STIMULATION OF PROLIFERATIVE ACTIVITY OF LIVER CELLS OF INTACT MICE BY LYMPHOCYTES OF PARTIALLY HEPATECTOMIZED DONORS

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UDC 612.35.014.3:612.6-06:612.112.94:612.35-089

Transplantation of living spleen cells of partially hepatectomized mice into intact syngenic recipients caused stimulation of the proliferative activity of the hepatocytes and reticulo-endothelial cells of the liver. This phenomenon was not reproduced when living spleen cells of intact mice or killed spleen cells of hepatectomized mice were transplanted. The fraction of small lymphocytes obtained from the spleen of the hepatectomized mice preserved these stimulating properties.

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The role of lymphocytes in reparative regeneration has not yet been adequately studied. Experimentally induced hypoplasia of the lymphoid system has been shown to be accompanied by inhibition of regeneration of the internal organs, notably of the liver [6-9]. Observations have been made showing that the degree of inhibition of liver regeneration is directly proportional to the lymphocytopenia developing in the experimental animals [6]. However, it is not yet clear whether this inhibition of regeneration is caused by changes in the lymphoid tissue or by other factors.

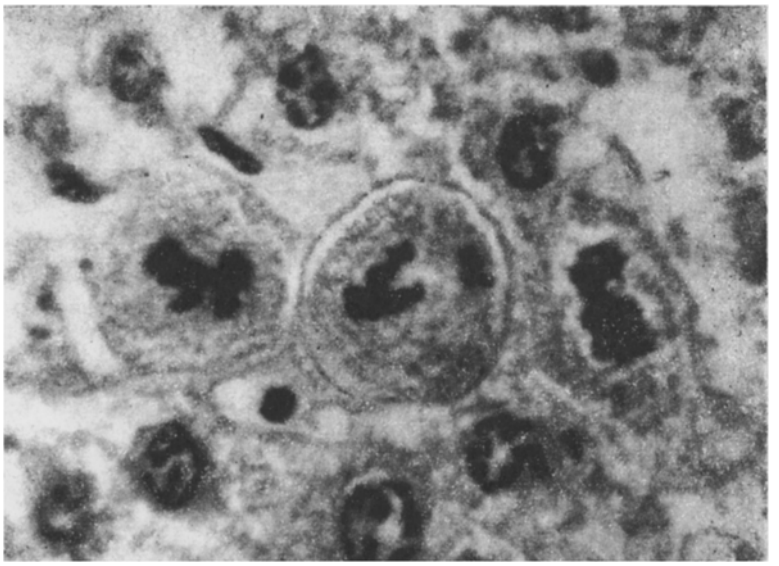

Fig. 1. Stimulation of mitotic activity in hepatocytes of recipients after transplantation of suspension of spleen cells from partially hepatectomized donors. Hematoxylin-eosin, 630 ×.

Laboratory of Growth and Development, Institute of Experimental Biology, Academy of Medical Sciences of the USSR; Laboratory of Immunology, Moscow Research Institute of Epidemiology and Microbiology (Presented by Academician N. A. Fedorov, Academy of Medical Sciences of the USSR.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 7, pp. 91-94, July, 1969. Original article submitted December 24, 1968.

TABLE 1. Mitotic Index of Reticulo-Endothelial Cells of Liver of Recipient Mice 43-45 h after Transplantation of Lymphocytes of Partially Hepatectomized Donor Mice

Group of animals	Donor interval	Number of recipients	Mean mitotic index (in %)	95% Confidence interval
Control	–	24	0.56	0.41-0.71
Recipients of living spleen cells of intact mice	–	28	0.74	0.39-1.09
Recipients of killed spleen cells of hepatectomized mice	17	12	0.81	0.62-1.00
Recipients of living spleen cells of hepatectomized mice	17	22	3.00	2.35-3.65
Recipients of fraction of small lymphocytes obtained from spleen of hepatectomized mice	17	14	2.43	1.67-3.19

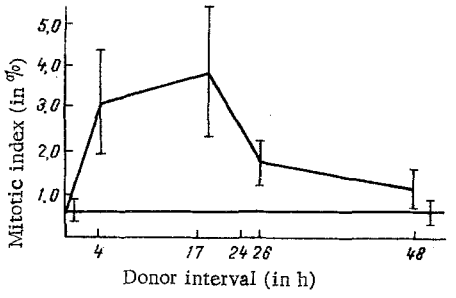

Fig. 2. Changes in mitotic index of reticulo-endothelial cells of liver in recipients 43-45 h after transplantation of lymphocytes of partially hepatectomized donors as a function of the time interval between operation and sacrifice (donor interval).

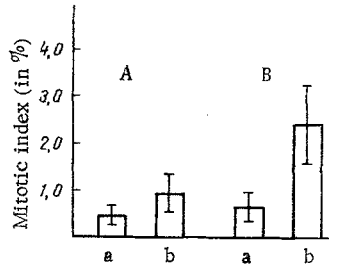

Fig. 3. Mitotic index of reticulo-endothelial cells and connective-tissue cells of liver (A) and kidney (B) of control (a) and experimental (b) mice.

The object of the present investigation was to study: 1) whether the properties of the lymphoid cells are modified during liver regeneration, and 2) whether proliferative processes can be induced in the liver of intact animals by transplantation of lymphocytes from partially hepatectomized donor into these animals.

EXPERIMENTAL METHOD

Experiments were carried out on 330 male mice of lines CC57Br, CC57Wt, and CBA, weighing 17-23 g. Two-thirds of the liver was removed from the group of mice serving as future donors of lymphocytes by the method of Higgins and Anderson [10]. The hepatectomized mice were sacrificed 2, 4, 17, 26, and 48 h after the operation (the donor interval) by means of chloroform vapor, and a suspension of spleen cells was prepared in medium No. 199 [2, 3] and transplanted intravenously into intact syngenic recipients in a dose of 80 million cells per animal. The recipients were autopsied 43-48 h after transplantation, always at 7-8 A.M. One group of recipients received an injection of colcemide in a dose of 5 mg/kg body weight 4 h before sacrifice. The liver and kidney were taken for histological examination. Mitotic activity was determined as described previously [1] in 7500 liver cells, 13,000 tubular epithelial cells of the kidney, and 4000 reticulo-endothelial cells of the liver and connective-tissue cells of the kidney. The mitotic index was expressed in promille. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

Determination of the mitotic index of the liver cells showed stimulation of cell division of the hepatocytes (Fig. 1) and reticulo-endothelial cells (Tables 1 and 2) in the recipients 43-45 h after injection of lymphoid cells of partially hepatectomized donors.

TABLE 2. Mitotic Index of Hepatocytes of Recipient Mice 48 h after Transplantation of Lymphocytes of Partially Hepatectomized Mice and 4 h after Injection of Colcemide

Group of animals	Donor interval	Number of recipients	Mean mitotic index (in %)	95% Conf. interval
Control	–	13	4.78	1.40-8.20
Recipients of living spleen cells of intact mice . . .	–	11	3.88	1.67-5.03
Recipients of living spleen cells of hepatectomized mice	2	13	10.30	3.49-16.84
The same	17	15	12.76	6.74-18.80

As Table 1 shows, living lymphocytes of intact donors and spleen cells from hepatectomized donors killed by freezing three times to −70° do not possess these stimulant properties. This fact indicates that 1) lymphocytes acquire stimulant properties as a result of certain changes produced by partial hepatectomy, and 2) the observed stimulation of proliferative processes in the liver is a function of living lymphocytes.

The fact will be noted that the fraction of small lymphocytes obtained from the spleen of partially hepatectomized donors completely retains its ability to stimulate proliferative processes in the liver. It may be assumed that the stimulant properties belong in fact to the lymphocytes.

The study of the dynamics of changes in the properties of the spleen cells following partial hepatectomy showed that the lymphocytes became capable of stimulating cell division very soon after the operation. A marked increase in the intensity of proliferation in the hepatocytes, for instance, was observed in recipients following transplantation of lymphocytes from a donor sacrificed 2 h after partial hepatectomy (Table 2).

Spleen cells taken from donors 4 and 17 h after partial hepatectomy showed the strongest stimulant activity relative to the reticulo-endothelial cells. This property became weaker 26 h after the operation and disappeared almost completely 48 h thereafter (Fig. 2). In other words, the stimulant properties were greatly reduced in donors of lymphocytes sacrificed at a time when, according to the literature, intensification of synthetic processes and an increase in the number of mitoses in the partially extirpated liver are observed [5].

The response of the recipient to transplantation of donor's lymphocytes was characterized by only partial organ specificity, for a significant increase in the mitotic index was observed also in the connective-tissue cells of the recipient's kidney. Nevertheless, this increase was much smaller than that observed in the reticulo-endothelial cells of the liver (Fig. 3).

These results may be summarized by stating that the transplantation of lymphocytes from partially hepatectomized animals into an intact recipient stimulates proliferative activity of the liver cells in the recipient. The ability of lymphocytes to transmit these changes develops early. The mechanism of the stimulant action of lymphocytes on the Kupffer cells hepatocytes of the recipient's liver have not yet been explained.

It may be postulated that the donor's lymphocytes, having acquired certain new properties, are themselves transformed into Kupffer cells. This hypothesis as regards the hepatocytes seems to be improbable. The possibility likewise is not ruled out that lymphocytes transmit information to the liver cells in some manner as yet unknown along the lines of the transmission of immunologic information. The possibility of transmission of immunologic information by the lymphocytes of an immune donor to the lymphocytes of a nonimmune recipient has been demonstrated experimentally by Fontalin and co-workers [4].

The results obtained significantly broaden the existing views regarding the ability of lymphocytes to receive and transmit information and they give conclusive evidence of their participation in the regulation of regeneration of the liver.

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