MORPHOLOGICAL AND FUNCTIONAL CHANGES IN THE IMMUNE SYSTEM DURING REPARATIVE REGENERATION OF THE LIVER

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During reparative regeneration in the liver functional activity of the immune system is increased. Resection of the liver is accompanied by sharp changes in the structure of the thymus and spleen. The thymus-dependent parts of the spleen are particularly reactive. Resection of the liver in rats is accompanied by an increase in the number of stem cells in the bone marrow, as determined by the splenic colonies method. The number of colony-forming cells in the spleen of recipients of lymphocytes taken from the hepatectomized animals is significantly greater than in the spleen of recipients of lymphocytes from intact rats. If the limb is screened, ability to form endogenous colonies also is increased in partially hepatectomized rats compared with intact animals. Resection of the liver in rabbits is accompanied by a significant increase in immunological reactivity on the first to third days after the operation. The reactions of leukergia and leukocytolysis are sharply intensified during this period.

KEY WORDS: *liver; regeneration; immune system; splenic colonies.*

Investigations of regeneration of the liver after partial hepatectomy have demonstrated the special importance of the lymphoid tissue. During regeneration, immune reactions have been shown to be intensified [1, 3, 6] and a definite role in the mechanism of cell division in the regenerating organ has been ascribed to lymphocytes [1-3, 7, 10-12].

Meanwhile many aspects of the role of the immune system in regeneration of the liver still awaits adequate study.

The object of this investigation was a morphological and functional study of the immune system in rabbits (56) and rats (30) after resection of one-third of the liver.

EXPERIMENTAL METHOD

The animals were killed on the 2nd, 3rd, 6th, 14th, 21st, 30th, and 120th days after the operation. Changes in the parenchyma of the liver were judged from the presence of organ-specific enzymes (histidase and urocaninase) in the blood. Histamine was determined in the blood and the regenerating liver. A general blood analysis was carried out, the serum protein spectrum was studied, the index of neutrophil vulnerability (INV) was determined *in vitro* [8], and the nonspecific and specific factors of cellular immunity — leukergia and leukocytolysis, and allergenoleukergia and allergenoleukocytolysis in the presence of liver antigen were studied [4, 12]. The kinetics of stem cells from the bone marrow [14] was studied in intact and partly hepatectomized rats. In some of the totally irradiated animals the hind limb was screened, whereas bone marrow from intact donors and donors undergoing hepatectomy was transplanted into other animals.

Squash preparations of the spleen and lymph nodes, liver, thymus, and bone marrow were examined. Paraffin sections were stained with hematoxylin—eosin, cresyl violet, and Schiff's reagent.

The mitotic activity and nucleo-cytoplasmic ratio of the mononuclear hepatocytes were determined. The numerical results were subjected to statistical analysis.

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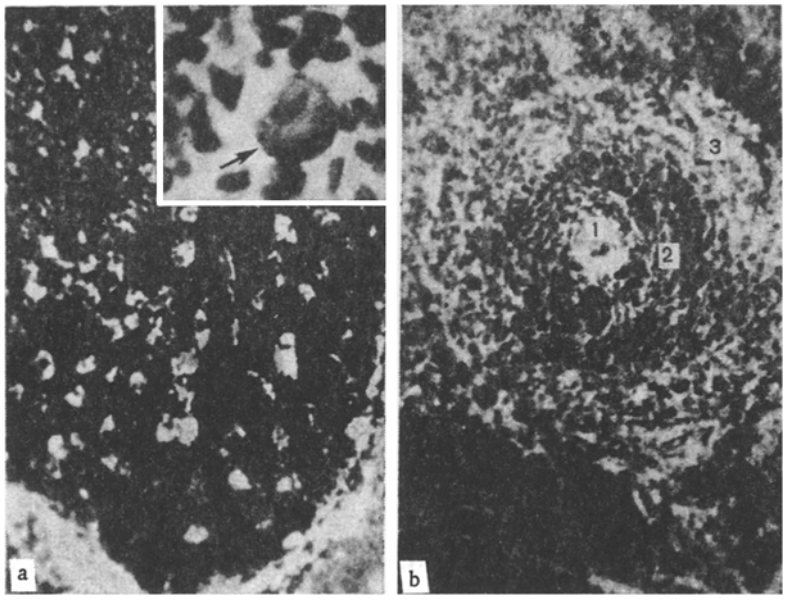

Fig. 1. Thymus and spleen of rabbit 48 h after resecton of the liver. a) Multiple "windows" in the cortex of the thymus; PAS-positive cell shown in top right hand corner; b) spleen: lymphoid follicle in center. 1) Artery; 2) T-zone; 3) thymus-independent zone. Shabadash's stain, counterstained with hematoxylin, 600×.

TABLE 1. Number of Cells of Plasma Series (in %) in Squash Preparations of Spleen and Lymph Nodes during Reparative Regeneration of Liver in Rabbits

Time after operation, days	n	Plasma cells				Plasmablast		Blast cells	
		immature		mature					
		spleen	lymph node	spleen	lymph node	spleen	lymph node	spleen	lymph node
Normal	16	8±2,0	2±0,2	10±1,6	5±1,0	4±0,5	7±0,8	3±0,5	8±2,0
1	5	5±1,4	2±0,1	1±0,1	1±0,1	2±0,1	20±1,4	2±0,1	10±2,0
3	5	12±0,8	15±1,9	1±0,1	4±0,1	9±1,9	26±1,4	4±0,8	37±8,0
6	5	2±0,1	4±0,8	4±0,8	4±0,1	10±1,0	20±1,0	—	28±1,0
14	5	25±4,0	4±0,8	4±0,4	5±0,5	4±0,4	15±1,4	—	1±0,1
21	5	18±2,0	7±0,5	17±2,0	7±2,0	12±0,5	14±2,0	1±0,3	1±0,3
30	5	8±0,9	4±0,9	13±0,4	8±0,9	4±0,4	4±0,4	4±0,4	3±0,9
120	11	7±2,0	2±0,1	6±1,0	6±1,0	4±0,7	8±1,0	2±0,5	10±2,0

EXPERIMENTAL RESULTS

The experiments showed that 48 h after partial hepatectomy mitotic activity of the hepatocytes (5.3‰ in the intact animals) was increased in the liver of the rabbits. The mitotic index fell on the third and sixth days (2.1 and 1.3‰, respectively). On the 14th, 21st, 30th, and 120th days the mitotic index varied within the limits of its level in the control animals. Before the seventh day after resection the nucleo-cytoplasmic ratio was almost doubled.

Proliferative processes in the liver were accompanied by lymphocytic infiltration along the course of the portal tracts and central veins, which could be detected until the 21st day after resection.

On the second to third day after the operation a high histamine content was found in the liver (from 2.28 ± 0.25 to 10.15 ± 0.8 μg/g; 1.55 ± 0.11 μg/g in the control). Considering that histamine accelerates the blood flow, increases membrane permeability, splits glucose, and dilates blood vessels an increase in its concentration in the regenerating liver can be

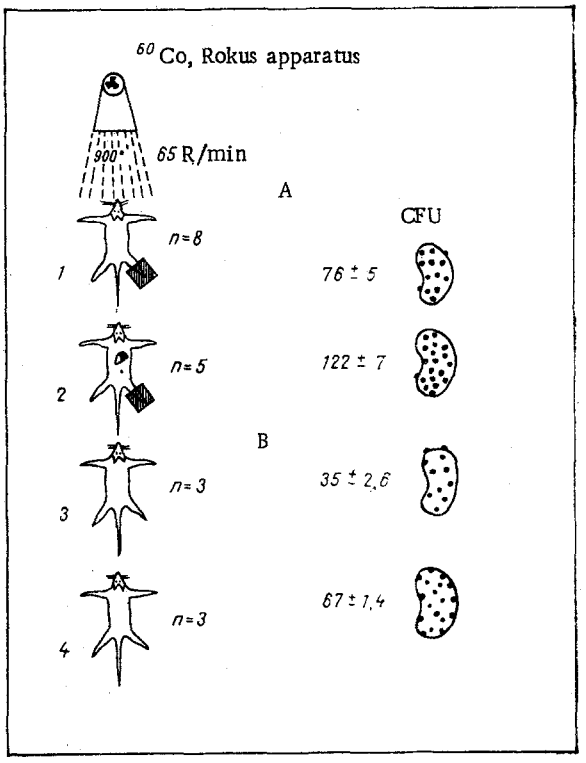

Fig. 2. Number of colony-forming units in spleen of rats on eighth day after lethal irradiation. A) Rats with screened hind limb: 1) with intact liver; 2) after resection of one-third of liver. B) Total irradiation of rats receiving bone marrow cells from intact rats (3) and rats receiving bone marrow cells from partially hepatectomized rats (4). Experimental conditions illustrated on left, results on right: number of colonies formed per spleen.

TABLE 2. Leukergia and Leukocytolysis (in %) during Reparative Regeneration of Liver in Rabbits

Time after operation, days	n	Leukergia		Leukocytolysis	
		spontaneous	allergeno-leukergia	spontaneous	allergenoleu-kocytolysis
Normal	16	1,8±0,40	3,2±0,4	3±0,2	8,3±1,6
1	5	12,7±3,40	25,6±4,7	6,6±0,8	16,7±1,5
3	5	7,5±1,20	19±2,0	38,2±1,4	50,2±2,5
6	5	25,3±5,50	46±13	0	12,7±3,0
14	5	0,3±0,01	14,7±2,3	2,3±0,2	13,3±1,9
21	5	0,3±0,06	8,2±0,5	2±0,1	1,4±0,4
30	5	1,7±0,20	1,5±0,4	5,1±1,0	10,8±3,0
120	11	1,9±0,20	3,3±0,8	3,6±0,1	7,8±0,1

Legend. Reaction considered to be positive if data exceed control by more than twice.

regarded as an adaptive reaction, favoring proliferation of its cells.

A reduction in the number of cells in the thymus was observed 48 h after resection of the liver. Multiple "windows" appeared in the cortex of the thymus, with degeneratively changed thymocytes visible in their clear spaces (Fig. 1a). The decrease in the relative weight of the thymus and obliteration of the boundary between the cortex and medulla could be due not only to destruction of the cells, but also to their migration into the blood stream and lymphoid organs [5]. On the 2nd to 14th days after the operation the volume and number of Hassall's corpuscles were increased and PAS-positive granules appeared in their cells.

The number of large PAS-positive cells in the cortex was increased and they often formed colonies and surrounded material of colloid type (Fig. 1a). These changes indicate an increase in the hormonal activity of the thymus in the early stages after partial hepatectomy [9].

In the red pulp of the spleen dilatation of the venous sinuses, swelling of the capillary endothelium, an increase in the number of leukocytes, and erythrophagia were observed 48 h after resection of the liver. There were fewer cells in the center of the lymphoid follicles and in their outer part (the zone of small lymphocytes), whereas near the arteries they were more numerous (T zone; Fig. 1b), possibly as a result of migration of thymocytes into that zone and proliferation of its cells.

After the third day, against the background of clearly outlined T zones and a marked macrophagal reaction in the outer part of the follicles, many blast cells, plasmablasts, and plasma cells appeared.

Examination of squash preparations of the spleen and lymph nodes 48 h after resection of the liver showed an increase in the number of plasmablasts in the lymph nodes, on the third day an increase in the number of plasmablasts and plasma cells in the lymph nodes, and by the 14th day an increase in the number of immature plasma cells in the spleen (Table 1). The increase in the number of antibody-forming cells coincided with hypergammaglobulinemia.

The study of the colony-forming capacity of the bone marrow in rats revealed stimulation of stem cells after resection of the liver.

After lethal irradiation of the partially hepatectomized rats with the bone marrow of the right hind limb screened, and also with transplantation of bone marrow ($2 \cdot 10^6$ nucleated cells) into the irradiated recipients from partially hepatectomized donors, the number of surface hematopoietic colonies in the spleen was almost twice their number in the control group (Fig. 2).

In rabbits 1-3 days after hepatectomy hyperhistaminemia was accompanied by a sharp increase in the intensity of nonspecific and specific factors of cellular immunity (Table 2), indicating an increase in immunological reactivity, intensification of phagocytosis, and sensitization of the blood leukocytes to the liver antigen. The leukocytolysis disappeared after 6 days and the positive allergenoleukergia after 21 days. A high (more than twice the normal level) INV coincided with positive reactions of allergenoleukergia. Disappearance of the organ-specific enzymes from the blood was observed 21 days after the operation, whereas their normal concentration in the liver was restored after 4 months.

After resection of the liver morphological and functional changes thus appear in the immune system of the body. The thymus-dependent part (cortex of the thymus, T zones of the spleen) are the most reactive. The increase in immunological reactivity and intensification of phagocytosis, aimed at the recognition and elimination of foreign substances or of the host's own genetically changed cells, and reparative processes in the liver are all interdependent. These experiments point to the existence of a complex mechanism of interaction between the immune system and repair processes in the liver, aimed at the restoration of homeostasis in the animal.

LITERATURE CITED

1. A. G. Babaeva, in: Regeneration and Cell Division [in Russian], Moscow (1968), pp. 1-11.
2. A. G. Babaeva, N. A. Kraskina, and L. D. Liozner, Tsitologiya, No. 12, 1511 (1969).
3. A. G. Babaeva, Immunological Mechanisms of Regulation of Regenerative Processes [in Russian], Moscow (1972).
4. A. F. Blyuger and Kh. M. Veksler, in: Advances in Hepatology [in Russian], No. 2, Riga (1968), pp. 191-225.
5. P. D. Gorizontov, in: Homeostasis [in Russian], Moscow (1976), pp. 43-45.
6. D. N. Evnin, Byull. Eksp. Biol. Med., No. 9, 1 (1970).
7. L. I. Osokina and A. M. Yartseva, in: Current Problems in Gastroenterology [in Russian], No. 6, Moscow (1973), pp. 202-204.
8. V. A. Fradkin, Sov. Med., No. 9, 41 (1962).
9. E. Z. Yusfina and A. N. Yakovleva, Patol. Fiziol., No. 2, 2 (1969).
10. R. G Burwell, Lancet, 2, 69 (1963).
11. P. R. J. Burch, An Inquiry Concerning Growth, Disease and Aging, Univ. Toronto Press, Toronto (1969).

12. A. J. S. Davies, E. Leuchars, S. M. A. Doak, et al., Nature (London), 201, 1097 (1961).
13. L. Fleck, Schweiz, Med. Wschr., 76, 175 (1946).
14. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).