EFFECT OF TIGHTNESS OF THE DNA–PROTEIN BOND IN SPLENIC T AND B LYMPHOCYTES OF C3HA MICE DURING CHEMICAL HEPATOCARCINOGENESIS

S. N. Khramtsova, R. P. Alekhina,
G. I. Potapova, and V. S. Shapot*

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A characteristic feature of the action of a malignant tumor of the body is disturbance of function of the immune system. However, the biochemical mechanisms lying at the basis of weakening of the immune function during tumor growth have not yet been explained.

The normal immune response is closely connected with active proliferation of lymphoid cells, so that the study of the various phenomena associated with disturbance of division of lymphocytes during tumor growth is extremely important.

The writers previously found marked changes in turnover of purine nucleosides and nucleotides in lymphocytes from the thymus and spleen of tumor-bearing animals, which led to arrest of DNA synthesis at the stage of precursor formation [1-3, 10]. However, in some cases besides depression of the immune response, a dramatic increase in the intensity of the DNA synthesis was found in the spleen of tumor-bearing animals [2, 3]. These data indicated that a certain proportion of lymphocytes could be injured after commencing the division cycle, where cells are most vulnerable to harmful agents.

To discover any possible disturbances in the DNA of dividing lymphocytes the method of nucleoprotein-celite (NPC) chromatography was used, for it enables nucleic acids to be fractionated on the basis of the tightness of their bond with proteins in nucleoprotein complexes and the proliferative status of the cell population to be evaluated [7].

In the investigation described below changes in the tightness of the DNA–protein bond in the nuclei of splenic T and B lymphocytes from C3HA mice at different stages of chemical hepatocarcinogenesis were studied. Activity of T and B suppressor cells, important regulators of proliferation of lymphoid cells, was determined at the same times [9, 11].

EXPERIMENTAL METHOD

Experiments were carried out on male C3HA mice. A hepatoma was induced by orthoaminoazotoluene (OAAT). Every month a pellet containing 10 mg of OAAT, moistened with glycerin, was implanted subcutaneously into the mice (seven times altogether). A mock operation was performed on the control animals.

Splenic lymphocytes were labeled by intraperitoneal injection of 100 μCi of ^{3}H-thymidine (experiment) and ^{14}C-thymidine (control) into each animal for 3 days before sacrifice. Fractions of spleen cells enriched with T and B lymphocytes were obtained by the method described previously [10].

Fractionation of DNA according to the tightness of its bond with proteins was carried out by the method of NPC chromatography [4, 5]. Lymphocytes (10^6) were lysed and layered above a column of celite 545, cooled to 2°C. Proteins, including those in the composition of nucleoproteins, bound virtually irreversibly with the adsorbent under these circumstances. Subsequent extraction of nucleic acids from the complex with proteins, and their fractionation according to tightness of the bonds with them, were carried out by elution in a gradient of LiCl and urea, and later in a temperature gradient with maximal LiCl and urea concentrations of 4 and 8 M respectively [7].

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

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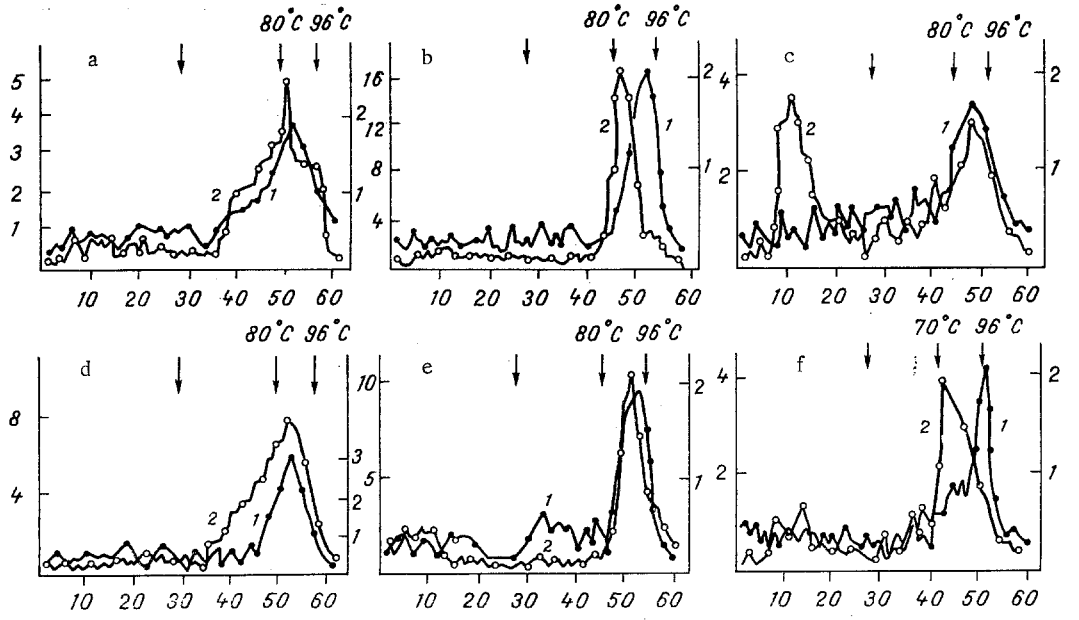

Fig. 1. NPC chromatography of DNA from T lymphocytes (a-c) and B lymphocytes (d-f) from spleen of C3HA mice at different stages of chemical hepatocarcinogenesis. Abscissa, nos. of fractions; ordinate: on left) number of cpm·10^{-3} for ^{3}H-thymidine, on right) number of cpm·10^{-2} for ^{14}C-thymidine. 1) Control, 2) experiment; a, d) 1 week, b, e) 3 weeks, c, f) 8 months after beginning of treatment. Arrows indicate beginning and continuation of temperature gradient.

Activity of T suppressors was studied by the method in [13]. Mice were given an intravenous injection of $4 \cdot 10^{9}$ sheep's red blood cells (SRBC). The spleen was removed 8-10 days later, a cell suspension prepared, and injected in a dose of $2 \cdot 10^{7}$ cells together with $2 \cdot 10^{8}$ SRBC intravenously into intact syngeneic recipients. The number of antibody-forming cells (AFC) in the recipients was counted 5 days later and expressed per 10^{8} karyocytes [12].

To determine B suppressor activity, lymph node cells (10^{7}) from donor mice were injected into intact mice simultaneously with $2 \cdot 10^{8}$ SRBC, and the intensity of the recipients' immune response was determined 5 days later by counting the number of AFC in their spleen [6].

EXPERIMENTAL RESULTS

The distribution of DNA of T lymphocytes of normal animals and animals with different stages of carcinogenesis is shown in Fig. 1a-c. Control and experimental cells were lysed and chromatographed in a mixture. Injection of radioactive label for 3 days before the experiment began labeled the whole pool of proliferative cells, i.e., all stages of the cell cycle. It will be clear from Fig. 1 that DNA of control lymphocytes was firmly bound with proteins and was eluted from the column by a solution of 4 M LiCl and 8 M urea at 95°C. Small differences in the chromatographic position of DNA from the control and experimental T lymphocytes were observed in the early stages of carcinogenesis (before 3 months) already. The strength of DNA-protein interactions in the experimental cells was weakened (the elution temperature was lowered by 10°C). Sudden changes in the DNA chromatogram profiles of the T lymphocytes were found by the 8th month of carcinogenesis, i.e., by the time of appearance of the first hepatoma. About 50% of DNA which had incorporated the label was in a form characterized by weak binding with protein: It was eluted from the column at 4°C by LiCl and urea in concentrations of 1.5 and 3 M respectively. This drastic weakening of the DNA—protein bond was accompanied by transition of the cells from division to resting [4].

NPC chromatograms of splenic B lymphocytes of mice in the control and at different stages of carcinogenesis are shown in Fig. 1d-f. The NPC chromatogram of T and B lymphocytes corresponding to the 3rd month of carcinogenesis coincided with the chromatographic position of DNA from cells taken from the animals 3 weeks after the beginning of carcinogenesis. Visible differences between chromatograms of B lymphocytes were observed only by the 8th month of carcinogenesis, and took the form of weakening of the DNA—protein bond (the elution temperature was lowered by 26°C).

TABLE 1. Changes in Antibody Formation in Spleen of C3HA Mice in Response to Transplantation of Spleen or Lymph Node Cells from Mice with Developing Hepatoma (M ± m)

Time of investigation	Number of AFC			
	transplantation of spleen cells		transplantation of lymph node cells	
	control	experiment	control	experiment
0	30 970±1 347	—	43 300±2 456	—
1 weeks	33 130±1 240	34 300±2 147	42 500±3 800	42 200±4 050
3 weeks	38 000±3 560	23 900±1 260	45 000±5 400	46 800±2 670
3 months	36 700±4 100	9 130±540	34 000±2 400	43 000±2 540
8 months	14 000±1 250	4 916±450	31 000±3 400	43 000±2 370
12 months	20 000±1 890	9 360±780	27 150±1 900	44 000±2 870

Data showing the action of specific T suppressor and B suppressor cells on the number of AFC at different stages of carcinogenesis in C3HA mice are given in Table 1. Transplantation of spleen cells from intact mice considerably depressed AFC formation in the spleen of normal recipients (the number of AFC without injection of spleen cells was 41,000 ± 4300). Transplantation of spleen cells from mice at different stages of carcinogenesis also depressed AFC production, but in this case a much greater degree of suppression was observed. By the 8th month AFC formation in the experimental mice was inhibited by 2.8 times compared with that in normal mice of the same age. Suppressive activity of the B cells at the time of appearance of hepatomas was virtually unchanged compared with the original values, but at the same time it was 1.4 times less than in control mice of the same age.

Lowering of the elution temperature of firmly bound DNA of T lymphocytes observed in the early stages of carcinogenesis may have been due to the effect of the carcinogen on chromatin. Similar weakening of the firm DNA–template bond in proliferating cells also was observed in response to the cytostatic action of various substances *in vitro* [8]. Transition of half of the dividing T cells from the cycle into the resting state by the 8th month of the experiment could be the result of the action both of the carcinogen and of changes in homeostasis caused by a tumor which had already formed; the second suggestion seems more likely, because the animals received the last dose of carcinogen 2 months before sacrifice. The possibility of participation of T suppressors in this phenomenon likewise evidently cannot be ruled out. Weakening of the DNA–protein bond in B lymphocytes took place much later and was less marked in degree than in T lymphocytes, indicating that DNA of the latter is more vulnerable during carcinogenesis.

Weakening of DNA–protein interactions is thus observed in nuclei of splenic lymphocytes of C3HA mice during chemical hepatocarcinogenesis, and this weakening is more marked in T lymphocytes. By the time of appearance of hepatomas, B suppressor activity in the lymph nodes shows no significant change, whereas antigen-specific activity of T suppressors in the spleen of these mice is considerably increased.

LITERATURE CITED

1. N. I. Belyanchikova, G. I. Vornovitskaya, S. N. Khramtsova, et al., Byull. Éksp. Biol. Med., No. 10, 476 (1981).
2. G. I. Vornovitskaya, E. S. Gershtein, and V. S. Shapot, Biokhimiya, 45, 1113 (1980).
3. G. I. Vornovitskaya, S. N. Khramtsova, T. V. Nuzhnaya, and V. S. Shapot, Vest. Akad. Nauk SSSR, No. 3, 1 (1982).
4. A. V. Likhtenshtein, M. M. Zaboikin, V. N. Moiseev, and V. S. Shapot, Dokl. Akad. Nauk SSSR, 245, 1005 (1979).
5. A. V. Likhtenshtein, R. P. Alekhina, M. M. Zaboikin, I. B. Zborovskaya, et al., Vest. Akad. Med. Nauk SSSR, No. 6, 50 (1980).
6. R. V. Petrov, R. M. Khaitov, and A. A. Batyrbekov, Dokl. Akad. Nauk SSSR, 226, 1446 (1976).
7. N. I. S'yakste, M. M. Zaboikin, A. V. Likhtenshtein, and V. S. Shapot, Mol. Biol., No. 6, 1321 (1981).
8. N. I. S'yakste, T. G. S'yakste, A. V. Likhtenshtein, and V. S. Shapot, Éksp. Onkol., 5, 63 (1983).
9. R. M. Khaitov, in: Progress in Science and Technology. Series: Immunology [in Russian], Vol. 13, Moscow (1984), p. 5.
10. S. N. Khramtsova, G. I. Potapova, and V. S. Shapot, Byull. Éksp. Biol. Med., No. 8, 217 (1984).

11. W. L. Farrar and K. D. Elgert, J. Immunol., 120, 1354 (1978).
12. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
13. R. L. Whisler and J. D. Strobo, J. Exp. Med., 144, 398 (1976).

EFFECT OF SOME POLYSACCHARIDES IN CYCLIC NUCLEOTIDE LEVELS AND PHOSPHODIESTERASE ACTIVITY IN ORGANS OF MICE WITH LEWIS' LUNG CARCINOMA

I. G. Veksler and S. G. Antonenko

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The problem of restoring disturbed activity of the immune system is an important factor in the treatment of several diseases accompanied by immunosuppression or disturbance of immune surveillance. In the combination treatment of malignant neoplasms great attention is paid to the use of various immunomodulators, which weaken immunosuppression and effectively enhance the body's immune response to the tumor [1, 5, 6]. According to some workers [3, 8, 9] the action of immunomodulators and other physiologically active substances is exerted through a system of universal cellular mediators, namely cAMP and cGMP. Much evidence has been obtained as a result of attempts to determine the role of cyclic nucleotides and enzymes responsible for their formation (adenylate cyclase, guanylate cyclase) and degradation (phosphodiesterase) in various pathological processes, including malignant growth.

This paper describes the results of a study of the effect of the bacterial polysaccharide prodigiosan and the yeast cell membrane biopolymer zymosan on the absolute and relative levels of cAMP, cGMP, and cAMP-dependent phosphodiesterase (PDE) in the thymus, spleen, and lungs of healthy mice and of mice with metastasizing Lewis' lung carcinoma.

EXPERIMENTAL METHOD

Experiments were carried out on male C57BL mice weighing 20-23 g. Lewis' lung carcinoma was transplanted intramuscularly into the hind limb in a dose of $2 \cdot 10^5$ tumor cells. A readily palpable tumor developed at the site of implantation after 9-12 days and metastases of this tumor in the lungs were found after 9-21 days. The test substances were injected intraperitoneally into the experimental animals on the 14th day, in 0.5 ml of physiological saline and in the following doses: zymosan (from Tallin Pharmaceutical Chemical Factory) 25 mg/kg, prodigiosan (from the Department of Microbiology, Central Postgraduate Medical Institute, Moscow) 2.5 mg/kg. Both substances, according to data obtained previously [4], effectively raised the levels of several immunologic parameters in the above doses. Animals of the control group (transplantation of the tumor) received physiological saline in a volume of 0.5 ml at the same time. All the animals were killed on the 21st day. The tumor at the site of implantation was weighed, the number of metastases in the lung and the mean number of metastases in the group were determined, the number of animals without metastases was counted, and the cAMP and cGMP concentrations and PDE activity were determined in tissue from the thymus, spleen, and lung. The cyclic nucleotide concentration was determined by radioimmunoassay using standard kits from Amersham Corporation (Great Britain). Radioactivity of the samples was studied in an SL-30 liquid scintillation counter (Intertechnique, France). PDE activity was determined by paper chromatography, using 8-^{3}H-cAMP as the substrate [11]. Values obtained on intact mice of the same strain, sex, and age were used as normal values. The numerical results were subjected to statistical analysis with calculation of the level of significance (P) and the coefficient of correlation (r).

R. E. Kavetskii Institute for Problems in Oncology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 605-607, November, 1985. Original article submitted November 18, 1984.

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