DEGREE OF HYPERTROPHY OF THE PORTAL DRAINAGE LYMPH NODES AS A MEASURE OF THE STRENGTH OF THE GRAFT-VERSUS-HOST REACTION IN THE MOUSE LIVER

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The liver has a detoxifying function in the body, it takes part in protein, hormone, and vitamin metabolism, it acts in the embryonic period as a hematopoietic organ, it regulates the blood erythropoietin level, and serves as a blood depot [1]. The role of the liver in immunologic reactions and, in particular, in transplantation immunity has not yet been adequately studied. In recent years a number of workers have attempted to study liver functions in the development of the response of cellular immunity, of a model of the local graft-versus-host reaction (GVHR) in the liver of F_1 hybrid dogs and rats developing after transplantation of allogeneic bone marrow or parental lymphoid cells into the liver [2-6, 8]. The strength of the GVHR was judged by the number of foci of paravascular infiltration (PVI) formed in the liver [3, 7]. This test cannot be regarded as strictly specific, for under normal conditions and in various pathological states a definite number of PVI foci is found in the liver. Moreover, to detect PVI histological sections have to be stained by complex methods and counted under a microscope, with the result that it is difficult to assess the strength of the GVHR in the liver.

The object of this investigation was to identify the drainage lymph nodes (DLN) in the anterior lobe of the liver and to work out a method of quantitative estimation of the strength of GVHR in the liver based on hypertrophy of the portal DLN (PDLN), developing after intrahepatic injection of parental immunocompetent cells.

EXPERIMENTAL METHOD

Male (CBA × C57BL/6)F_1 mice weighing 20 g, obtained from the Stolbovaya Inbred Animals Nursery, Academy of Medical Sciences of the USSR, were used as recipients. Male C57BL/6, CBA, and AKR mice weighing 23 g were used as donors. A cell suspension was prepared in the usual way from the spleen and its viability determined by the trypan blue test (not more than 15% of dead cells). The suspension of spleen cells (SC) was injected through a midline laparotomy incision with a microsyringe in a dose of 0.02 ml, containing 5×10^6, 10×10^6, or 15×10^6 living SC beneath the capsule of the anterior lobe of the liver of the F_1 hybrids. Control animals were given 0.02 ml of medium 199. The postoperative wound was sutured with Kapron without drainage. All manipulations were carried out under intraperitoneal hexobarbital anesthesia. The mice were killed on the 5th, 7th, 10th, 15th, 21st, and 30th days with chloroform vapor, the PDLN were removed and dehydrated in acetone and weighed with an accuracy of 0.01 mg. The strength of GVHR in the liver was determined by comparing the weight of the PDLN in the experimental and control animals. The mice were irradiated on the RUM-17 apparatus in a dose of 850 rads (voltage 210 kV, current 15 mA, filters 0.5 mm Cu and 1.0 mm Al, field 20 × 20 cm, focus distance 40 cm, dose rate 62 rads/min). The irradiated animals received antibiotics together with cottage cheese for 7 days. The lymphatic system of the anterior lobe of the liver was injected with Gerota's mass in the usual way. Numerical results were subjected to statistical analysis by Student's t test.

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TABLE 1. Degree of Hypertrophy of Portal PDLN (on 7th day) (M ± m) in Intact and Irradiated (CBA × C57BL/6)F_1 Mice after Injection of 10×10^6 SC of Different Genotypes into the Liver

Series of experiment	Group	Number of recipients	Strain of donor	Weight of portal lymph nodes, mg	P_{1-2}	P_{2-3}	P_{1-3}	P_4
I	1	15	CBA	4,07±0,4118 (2,41—5,51)	>0,05	—	—	—
	2	15	C57Bl/6	3,8±0,4118 (2,15—7,53)	—	<0,05	—	—
	3	10	AKR	2,35±0,1621 (2,3—4,35)	—	—	<0,05	—
II	1 *	10	C57Bl/6	1,3±0,09478 (0,8—2,0)	<0,001	—	—	—
	2	23	C57Bl/6	2,67±0,1537 (1,93—4,2)	—	<0,001	—	—
	3	10	Control	1,49±0,0481 (1,28—1,7)	—	—	<0,001	—
	4 **	11	C67Bl/6	1,74±0,0297 (1,18—2,09)	—	—	—	>0,5
III	1	10	CBA×C57Bl/6)F_1	1,23±0,1178 (0,92—1,62)	>0,5	—	—	—
	2	10	Control	1,11±0,0179 (0,84—1,29)	—	—	—	—

Legend. *) Recipients irradiated in a dose of 850 rads; **) SC injected intraperitoneally; 0.02 ml of medium 199 injected intrahepatically into control mice; here and in Table 2, limits of variations shown between parentheses.

TABLE 2. Dependence of Degree of Hypertrophy of PDLN in (CBA × C57BL/6)F_1 Mice on Dose of CS from C57BL/6 Mice Injected into the liver (M ± m)

Number of mice in experiment	Group	Number of mice	Time of reading reaction, days					P_1	P_2	P_3	P_4	P_5
			5	7	10	15	21					
0,2 ml of med.199	1	100	0,89±0,60 (0,73—1,14)	1,45±0,04 (1,14—1,73)	0,85±0,11 (0,68—1,14)	1,0±0,02 (0,8—1,32)	0,9±0,02 (0,9—1,1)					
5	2	70	1,69±0,03 (1,38—2,13)	3,1±0,09 (2,28—3,7)	2,53±0,21 (1,63—3,54)	2,19±0,22 (1,23—2,62)	1,58±0,07 (1,14—2,2)	<0,05	<0,05	<0,05	<0,05	<0,05
10	3	80	2,76±0,15 (2,0—3,45)	3,64±0,13 (2,6—4,51)	3,0±0,26 (1,8—3,95)	2,39±0,09 (1,76—2,8)	1,76±0,01 (1,57—1,96)	<0,05	<0,05	<0,05	>0,05	>0,05
15	4	83	3,34±0,15 2,25—4,2)	2,21±0,09 (1,58—2,71)	3,14±0,27 (2,13—4,65)	3,13±0,19 (2,05—3,75)	1,4±0,096 (1,12—1,8)	<0,05	>0,05	>0,05	<0,05	>0,05

Legend. P_1, P_2, P_3, P_4, P_5) Comparison of groups 1-2, 2-3, 3-4 respectively on 5th, 7th, 10th, 15th, and 21st days of GVHR.

EXPERIMENTAL RESULTS

The lymphatic system of the visceral surface of the liver was injected with Gerota's mass in 20 animals. The contrast material accumulated in two lymph nodes lying on the right of the portal vein, 0.3-0.4 mm below the hilus of the liver, above the head of the pancreas (the whole of the intestine had to be displaced to the left). Other lymph nodes were not contrasted with Gerota's mass.

As Table 1 shows, intrahepatic injection of SC of the parental genotype was accompanied by specific hypertrophy of PDLN, significantly greater than after injection of SC of the 3rd strain (AKR mice; series I, groups 1, 2, and 3), and also syngeneic SC (series III, groups 1 and 2). Intraperitoneal injection of parental SC did not cause significant hypertrophy of PDLN compared with the control, and this result was independent of the dose of SC injected (series II, groups 3 and 4; $P > 0.05$). Lymphoid cells from both parental lines led to an equal degree of hypertrophy of PDLN. Irradiation of recipients in a dose of 850 rads abolished the development of hypertrophy of the PDLN, indicating that radiosensitive cells of the recipient play a role in the hypertrophy of PDLN (series II, groups 1 and 2).

It will be clear from Table 2 that hypertrophy of the PDLN reached a peak on the 5th-7th days of the reaction, and at these times the degree of hypertrophy of PDLN depended on the dose of SC injected. This dependence diminished for 10 days, but starting with the 15th

day the weight of the PDLN decreased and by the 30th day it was equal to the control value. Doses of 5×10^6-10×10^6 living SC of the parental genotype were thus optimal for induction of the GVHR in the liver, but on the 5th-7th days they were optimal for reading the reaction.

The results show that intrahepatic injection of SC of the parental genotype into F_1 hybrids is accompanied by specific hypertrophy of PDLN caused by the development of a GVHR in the liver. On the 5th-7th days of the reaction the degree of hypertrophy of PDLN depends on the dose of cells injected. Doses of 5×10^6-10×10^6 living SC of the parental genotype are optimal for induction of the GVHR in the liver and development of hypertrophy of the PDLN. On this basis, a new method of quantitative analysis of the strength of GVHR in the liver, allowing the role of the liver to be studied in transplantation immunity, can be suggested.

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ULTRASTRUCTURAL LOCALIZATION OF α-FETOPROTEIN SYNTHESIS IN THE REGENERATING MOUSE LIVER

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After partial hepatectomy or administration of various hepatotoxins there is a sharp but transient rise in the α-fetoprotein (AFP) level in the blood, and cells containing AFP can be found at these times in liver sections. By means of ordinary immunomorphological methods the AFP-containing cells have been characterized as typical differentiated hepatocytes. In adult mice after treatment with hepatotoxins as a rule such cells are few in number and they are located mainly at the boundary with the injured tissue [3, 5, 6]. These observations suggest that during regeneration of the mouse liver temporary derepression of AFP synthesis takes place in mature differentiated hepatocytes. However, it is only through the use of an immunoenzyme technique for localizing antigens at the electron-microscopic level that cells synthesizing AFP can be reliably differentiated from cells passively accumulating AFP as a result of toxic injury. This technique was used previously to reveal AFP-synthesizing cells in human and murine hepatomas, in the human fetal liver, and in the liver of animals during chemical carcinogenesis [8, 9, 13, 14].

This paper describes the use of an electron-microscopic immunoperoxidase method [10] to identify and characterize AFP-synthesizing cells during regeneration of the mouse liver.

EXPERIMENTAL METHOD

Regeneration of the liver was induced in SWR mice aged 2-3 months by poisoning with CCl_4 vapor [2]. The animals were killed 72 h after poisoning. Rabbit antisera (AS) against a purified preparation of mouse AFP were exhausted with a sorbent of normal mouse serum on a basis of sepharose 4B-CNBr, after which monospecific antibodies (AB) against AFP were iso-

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