5. M. Fox and H. E. Wahman, Invest. Urol., 5, 512 (1968).
6. G. M. Higgins and R. M. Anderson, Arch. Pathol., 12, 186 (1931).
7. M. E. Pliskin and R. T. Prehn, J. Reticuloend. Soc., 17, 290 (1975).
8. B. Radoševič-Stašic and D. Rukavina, Period. Biol., 81, 151 (1979).

EFFECT OF SPLENIC LYMPHOCYTES FROM DONORS POISONED WITH CARBON TETRACHLORIDE ON MITOTIC ACTIVITY AND α-FETOPROTEIN PRODUCTION OF LIVER CELLS IN SYNGENEIC RECIPIENTS

L. Ya. Shipova, V. S. Poltoranina, and A. P. Suslov

UDC 612.359.014.2:612.6]-06:[612.94:612.411].014.46:615.917:547.412.133

KEY WORDS: regeneration of the liver; mitotic index; proliferation; α-fetoprotein (AFP); lymphocyte.

Mitotic activity of mouse liver cells is increased after intravenous transplantation of splenic lymphocytes from partially hepatectomized syngeneic donors into the animals [2]. Mitotic activity of hepatocytes also is increased during regeneration of the liver after poisoning with carbon tetrachloride (CCl_4) [7]. This process is accompanied by the appearance of an embryo-specific protein — α-fetoprotein (AFP) — in the animal's blood serum [1].

It was interesting to discover whether lymphocytes of CCl_4-treated donors would affect mitotic activity and AFP production in the recipients' liver, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

SWR mice aged 2-3 months, used as lymphocyte donors, were given one or 11 doses of an 8% solution of CCl_4 in sunflower oil (one dose was 0.1 ml of the preparation [1]) perorally. The CCl_4 was given twice a week. Cell suspensions in medium 199 were prepared from the spleens of the experimental and intact donors 16 h after administration of CCl_4. The washed cell suspension was injected into the caudal vein of intact recipient mice of the same strain in doses of $2 \cdot 10^7$ or $7 \cdot 10^7$ lymphocytes in 0.7 ml medium 199 (Table 1). Intact animals and mice receiving 0.7 ml of medium 199 intraperitoneally also were investigated.

The animals were decapitated 50 h after transfer of the lymphocytes. Colchicine solution was injected into the animals 4 h before sacrifice in a dose of 5 mg/kg body weight.

Pieces of liver 3-4 mm thick were fixed in a mixture of acetone-formalin—0.03 M phosphate buffer, pH 6.1-6.2 (9:5:6) and embedded in paraffin wax [3].

The mitotic index in the hepatocytes was determined in liver sections 3 μ thick, stained with hematoxylin, by counting from 2600 to 25,000 hepatocyte nuclei [5].

AFP was revealed in liver sections by an indirect immunoperoxidase method, using monospecific antibodies against AFP and a preparation of donkey antibodies against rabbit γ-globulin, conjugated with horseradish peroxidase [9].

Monospecific antibodies against AFP were obtained from rabbit antisera against mouse AFP on sepharose 4B—AFP sorbent. The specificity of staining was verified by means of an antibody preparation neutralized with the equivalent quantity of pure AFP. Treatment of a serial section with antiserum against mouse γ-globulin served as the control for nonspecific uptake of serum proteins by the hepatocytes [8].

The AFP concentration in the animals' blood sera was determined by double immunodiffusion in gel [4] with a standard test system.

Laboratory of Immunochemistry and Diagnosis of Tumors, 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. N. Blokhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 6, pp. 99-101, June, 1982. Original article submitted November 9, 1981.

0007-4888/82/9306-0817$07.50

TABLE 1. Mitotic Activity of Hepatocytes and Detection of AFP-containing Cells in Recipient Mice

Treatment of donors	Number of lymphocytes injected (recipients)	Mitotic index of hepatocytes, %	Presence of AFP-containing cells in liver sections
One dose of CCl_4	$2 \cdot 10^7$	0,11±0,01	2/4
	$7 \cdot 10^7$	3,8±0,9	4/9
11 doses of CCl_4	$7 \cdot 10^7$	1,3±0,3	2/5
Intact animals	$7 \cdot 10^7$	0,08±0,005	1/4
	0.7 ml of medium 199	0,1±0,02	3/3
	Intact animals* [2]	0,05±0,004	0/4
		—	0/50

Legend. Numerator gives number of animals with AFP-containing cells, denominator gives total number of animals in group studied.

*Intact mice not receiving colchicine; mitotic index in hepatocytes of this group of animals was not determined.

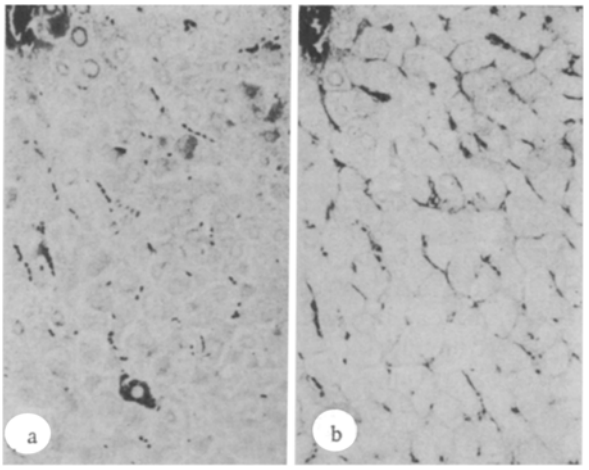

Fig. 1. Detection of AFP in liver of recipient mice. Parallel sections treated with antibodies against mouse AFP (a) and against mouse γ-globulin (b).

EXPERIMENTAL RESULTS

Intravenous injection of $7 \cdot 10^7$ lymphocytes from donors receiving one dose of CCl_4 led to a marked increase (by 75 times) in the mitotic index of hepatocytes in the recipients' livers (Table 1). Injection of the same lymphocytes in a dose of $2 \cdot 10^7$ increased the mitotic index much less (two-fold). Consequently, the observed effect was dose-dependent. Injection of $7 \cdot 10^7$ lymphocytes from donors receiving 11 doses of CCl_4 increased the mitotic index by about 25 times, although significantly less ($P < 0.001$) than after poisoning by a single dose. Injection of $7 \cdot 10^7$ lymphocytes from intact donors into mice of the control group, like injection of medium 199 alone in the equivalent volume, increased the mitotic index by 1.5-2 times ($P < 0.01$; Table 1).

Immunoperoxidase investigation of liver sections from 54 intact mice revealed no cells containing AFP. In the remaining groups AFP-containing cells were found in some of the animals (Table 1). AFP was found in the cytoplasm of solitary hepatocytes, the number of which did not exceed 0.02-0.03% of the total number of hepatocytes (Fig. 1). No general rule was found as regards the arrangement of AFP-containing cells in the structure of the liver. No

correlation was found between the intensity of the proliferative process induced by lymphocyte transfer and the number of AFP-positive cells, for any of the procedures used in the experimental and control groups induced the appearance of hepatocytes containing AFP. The number of cells containing AFP was about equal whether the increase in the mitotic index was by 75 times or 1.5 times.

AFP likewise was not found in the sera of any of the recipients by the gel-diffusion method.

Transfer of $7 \cdot 10^7$ lymphocytes from donors receiving a single dose of CCl_4 thus stimulates by 2 orders of magnitude an increase in the mitotic index of intact mice. The effect caused by transfer of lymphocytes from partially hepatectomized donors was much weaker. It is not yet clear with what this difference in the stimulation of mitotic activity of the hepatocytes is associated.

The discovery of AFP-containing cells after such minimal and nonspecific procedures as injection of culture medium may perhaps be due to the fact that there is a certain number of AFP-producing cells which remain undetected, being outside the limits of sensitivity of the method. In that case an extremely small physiological increase in the level of AFP production leads to the discovery of these cells. The results are in good agreement with data in the literature [6] indicating that AFP production is independent of the phase of the mitotic cycle of the hepatocytes and that the appearance of AFP-producing cells is unconnected [7] with the intensity of proliferation in the hepatocytes.

The use of a model of transfer of stimulation of proliferative activity of hepatocytes by lymphocytes thus yielded data confirming the absence of any direct connection between the appearance of AFP-producing cells and the intensity of proliferative processes in the liver.

The authors are grateful to A. K. Yazova for providing the pure preparation of mouse AFP and the test system for mouse AFP.

LITERATURE CITED

1. R. D. Bakirov, Byull. Eksp. Biol. Med., No. 2, 45 (1968).
2. A. G. Babaeva, N. A. Kraskina, and N. V. Yudina, Byull. Eksp. Biol. Med., No. 1, 69 (1980).
3. A. S. Gleiberman, Byull. Eksp. Biol. Med., No. 5, 626 (1978).
4. A. I. Gusev and V. S. Tsvetkov, Lab. Delo, No. 2, 43 (1961).
5. V. I. Gel'shtein, "Appearance and progression of experimental liver tumors," Doctoral Dissertation, Moscow (1966).
6. M. N. Lazareva, in: Oncodevelopmental Biology and Medicine, Vol. 2, Amsterdam (1981), pp. 89-99.
7. V. S. Poltoranina, Byull. Eksp. Biol. Med., No. 2, 212 (1981).
8. N. V. Engelgardt, A. I. Gusev (A. J. Goussev), L. Ya. Shipova (L. Ja. Shipova), et al., Int. J. Cancer, 7, 198 (1971).