CHANGES IN THE NUMBER OF STEM CELLS IN THE SPLEEN OF PARTIALLY HEPATECTOMIZED MICE AT VARIOUS TIMES DURING REGENERATION OF THE LIVER

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Suspensions of spleen cells from intact and partially hepatectomized mice were injected into lethally irradiated (CBA × C57BL/6j) F_1 hybrid mice 24 h after irradiation. The number of colonies of hematopoietic cells in the recipients' spleen and the mitotic activity in their liver were determined on the eighth day after transplantation of the cells. The number of colonies in the spleen of the hepatectomized mice showed a significant decrease 19-21 h and an increase 48 h after the operation. Injection of spleen cells into the irradiated mice stimulated proliferative activity in the liver. The donors' spleen cells had the greatest stimulant effect 17 h after partial hepatectomy. Acquisition of the ability to induce proliferation in the liver of an intact recipient by the spleen cells of partially hepatectomized mice is independent of the level of hematopoietic stem cells in the donors' spleen.

A suspension of lymphoid cells from the spleen of partially hepatectomized mice has been shown to stimulate mitotic activity of the liver and reticulo-endothelial cells of intact syngeneic recipients [1, 2]. The lymphoid cells possess this property only at certain periods after the operation (4-26 h), and it is completely lost 48 h after the operation [1].

It was decided to investigate how the cell composition of the spleen changes at these times of regeneration and whether the stimulant activity of the splenic lymphocytes is connected with an increase in the number of stem cells, i.e., of undifferentiated cells capable of active division, among them.

EXPERIMENTAL METHOD

The mice used were (CBA × C57BL/6j) F_1 hybrids weighing 20-22 g (195 males and 40 females). Two-thirds of the liver was removed from the animals by the usual method [3], and the mice were sacrificed 4, 17-18.5, 19-21, and 48-49 h after the operation. A suspension of spleen cells from three to seven of the hepatectomized animals was prepared and injected intravenously (in a dose of 1×10^6) into lethally irradiated recipients of the same sex. The recipients were irradiated with Cs^{135} γ rays on the "Stebel'-3A" apparatus in a dose of 900 R (rate 900 R/min) 24 h before transplantation of the cells. From 10 to 15 animals were used in each group. The recipients were sacrificed on the eighth day after injection of the cells, the spleen was fixed in Clark's solution (a mixture of glacial acetic acid with ethanol in the ratio 1: 3), and the number of colonies formed was counted [4]. Irradiated recipients receiving injections of spleen cells from intact mice and from mice undergoing a mock operation were used as the controls. With the dose of irradiation given, the number of spontaneous colonies of hematopoietic cells in the spleen of the irradiated recipients (irradiation control) averaged 0.4 per mouse. Mitotic activity in the liver was determined by the method used previously, by counting the number of dividing nuclei in 1000 cells [1, 2]. The mitotic index was expressed in

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TABLE 1. Number of Colonies in Spleen of Irradiated Mice Receiving Injections of Spleen Cells from Partially Hepatectomized Mice

Time after operation (in h)	Group of recipients	No. of recipients	Expt. No.	No. of colonies in spleen (M± m)	P
4	Control	10	1	8,9±1,4	0,2
	Experimental	10		11,6±0,48	
	Control	5	2	9,4±0,5	
	Experimental	9		10,8±1,4	0,2
17—18½	Control	13	1	8,5±1,0	0,02
	Experimental	10		12,0±1,1	
	Control	2	2	9,5±0,5	0.4
	Experimental	7		10,4±1,0	
19—21	Control	6	1	8,2±0,6	
	Experimental	6		5,5±0.8	0,02
	Control	10	2	8,9±1,4	0,28
	Control (suspension of cells from donors undergoing mock operation)	9		6,7±1,2	
	Experimental	16		5,4±0,4	0,002
48	Control	7	1	8,5±0,5	0,001
	Experimental	10		14,4±1,0	
	Control	13	2	8,5±1,0	0,004
	Experimental	13		12,2±0,6	

TABLE 2. Mitotic Index (in ‰) of Hepatocytes and Reticulo-Endothelial Cells in Liver of Irradiated Mice after Receiving Injections of Spleen Cells from Partially Hepatectomized and Intact Donors

Group of animals	Time after operation (in h)	No. of mice	Mitotic index	
			in hepatocytes	in reticulo-endothelial cells
Irradiated	—	10	0,06±0,04	0,13±0,05
Recipients of spleen cells of normal mice	—	10	0,02±0,02 $P<0,1$	0,56±0,18 $P<0,005$
Recipients of spleen cells of hepatectomized donors	17	11	0,28±0,11 $P<,0001$	0,93±0,19 $P<0,001$
Recipients of spleen cells of hepatectomized donors	48	10	0,02±0,02 $P>0,1$	0,62±0,21 $P<0,001$

promille. The significance of differences between the numerical results was determined by the Fisher—Student method.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1, which shows that the number of stem cells in the spleen changed significantly in the course of regeneration in the liver. The number of stem cells in the spleen of the experimental mice was approximately the same as in the control 4 h after partial hepatectomy. A statistically significant increase in the number of stem cells was observed in one group of animals 17-18.5 h after the operation, but the difference in the other group of mice at this time was not significant. From 19 to 21 h after partial hepatectomy the number of stem cells in the spleen fell significantly. By contrast, a significant increase in the number of stem cells was observed in the spleen of the experimental mice 48-49 h after the operation.

The study of the mitotic activity in the liver of the irradiated animals showed that it was sharply reduced in the irradiated mice not receiving injections of spleen cells. Transplantation of spleen cells from intact mice stimulated proliferation in the recipients' liver, but only in the reticulo-endothelial cells. By contrast, the spleen cells from hepatectomized mice led to increased proliferation of both reticulo-endothelial cells and of hepatocytes. The effect was most marked when spleen cells obtained 17 h after partial hepatectomy were used (Table 2). The experimental results confirmed earlier observations on the times after resection of the liver when the lymphocytes begin to exert their stimulant action [1].

It must be emphasized that the lymphocytes also exerted their stimulant activity in lethally irradiated recipients. However, the results show that this stimulant activity of the splenic lymphocytes of the hepatectomized animals is not determined directly by the number of stem cells in the spleen at the time of investigation. The ability of the lymphocytes to stimulate proliferation was lost after 48 h at a time when the number of stem cells in the animals' spleen was increased. Their stimulant activity could be high when the number of stem cells was reduced, increased, or normal (i.e., 4, 17-18.5, and 19-21 h after the operation).

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