Reactions of Pulmonary Phagocytes Following Partial Hepatectomy

I. V. Plyushch, D. D. Tsyrendorzhiev, and D. N. Mayanskii

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Zymosan administration into the trachea of rats led to intensified infiltration of the lungs by macrophages which migrated to alveolar and bronchial cavities, and the inflow of these cells was accompanied by their increased chemiluminescence and by elevation in acid phosphatase and cathepsin D activities. After partial hepatectomy, the zymosan-induced inflow of cells into the airways was much less pronounced, and no enzyme activation was observed, but the chemiluminescence of airway cells remained high.

Key Words: *pulmonary macrophage; neutrophil; phagocyte; hepatectomy; zymosan*

The liver plays an important part in the regulation of specific immunity [1,4]. It synthesizes proteins inhibiting the immune response, including α-fetoprotein [13], L-arginase [8], and a number of others [15]. These factors suppress various humoral and cell-mediated reactions, in particular those of transplantation immunity such as the graft-versus-host reaction and rejection of allogeneic heart, kidney, or skin [6]. Immunosuppression is more pronounced when the liver is undergoing reparative regeneration, in which case it is likely to be associated with Kupffer's cells given that at particular periods of liver regeneration these cells begin producing prostaglandin E_2 - a universal inhibitor of immune reactions [8].

The regulatory activity of the liver extends to the effector phase of immunity manifested in an inflammatory response. It has been shown that inflammatory infiltration is inhibited during reparative regeneration of the liver. Since infiltration depends on the reactivity of phagocytes, which are effectors of inflammation, we deemed it useful to compare the reactions of pulmonary phagocytes to a phlogogenic (inflammatory) stimulus in normal, sham-operated, and partially hepatectomized (PHE) rats.

Laboratory of Pathological Physiology and Human Ecology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. (Presented by V. P. Kaznacheev, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

The study was conducted on 50 female Wistar rats (body weight 180-200 g) obtained from the nursary of Vektor Company (Novosibirsk). Partial hepatectomy with removal of 2/3 of the liver was performed as described by Higgins and Anderson [12]. Intact and sham-operated rats served as controls. The 50 rats were divided into four groups. Group 1 was administered 0.5 ml of 0.85% NaCl intratracheally and group 2, 0.5 ml of a suspension of zymosan granules in 0.85% NaCl (5 mg zymosan/kg body weight) by the same route. Groups 3 and 4 received the indicated intratracheal dose of zymosan granules 24 h after the sham operation and partial hepatectomy, respectively. One hour before their sacrifice on days 2, 3, and 5 after the introduction of zymosan granules, all rats were injected intravenously with 0.4 ml of a colloidal charcoal (Gunter Wagner) suspension.

Cells were recovered from the airways by washing the lungs three times through the trachea with 5 ml of medium 199 containing added heparin (5 units/ml). Airway cell numbers were counted in Goryaev's chamber. Differential cell

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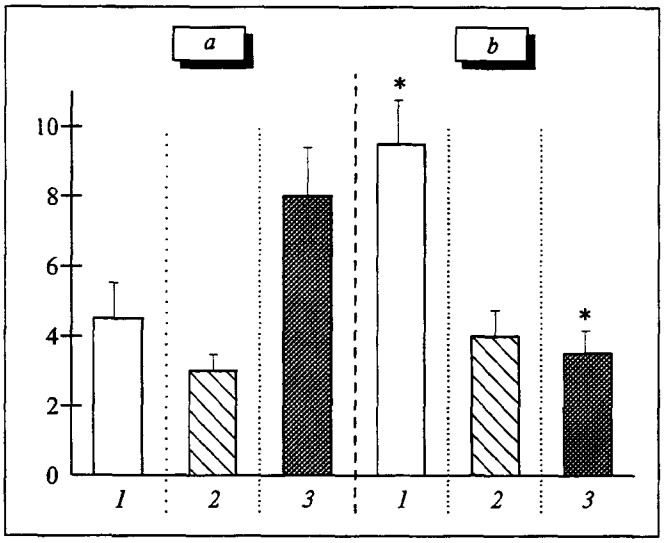

Fig. 1. Phagocytizing macrophages in the pulmonary interstitium of partially hepatectomized rats before (*a*) and 5 days after (*b*) intratracheal administration of zymosan. Ordinate: number of phagocytizing macrophages per field of vision. Here and in Fig. 3: *1*) intact rats; *2*) sham-operated rats; *3*) partially hepatectomized rats. *$p<0.05$ in comparison with hepatectomized rats before zymosan administration.

counts were done in centrifugal preparations stained by the Romanowsky-Giemsa method. The chemiluminescence of airway cells was recorded with a SKIF-0301 chemiluminometer (Krasnoyarsk, Russia). To each of its cuvettes containing Hanks' balanced salt solution without phenol red (pH 7.4), 0.1 ml of luminol (10^{-3} M) and 0.1 ml of the suspension containing 2×10^5 airway cells were added to a final volume of 1 ml. Chemiluminescence intensity was measured every minute for 30 min and expressed in counts per minute per cell (cpm/cell) [2]. In hematoxylin and eosin-stained 5 μ sections of lung tissue, charcoal-loaded pulmonary interstitial macrophages were counted and the lung tissue area per vision field was measured at ×1000 magnification using a standard ocular insert with a morphometric grid. In addition, acid phosphatase (EC 3.1.3.2) and cathepsin D (EC 3.4.23.5) activities were estimated in 10% lung tissue homogenates prepared after perfusing the lungs *in situ* with 0.85% NaCl cooled to 2-4°C. Protein in the homogenates was determined by Lowry's method. The results were subjected to statistical treatment using Student's *t* test.

RESULTS

Intratracheal introduction of zymosan granules to intact rats (group 2) led to enhanced migration of phagocytic cells to the airways (Table 1). Five days after zymosan stimulation, the number of airway cells in this group was 8 times that in group 1 ($p<0.05$), mainly as a result of increased inflow of monocytes/macrophages. In PHE rats (group 4), zymosan-stimulated migration of macrophages and neutrophils 5 days after zymosan administration was inhibited: the number of airway cells in this group was almost 4 times lower than in groups 1 and 3 ($p<0.05$).

Five days after zymosan granules were introduced into the trachea, the lungs of group 2 rats contained twice as many interstitial macrophages phagocytizing colloidal charcoal particles as did the lungs of group 1 rats ($p<0.05$). At 24 h after hepatectomy, the number of phagocytizing cells exceeded by a factor of 1.8 their number in rats of control group 2 ($p<0.05$). Zymosan administration to PHE rats, rather than increasing the inflow of phagocytic pulmonary interstitial macroph-

Table 1. Counts of Cellular Elements in Bronchoalveolar Fluid Samples from Normal and Hepatectomized Rats Following Intratracheal Administration of Zymosan

Group	Time after administration of zymosan granules, days	Cellular elements, 10^6/g tissue		
		total	alveolar macrophages	neutrophils
1	0	0.83±0.10	0.67±0.08	0.04±0.01
2	2	2.24±0.18	1.04±0.13	0.43±0.10
3	2	5.34±0.67	2.40±0.21	1.34±0.44
4	2	5.64±1.07	3.53±0.64	1.41±0.56
2	3	3.99±0.68	2.35±0.46	0.73±0.28
3	3	3.75±0.79	2.49±0.61	0.61±0.09
4	3	3.16±0.72	2.05±0.33	0.46±0.15
2	5	6.17±2.78*	4.13±1.66*	0.20±0.09*
3	5	6.55±1.83	5.38±1.69	0.49±0.17
4	5	1.64±0.22**	1.40±0.27**	0.10±0.05**

Note. *$p<0.05$ in comparison with group 1; **$p<0.05$ in comparison with group 3.

ages, led to a paradoxical reduction of their number by nearly a half ($p<0.05$; Fig. 1).

Intratracheal introduction of 0.85% NaCl (group 1) had little or no effect on lung tissue structure. In contrast, zymosan granules elicited a diffuse inflammatory infiltration of the lungs with a consequent thickening of interalveolar septa, so that the surface area of lung tissue, as measured in histological sections, was increased by one-third. It is noteworthy that 5 days after zymosan administration to PHE rats (group 4), the lung tissue surface area was increased by only 12%, being significantly smaller than in group 2 ($p<0.05$).

In group 2, total acid phosphatase activity 3 and 5 days after the introduction of zymosan granules was 1.8 and 1.5 times higher, respectively, than before their introduction ($p<0.05$). A similar observation was made for sham-operated rats (group 3). In group 4, by contrast, total acid phosphatase activity after zymosan stimulation remained virtually unchanged and was almost 3 times lower than in groups 2 and 3 ($p<0.01$). Cathepsin D activity likewise changed little in response to zymosan stimulation in this group (Table 2).

As shown in Fig. 2, the chemiluminescence of airway cells in the three groups was almost equal prior to zymosan administration but had increased in all groups by day 2 after stimulation. On day 5, it was again low in groups 2 and 3 while reaching a still higher level than on day 2 in group 4, where its value exceeded about 16-fold the values in groups 2 and 3 ($p<0.01$) (Fig. 2).

Thus, the introduction of zymosan granules directly into the airways elicited a powerful inflow

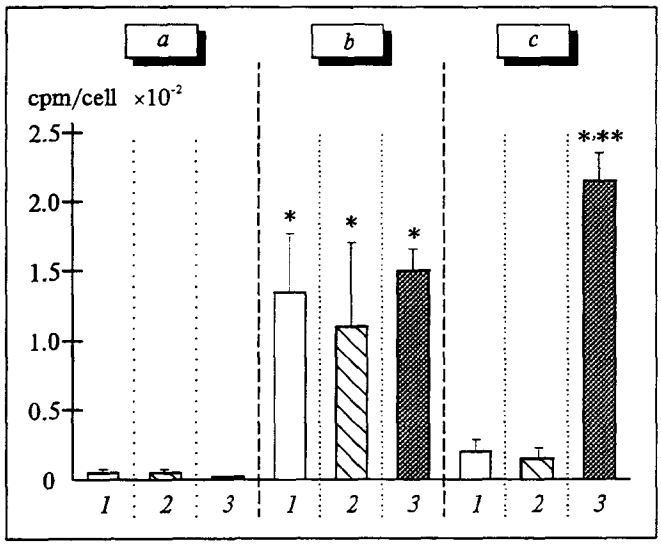

Fig. 2. Chemiluminescence of airway cells from hepatectomized rats before (*a*) and 2 (*b*) and 5 (*c*) days after intratracheal administration of zymosan. *$p<0.05$ in comparison with the same group before zymosam administration; **$p<0.01$ in comparison with the intact and sham-operated groups 5 days after zymosam administration.

of phagocytic cells, with a nearly 20-fold rise in neutrophils and a more than 6-fold rise in macrophages. Ultimately, the total number of cells migrating into the airway lumens was almost 8 times above normal, at a 20:1 ratio of macrophages to neutrophils. The entry of these cells into alveolar and bronchial cavities was accompanied by intensive infiltration of pulmonary interstitium.

The increase in the number of airway cells was attended by growth of their destructive potential, as indicated, first, by their enhanced chemiluminescence (which reflects the phagocyte's capacity to generate

Table 2. Total Activity of Lysosomal Enzymes in Lung Tissue Homogenates from Normal and Hepatectomized Rats after Intratracheal Administration of Zymosan

Group	Time after administration of zymosan granules, days	Acid phosphatase, mmol inorganic P/min/g protein	Cathepsin D, μmol tyrosine/min/g protein
2	0	7.60±1.31	2.05±0.92
3	0	7.70±2.39	1.42±0.48
4	0	5.41±1.46	2.01±0.29
2	2	7.06±1.45	1.89±0.24
3	2	8.72±1.95	1.69±0.34
4	2	5.72±1.41	2.29±0.27
2	3	13.33±0.79*	8.67±1.12*
3	3	16.24±1.43*	7.18±2.30*
4	3	5.64±1.12**	2.90±1.25**
2	5	11.17±1.62*	3.43±0.66
3	5	9.59±0.52	2.71±0.24
4	5	8.99±0.89	2.80±0.23

Note. *$p<0.05$ in comparison with activity before zymosan administration (0); **$p<0.01$ in comparison with activity in groups 2 and 3.

reactive oxygen species [10]) and, second, by the elevated total activity of the lysosomal enzymes acid phosphatase and cathepsin D [7].

On the other hand, 24 h after partial hepatectomy, i.e., when DNA synthesis in hepatocytes was at its peak [12], the magnitude of zymosan-induced phagocyte migration to the airways was consistently decreased almost 4-fold; the macrophage to neutrophil ratio was 14:1. Lung tissue was infiltrated to a lesser extent and zymosan was less effective in inducing lysosomal enzyme activity while leading to a fairly large increase in the chemiluminescence of airway cells, which presumably may be associated with depression of the pulmonary antioxidant system following the acute impairment of structural homeostasis [5].

To sum up, the responses of pulmonary phagocytes to a phlogogenic stimulus during reparative regeneration of the liver are inhibited, and this results in diminished inflow of these cells to the lung tissue and thus into the airway lumens. Possibly, the growth potential of phagocytes is mobilized, whereas their phlogogenic potential is partly blocked during the emergency restitution of homeostasis after partial hepatectomy.

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