cytes, but proliferation of any other cells was not found. The same role, i.e., tumor growth on account of pericyte proliferation, was found by the writers previously when studying a connective-tissue tumor of a different type — a desmoid fibroma [6].

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DEPENDENCE OF MONONUCLEAR INFILTRATION OF THE LIVER ON HEPATOCYTE PROLIFERATION

D. N. Mayanskii, V. I. Shcherbakov, and T. G. Komlyagina

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After injection of microbial polysaccharides into mice, multiple foci of mononuclear infiltration are formed in the liver. Their origin is linked with primary stimulation of Kupffer cells (KC) and secondary "recruiting" of mononuclear cells — monocytes and macrophages, lymphocytes and their derivatives from the bloodstream and lymph [6]. Foci of mononuclear accumulation are evidently formed, on the one hand, under the influence of chemoattractants, secreted initially by stimulated hepatic mononuclear phagocytes (MP), and on the other hand, by induction of medullary monocytopoiesis [3]. It has been shown that restoration of the structure of the liver after partial resection depends on the initial functional state of the KC [4]. The proliferating parenchyma of the liver is also known to inhibit the fibroplastic process (FP) in the same organ [5]. However, the mechanisms of this effect have not been studied. Realization of FP largely depends on reactivity of resident marcophages in the stroma [2].

It was accordingly decided to study how reactivity of MP in the liver stroma and the MP system as a whole change when hepatocyte proliferation is disinhibited. The investigation described below was devoted to these problems.

Laboratory of Pathophysiology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk.

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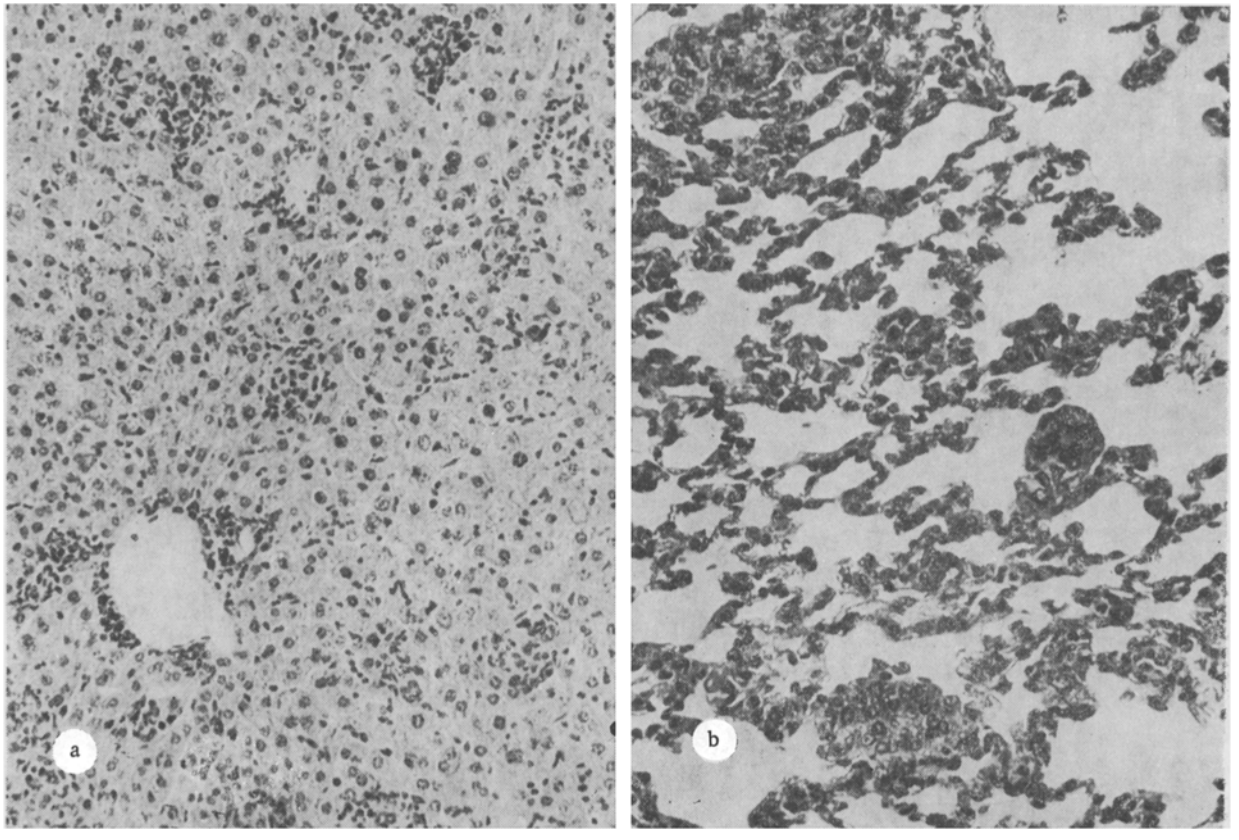

Fig. 1. Sections of mouse liver (a) and lung (b) 5 days after injection of zymosan: discrete foci of MI. Hematoxylin—eosin, 250 ×.

TABLE 1. Effects of Different Doses of Zymosan 3 days after Stimulation

Doze of zymosan, mg	Number of leukocytes (x 10^3)	Leukocyte formula (x 10^3)			Area of section of liver occupied by foci of MI, %
		granulocytes	lymphocytes	monocytes	
2	4,3±0,16**	1,1±0,1	2,4±0,12*	0,78±0,04*	11,9±1,25
0,2	5,2±0,2*	1,1±0,1	3,5±0,13*	0,6±0,05*	1,7±0,15
0,02	6,5±0,4	1,2±0,13	4,8±0,28	0,5±0,05	0,2±0,035
Control (physiological saline)	6,2±0,22	1,4±0,07	4,5±0,16	0,3±0,02	—

Legend. *P < 0.01 compared with control. [**Not defined in Russian original.]

TABLE 2. Area of Liver Section (in %) Occupied by Foci of MI in Control (MH) and Experiment (zymosan followed after 2-4 h by PH) in Zymosan-Stimulated Mice

Time after injection of zymosan, days	Control	Experiment	P
2	3,49±0,24	—	
5	14,9±1,33	1,6±0,3	<0,01
9	35,4±2,52	16,76±2,56	<0,01
15	14,05±0,88	29,4±1,84	<0,01
21	3,52±0,32	21,08±1,44	<0,01
35	—	8,33±0,55	

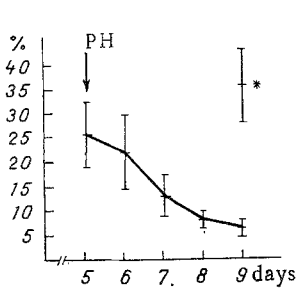

Fig. 2

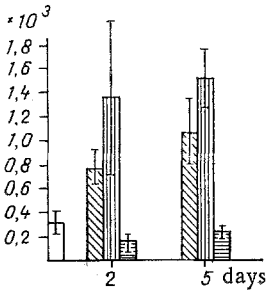

Fig. 3

Fig. 2. Area of section of liver (in %) occupied by foci of MI (mice subjected to PH 5 days after stimulation by zymosan). *Control animals undergoing mock operation.

Fig. 3. Absolute number of monocytes in blood of mice 2 and 5 days after stimulation by zymosan. Unshaded column — intact control, obliquely shaded columns — mock operation, vertically shaded columns — PH, horizontally shaded columns — injection of hydrocortisone.

EXPERIMENTAL METHOD

Male (CBA × C57BL)F_1 mice weighing 16-22 g were used. Zymosan was injected intravenously into the animals in a dose of 2 mg in 0,5 ml of 0.85% NaCl. Two-thirds of the liver was removed from some mice 2-4 h, and others 1 and 5 days after injection of zymosan, by the method of Higgins and Anderson [7]. A mock hepatectomy (MH) was performed on the control animals. Other mice were given an intraperitoneal injection of hydrocortisone acetate (Gedeon Richter, Hungary) in a dose of 125 mg/kg. The animals were killed 5-10 at a time 1, 2, 3, 5, 9, 15, 21, and 35 days after injection of zymosan. Sections of liver, lung, and spleen 5-8 μm thick were stained with hematoxylin and eosin. The area of the foci of infiltration in liver sections was determined by means of a special morphometric grid [1]. The number of leukocytes and the leukocyte formula were counted in blood from the retro-orbital sinus. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Foci of mononuclear infiltration (MI) were formed in the liver of mice of group 1, 2 days after injection of zymosan and their number and size increased up to a maximum on the 9th day, when they occupied about 32.8% of the total measured area of the section (Fig. 1a). Later the infiltration regressed, and after 2 months only isolated concentrations of mononuclear cells were visible. The number of monocytes in the blood and the degree of infiltration of the liver depended directly on the dose of zymosan (Table 1). In the mice of group 2, undergoing partial hepatectomy (PH) 2-4 h after injection of zymosan, the development of MI in the liver was sharply inhibited. In these mice, 2 days after PH, no foci of MI were present in the liver, whereas in control animals undergoing MH, they appeared at the same times as in intact animals (Table 2). The first signs of infiltration were observed on the 5th day and its intensity was many times lower than in the control. On the 9th day infiltration reached a maximum in the control, whereas in the experimental series it was only 50% of this value. The peak of infiltration in the experimental series occurred on the 15th day after injection of zymosan. Meanwhile regression of MI in the control took place more rapidly than in the experiment. In mice of group 3 the time for performance of PH was chosen on the following grounds. After 1 day disinhibition of the MP system takes place after injection of zymosan, but no foci of MI are able to form in the liver. After 5 days foci of MI are well defined, but have not yet reached their peak, which occurs on the 9th day. When PH was performed 1 day after injection of zymosan, just as in the previous series, the first foci of MI did not appear until the 5th day. If, however, PH was performed 5 days after stimulation by zymosan, there was not only delay, but actual regression of the already formed mononuclear foci (Fig. 2). For instance, whereas at the time of PH the foci of MI occupied about 25% of the total area of the section, 4 days after PH and, correspondingly, 9 days after injection of zymosan, only 6.33 ± 0,65% of the area of the section was occupied by infiltration, i.e., only about one-quarter as much. For comparison, in mice undergoing MH in this series of experiments, by the 9th day after stimulation with zymosan, infiltration increased and occupied 30-35% of

the area of the section. It was noted that PH did not abolish the monocytosis which is usually observed after stimulation with zymosan (Fig. 3). In other words, disinhibition of monocytosis took place but there was no fixation of fresh portions of blood monocytes in the liver tissue. The first suggestion which arose was that disturbance of monocyte "recruiting" in the liver could be connected with certain changes in the microenvironment in the intensively regenerating organ. However, it was found that after PH, infiltration induced by zymosan was inhibited not only in the liver itself, but also in the lung, where excessive proliferation of the parenchyma did not occur (Fig. 1b). In other words, initiation of the liver regeneration program was accompanied by depression of reactivity of resident macrophages, not only in the liver, but also in other organs with elements of the reticuloendothelial system (in particular, the lungs). This, in turn, created conditions impeding the development of inflammation during the period of intensive restoration of structural homeostasis, an important adaptive factor for the animal enabling it to recover from the emergency situation.

By contrast with PH, after injection of hydrocortisone not only was the induction of MI in the liver by zymosan inhibited, but so was monocytopoiesis. For instance, 2 and 5 days after injection of hydrocortisone the number of monocytes in the blood fell, whereas after PH, their number in zymosan-stimulated mice was significantly higher than in mice undergoing MH and stimulated by zymosan (Fig. 3). It is thus unlikely that inhibition of MI after PH in zymosan-stimulated mice was due to the effect of stress-induced mobilization of endogenous glucocorticoids. If this mechanism had played an essential role, depression of MI in the control in animals undergoing MH and in the experimental series in mice undergoing PH would have been about equal. Moreover, if the glucocorticoid concentration in zymosan-stimulated mice was increased artificially, the formation of MI in the liver was inhibited just as after PH. However, this was combined with a sharp decrease in the number of monocytes in the blood, whereas after PH the blood monocyte count of zymosan-stimulated mice was almost five times higher than in intact animals. This is evidence that imhibition of MI formation after resection of the liver and injection of hydrocortisone is effected by different mechanisms.

It can be postulated on the basis of these results that intensively proliferating epithelium, in this case regenerating hepatocytes, is the source of a factor (factors) which temporarily blocks reactivity of resident macrophages, and they lose their ability to perform the role of organizers of mononuclear infiltration. There is evidence in the literature that after major operations, leading to more intensive regeneration of epithelium, the functional activity of the macrophages is depressed. After partial hepatectomy, pneumonectomy, unilateral nephrectomy, or limb amputation, migration of macrophage precursors into the peritoneal cavity is inhibited after injection of phlogogenic irritants into it [10]. A similar effect also is observed during infiltrative growth of tumors *in vivo* [8, 9]. The molecular mechanisms of the effect of proliferating epithelium on such a universal reactive process as mononuclear infiltration of the stroma calls for special study.

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