MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN LYMPHOID AND HEMATOPOIETIC TISSUE IN RATS WITH ZAJDELA'S HEPATOMA

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Relations between the malignant tumor and host organism are characterized, on the one hand, by rapid division of the tumor cells, liberation of metabolic and breakdown products into the blood stream, the spread of the cells through the body, their invasion of the surrounding tissues, and their growth at the expense of metabolic products of normal tissues, and on the other hand, by a reduction in the reactivity of the host, or immunodepression. The functioning of the immune system is closely linked with the proliferative capacity and differentiation of its cells. The writers previously studied the proliferative activity of lymphoid tissues in animals with tumors as reflected in the rate of incorporation of ^{14}C-thymidine into DNA, thymidine kinase activity, and the intracellular TTP content [1].

We now know that the lymphoid system is sensitive to disturbances of purine metabolism. Congenital adenosine deaminase (AD) deficiency, for instance, is accompanied by simultaneous disturbance of both humoral and cellular immunity [8], whereas purine nucleoside phosphorylase (PNP) deficiency is accompanied by disturbance predominantly of cellular immunity [7]. Changes in the activity of these enzymes in the lymphocytes are associated with a disturbance of their differentiation [5].

Significant changes in activity of the enzymes of purine metabolism have also been observed in the lymphocytes of cancer patients and the AD/PNP ratio correlated with the immune status [11]. Furthermore, in animals with tumors the hematologic parameters are sharply altered [12], and immune reactions of the host to tumor growth are closely linked with the state of the hematopoietic system [2].

The object of this investigation was to study morphological changes in the tissues of rats with transplantable allogeneic Zajdela's ascites hepatoma, and also AD and PNP activity in lymphocytes of the thymus and spleen, and erythrocytes of the spleen and blood.

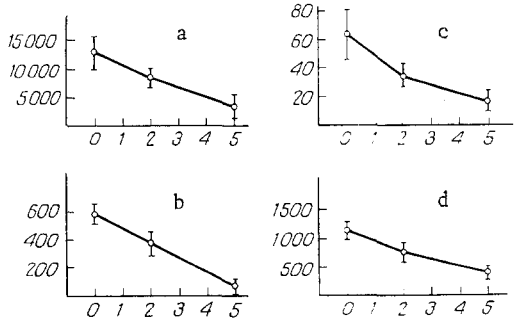

Fig. 1. Changes in cell composition of thymus of rats with Zajdela's ascites hepatoma: a) small and medium-sized lymphocytes, b) blast cells, c) mitoses, d) large lymphocytes. Abscissa, days of growth of hepatoma; ordinate, number of cells ($\times 10^5$).

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TABLE 1. Changes in Composition of Spleen Cells in Wistar Rats Associated with Growth of Zajdela's Ascites Hepatoma (M ± m)

Days of growth of tumor	Weight, mg	Number of cells $\cdot 10^5$						
		total	dividing granulocytes	nondividing granulocytes	monocytes	erythroid series	lymphocytes	tumor cells
0	726±31,4	8959,24±1205,11	50,741±11,73	345,07±39,32	141,08±19,46	327,83±53,54	7568,83±1034,41	—
2nd	1236±72,36	15228,82±1007,96*	11,97±5,99*	443,65±81,62*	1772,23±283,18*	1023,32±536,03	10453,94±695,1	715,71±82,1
5th	2500±93,12*	25148,45±2123,45	88,1±82,37*	1378,95±334,03*	1385,85±99,0*	3127,65±986,45*	15205,05±936,45*	1549,89±168,63*

*P < 0.05 relative to control.

TABLE 2. AD and PNP Activity in Lymphoid and Hematopoietic Cells of Normal Rats and Rats with Zajdela's Hepatoma (M ± m)

Parameter	Experimental conditions	Thymus	Splenic lymphocytes	Splenic erythrocytes	Blood erythrocytes
AD, units	Normal	238,0±27,6	35,7±4,3	71,3±9,5	0
	Hepatoma:				
	1st day of growth	380,5±31,2	17,4±3,0	105,7±8,9	0
	3rd " "	210,5±20,4	23,6±2,5	63,7±3,8	0
	5th " "	174,5±4,3	17,4±1,5	45,0±4,3	0
PNP, units	Normal	6,2±0,5	9,9±1,8	22,8±1,2	0,87±0,02
	Hepatoma:				
	1st day of growth	8,5±0,9	8,5±1,4	23,5±1,6	1,04±0,09
	3rd " "	9,7±0,7	11,3±1,6	27,4±2,5	0,99±0,09
	5th " "	10,4±0,7	4,8±0,9	27,4±2,5	8,26±0,46
AD/PNP	Normal	38,3	3,6	3,1	—
	Hepatoma:				
	1st day of growth	44,7	2,0	4,5	—
	3rd " "	21,8	2,1	2,7	—
	5th " "	16,9	3,6	1,6	—

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-290 g. Zajdela's ascites hepatoma was transplanted intraperitoneally in a dose of 10^7 cells. In normal rats and on the 2nd and 5th days after transplantation, blood was taken from the tail for determination of the leukocyte formula and leukocyte count. After decapitation of the rats the spleen was removed and weighed and the absolute number of nucleated cells in the whole organ was counted. Squash preparations of the spleen, thymus, liver, and bone marrow and blood films were stained with azure-eosin by Romanowsky's method. Differential counting extended to 1000 cells in the preparations. The classification suggested by Chertkov and Vorob'ev [3] was used in the determination.

Activity of enzymes of purine metabolism was determined in the control animals and on the 1st, 3rd, and 5th days after transplantation of the hepatoma in lysates of lymphocytes, splenic and blood erythrocytes, and in cytosol fractions of the thymus obtained after centrifugation at 20,000g in 0.1 M phosphate buffer, pH 7.0, with 10 mM mercaptoethanol. Splenic lymphocytes and blood erythrocytes were obtained in a Ficoll–Verografin gradient [6]. The lymphocytes were disintegrated by freezing and thawing twice, and the erythrocytes were lysed in bidistilled water for 30 sec; the thymus was disintegrated in a Potter's homogenizer. Activity of the enzymes was determined on a Beckman (USA) spectrophotometer in a constant-temperature cuvette at 37°C. AD activity was recorded on an automatic Linear Recorder on the basis of the initial velocity of conversion of adenosine to inosine at 265 nm [9]. The incubation mixture contained $8 \cdot 10^{-5}$ M adenosine in initial buffer and 30-60 μg protein. PNP activity was determined similarly from conversion of guanosine into guanine at 252 nm [10]. The incubation mixture contained $8 \cdot 10^{-5}$ M guanosine in initial buffer and 300-500 μg protein. Activity of the enzyme was expressed in nanomoles of converted substrate per minute per mg protein (units). Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

On the 5th day of tumor growth in the rats the weight of the thymus was reduced from 376.5 to 234.6 mg, and this was accompanied by a reduction by half in the absolute number of cells in the organ from 15,088.764 ± 3165.042 under normal conditions to 7461.732 ± 1005.916 (all results of morphological analysis are expressed in values multiplied by 10^5). Data on changes in the number of small and medium lymphocytes, which constitute the great majority of the population, are given in Fig. 1. The number of actively proliferating blast cells also was reduced by 18.1 times and the number of large lymphocytes by 3.3 times. With growth of the tumor, many pycnotic cells appeared in the thymus.

Data on changes in the cell composition of the spleen in rats with a rapidly growing ascites hepatoma are given in Table 1. The weight of the spleen and absolute number of cells in the organ were increased almost threefold. The main increase in the number of cells took place on account of an increase in the absolute number of lymphocytes (twofold), but their relative proportion of the total population fell from 84.5 to 60%. The increase

in the number of spleen cells also was connected with an increase in the number of blast cells and of dividing and nondividing granulocytes, monocytes, and plasma cells. The number of juvenile forms of the erythrocytic series was considerably increased. On the 2nd day after transplantation of the tumor, tumor cells appeared in the spleen and accounted for 4.6% of the total cell population, and by the 5th day this proportion had increased to 6.2%.

The study of the blood cell composition in control rats and rats with tumors showed that growth of the tumor was accompanied by an increase in the number of leukocytes, to reach a mean value of 48,428 ± 1664/mm^3 blood by the time the animals died (5th day), compared with 20,137 ± 910 in the control. The increase in the number of leukocytes was connected mainly with an increase in the number of neutrophils and monocytes. A shift to the left was observed among the neutrophils. With continuing growth of the tumor the rats developed anemia, reflected in a fall in the erythrocyte count by 2.3 times (from 8,383,000 ± 404,826 in the control animals to 3,774,000 ± 510,452 on the 5th day). Meanwhile anisocytosis and poikilocytosis were observed and polychromatophilic erythrocytes appeared.

No appreciable changes in the cell population were observed in the bone marrow and inguinal lymph nodes, except for a small increase in the number of cells of the erythroid series in the bone marrow and the appearance of a few tumor cells. In squash preparations of the liver there was an increase in the number of mature neutrophils to 12%. Tumor cells accounted for 19.1% of the total population.

Data on changes in activity of enzymes of purine metabolism in the lymphoid and hematopoietic cells of rats at different stages of growth of Zajdela's hepatoma are given in Table 2.

AD activity in the thymus increased sharply 24 h after transplantation of the tumor, after which it fell significantly toward the 5th day. PNP activity was increased by 1.5 times by this time. Activity of the enzymes in splenic lymphocytes was reduced by 50% compared with normal, but in the splenic erythrocytes a decrease in AD was observed only when the animals were near death. A significant decrease in the AD/PNP ratio was observed in the thymus and splenic erythrocytes. The AD/PNP ratio in the total splenic lymphocyte population did not change significantly, possibly on account of differences in metabolism of the T and B lymphocytes. In the blood erythrocytes PNP activity was sharply increased on the 5th day of tumor growth and this correlated with the picture of anemia and the fall in the hematocrit index in rats with Zajdela's ascites hepatoma.

Growth of Zajdela's ascites hepatoma in Wistar rats is thus accompanied by involution of the thymus and by the appearance and development of a leukemoid reaction and anemia. Dissemination of tumor cells in many organs is observed: spleen, liver, bone marrow, lymph nodes. AD activity and the AD/PNP ratio were significantly reduced in the thymus and spleen cells of the tumor-bearing animals, reflecting a disturbance of differentiation and a fall in the effectiveness of the immune response.

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