
13. B. Pogo, J. Cell Biol., 53, 635 (1972).
14. C. Plotz, A. Knowlton, and C. Ragan, Am. J. Med., 8, 597 (1952).
15. W. Sealy, Surgery, 68, 906 (1970).
16. H. Spengeler, K. Brunner, and A. Weck, Schweiz, Med. Wochenschr., 102, 1167 (1972).

ELECTRON-CYTOCHEMICAL AND MORPHOMETRIC INVESTIGATION OF ENZYME ACTIVITY IN THYROCYTE MITOCHONDRIA DURING CARCINOGENESIS

N. P. Dmitrieva, S. B. Stefanov, and E. A. Amirkhanyan

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Activity of cytochrome oxidase and succinate dehydrogenase in the mitochondria of thyroid gland cells of rats was studied by electron-histochemical and morphometric methods during experimental carcinogenesis. The activity of these enzymes in the mitochondria was shown to vary depending on the stage of malignant transformation: In the early stages it was close to normal, but later (precancer) it fell sharply and approached the level observed in the mitochondria of cancer cells. A marked decrease in the activity of the enzymes studied in the morphologically altered mitochondria of cancer cells may be a qualitative characteristic of these cells.

KEY WORDS: *mitochondria; cytochrome oxidase; succinate dehydrogenase; malignant transformation; thyroid gland.*

A previous investigation showed marked changes in the size, shape, and ultrastructure of the mitochondria in the cells during experimental carcinogenesis [1]. It was therefore interesting to discover whether the structural changes observed in the mitochondria correlate with their cytochemical features. Almost all the enzymes are known to be located in mitochondria and some of them participate in the formation and transformation of energy. Among the most important oxidoreductases are succinate dehydrogenase (SD) and cytochrome oxidase (CO). Changes in the intensity of the reaction for SD in the mitochondria of thyroid gland cells during malignant transformation were established by the writers electron-cytochemically [2].

The object of this investigation was to study the ultrastructural localization of CO and to determine quantitative changes in the SD and CO activity in the mitochondria of thyrocytes during experimental carcinogenesis.

EXPERIMENTAL METHOD

Intact and hyperplastic thyroid glands of noninbred albino rats and tumors in them were used as the test objects. Hyperplasia and tumors were induced in the thyroid gland by prolonged daily administration of 6-methylthiouracil to the experimental animals. After 6-12 months, hyperplasia developed progressively in the thyroid gland of these animals. As a rule, tumors diagnosed as thyroid adenocarcinomas appeared after 14-20 months. The late stages of hyperplasia, preceding the appearance of a carcinoma, were regarded, as by most

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