Functional State of Pulmonary Macrophages after Partial Hepatectomy in Mice

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Partial hepatectomy leads to qualitative and quantitative changes in the pulmonary compartment of the mononuclear phagocyte system during different periods after surgery. These changes have compensatory and adaptive nature because of loss and subsequent partial recovery of the hepatic compartment of the mononuclear phagocyte system.

Key Words: *mononuclear phagocyte system; pulmonary and hepatic compartments; partial hepatectomy*

Differentiation of the mononuclear phagocyte system (MPS) from the reticuloendothelial system [11] stimulated studies of the functional relations between its central and peripheral compartments [10]. The nature of these relations between the peripheral compartments of MPS and the mechanisms initiating and maintaining them are less studied. Partial hepatectomy (PHE) model can be used for the studies of this problem, because the liver harbors the largest MPS compartment. It is hypothesized that reduction of hepatocyte count without their destruction and, proportional decrease in the number of Kupffer cell after PHE create a potent stimulus modulating the status of the entire MPS without creating a chemattractant gradient in it by the degradation products, similar to that observed in toxic damage to the liver [3]. The development of reparative regeneration in the liver parenchyma (a process compensating for the count and functions of lost cells of this peripheral MPS compartment) after PHE is presumably paralleled by changes in another large peripheral (*e.g.*, lung) compartment, in which phagocyte status is not modulated by hepatocyte degradation products under conditions of PHE.

We studied possible functional relations between the peripheral (liver and lungs) components of MPS during reparative regeneration of the liver after PHE.

MATERIALS AND METHODS

The study was carried out on 63 male (CBA×C57Bl/6)F_1 mice aged 2 months (20-22 g) from Breeding Center of Vector Company (Novosibirsk). Partial hepatectomy was carried out by excising the middle and left lobes [7] under ether narcosis between 9.00 and 11.00 a.m. [2]. Group 1 were intact animals, group 2 were sham-operated (SO), and group 3 were animals subjected to PHE.

Pulmonary phagocytes were examined 24 and 36 h, 3 and 5 days after PHE. Phagocytic cells in the bronchoalveolar lavage fluid were detected as described previously [8], after centrifugation, the cells in specimens were counted in a Goryaev chamber.

The MPS cell clearance function was studied by a previously described method [6]: the animals were injected with 0.1 ml colloid charcoal (Gunter Wagner) in the lateral caudal veins, after which phagocytes containing charcoal were counted on

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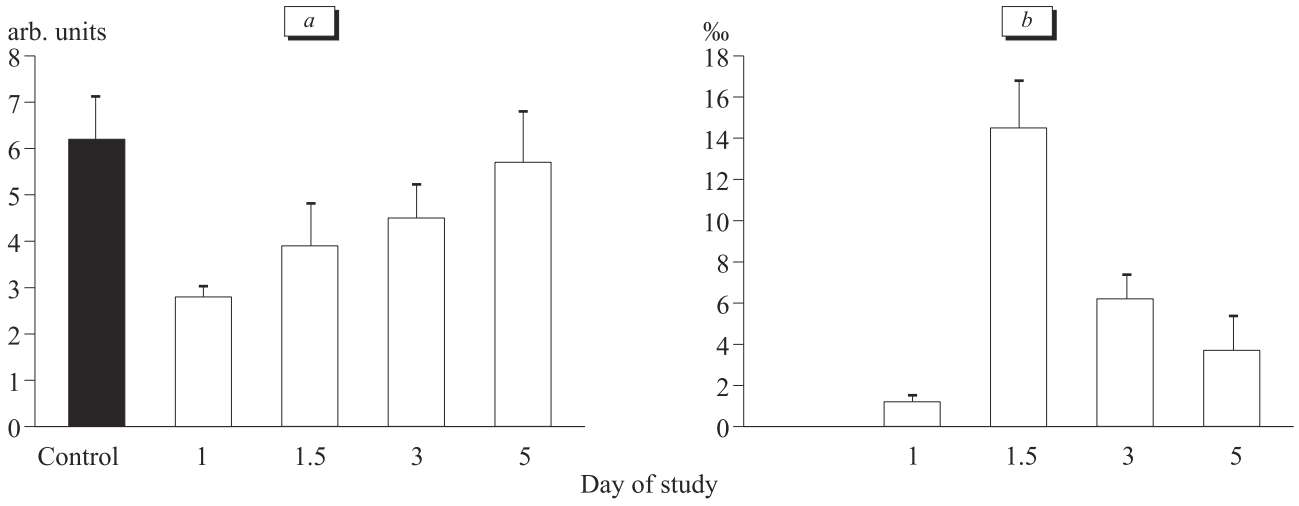

Fig. 1. Liver weight (*a*) and mitotic index of hepatocytes (*b*) over the course of reparative regeneration of the liver.

histological sections (per μ^2) of the lungs prepared by the common methods [1].

The oxidative metabolic capacity of phagocytes was studied by the chemiluminescent method [9] using SKIF-306M biochemiluminometer (Nauka). Serum concentration of endotoxin (*E. coli* lipopolysaccharide) was measured using Limulus Amebocyte Lysate test kit (Associates of Cape Cod Inc.).

Mitotic cells were counted at magnification 1000 in liver sections (in 3000 cells) stained with hematoxylin and eosin. The relative weight of the liver was evaluated using the hepatorenal index [5].

The probability of differences between the means was evaluated using Student's *t* test; the differences were considered significant at $p<0.05$.

RESULTS

The dynamics of hepatocyte division after PHE somewhat differed from the dynamics of liver weight (Fig. 1). Presumably, liver weight increased on days 3 and 5 after PHE, despite the decrease in mitotic activity in the liver parenchyma, was realized through the regeneration hypertrophy mechanism.

The clearance function of MPS sharply decreased (Fig. 2) and serum level of endotoxin increased in SO animals during the first 24 h (Fig. 3), which was presumably a reaction to intervention. Later, these parameters normalized, which presumably reflected urgent adaptive activation of MPS [3], mainly at the expense of its hepatic compartment (Kupffer cells) and hepatic sinusoidal endothelial cells characterized by high capacity to adsorption pinocytosis by which colloid charcoal was captured [4]. Unchanged macrophage count in the bronchoalveolar lavage fluid (Table 1), count of active phagocytic macrophages in the lungs (Table 2), and chemiluminescent response of their macrophages (Fig. 4) during this and subsequent periods after SO indicate that pulmonary phagocytic cells in SO animals are not involved in this process, presumably due to high reserve potential primarily of the hepatic compartment of MPS.

Decrease of liver weight by $^2/_3$ and proportional decrease in the number of Kupffer cells and sinusoidal endothelial cells after PHE impaired clearance of colloid charcoal (Fig. 2) and increased endotoxin content (Fig. 3) 24 and 36 h after surgery. Despite activation of the reparative processes in the liver parenchyma, mitotic activity (Fig. 1, *a*) and liver weight (Fig. 1, *b*) were not maximum, especially on day 1. But the highest concentrations of actively phagocytosing cells in the lungs were observed during these periods (Table 2). The peak of macrophage concentration in the bronchoalveolar lavage fluid after PHE was somewhat delayed (Ta-

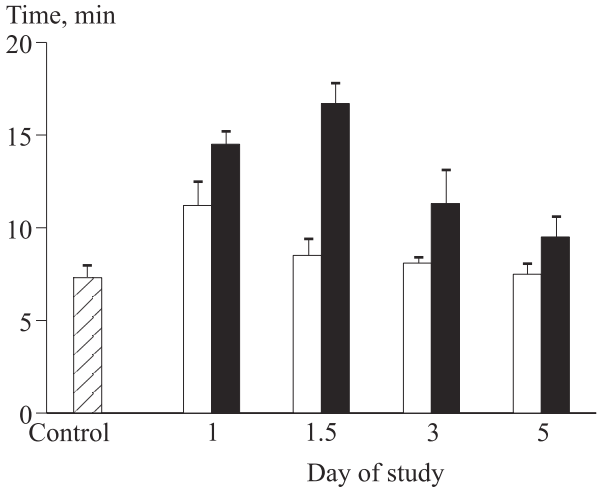

Fig. 2. Colloid charcoal half-life in circulating blood of intact controls, SO mice (light bars), and mice subjected to PHE (dark bars).

TABLE 1. Total Counts of Cells, Counts of Monocytes/Macrophages and Neutrophils in Bronchoalveolar Lavage Fluid after PHE (*M*±*m*)

Day after operation	Group	Number of phagocytic cells, $\times 10^6$/g lung weight	
		monocytes/macrophages	neutrophils
0	Control (*n*=7)	0.13±0.04	0.0040±0.0009
1	SO (*n*=5)	0.15±0.02	0.010±0.002*
	PHE (*n*=6)	0.18±0.05	0.016±0.001*
1.5	SO (*n*=5)	0.16±0.01	0.008±0.001*
	PHE (*n*=6)	0.49±0.03*+	0.020±0.003*+
3	SO (*n*=5)	0.18±0.03	0.007±0.001
	PHE (*n*=6)	0.35±0.02*+	0.013±0.003*
5	SO (*n*=5)	0.16±0.01	0.0060±0.0007
	PHE (*n*=6)	0.23±0.04*	0.0070±0.0008

Note. Here and in Table 2: $p<0.05$ *vs.* *control, +SO.

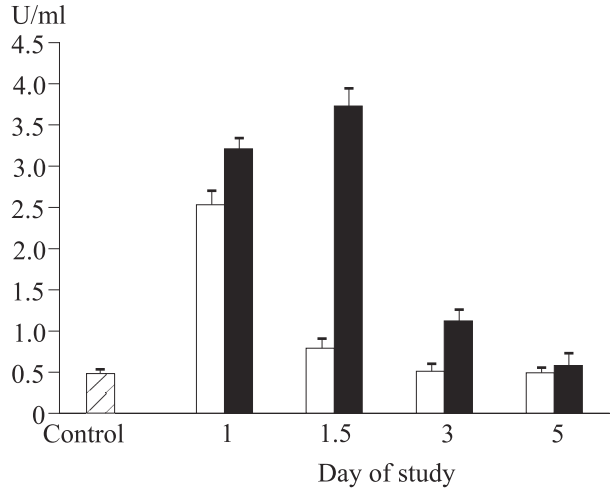

Fig. 3. Serum endotoxin level in (CBA×C57BL/6)F_1 mice after SO (light bars) and PHE (dark bars).

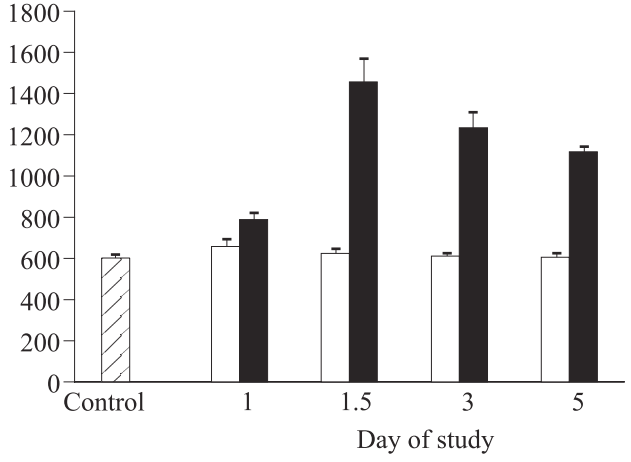

Fig. 4. Summary chemiluminescent response of pulmonary phagocytic cells over the course of reparative regeneration of the liver after SO (light bars) and PHE (dark bars).

ble 1), which was logical because they were involved in the realization of clearance processes in the pulmonary microcirculation.

With intensification of reparative processes in the liver parenchyma and recovery of the populations and endocytosis activities of Kupffer cells and sinusoidal endothelial cells, the concentration of endotoxin decreased and clearance of colloid charcoal was accelerated (Figs. 2, 3). Presumably, the increase in neutrophil and macrophage release into the bronchoalveolar space was adaptive and nonspecific, because the reduction of the MPS clearance potency at the expense of its intraorgan and strongest (hepatic) compartment required more intensive work, of particular the compartment performing the barrier functions directly at the interface with the environment (in alveoles and bronchi).

It seems that the lysosomotropism and impossibility of biodegradation of colloid charcoal, presumably re-captured by phagocytes after death of some cells initially phagocytosing it were the leading factors of somewhat different kinetics of its elimination in comparison with the endotoxin (Fig. 2, 3). Presumably, the same factors were responsible for longer increase in functional activity of pulmonary phagocytic cells, because reduction of charcoal clearance from the blood correlated by its dynamics with the reduction of their summary chemiluminescent response (Fig. 4) and phagocytic activity of pulmonary interstitial macrophages (Table 2), but not with the dynamics of reparative regeneration in the liver (Fig. 1), because its macrophages could also be loaded with colloid charcoal. This led to the above changes in the pulmonary compartment of MPS.

TABLE 2. Absorption Activity of Pulmonary Interstitial Macrophages after PHE ($M \pm m$)

Day after operation	Group	Count of active pulmonary phagocytic macrophages per mm^2 lung tissue
0	Control (n=7)	158.3±27.4
1	SO (n=5)	172.5±14.5
	PHE (n=6)	325.6±33.7*+
1.5	SO (n=5)	163.3±9.7
	PHE (n=6)	478.6±43.1*+
3	SO (n=5)	165.4±8.5
	PHE (n=6)	213.7±55.2
5	SO (n=5)	161.5±9.5
	PHE (n=6)	183.5±29.5

Increased count and functional activity of the pulmonary compartment of MPS during the first two periods of observation after PHE were presumably caused by activation of pulmonary resident macrophages because of increased load with endogenous endotoxin and, presumably, mobilization of monocytes from the bone marrow, which was experimentally realized by increased capture of exogenous endotoxin and colloid charcoal. These parameters of the MPS pulmonary compartment decreased with intensification of reparative processes in the liver. However, the methodological approaches used in the study failed to provide evidence indicating that the increase in the counts of actively phagocytosing interstitial and bronchoalveolar fluid macrophages and the clearance function of MPS towards exogenous endotoxin and charcoal were realized also due to an increase in the cell count in this compartment, and not just functional activity of cells.

Hence, the findings indicate an obvious relationship between the two peripheral compartments of the MPS, which is compensatory and adaptive in the MPS pulmonary compartment during loss and then recovery of the counts of hepatic compartment of the MPS and endocytosing endothelial cells of hepatic sinusoids.

REFERENCES

1. O. V. Volkova and Yu. K. Eletskii, *Fundamentals of Histology and Histological Methods* [in Russian], Moscow (1971).
2. L. D. Liozner, *Regeneration and Development* [in Russian], Moscow (1982).
3. V. A. Shkurupiy, *Byull. Eksp. Biol. Med.*, **99**, No. 6, 754-756 (1985).
4. V. A. Shkurupiy, Yu. N. Kurunov, and N. N. Yakovchenko, *Lysosomotropism is a Problem of Cell Physiology and Medicine* [in Russian], Novosibirsk (1999).
5. V. I. Shcherbakov, T. G. Komlyagina, and D. N. Mayanskii, *Byull. Eksp. Biol. Med.*, **99**, No. 3, 288-290 (1985).
6. B. Benaceraff, G. Biozzi, and B. Halpern, *Physiopathology of RES*, New York (1957), P. 52-79.
7. G. Higgins and R. Anderson, *Arch. Pathol.*, **12**, 186-202 (1931).
8. Q. N. Myrvik, E. S. Leake, and B. Farris, *J. Immunol.*, **86**, 128-132 (1961).
9. T. Tono-oka, N. Ueno, and T. Matsumoto, *Clin. Immunol. Immunopathol.*, **26**, No. 1, 66-75 (1983).
10. R. Van Furth, *Inflammation*, **6**, No. 1, 39-53 (1982).
11. R. Van Furth and Z. A. Cohn, *J. Exp. Med.*, **128**, 415-435 (1968).