Reactivity of Alveolar Macrophages during Granulomatous Inflammation of the Lungs under Conditions of Acute Massive Blood Loss

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Reactivity of mouse alveolar macrophages by their ability to phagocytize killed *St. aureus* bacteria and production of reactive oxygen metabolites (nitro blue tetrazolium test) in response to zymosan administration was studied under normal conditions and after acute massive blood loss. Zymosan-induced granulomatous inflammation of the lungs during acute massive blood loss 2-fold inhibited the increase in oxidative metabolism of alveolar macrophages. Suppressed production of toxic oxygen radicals in alveolar macrophages was accompanied by accelerated recovery of cells on the surface of the respiratory tract.

Key Words: *alveolar macrophages; nitro blue tetrazolium test; phagocytosis; blood loss; zymosan; granulomatous inflammation*

Stress exposure (cold and long-term swimming) is followed by pronounced functional changes in the system of mononuclear phagocytes, *e.g.* suppression of liver macrophages and activation of lung macrophages [4]. The observed changes affect the type of inflammation mediated by these cells. Published data show that stress can modify various stages of the inflammatory process [3]. Our previous studies showed that intensive physical exercise (long-term swimming) decreases the severity of destructive changes in the lungs after intratracheal instillation of silver nitrate [2]. Similarly to other stress factors, acute massive blood loss (AMBL) affects functional activity of phagocytes and modifies the type of inflammation. Engulfing activity of organotypic macrophages is violated during the posthemorrhagic period [7]. It remains unclear whether these changes concern functional activity of the phagocytic system. Here we studied reactivity of alveolar macrophages (AMP) after AMBL and assayed cell reactions on the surface of the respiratory tract during zymosan-induced granulomatous inflammation of the lungs (GIL) and AMBL.

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MATERIALS AND METHODS

Experiments were performed on male (CBA×C57Bl)F_1 mice. Group 1 included intact animals. GIL in group 2 mice was produced by intravenous treatment with the suspension of zymosan granules in a dose of 100 mg/kg. Zymosan is an insoluble polysaccharide complex isolated from the wall of *S. cerevisiae* yeast fungi (Biolar) [5]. The animals were killed under ether anesthesia on days 2, 5, and 9 after the induction of inflammation. AMBL in group 3 mice was modeled by puncture of the retroorbital sinus (2.5% body weight, 27% circulating blood volume). The study was conducted 6 h and 2, 5, and 9 days after blood loss. Group 4 animals received zymosan immediately after AMBL. Group 3 and 4 mice were killed in the same periods. Mouse lung cells were isolated [7]. Functional activity of AMP was estimated by the ability to phagocytize killed *St. aureus* (Kharkov Company of Bacterial Preparations) and produce reactive oxygen metabolites (nitroblue tetrazolium test, NBT test) [6].

The results were analyzed by Student's *t* test.

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RESULTS

Administration of zymosan significantly changed the ratio between cells on the surface of the respiratory tract in group 2 mice (Fig. 1). The number of neutrophils in the bronchoalveolar lavage fluid progressively increased and by the 9th day this parameter 10-fold exceeded the normal (Fig. 1). Moreover, zymosan modified functional activity of AMP. Engulfing activity of AMP and their ability to produce oxygen radicals increased on days 5 and 9, respectively (Fig. 2). After treatment with zymosan the population of AMP was more potent in phagocytizing killed bacteria (by 40%) and reducing NBT. Microscopy of histological preparations of the lungs revealed signs of alveolitis: decreased airiness of the lung tissue, thickening of alveolar septa, and formation of granulomas.

In group 2 mice AMBL stimulated migration of cells to the respiratory tract. The number of cells in the lavage fluid 6 h after AMBL increased by 50% (Table 1). Cell count in the lavage fluid remained high to the 5th day, which was related to an increase in the number of AMP. The number of neutrophils in bronchoalveolar lavage fluid significantly increased only 9 days after AMBL. Functional activity of AMP underwent biphasic changes after AMBL. Phagocytic activity of macrophages progressively decreased, but then increased (Table 1). On day 2 of the posthemorrhagic period AMP were less potent than control cells in phagocytizing staphylococci (by 30%). Phagocytic activity of AMP returned to normal and 1.3-fold exceeded the control level on days 5 and 9, respectively. Biocidal function of AMP was high after AMBL, decreased to normal in the follow-up period, but increased by the end of observations. Parameters of the NBT test increased by 1.5 times 6 h after AMBL, progressively decreased to the 5th day, but 2.2-fold exceeded normal on day 9 (Table 1).

AMBL modified the reaction of lung cells to phlogogenic zymosan (Fig. 1). In group 4 mice the rate of neutrophil influx into the lungs was higher than in group 2 animals. Leukocyte count in the respiratory tract of mice with AMBL and control animals with inflammation reached maximum on days 5 and 9 after

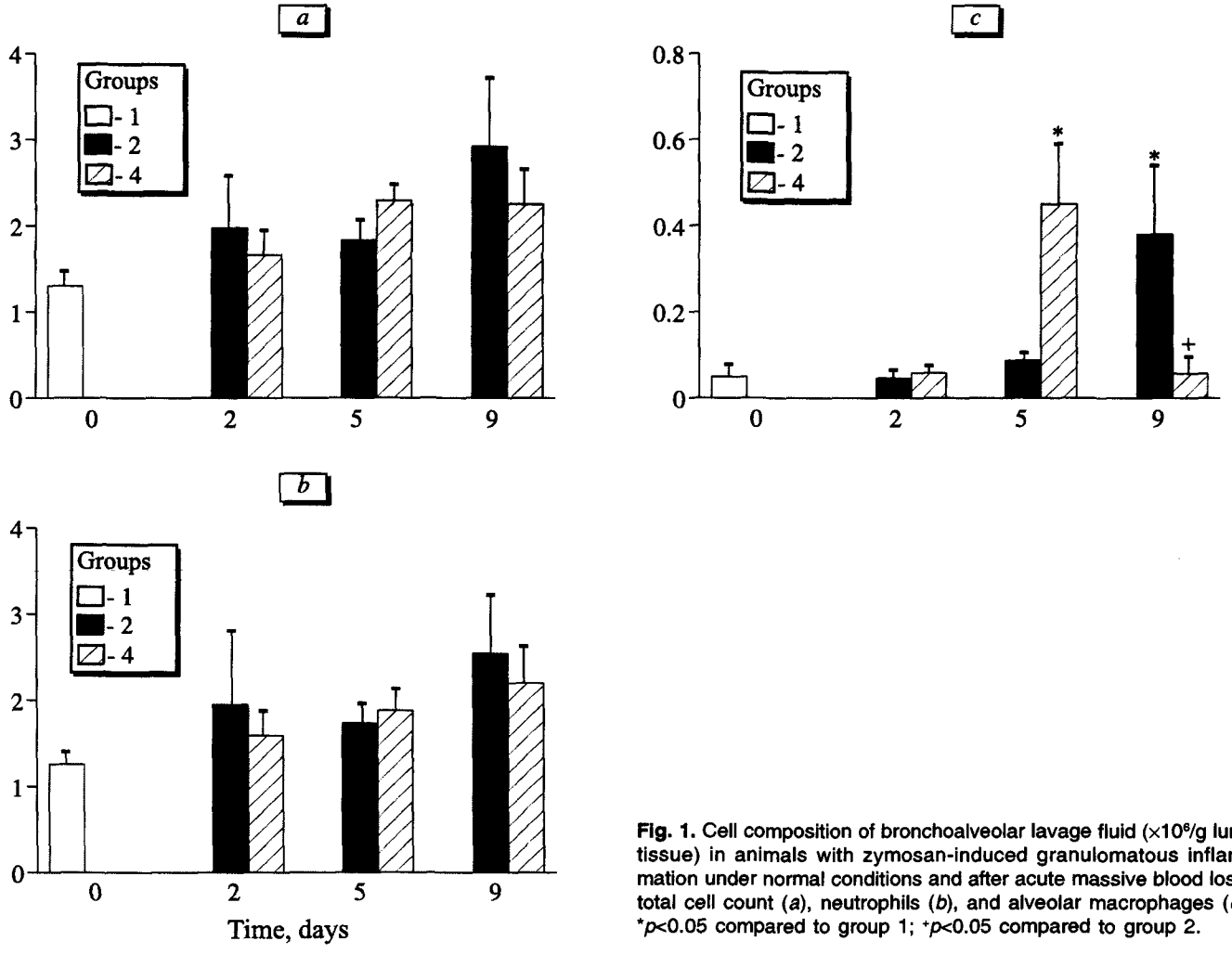

Fig. 1. Cell composition of bronchoalveolar lavage fluid (×10⁶/g lung tissue) in animals with zymosan-induced granulomatous inflammation under normal conditions and after acute massive blood loss: total cell count (*a*), neutrophils (*b*), and alveolar macrophages (*c*). *$p<0.05$ compared to group 1; +$p<0.05$ compared to group 2.

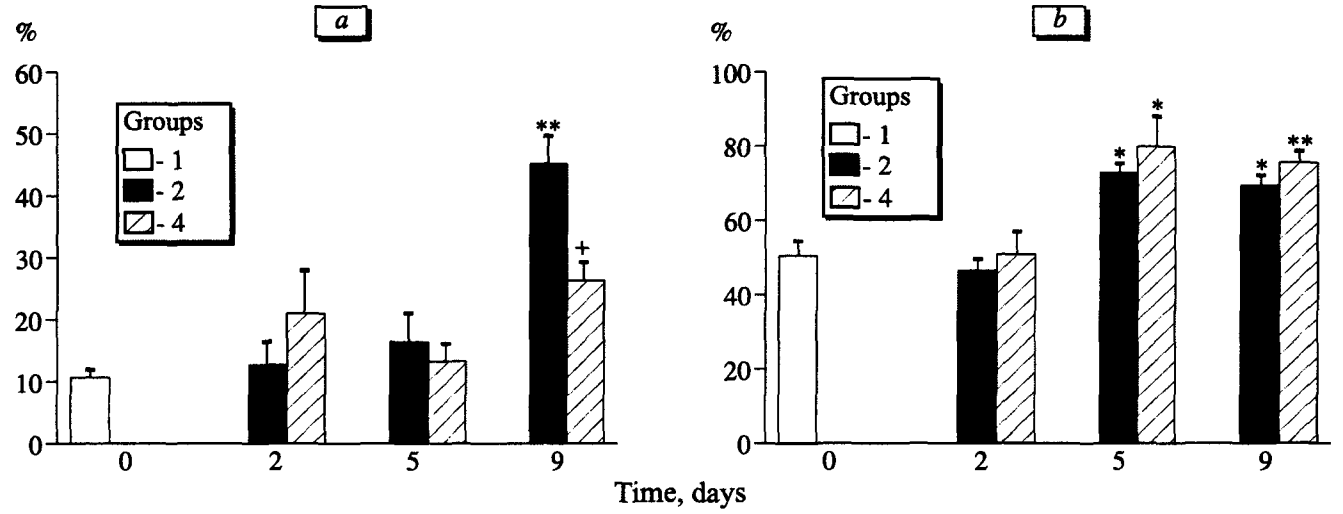

Fig. 2. Number of NBT-positive and phagocytizing cells in the population of alveolar macrophages in animals with zymosan-induced granulomatous inflammation under normal conditions and after acute massive blood loss: NBT test (*a*) and phagocytosis of killed *St. aureus* (*b*). *$p<0.01$ and **$p<0.001$ compared to group 1; +$p<0.05$ compared to group 2.

zymosan injection, respectively. AMBL had no effect on the number of free macrophages lying on the surface of the respiratory tract in mice with GIL. However, AMBL inhibited production of reactive oxygen metabolites by these cells (Figs. 1 and 2). On day 9 after zymosan injection parameters of the NBT test in mice of groups 2 and 4 increased by 4 and 2 times, respectively. AMBL did not modify the increase in phagocytic activity of AMP in mice with GIL.

Our results show that the ability of AMP to phagocytize microorganisms decreases, while generation of reactive oxygen metabolites increases in the posthemorrhagic period. The reduced ability to cleanse the blood suggests that these changes are associated with the increase in blood endotoxin concentration. The increase in the oxidative metabolism of AMP can serve as a compensatory reaction directed towards protection of the organism from infection. Generation of oxygen radicals by macrophages plays a role not only in the microbicidal and cytotoxic effect, but also in immunoregulatory activity. Superoxide anion radicals initiate the production of chemotactic cytokines and increase mitogen-stimulated proliferation of lymphocytes [1]. High phagocytic activity of AMP on days 5 and 9 of the posthemorrhagic period is probably related to compensatory activation of granulomonocytopoiesis, since colony-stimulating factors promote the increase in functional activity of phagocytes. The influx of neutrophils into the lungs 9 days after AMBL can be associated with increased production of macrophageal inflammatory protein-2 with chemotactic properties. Published data show that AMBL stimulates biosynthesis of cytokines in AMP (tumor necrosis factor-α, interleukin-β, and macrophageal inflammatory protein-2) due to activation of transcriptional factors [10]. We showed that reactivity of AMP decreases after AMBL, which manifested in blockade of a zymosan-induced in-

TABLE 1. Cell Composition of Bronchoalveolar Lavage Fluid and Functional Activity of AMP in Group 3 Mice ($M\pm m$)

Parameter	Intact animals (n=5)	Time after AMBL			
		6 h (n=5)	2 days (n=5)	5 days (n=5)	9 days (n=5)
Cell count in lavage fluid, $\times10^6$/g lung tissue					
total	1.3±0.16	1.99±0.21*	1.82±0.32	1.81±0.13*	1.56±0.16
macrophages	1.26±0.14	1.89±0.19	1.75±0.29	1.74±0.14*	1.41±0.17
neutrophils	0.049±0.021	0.074±0.029	0.049±0.036	0.052±0.005	0.14±0.02**
AMP					
NBT, %	10.67±1.21	15.0±1.6*	13.2±0.86	12.67±0.33	22.6±4.7*
phagocytosis	50.5±4.09	46.8±3.51	38.2±3.3**	58.4±5.68	74.4±4.35**

Note. *$p<0.05$ and **$p<0.01$ compared to intact animals.

crease in oxidative metabolism of these cells. The inhibition of toxic oxygen radical production by AMP in mice with GIL and AMBL was accompanied by accelerated recovery of cells on the surface of the respiratory tract. The development of tolerance to phlogogenic agents after sublethal blood loss in rats was reported previously [8,9].

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