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REGENERATION OF THE LIVER AT DIFFERENT PERIODS OF MONONUCLEAR INFILTRATION INDUCED BY ZYMOSAN GRANULES

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UDC 616.36-008.953.3-092.9-07:616.36-003.93

KEY WORDS: regeneration; mononuclear infiltration; liver; Kupffer cells.

After injection of 2 mg of zymosan granules (ZG) into mice they are initially ingested by Kupffer cells (KC), which induces the development of focal, and later of diffuse, areas of mononuclear infiltration in the liver [4]. Regression of mononuclear infiltration is not complete until at least 2 months after injection of ZG. The areas of infiltration contain liver macrophages with high acid phosphatase activity. The course of reparative regeneration of the liver depends essentially on the reactivity of KC [1, 2].

The aim of this investigation was to discover how the liver, with areas of mononuclear infiltration, developing in response to activation of KC by ZG, regenerates.

EXPERIMENTAL METHOD

Experiments were carried out on 250 male (CBA × C57BL)F_1 mice weighing 18-22 g. ZG were injected intravenously into the experimental animals in a dose of 2 mg per animal in 0.5 ml of 0.85% NaCl. In the control, 0.5 ml of 0.85% NaCl was injected. Partial resection of the liver (PRL) by the method in [6] was performed under ether anesthesia between 9 and 10 a.m., 1 and 5 days and 2 months (series I, II, and III of experiments respectively) after injection of ZG. The animals were killed 24, 30, 36, 42, 48, 54, 60, 66, 72, and 96 h and 11 and 16 days after the operation. An intraperitoneal injection of 1 μCi of ^{3}H-thymidine/g body weight (specific radioactivity 20 Ci/mmole) was given to the mice 1 h before sacrifice. Liver sections, stained with hematoxylin and eosin, were coated with liquid photographic emulsion (Photographic Chemical Research Institute project) and exposed in darkness at 4°C for 3 weeks. To determine the mitotic index (MI) of the hepatocytes (in %) 5000 hepatocytes were counted in liver sections stained with hematoxylin and eosin. To calculate the index of labeled nuclei (ILN) of the hepatocytes (in %) 3000 nuclei were counted on autoradiographs. The percentage regeneration capacity of the liver was determined by the equation [5]:

$$\text{Percentage regeneration capacity} = \frac{P_1 - P_2}{P_3} \times 100\%,$$

where P_1 is the weight of the liver at sacrifice, P_2 the weight of the residual liver after PRL, and P_3 the weight of the part of the liver removed during resection.

The numerical results were subjected to statistical analysis by Student's t test.

Laboratory of Pathophysiology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 288-290, March, 1985. Original article submitted July 13, 1984.

0007-4888/85/9903-0275$09.50

TABLE 1. ILN (in %) of Hepatocytes of Regenerating Liver after Injection of ZG 1 Day (experiment 1) and 5 Days (experiment 2) before PRL (M ± m)

Time after operation, h	Control	Expt. 1	Expt. 2
24	1,7±0,46	2,1±0,07	1,9±0,52
30	5,7±0,21	5,7±0,82	20,4±1,02**
36	31,5±1,13	35,1±1,72	3,8±0,24**
42	9,5±0,66	20,1±2,51**	12,4±1,56
48	7,2±0,15	11,1±0,98**	7,6±0,37
54	4,2±1,96	7,9±1,07	8,6±0,37*
60	6,3±0,77	13,7±0,83**	7,5±0,57
72	3,2±0,95	2,0±0,09	2,4±0,34
96	1,5±0,30	1,8±0,31	1,5±0,12

Legend. Here and in Table 2: in control 0.85% NaCl was injected 24 h before PRL; *P < 0.05, **P < 0.01 compared with control.

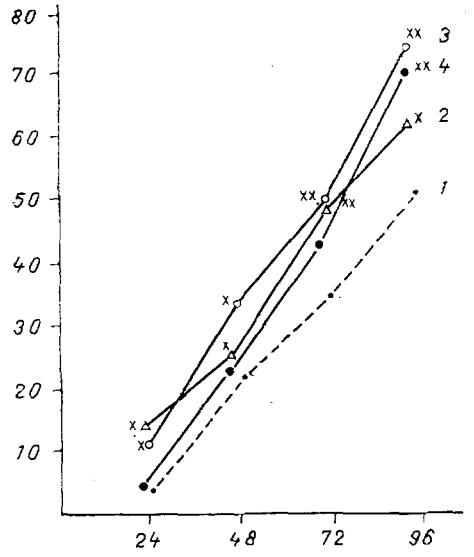

Fig. 1. Percentage of regeneration of the liver in control (1) and after injection of ZG 1 day (2), 5 days (3), and 2 months (4) before PRL. Abscissa, time after PRL (in h); ordinate, regeneration capacity, percent *P < 0.05, **P < 0.01.

EXPERIMENTAL RESULTS

In the experiments of series I resection of the liver was performed 24 h after injection of ZG, i.e., when the system of mononuclear phagocytes was disinhibited, but mononuclear infiltration was not yet present in the liver. After 24 h the percentage regeneration capacity of the liver was 3.8 times higher than in the control. In the later stages it gradually increased, and after 4 days it was almost 1.2 times higher than in the control (Fig. 1).

ILN of the hepatocytes in the control (Table 1) rose after 24-30 h, to reach a peak 36 h after PRL, after which it fell until 54 h. After 60 h a second peak of ILN was observed, although it was lower than the first, and after it ILN fell until 96 h. In the experimental series ILN of the hepatocytes, just as in the control, rose after 24-30 h and reached a peak after 36 h; this peak was higher than in the control. By 54 it had fallen, and after 60 h a second peak of ILN, twice as high as in the control, was observed.

MI in the control (Table 2) rose 30 h after PRL to reach a peak by 48 h. A second rise of MI was observed after 60 h, and this was followed by a gradual fall until 96 h. In the experimental group MI rose as early as 24 h after PRL, to reach a peak after 48 h. A second rise of MI was recorded after 60-72 h, and this was followed by a sharp fall in this parameter.

TABLE 2. MI (in %) of Hepatocytes of Regenerating Liver after Injection of ZG 1 Day (experiment 1) and 5 Days (experiment 2) before PRL (M ± m)

Time after operation h	Control	Expt. 1	Expt. 2
24	—	0,15±0,09	1,4±0,17
30	0,12±0,05	0,24±0,04	1,5±0,35**
36	0,87±0,29	1,29±0,29	3,6±0,25**
42	11,7±0,64	11,35±0,64	19,8±0,86**
48	14,9±0,54	18,0±1,60	14,4±1,58
54	3,2±0,43	5,9±0,70*	4,4±1,22
60	8,0±0,86	8,7±0,48	14,1±1,90*
72	5,15±0,42	9,2±0,88**	6,7±1,14
96	2,9±0,34	0,6±0,11**	1,8±0,49

TABLE 3. ILN (in %) and MI (in %) of Hepatocytes of Regenerating Liver after Injection of ZG 2 months before PRL (M ± m)

Time after operation, h	ICN		MI	
	control	expt.	control	expt.
24	0,7±0,15	0,9±0,16	—	—
30	15,7±1,81	21,6±3,95	—	—
36	17,3±0,90	35,0±3,76*	1,7±0,40	2,3±0,54
48	47,8±5,37	15,9±3,05*	6,2±0,95	18,7±2,9*
54	19,6±4,89	10,7±2,62	14,0±1,80	2,6±0,8*
60	9,5±1,4	21,7±2,40*	3,1±0,80	10,4±1,43*
66	15,3±2,1	9,7±1,49	7,4±0,50	15,5±1,76*
72	10,8±1,9	7,9±0,86	10,1±1,40	11,9±1,30
96	7,7±1,30	4,0±0,90	2,5±0,23	0,9±0,13*

Legend. In control, 0.85% NaCl was injected 2 months before PRL. *P < 0.01 compared with control.

In the experiments of series II PRL was performed 5 days after injection of ZG, when areas of infiltration occupied 20-25% of the area of the liver section. After 24 h the percentage regeneration of the liver was 3.5 times higher than in the control. Later this parameter increased, and after 4 days it was 1.5 times greater than the control value (Fig. 1).

ILN of the hepatocytes rose 24 h after PRL, reached a maximum quickly by 30 h, and then fell sharply until 36 h. A second peak of ILN was recorded after 54 h, and this was followed by a gradual decline until 96 h (Table 1).

MI in this series (Table 2) also was quite high 24 h after PRL, and 30 and 36 h thereafter it was 12.5 and 4.13 times higher respectively than the control level; it reached a peak after 42 h. A second peak of MI was recorded 60 h after PRL.

In the experiments of series III PRL was performed 2 months after injection of ZG, when mononuclear infiltration in the liver had virtually disappeared.

The percentage regeneration capacity 24-28 h after PRL differed only a little from the control value, and after 72-96 h it was higher than in the control (Fig. 1).

ILN after 24-30 h was a little higher than in the control and reached a peak after 36 h. This was followed by a decrease in this parameter until 48-54 h, and by 60 h a second peak, twice as high as in the control, was observed (Table 3).

MI reached a maximum after 48 h, compared with after 54 h in the control (Table 3). A second rise of MI was observed 66 h after PRL, and the number of mitoses was twice as high as in the control.

Proliferation of hepatocytes and regeneration of the liver were thus intensified most when PRL was performed at the peak of mononuclear infiltration of the liver, namely 5 days

after injection of ZG. It has not been established that macrophages control proliferation of lymphocytes [7], fibroblasts, smooth-muscle cells, endothelium of blood vessels [8], and hematopoietic stem cells [10]. Stimulation of KC of the liver by prodigiosan or ZG [3] or by *E. coli* endotoxin [9] stimulates hepatocyte proliferation in the intact liver. The data indicate that not only activated KC, but also areas of mononuclear infiltration potentiate hepatocyte regeneration.

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