FUNCTIONAL CHANGES AFFECTING THE MONONUCLEAR PHAGOCYTE SYSTEM IN EXPERIMENTAL CIRRHOSIS OF THE LIVER

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The development of cirrhosis of the liver is largely dependent on functional activity of organotypic macrophages and the mononuclear phagocyte system (MPS) as a whole [2]. There are indications in the literature of the depressed functional activity of the liver macrophages (Kupffer cells) in cirrhosis [1]. At the same time, we do not know how the functional activity and reactive properties of macrophages in other situations change in cirrhosis of the liver.

The aim of this investigation was to study combined functional changes in the hepatic, pulmonary, and other components of MPS in cirrhosis of the liver induced in rats by carbon tetrachloride (CCl_4).

EXPERIMENTAL METHOD

Experiments were carried out on 120 male Wistar rats weighing 180-200 g. The rats of group 1 were given an injection of vegetable oil through a gastric tube twice a week for 14 weeks in a dose of 0.25 ml/100 g body weight (control). The animals of group 2 received the oil, but 6 days before sacrifice they were given an intravenous injection of zymosan in a dose of 10 mg/100 g body weight, in the form of a 2% suspension in physiological saline. Cirrhosis of the liver was induced in the animals of group 3 by injecting a 10% solution of CCl_4 in vegetable oil by gastric tube in a dose of 0.25 ml/100 g body weight twice a week for 14 weeks. Cirrhosis of the liver was induced in the animals of group 4, but after 14 weeks and 6 days before sacrifice they were given an injection of zymosan.

The total ingestive activity of PSM was determined by studying clearance of colloidal carbon from the blood and expressed as "K indices" [6]. Bronchioalveolar washings (BAW) were obtained from the rats by lavage [4], and the absolute number of cells in them was counted by means of a Goryaev's chamber, and films were made. The films were stained by the Romanovsky–Giemsa method and the relative percentages of cells calculated and the absolute number of monocyte-macrophages counted. Peritoneal washings were obtained by the usual method [5] and the number of monocyte-macrophages in them was counted in the same way. To determine the absolute number of monocyte-macrophages in the spleen, squash preparations were made of the organ, after which a spleen cell suspension was obtained and the total number of cells counted in a Goryaev's chamber. Squash preparations of the spleen were stained by the Romanovsky–Giemsa method and the relative percentage of monocyte-macrophages per 500 splenocytes was counted. Ingestive activity of alveolar and peritoneal macrophages was determined in a monolayer by Vétvička's method [10]. Methacrylate granules (MG) 0.9 μ in diameter (produced by the Research Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences) were used as the object of phagocytosis. To obtain a monolayer the cells were sedimented for 1 h at 37°C in nutrient medium 199 with 10% bovine serum, and the resulting monolayer was washed 3 times and incubated with MG. Incubation was carried out for 1 h at 37°C in nutrient medium 199 with serum. MG was added at the rate of 100 particles per cell. The results were estimated under the microscope and the percentage of cells showing active phagocytosis was determined. Ability to generate O_2^- was judged from the results of the nitro-BT test. The monolayer of alveolar and peritoneal macrophages was incubated with

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TABLE 1. Parameters of Functional Activity of Different Parts of the Mononuclear cyte System (M ± m)

Group of animals	Absolute number of macrophages			Reduction of nitro-BT		Phagocytosis of methacrylate granules	
	BAW, 10^6/g weight of lungs	peritoneal, $\times 10^6$	splenic, $\times 10^8$	by alveolar macrophages, %	by peritoneal macrophages, %	by alveolar macrophages %	by peritoneal macrophages, %
1-	2,12±0,01	11,0±1,2	1,86±0,29	10,6±1,14	53,6±1,3	42,0±5,09	59,6±3,1
2-	3,08±0,02	18,0±1,9	9,51±1,31	18,68±1,14	61,1±2,7	48,4±6,3	67,3±2,9
3-	3,18±0,03*	11,8±1,5	3,16±0,31*	15,4±0,09*	32,6±3,9	50,75±5,5	56,2±4,1
4-	2,93±0,15	13,3±3,5	13,84±4,68	21,4±1,67	50,5±9,5	47,9±1,06	39,5±6.0

Legend. In all cases the number of animals was not less than 5. *$p < 0.05$ compared with control.

0.2% nitro-BT (Chemapol, Czechoslovakia) in Hanks' solution for 30 min at 37°C. The number of macrophages with diformazan deposits was then counted (in per cent). The number of cells phagocytosing carbon in the liver and lungs was determined in histological sections stained with hematoxylin and eosin. The area of the preparation occupied by zymosan-induced infiltration was estimated with the aid of an ocular attachment with a regular test grid (16 × 16), by counting the number of corners of the grid coinciding with the object.

EXPERIMENTAL RESULTS

The half-elimination time of colloidal carbon from the blood in the group of control animals was 11.8 ± 1.2 min. Macrophages of the liver, lungs, and spleen, but not peritoneal macrophages, took part in the ingestion of colloid.

In animals of the control group the potential reserves of MPS were found to be quite high. Under the influence of zymosan stimulation the rate of clearance of the blood from colloidal carbon was increased almost fourfold (the half-elimination time of carbon was reduced from 11.8 ± 1.2 to 2.9 ± 0.2 min). The number of phagocytic cells in the liver was increased by 10%, in the lungs by 48%, and in the spleen by 90% (Table 1). Under the influence of zymosan the macrophage populations increased in number: the number of monocyte-macrophages in the bronchoalveolar and peritoneal washings was increased by about 1.5 times, but in the spleen by more than 5 times (Table 1). The results of the nitro-BT test and ingestion of MG by alveolar and peritoneal macrophages also increased (Table 1). Zymosan stimulation of the macrophages led to the development of foci of mononuclear infiltration. In the liver foci of zymosan-induced infiltration occupied up to 10% of the area of the section, and 19% in the lungs. The clearing function of MPS in the animals of group 3 was sharply reduced and the rate of clearance of the blood from colloidal carbon fell by more than half (Fig. 1). The number of phagocytic macrophages in the liver was reduced by almost 75%. Simultaneously with functional insufficiency of the hepatic portion, activity of the extrahepatic regions of the MPS was intensified in cirrhosis. The number of carbon-loaded macrophages in lung sections was increased more than tenfold. The absolute number of macrophages in BAW was 1.5 times greater than in the control. Parameters of the nitro-BT test and ingestion of MG were increased by 1.5 and 1.1 times, respectively. There was also a tendency for the number of peritoneal macrophages to increase (Table 1). Similar changes developed in the splenic portion of MPS.

The response to zymosan was essentially transformed in cirrhosis of the liver. Clearance of the blood from colloidal carbon was virtually not increased. The ingestive activity of the hepatic macrophages, when depressed during cirrhosis, likewise did not increase as a result of the action of zymosan.

Zymosan-induced infiltration of the liver was reduced by more than 50 times. Zymosan did not increase the number of alveolar macrophages in the animals with cirrhosis; the ingestive function of the macrophages of the interstices of the lungs likewise was not increased. Zymosan-induced infiltration in the lungs virtually did not develop in cirrhosis. Phagocytosis of MG by macrophages of BAW and peritoneal washings not only was not increased by zymosan, but was actually depressed. In the spleen, the percentage of macrophages was increased in response to zymosan by only 1.14 times, whereas the number of macrophages which

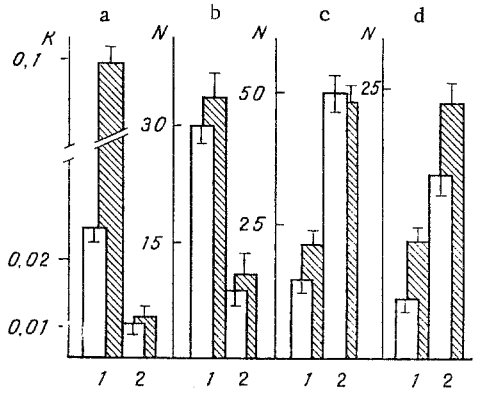

Fig. 1. Ingestion of colloidal carbon by macrophages of the liver, lungs, and spleen and total ingestive activity of MPS in cirrhosis of the liver. Unshaded columns — without zymosan stimulation; shaded columns — with zymosan stimulation; a) clearance from colloidal carbon (K denotes "K indices"); b) number of macrophages in liver, c) the same in lungs, in one field of vision (N); d) number of macrophages loaded with carbon, per 500 splenocytes (N).

had ingested colloidal carbon was increased by 1.5 times (compared with 1.7 and 1.9 times respectively in the control). The response of peritoneal macrophages to zymosan also was depressed (Fig. 1; Table 1).

Thus in cirrhosis of the liver the phagocytic activity of the hepatic macrophages and the clearing function of MPS are depressed. In response to injection of zymosan the ingestive activity of the hepatic macrophages virtually did not increase in cirrhosis, but zymosan-induced infiltration developed in minimal volume. In the lungs and the other extrahepatic portions of MPS, the number and functional activity of the macrophages increased during cirrhosis of the liver. Additional stimulation of the extrahepatic macrophages was not accompanied by the increase in their activity that was observed in the control animals.

Liver macrophages (Kupffer cells) are known to be a filter in the path of endotoxins of the intestinal microflora, carried by the portal blood. Under physiological conditions they retain the endotoxin virtually entirely [7]. In chronic hepatitis and cirrhosis the ingestive and endotoxin-detoxicating function of the Kupffer cells is sharply depressed and systemic endotoxinemia develops [8]. Activation of the extrahepatic components of MPS can evidently be explained by the stimulating action of endotoxins which pass freely through the damaged filter. A similar situation also arises in other versions of insufficiency of the hepatic component of MPS, such as after partial resection of the liver [9].

In the modern view macrophages are important regulators of fibroblast functions [3]. In response to stimulation they secrete collagenase, which splits performed collagen, thereby preventing it from accumulating in the zone of inflammation. The sharp decrease in reactivity of the hepatic macrophages which we found may be responsible for the insufficiency of collagenolysis and the transition of the inflammatory process in the liver into a phase of irreversible cirrhotic changes.

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