Phlogogenic and Hemopoiesis-Stimulating Activities of Liver and Lung Macrophages Upon Liver Regeneration

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The phagocytizing and colony-stimulating activities of liver macrophages are increased 36 h after partial hepatectomy. Although the blood count of phagocytizing cells is increased, the rate of the formation of zymosan-induced granulomas in the liver and lungs is decreased. Administration of zymosan after partial hepatectomy inhibits granulomonocytopoiesis in the bone marrow and reduces colony-stimulating activity of Kupffer cells and phagocytizing activity of liver and lung macrophages.

Key Words: *liver regeneration; granulomatous inflammation; granulomonocytopoiesis; phagocyte; zymosan*

Inflammatory infiltration of the liver is markedly reduced against the background of intense proliferation of hepatocytes [4]. This may be associated with modulations of the activity of liver macrophages (Kupffer cells) that, on the one hand, are the key effectors of inflammation [3] and, on the other, are involved in the regulation of reparative regeneration of the liver [11]. In order to elucidate the phagocytosis-related mechanisms responsible for anti-inflammatory activity of the regenerating liver we studied phlogogenic (inflammatory) reactions of Kupffer cells. Bearing in mind a strong association between the liver and the lungs through the "macrophagal channel" [14], we assessed the intensity of inflammatory infiltration of the lungs and the phlogogenic reactions of lung phagocytes in regenerating liver.

MATERIALS AND METHODS

Experiments were performed on eighty (CBA×C57Bl/6) F_1 male mice weighing 20-25 g. A suspension of zymosan granules (ZG) in 0.85% NaCl (0.25 ml) was injected intravenously in a dose of 100 mg/kg body weight (2 mg/mouse) to stimulate phagocytes and induce granulomatous inflammation. Prior to injection of ZG, the mice were divided into 3 groups: intact (group 1), sham-operated (SO, group 2), and partially hepatectomized (PHE, group 3). Hepatectomy (2/3 of the liver was excised) was performed as described elsewhere [12]. Groups 1 and 2 served as the control. Zymosan granules were injected 36 h after surgery, when the rate of DNA synthesis in hepatocytes reached the maximum [2]. The reaction of phagocytes was estimated before and on day 2 and 5 after injection of ZG.

The ability of mononuclear phagocytes to eliminate foreign material (colloid charcoal) from the circulation was assessed as described [6]. The count of phagocytizing macrophages and the intensity of internalization of foreign particles were determined using histological preparations of hepatic and pulmonary tissues. The animals were given an intravenous injection of charcoal suspension (Gunter Wagner, 0.1 ml, 2 mg/kg) 1 h prior to sacrifice. The intensity of inflammatory infiltration of the liver and lungs was evaluated from the number of granulomas per square millimeter of tissue and the mean area of one granuloma.

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Proinflammatory activity of Kupffer cells was assessed by the ability of their cytosolic extracts to stimulate the growth of granulocyte-monocyte colonies in a cell culture initiated from the bone marrow of intact mice (colony-stimulating activity, CSA).

The content of precursor stem cells giving rise to granulocytes and macrophages (colony-forming units granulocyte-monocyte) was determined by the number of granulocyte and monocyte colonies grown in a culture of bone marrow cells [1].

The neutrophil and macrophage counts in bronchoalveolar lavage were determined after measuring the total cell content, centrifugation, and staining by the method of Romanowsky—Giemsa. For determination of the total and differential leukocyte counts blood was collected from the retro-orbital sinus.

The results were analyzed using conventional statistical methods and Student's *t* test.

RESULTS

Prior to administration of ZG, the number of charcoal-loaded Kupffer cells per mm² of lung and liver tissue was the same in PHE and intact mice. However, the phagocytizing activity of Kupffer cells in PHE mice was 1.6- and 1.3-fold higher than in intact and SO mice, respectively. In the lungs of PHE mice, the number of interstitial macrophages was 3- and 7-fold as high as that in intact and SO mice, respectively. The phagocytizing activity of these cells was similar in all groups (Table 1). The clearance rate was slightly lower in PHE mice.

Prior to injection of ZG, CSA of Kupffer cells from PHE mice was 7- and 3-fold as high as that in intact and SO mice (Fig. 1). Irrespective of the high CSA activity, the amount of colony-forming units of granulocyte-monocyte (CFU-GM) in the bone marrow of PHE mice was 1.4-fold lower than in SO animals (Fig. 2). The total count of circulating leukocytes, including monocytes and neutrophils, in PHE mice did not differ from that in the control.

Five days after intravenous administration of ZG, phagocytizing activity of Kupffer cells of intact and SO mice increased 2- and 1.7-fold compared with the original level. However, in PHE mice this parameter remained virtually the same (Table 1).

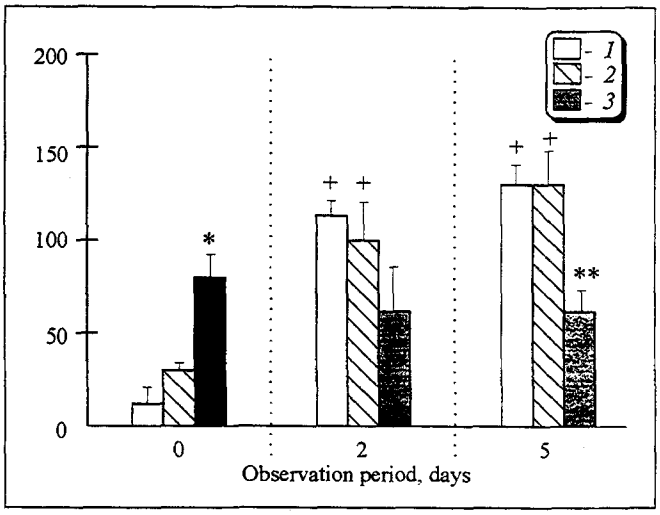

Fig. 1. Colony-stimulating activity of Kupffer cell extracts before and after administration of zymosan to intact (*1*), sham-operated (*2*), and partially hepatectomized (*3*) mice. Here and in Fig. 2: ordinate: number of colony-stimulating units per 10^5 bone marrow cells. *$p<0.05$, **$p<0.01$ compared with *1* and *2*; +$p<0.05$ compared with that prior to administration of zymosan.

TABLE 1. Phagocytizing Activity of Hepatic and Pulmonary Tissues Before and After Intravenous Administration of ZG to Intact, Sham-Operated (SO), and Partially Hepatectomized (PHE) Mice (*M±m*)

Days after administration of ZG	Mice	Liver		Lungs	
		number of charcoal-loaded Kupffer cells/mm² tissue	phagocytizing activity of a Kupffer cell, arb. units	number of charcoal-loaded interstitial macrophages/mm² tissue	phagocytizing activity of an interstitial macrophage, arb. units
0	Intact (8)	707.7±61.5	22±1	162.8±32.9	670±0.6
	SO (5)	492.3±61.5	27±2	81.2±11.7	6.0±1.0
	PHE (5)	615.4±61.5	36±2**	540.9±139.4**	7.0±0.8
2	Intact (5)	215.4±21.5*	32±2*	884.3±88.6*	8.0±0.4
	SO (5)	200.6±27.7*	30±2	436.3±82.2*	5.8±0.8
	PHE (5)	224.6±27.7*	35±4	350.8±76.9	4.4±1.6
5	Intact (5)	477.0±67.6	46±3*	443.1±70.8*	12.8±2.8*
	SO (5)	480.0±64.6	40±2*	556.3±135.4*	14.0±2.6*
	PHE (5)	670.8±55.4	42±3	717.6±137.5	5.6±0.4

Note. Number of animals is given in parenthesis. $p<0.05$: *compared with that before administration of ZG, **compared with intact and SO mice.

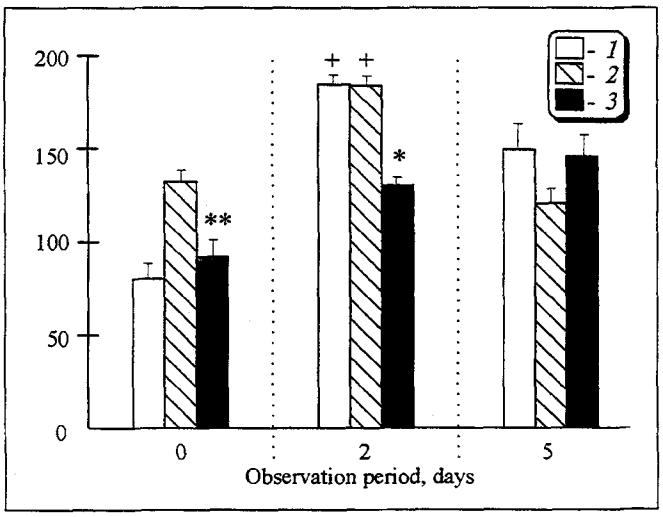

Fig. 2. The amount of CFU-GM in the bone marrow before and after administration of zymosan to intact (*1*), sham-operated (*2*), and partially hepatectomized (*3*) mice. $p<0.01$: *compared with *1* and *2*, **compared with *2*, +compared with that prior to administration of zymosan.

Zymosan stimulated the phagocytizing activity of lung macrophages in intact and SO mice: 2- and 2.3-fold, respectively, compared with the original level, without changes in it in PHE mice. Five days after administration of ZG, the amount of phagocytizing interstitial macrophages in intact and SO mice increased 2.7- and 6.7-fold compared with the original level. In PHE mice, the amount of phagocytizing macrophages in the lungs remained practically unchanged (Table 1).

Administration of ZG induced focal granulomatous inflammation in the liver of intact mice. Two days after administration of ZG, intact mice developed 5-12 granulomas/mm^2 hepatic tissue, the mean area of a granuloma being $(0.9\pm0.1)\times10^{-3}$ mm^2. On day 6 after administration of ZG, the number and the area of granulomas increased 3.4- and 2.7-fold, respectively, compared with those on day 2. Similar changes in the number and area of granulomas were observed in SO mice (Table 2).

On day 3 after ZG administration, the number of granulomas per mm^2 hepatic tissue in PHE mice was 2.7- and 2.6-fold lower than in intact and SO mice, respectively. On day 5, the number of granulomas remained practically unchanged, being 6.1- and 6.5-fold lower than in intact and SO mice. There were no differences in the mean area of granulomas in PHE and intact mice (Table 2).

Administration of ZG induced granulomas not only in the liver but also in the lungs. In PHE rats, granulomatous inflammation was inhibited both in the liver and in the lungs. Five days after ZG administration, their number decreased 9.7- and 5.0-fold compared with intact and SO rats. However, the mean size of pulmonary granulomas did not differ in PHE and intact rats (Table 2).

Zymosan-induced granulomatous inflammation in lungs was accompanied by accumulation of phagocytes in the respiratory tract. On day 2 after administration of ZG, the total cell content in bronchoalveolar lavage was 5-fold as high as the basal content. This was associated predominantly with an increase in the number of macrophages. Administration of ZG caused no increase in the cell content of bronchoalveolar lavage. Moreover, on day 6, this parameter was 1.4-fold lower than that in SO mice (Table 3).

On day 6 after administration of ZG, the total blood leukocyte count in PHE mice was 2-fold higher

TABLE 2. Inflammatory Infiltration of Lungs and Liver After Intravenous Administration of ZG to Intact, Sham-Operated (SO), and Partially Hepatectomized (PHE) Mice (n=5)

Days after administration of ZG	Mice	Liver		Lungs	
		number of granulomas/mm^2 tissue	area of a granuloma, $\times10^{-3}$ mm^2	number of granulomas/mm^2 tissue	area of a granuloma, $\times10^{-3}$ mm^2
2	Intact	9.0±1.6	0.9±0.1	24±5	1.7±0.2
	SO	8.5±1.5	0.8±0.2	6±2	1.9±0.2
	PHE	3.3±1.0**	0.6±0.2	13±2	1.5±0.3
5	Intact	30.5±2.5*	2.3±0.4*	58±7*	1.4±0.2
	SO	32.5±5.5*	2.2±0.3*	31±6*	1.4±0.1
	PHE	5.0±1.5**	1.7±0.4	6±2**	1.0±0.3
14	Intact	25.9±2.7	1.8±0.1	17±6	1.5±0.3
	SO	11.9±2.6	1.2±0.2	10±3	1.7±0.2
	PHE	17.1±3.1	1.5±0.1	9±2	1.0±0.1

Note. $p<0.05$: *compared with that 2 days after administration of ZG; **compared with intact and SO mice.

TABLE 3. Cell Content in Bronchoalveolar Lavage Before and After Intravenous Administration of ZG to Intact, Sham-Operated (SO), and Partially Hepatectomized (PHE) Mice

Days after administration of ZG	Mice	Total cell count	Alveolar	
			macrophages	neutrophils
		$\times 10^6$ cells/g tissue		
0	Intact (8)	0.18±0.04	0.16±0.04	0.008±0.002
	SO (10)	0.54±0.10	0.48±0.10	0.017±0.008
	PHE (10)	0.46±0.04**	0.42±0.04**	0.010±0.004
1	Intact (5)	0.84±0.19*	0.64±0.20*	0.066±0.030
	SO (5)	0.54±0.14	0.48±0.14	0.032±0.008
	PHE (5)	0.33±0.04	0.32±0.01	0.018±0.007
2	Intact (5)	0.54±0.23	0.31±0.08	0.167±0.060*
	SO (5)	0.24±0.05	0.16±0.02	0.063±0.019
	PHE (5)	0.28±0.03	0.21±0.03	0.044±0.014
5	Intact (5)	0.18±0.03	0.14±0.02	0.012±0.003
	SO (5)	0.38±0.03	0.32±0.04	0.023±0.005
	PHE (5)	0.27±0.03***	0.20±0.02***	0.012±0.005

Note. $p<0.05$: *compared with that before administration of ZG; **compared with intact mice; ***compared with SO mice.

than that in SO mice: $7.7\pm1.2\times10^9$ *vs.* $3.9\pm0.5\times10^9$ cells/liter, $p<0.05$. This was due to increased neutrophil count: $5.76\pm1.06\times10^9$ *vs.* $2.37\pm0.24\times10^9$ cells/liter. The monocyte count did not differ from that in the control.

On day 3 after administration of ZG, the amount of CFU-GM in the bone marrow of intact mice increased 2.2-fold, while in PHE mice it increased only 1.4-fold, the increase being significantly lower than in the control (Fig. 2).

By the 5th day after administration of ZG, CSA of extracts prepared from Kupffer cells of intact mice increased 11-fold compared with basal CSA. A similar tendency was observed in SO mice. However, CSA of Kupffer cells from PHE mice did not change after administration of ZG, on day 5 being 2-fold lower than in the control (Fig. 1).

Thus, immediately after PHE (upon intense proliferation of hepatocytes), phagocytizing activity and CSA of resident liver macrophages increase. At the same time, the number of phagocytizing macrophages in pulmonary interstitium increases, partially compensating for the deficiency of liver monocyte-phagocytes and proving an adequate elimination of foreign materials from the circulation of PHE mice. Although CSA of Kupffer cells increases after PHE, the CFU-GM content in the bone marrow declines.

An increase in phagocytizing activity of Kupffer cells and interstitial macrophages in the lungs after PHE coincided with suppression of ZG-induced granulomatous inflammation in the liver and lungs. The count of circulating phagocytes (precursors of the inflammatory infiltrate cells) remains high, implying that phagocyte migration to the focus of inflammation is inhibited. The phagocytizing activity of liver and lung macrophages did not increase after administration of ZG. Zymosan induced no changes in CSA of Kupffer cells. Colony-stimulating activity is an integral parameter of the phlogogenic activity of these cells mediated by IL-1 and IL-6, which not only determine phlogogenic gradient *in situ*, but also regulate granulomonocytopoiesis in the bone marrow. The fact that zymosan did not stimulate CSA of Kupffer cells is consistent with a weak response of CFU-GM to ZG in PHE mice.

Suppression of inflammation in the lungs against the background of active proliferation of hepatocytes was observed not only after intravenous administration of ZG, but also after instillation of zymosan in the trachea [5].

From the published data it can be concluded that specific function of interstitial macrophages in the lungs and Kupffer cells in regenerating liver, inhibition of phagocyte migration to the foci of inflammation, and a decrease in the bone marrow granulomonocytopoiesis are associated with alterations developing after hepatectomy, including the production of transforming growth factor-β which exhibits an anti-inflammatory activity [15]. Liver proteins with immunosuppressor activity (α-fetoprotein, etc.) [13], the production of which increases after PHE, may act as immunosuppressors. It should be noted that the production of endotoxin increases considerably within the first 6 h after PHE [9]. This

endotoxin is a nonhepatic factor mediating the tolerance of phagocytes to a phlogogenic stimulus upon reparative regeneration of the liver. The endotoxin stimulates macrophagal production of IL-1 and IL-6 [7] that enhance the synthesis of the acute phase proteins in hepatocytes. The endotoxin also elevates blood concentration of glucose [10] and stimulates macrophagal production of prostaglandin E_2 [8]. This may inhibit phlogogenic and hemopoiesis-stimulating activities of lung and liver macrophages and suppress granulomatous inflammation in regenerating liver.

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p-Aminobenzoic Acid as a Stimulator of Angiogenesis

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p-Aminobenzoic acid (0.002 and 0.005% in a volume of 0.1 ml) stimulates angiogenesis in the yolk sac of chick embryos. It is more effective in a concentration of 0.005%, significantly increasing the length and branching of large vessels (by 27-33% and 35-43%, respectively) in comparison with the control (injection of 0.1 ml normal saline).

Key Words: *p-aminobenzoic acid; angiogenesis; yolk sac; chick embryos*

Angiogenesis is a reactive process occurring not only during embryogenesis but also in adult organism. It is triggered under various conditions by different pathophysiological factors. Angiogenesis has been regarded as a positive (for example, wound healing) or negative process (carcinogenesis, tissue ischemia, etc.). Modulation of angiogenesis with the use of stimulators and inhibitors may be helpful in the correction of various pathologies. In was reported that neovascularization is stimulated by heparin and copper-containing substances [8] and inhibited by protamine [10], cartilaginous extracts [9], emoxipine [4], α-interferon, etc.

We attempted to modulate angiogenesis with p-aminobenzoic acid (PABA). This substance stimulates phenotypic traits in animals [2] and plants [7]. Of special interest is the ability of PABA to induce selective modifications during ontogenesis; for example, it stimulates the development of the external segments of retinal photoreceptor cells [6] and promotes proliferation of stromal cells during healing of corneal wounds [5]. In the present

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