CHANGES IN RESISTANCE OF PARTIALLY HEPATECTOMIZED MICE TO TRANSPLANTABLE TUMORS IN THE FINAL STAGE OF REGENERATION OF THE LIVER

A. G. Babaeva and V. I. Demskii

UDC 616-006-092.9-06:616.36-089.873

When an ascites form of hepatoma 22a was transplanted intraperitoneally into A/he mice 1-12 days after partial hepatectomy two periods of inhibition of its growth were observed. The first 24 h after the operation and the second 3-10 days later. Animals most resistant to transplantation of this particular tumor (5-8 days after the operation) were resistant, although to a lesser degree, to intraperitoneal transplantation of sarcoma 37 and Ehrlich's adenocarcinoma.

KEY WORDS: *partial hepatectomy; regeneration; tumor growth.*

Lymphocytes in the early stages of regeneration of the liver and kidney acquire the ability to stimulate proliferative activity in the organs of intact animals [1, 2 8, 9]. It has been suggested that lymphocytes play an important role not only in the initiation of regeneration, but also in its completion [1]. In other words, the theory is that in the late stages of regeneration the lymphocytes are able to inhibit proliferation. Testing of this hypothesis has shown that transplantation of lymphocytes from the spleen of partially hepatectomized mice, removed after a wave of mitotic division of the hepatocytes, induces inhibition of mitotic activity in the regenerating liver of the donors. These results are in agreement with those of investigations which have shown inhibition of growth of transplantable hepatoma 22a if injected simultaneously with lymphocytes of normal and, in particular, of partially hepatectomized mice on the 6th-8th day after resection of the liver [7]. Meanwhile transplantation of the tumor directly into hepatectomized mice at various times after resection of half of a lobe of liver was not accompanied by inhibition of tumor growth [6]. However, the operation performed in these experiments was on a relatively small scale and did not give rise in full measure to the changes in the liver itself and in the system of immunogenesis that characterize the operation usually used in the model, namely removal of two thirds of the liver [2-4].

It was accordingly decided to study growth of a tumor transplanted at different stages of regeneration of the liver after extensive resection of that organ.

EXPERIMENTAL METHOD

Experiments were carried out on 250 female A/he mice weighing 18-20 g. Two thirds of the liver was removed from the experimental mice in the usual way, and at different times (1-12 days) after the operation ascites forms of hepatoma 22a, sarcoma 37, and Ehrlich's adenocarcinoma were transplanted intraperitoneally into them. For this purpose the ascites fluid containing tumor cells was diluted in sterile isotonic saline and injected intraperitoneally in a dose of $1 \cdot 10^6$ cells per mouse. Each experimental and control group consisted of 10 mice. The animals were kept in natural illumination with free access to food. The mice were killed 7 days after transplantation simultaneously in the control and four experimental groups, for which purpose the time of the operation was varied so that the time of transplantation and sacrifice in the experimental groups coincided with those in the control. All the ascites fluid was taken from the peritoneal cavity, its volume was measured, the

Laboratory of Growth and Development, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 3, pp. 326-328, March, 1977. Original article submitted August 9, 1976.

TABLE 1. Number of Tumor Cells in Partially Hepatectomized Mice Depending on Time of Transplantation of Tumor after Operation

Group of animals	Time of transplantation after operation, days	Hepatoma 22a			Sarcoma 37			Ehrlich's adenocarcinoma		
		number of tumor cells		P	number of tumor cells		P	number of tumor cells		P
		million	percent of control		million	percent of control		million	percent of control	
Control		251,9								
Experimental	1	99,0	39,4	0,0001						
	2	225,7	90,0	0,6892						
	3	116,2	46,2	0,005						
	4	58,7	23,3	0,0001						
Control		301,0			75,6			186,1		
Experimental	5	43,2	14,4	0,0001	11,3	14,9	0,0001	26,5	14,2	0,0001
	6	2,0	0,7	0,0001	24,6	32,5	0,017	41,4	22,2	0,0001
	7	41,9	13,9	0,0001	5,5	7,3	0,0001	60,0	32,2	0,0001
	8	19,2	6,4	0,0001	11,8	15,6	0,001	70,7	38,0	0,001
Control		254,2								
Experimental	9	53,8	21,2	0,0001						
	10	122,2	48,0	0,0037						
	11	189,0	74,5	0,087						
	12	203,1	80,0	0,177						

number of tumor cells was counted in a Goryaev chamber, and the absolute number of tumor cells from each animal was calculated. Films of ascites fluid were fixed with methanol, stained with Carazzi's hematoxylin, and counterstained with methylene blue. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The changes in growth of the hepatoma 22a when transplanted at different times after hepatectomy showed two periods of inhibition (Table 1). When the tumor was transplanted 24 h after the operation its growth was inhibited and the number of tumor cells was only 39.4% of the control ($P = 0.0001$). A tumor transplanted 2 days after hepatectomy grew the same as in intact animals. The number of cells in the ascites fluid of the experimental mice was 90% of the control (difference not significant). When the tumor was transplanted 3 days after the operation its growth again was inhibited and the number of tumor cells was only 46.2% of the control ($P = 0.005$). Inhibition was observed when the tumor was transplanted on the following days after the operation until the 10th day inclusive, when growth of the tumor was 48% of the control ($P = 0.0037$). Growth of the tumor transplanted into animals 11-12 days after hepatectomy again did not differ significantly from the control, the precise values being 74.5% ($P = 0.087$) and 80.0% ($P = 0.177$), respectively. Inhibition of tumor growth was maximal when it was transplanted 5-8 days after hepatectomy, when the mean number of cells was only 8.8% of the control.

Inhibition of growth of the tumor when transplanted 24 h after hepatectomy was evidently connected with the nonspecific increase in the animals' resistance as a result of stress caused by the operation [5]. Inhibition observed when the tumor was transplanted 3-10 days after the operation cannot yet be explained; it may tentatively be considered to be due to both nonspecific and specific changes in the system of immunogenesis developing after extensive hepatectomy [1-4].

To decide whether the processes developing in the second stage of inhibition of tumor growth were specific, additional experiments were carried out in which Ehrlich's adenocarcinoma and sarcoma 37, with less antigenic similarity to liver tissue than hepatoma 22a, were transplanted at the times of maximal resistance of the animals to transplantable tumors (5-8 days after resection of the liver). The results do not solve the problem unequivocally. Growth of both tumors (Table 1) was sharply inhibited, although by a rather lesser degree than growth of hepatoma 22a. The number of cells of sarcoma 37 and Ehrlich's adenocarcinoma

in the hepatectomized mice averaged 17.6 and 26.7%, respectively, of the number of tumor cells in the control. Both indices were significantly higher than in the experiments with transplantation of hepatoma 22a, but whether this was due to the different affinity of these tumors for liver tissue is not yet clear; the factors on which this sharp inhibition of their growth depends likewise remain unexplained.

It is noteworthy that in all the experiments there were some animals which did not develop tumors, although films made from the contents of their peritoneal cavity revealed single tumor cells. This indicates that inhibition of tumor growth in these experiments was partial at the times chosen for investigation.

The results are evidence that sharp inhibition of growth of a tumor can take place in partially hepatectomized animals if it is transplanted intraperitoneally at certain periods after extensive resection of the liver.

LITERATURE CITED

1. A. G. Babaeva, Immunological Mechanisms of Regulation of Repair Processes [in Russian], Moscow (1972).
2. A. G. Babaeva, N. A. Kraskina, and L. D. Liozner, Tsitologiya, No. 12, 1511 (1969).
3. A. G. Babaeva, N. Yu. Alekseeva, S. S. Gambarov, et al., Byull. Éksp. Biol. Med., No. 8, 106 (1973).
4. S. S. Gambarov, A. G. Babaeva, N. Yu. Alekseeva, et al., Byull. Éksp. Biol. Med., No. 12, 51 (1973).
5. P. D. Gorizontov, Arkh. Pat., No. 3, 13 (1976).
6. V. Ya. Fel', "Disturbance of cytodifferentiation during malignant change and the problem of immunological surveillance," Doctoral Dissertation, Leningrad (1975).
7. V. Ya. Fel' and A. M. Malygin, Tsitologiya, 16, 115 and 651 (1974).
8. M. E. Pliskin and R. T. Prehn, J. Reticuloend. Soc., 17, 290 (1975).

CHANGES IN SOME PROPERTIES OF TRANSPLANTABLE ADENOCARCINOMA OF THE COLON DURING PASSAGE

R. L. Turetskaya, G. I. Avdeev, N. I. Khatuntseva, I. A. Svet-Moldavskaya, and G. A. Frank

UDC 616.345-006.66-092.9

During serial passage of an adenocarcinoma (strain AKTOL) arising as a result of spontaneous malignant change in tissues of the embryonic intestine, transplanted into syngeneic adult mice, the rate of growth of subcutaneous grafts was increased, the ability to form multiple lung tumor nodules after intravenous inoculation appeared (it was absent in the first passages), and morphological anaplasia of the tumor was increased. These changes, evidence of progression of the tumor, were not directly connected with changes in its immunogenicity.

KEY WORDS: *transplantable adenocarcinoma; pulmonary metastases; immunogenicity.*

Previous investigations have shown that during serial passage of experimental tumors arising spontaneously or induced by chemical carcinogens and viruses the character and rate of their growth and also their antigenic properties change [4-9].

P. A. Gertsen Moscow Oncologic Research Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 3, pp. 328-330, March, 1977. Original article submitted July 26, 1976.