CHANGES IN PURINE METABOLISM AND IMMUNE RESPONSE IN THYMUS AND SPLEEN LYMPHOCYTES OF C3HA MICE WITH CHEMICALLY INDUCED HEPATOMAS

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

UDC 616-006-02:616.155.32-008.938.57-074

KEY WORDS: adenosine deaminase, purine nucleoside phosphorylase, 5'-nucleotidase, carcinogenesis, immune function.

The development of a tumor in the body is accompanied as a rule by weakening of the immune response. We now know that lymphoid cells are sensitive to a change in the concentration of products of purine metabolism, and disturbances of immunity in hereditary T- and B-cell deficiency, linked with a disturbance of lymphocyte differentiation and function, are accompanied by reduced activity of some enzymes of purine nucleoside and nucleotide metabolism: adenosinedeaminase (AD), purine nucleoside phosphorylase (PNP), and 5'-nucleotidase. Substantial changes in activity of enzymes of purine metabolism also have been found in the lymphocytes of cancer patients, and the ratio between activities of AD/PNP correlated with the immune status [14], and decreased as the disease progressed.

In previous research the writers found considerable deviations in the purine metabolism of thymocytes and the total fraction of splenic lymphocytes of mice and rats with rapidly growing transplanted hepatomas [2, 4]. To determine the role of these disturbances in the pathogenesis of malignant growth, it was necessary to study the biochemical characteristics and immune response of functionally different subpopulations of immunocompetent cells.

In the investigation described below activity of AD, PNP, and 5'-nucleotidase and also the DNA content in thymocytes and T- and B-lymphocytes in the spleen of C3HA mice with hepatomas induced by orthoaminoazotoluene (OAAT) were studied. The state of function of the immunocompetent cells was assessed by the blast transformation test (BTT) in response to various mitogens, production of antibody-forming cells (AFC), and also the number of rosette-forming cells (RFC) bearing receptors for the Fc-fragment of immunoglobulin (EA-RFC) and the C'3 component of complement (ECAC-RFC).

EXPERIMENTAL METHOD

Experiments were carried out on male C3HA mice. A hepatoma was induced with OATT, which was injected subcutaneously in the spinal region monthly in a dose of 10 mg per mouse (in glycerol). All tests were carried out 1 and 3 weeks and 3, 8, and 12 months after the beginning of carcinogenesis. Lymphocytes were isolated from the thymus and spleen in a Ficoll—Verografin gradient [6], after which the splenocytes were passed through a column with nylon wadding to separate them into T and B fractions [10, 13].

Activity of AD and PNP was determined in lysates of lymphocytes, and 5'-nucleotidase activity in intact cells by the method described previously [4]. Activity of the enzymes was expressed in micromoles substrate converted per minute, calculated per 10^9 cells (units). DNA was determined by the method in [7] and expressed in milligrams per 10^9 cells.

The number of AFC was estimated by the local hemolysis in agar method [11]. Mice were immunized intraperitoneally with 0.2 ml of a 5% suspension of sheep's red blood cells and the number of AFC in the spleen was counted on the 5th day per 10^8 nucleated cells. The BTT was carried out by Adler's method [1]; $5 \cdot 10^5$ cells in 0.2 ml were cultured in RPMI medium containing 20 mM HEPES, 2.9 g/liter glutamine, 40 units/liter of gentamicin, and 10% embryonic calf serum at 37°C in an atmosphere with 5% CO_2. Optimal stimulation of the lympho-

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

Laboratory of Biochemistry of Tumors, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 8, pp. 217-220, August, 1984. Original article submitted June 5, 1985.

 0007-4888/84/9808-1108$08.50

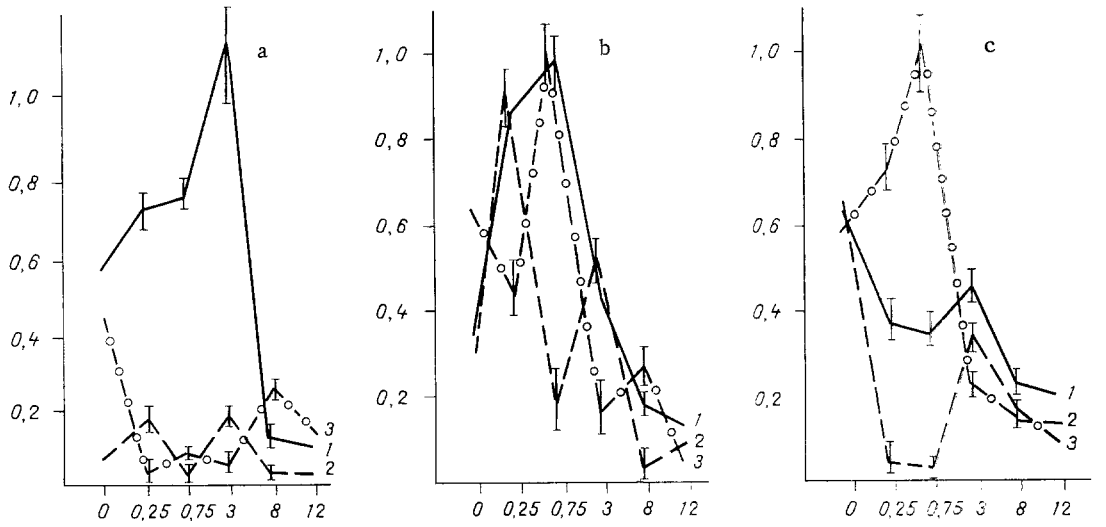

Fig. 1. Changes in AD, PMP, and 5'-nucleotidase activity in thymocytes (a), T lymphocytes (b), and B lymphocytes (c). Abscissa, time of investigation (in months); ordinate, enzyme activity (in units). 1) AD; 2) PMP; 3) 5'-nucleotidase.

cytes was observed with the following concentrations of ingredients in the medium: phytohemagglutinin (PHA) 2 μg/ml, concanavalin A (con A) 2.5 μg/ml, and lipopolysaccharide (LPS) 10 μg/ml. The proliferative activity of the lymphocytes in the BTT was estimated as incorporation into cell DNA of [^{3}H]thymidine, added at the rate of 1 μCi per cell 16 h before the end of the culture period. The results are represented as a stimulation index, calculated as the ratio of incorporation of isotope label (in cpm) in a cell culture containing the mitogen to incorporation in a sample not containing the mitogen.

The number of EA-RFC and EAC-RFC was determined by the method in [15] and expressed as a percengage (calculated per 100 karyocytes).

EXPERIMENTAL RESULTS

Data showing changes in AD, PNP, and 5'-nucleotidase activity in thymocytes and splenic T and B lymphocytes at different times of carcinogenesis are shown in Fig. 1.

AD activity in the thymus was sharply increased (doubled) by the 3rd month of carcinogenesis, after which it fell significantly by the 12th month. 5'-Nucleotidase activity was reduced by 75% at this time. AD and PNP activity in T lymphocytes was increased by 2.7 times by the end of the first week; AD activity remained at a high leveL until the 3rd week, whereas 5'-nucletidase activity increased by 1.6 times at this period. By the 12th month 5'-nucleotidase and AD activity was reduced, whereas PNP activity, starting with the 3rd week, changed inconsistently, but taken as a whole it was reduced by 3.2 times compared with normal.

A decrease in activity of all the enzymes was observed in the B lymphocutes for 12 months, with the exception of 5'-nucleotidase activity, which continued to rise until the 3rd week. A significant decrease in the AD/PNP ratio was observed in the thymus by the 12th month, but it remained close to normal in the T and B lymphocytes by this time (Table 1).

Hepatocarcinogenesis in mice is thus accompanied by significant changes in purine metabolism of lymphocyted. In the early stages an increase in AD activity and in the AD/PNP ratio was observed in the thymus, and an increase in AD, PNP, and 5'-nucleotidase activity in the T lymphocytes and in 5'-nucleotidase activity and the AD/PNP ratio in B lymphocutes. Subsequently the activity of all these three enzymes decreased.

As Table 1 shows, the DNA content in the thymocytes and T lymphocytes was increased by 1.4 times by the time of appearance of multiple tumor nodules in the liver, whereas in the B lymphocytes it was reduced by 2.5 times. However, in the early stages an increase in the DNA content was observed in all cells studied. These findings agree with results obtained by other workers who also observed an increase in the DNA content in lymphocyte nuclei during tumor growth [5].

TABLE 1. DNA Content and AD/PNP Ratio in Lymphocytes of C3HA Mice during Development of OAAT-induced Hepatomas

Parameter	Cells	Time of investigation					
		normal	1 week	3 weeks	3 months	8 months	12 months
AD/PNP	Thymocytes	5,8	6,3	19,5	10,0	4,0	2,0
	T lymphocytes	0,9	0,9	6,1	0,8	8,2	1,3
	B lymphocytes	0,9	5,0	7,3	1,4	1,2	1,1
DNA	Thymocytes	2,8±0,7	3,5±0,7	1,7±0,5	3,2±0,8	1,8±0,5	3,8±0,8
	T lymphocytes	2,2±0,9	3,2±0,5	3,8±0,5	3,3±0,7	2,5±0,7	3,1±0,6
	B lymphocytes	3,8±0,9	4,4±0,9	2,8±0,5	4,1±0,7	1,9±0,5	1,5±0,5

Legend. Data for 8 months indicate appearance of first tumor nodules in the liver, at 12 months to animals with a hepatoma.

TABLE 2. Parameters of Immune Response of Mouse Spleen Cells during Hepatocarcinogenesis (M ± m)

Parameter	Time of investigation				
	normal	1 week	3 weeks	3 months	8 months
BTT:					
to PHA	3,4±0,2	8,3±1,4	9,8±1,1	3,1±0,1	4,8±0,6
to con A	2,6±0,1	2,4±0,7	2,1±0,8	1,4±0,2	5,9±0,5
to LPS	3,4±1,1	3,1±0,9	2,7±0.3	1,0±0.1	6,0±0,2
AFC × 10^3	41±4,3	70,0±8,4	110,0±10,6	1,5±0.6	—
EAC-RFC	6	8	8	2,5	—
EA-RFC	14	23	19	12	—

Data on the state of the immune response in mice during hepatocarcinogenesis are given in Table 2. Blast transformation of mouse spleen lymphocytes in response to PHA was found to be increased until the 3rd week, which correlates with the increased AD activity and DNA content in the T lymphocytes at that time. An unexpectedly high response to con A and LPS compared with the normal state was observed toward the 8th month. Meanwhile the highest value of the AD/PNP ratio was observed in T lymphocytes (Table 1). Such a strong response to con A in the late stages of carcinogenesis could be due to an increase in the population of T suppressors or their precursors, for these cells carry an Fc receptor on their surface. We know from the literature that only cells carrying an Fc receptor respond to con A, whereas cells both with and without such receptors respond to PHA [3].

AFC production rose considerably toward the 3rd week of carcinogenesis and then dropped sharply. Similar changes took place in the number of EA-RFC, which was increased by 1.5 times by the 3rd week, but returned to the normal level by the 3rd month. The number of EAC-RFC was practically unchanged until the 3rd week, but was reduced by 2.4 times by the 3rd month. Similar changes in 5'-nucleotidase activity and the AD/PNP ratio in the B lymphocytes corresponded to these processes.

Significant disturbances of purine metabolism in the initial period of growth of OAAT-induced hepatomas in C3HA mice, manifested as an increase in AD, PNP, and 5'-nucleotidase activity and in the DNA content in lymphocytes, thus reflect activation of cellular immune mechanisms in the early stages of hepatocarcinogenesis. The subsequent fall in enzyme activity in the cells points to a disturbance of lymphocyte differentiation and a decrease in effectiveness of the immune response. The results of these experiments indicate that changes in reactivity of individual subpopulations of immunocompetent cells during the development of malignant tumors correlate clearly with changes in purine nucleoside and nucleotide metabolism in these cells.

LITERATURE CITED

1. W. Adler, in: Immunological Methods [Russian translation], Moscow (1979), p. 159.
2. N. I. Belyanchikova, G. I. Vornovitskaya, S. N. Khramtsova, et al., Byull. Éksp. Biol. Med., No. 10, 476 (1981).
3. B.D. Brondz and O. V. Rokhlin, Molecular and Cellular Bases of Immunologic Recognition [in Russian], Moscow (1978), p. 71.

4. G. I. Vorovitskaya, S. N. Khramtsova, and V. S. Shapot, Vestn. Akad. Med. Nauk SSSR, No. 3, 29 (1982).
5. Yu. A. Umanskii, in: Immunology of Chemical Carcinogenesis [in Russian], Kiev (1975), p. 104.
6. A. Boyum, Scand. J. Clin. Lab. Invest., 21, 97 (1968).
7. K. Burton, Biochem. J., 62, 315 (1956).
8. E. R. Giblett, A. J. Amman, D. W. Wara, et al., Lancet, 1, 1010 (1975).
9. E. R. Giblett, J. E. Anderson, F. Cohen, et al., Lancet, 2, 1067 (1972).
10. B. S. Handerwerger and R. H. Schwartz, Transplantation, 18, 544 (1974).
11. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
12. S. M. Johnson, M. E. North, and G. L. Asherson, Lancet, 1, 168 (1977).
13. M. N. Julius, E. Simpson, and L. A. Herrenberg, Eur. J. Immunol., 3, 645 (1973).
14. K. Ogawa, K. Tominaga, S. Taoka, et al., Gann, 69, 471 (1978).
15. E. M. Shevach, L. Ellman, J. M. Davie, et al., Blood, 39, 1 (1972).

HEPATOMA 27 AND RAT COLONIC EPITHELIAL CELLS HAVE THE SAME PREKERATIN PROTEIN PROFILE

S. M. Troyanovskii, V. A. Krutovskikh, and G. A. Bannikov

UDC 616.36-006-008.939.6:616.348-018.73-0080939.6]-074

KEY WORDS: hepatoma 27, colon, prekeratin proteins.

The prekeratins are a family of related proteins found in the composition of intermediate filaments of epithelial cells. One special feature of the prekeratins, which distinguishes them from proteins of the intermediate filaments of other types of cells (vimetin, desmin, proteins of neurofilaments and glial filaments), is their biochemical heterogeneity. It has also been shown that the prekeratin profiles of different epithelia also differ [8]. It has been suggested that the different profiles of these proteins determines, at least partially, differences in the morphological reactions of different epithelial cells [7].

However, for various reasons the scale of this heterogeneity and of the differences in prekeratins in different cells have so far proved difficult to estimate. This is due above all to the high degree of homology of the different prekeratins, which makes the use of polyclonal antisera for the characterization of individual proteins of this family difficult. In some cases the increased heterogeneity of the prekeratins can be attributed to partial proteolysis of these proteins during isolation.

By using monoclonal antibodies which we obtained against various antigenic determinants of prekeratin and also a number of biochemical techniques, we showed in this investigation that under conditions when isolation of prekeratins is not accompanied by proteolytic degradation, their profile is the same in colonic epithelial cells and in cells of a transplantable hepatoma.

EXPERIMENTAL METHOD

Prekeratin was isolated from the liver and colon of noninbred rats. In addition, primary cultures of transplantable rat hepatoma 27, obtained from the collection of tumor strains, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, were used. For electrophoretic investigation of proteins, cells (or pieces of organs) were either dissolved immediately in buffer for electrophoresis (cell lysates) or prekeratin was isolated from them beforehand. Prekeratin was isolated by the standard method [5], which consists of successive extraction of the tissue homogenate, first with a 1% solution of Triton X-100, and then with 1.5 M KCl. Prekeratin proteins with molecular weights of 40-60 kilodaltons (kD) are sedimented by this treatment in the residue. The technique of polyacrylamide gel elec-

Laboratory of Mechanisms of Carcinogenesis and Laboratory of Carcinogenic Substances, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR, N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 8, pp. 220-223, August, 1984. Original article submitted August 11, 1983.

