Size of Lymphocyte Subpopulations in the Spleen and Level of Hemopoietic Tissue Proliferation in Mice during Surgical Interventions

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The percent and absolute content of T helpers increases in the spleen of mice 4 and 17 h after resection of the liver or removal of a kidney, which is associated with an increase of the mitotic index of splenocytes. The number of T suppressors and the bone marrow cell mitotic index are unchanged. The T-helper/T-suppressor ratio in the spleen increases 1.7-2 times during surgery on the liver and kidney.

Key Words: *T helpers; T suppressors; regeneration; proliferation*

Lymphoid cells of the spleen (mainly T-lymphocyte subpopulations) are known to acquire the capacity, after some surgical interventions, of soon inducing proliferation in organs homologous to the operated donor organ in nonoperated syngeneic recipients [1-3,7,9]. This property, specifically after partial hepatectomy (PH) and unilateral nephrectomy (UN), manifests itself 4 and 17 h after the intervention [1,2].

However, it is still unclear whether the subpopulation composition of the spleen changes during this period and whether these changes are caused by an altered level of proliferation in the spleen proper and in the bone marrow as a possible source of the precursors of these cells, arbitrarily called morphogenetically active lymphocytes.

The few reports available on this topic do not answer these questions, as in one of them remote periods were selected for investigation [8] and in another the analysis disregarded the level of proliferation of these cells [4].

In this research the changes in the subpopulation composition of splenic lymphocytes were studied using the Parmokvant-2 device during the period of their highest morphogenetic activity towards lymphoid and hemopoietic tissue and the tissues of damaged organs.

MATERIALS AND METHODS

Experiments were carried out with male (CBA×C57Bl/6) F_1 mice weighing 16 to 18 g. Removal of two-thirds of the liver and UN were carried out routinely under Nembutal narcosis in a dose of 60 ml/kg [1,2]. The operated and control animals, each group consisting of at least 10 animals, were sacrificed between 10.30 and 11 o'clock by chloroform vapor 4 or 17 h after the procedure.

Fragments of the liver and kidney were fixed in Carnoy fluid and treated routinely for histologic examination. Paraffin slices 4 μ thick were stained with hematoxylin-eosin. Smears from the bone marrow and spleen were prepared as described previously [5], fixed with methanol, and stained with azure-eosin.

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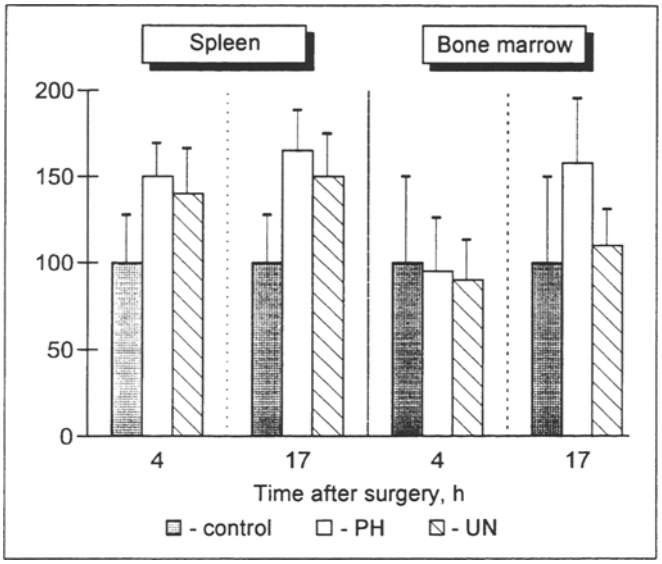

Fig. 1. Level of mitotic activity of splenic and bone marrow cells after surgical intervention. Ordinate: number of dividing cells in percent of the control. PH: partial hepatectomy, UN: unilateral nephrectomy.

Mitoses were counted per 4000 nuclear cells of the bone marrow and splenocyte suspension [5], 4000 hepatocytes, and 10,000 cells of the proximal portions of the urinary canaliculi. The mitotic index was expressed in promille or percent of the initial level of the parameter.

T helpers and T suppressors were counted using the Parmokvant-2 device for measuring the electrophoretic mobility of lymphocytes, from which the size of a lymphocyte subpopulation may be estimated [8].

The results were statistically processed after Fisher and Student [6]. Differences in the parameters were considered reliable at $p<0.05$.

RESULTS

Table 1 presents changes in the counts of T helpers and T suppressors and their ratio in the spleen 4 to 17 h after PH and UN.

The data indicate that both UN and PH are associated with an increase of the percent and absolute content of T helpers in the spleen. The number of T suppressors did not change. Hence, 4 h after the operation the T-helper/T-suppressor ratio increased twofold. This reflects significant changes in the immunoregulatory system. The data presented in Fig. 1 indicate that the restructuring in the immunoregulatory system of T cells went along with some changes in the proliferative activity of hemopoietic cells. For example, 4 and 17 h after the intervention (whether PH or UN), the mitotic index of splenocytes was observed to rise. The level of proliferative activity of bone marrow cells did not change after surgery. The high mitotic index of the lymphoid cells of the spleen gives reason to assume that the elevated count of T helpers in it may be a result of their multiplication. At the same time, additional evidence is needed to prove that the high mitotic index reflects a true in-

TABLE 1. Effect of Surgical Intervention on the Count of Immunoregulatory T Cells in Mouse Spleen ($M\pm2m$)

Group	Parameter	Time after intervention, h					
		4			17		
		T helpers	T suppressors	T-helper/T-suppressor ratio	T helpers	T suppressors	T-helper/T-suppressor ratio
Intact (control)	%	10±1.2	24.2±2.0	0.41±0.16	12.2±2.4	22.8±1.8	0.54±0.1
	abs., ×10	15.2±1.8	36.8±3.04	-	17.1±3.4	31.9±2.5	-
	% of control	100±11.8	100±8.3	100±39	100±19.9	100±7.8	100±18.5
Hepatectomy	%	19.2±2.8	22.3±3	0.86±0.2	21.3±3.0	20.8±2.4	1.02±0.18
	abs., ×10	27.3±4.0	31.7±4.3	-	27.5±3.9	26.8±3.1	-
	% of control	179.6±26.3	86.1±11.7	209.7±48.7	161±23	84±9.7	188±33.3
Nephrectomy	%	21.5±1.3	23.8±4.2	0.9±0.18	20.4±1.9	23.1±2.3	0.96±0.2
	abs., ×10	29.7±1.8	32.8±5.8	-	25.1±2.3	28.4±2.8	-
	% of control	197±12	89.1±15.7	219.5±43.9	146.9±13.5	89.0±8.8	177.8±37

TABLE 2. Changes in the Level of Proliferation of Hepatocytes and Epitheliocytes of the Proximal Part of the Urinary Canaliculi 17 h after Partial Hepatectomy (PH), Unilateral Nephrectomy (UN), and Sham Operation ($M\pm m$)

Time after surgery	Hepatocytes			Renal epithelium	
	PH	sham operation	UN	PH	UN
0 (intact control)	1.18±0.38	1.18±0.38	0	2.1±0.62	2.1±0.62
17 h	0	0	0	0	0

crease of proliferative activity rather than the discontinuation of mitosis.

Table 2 shows that a sham operation, PH, and UN are followed by complete suppression of liver and kidney cell proliferation 17 h after the intervention, evidently the result of postoperative stress. On the whole, the data indicate a clear-cut increase of the T helper count in the spleen during the period of the highest morphogenetic activity of lymphocytes after surgery on either the liver or kidney.

A rise of mitotic activity in the regenerating liver and hypertrophied kidney is observed 44 to 50 h after the operation, and since we know the duration of individual periods of the mitotic cycles, we can assume that the increase in the T-helper count is involved in the triggering of the proliferative wave in the regenerating organs. However, our findings do not mean that T helpers alone are responsible for the change of proliferation during regeneration processes and for the morphogenetic activity of lymphoid cells of the spleen of partially hepatectomized and unilaterally nephrectomized animals. Only further experiments with adoptive transfer of the fraction of identified lymphocyte subpopulations of operated animals will answer this question.

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