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Response to Airway Phagocytes to Lung Damage Before and After Strenuous Physical Exercise

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Quantitative ratios between alveolar polymorphonuclear leukocytes (PML) and macrophages and the ingesting and reducing potentials of these phagocytic cells were studied after lung damage in unstressed mice and mice that had just been stressed by strenuous physical exercise (swimming for 60 min). Three days after the lung damage induced in unstressed mice by $AgNO_3$ (0.1 ml instilled intratracheally), PML numbers in the airway lumens were significantly increased, while the bronchoalveolar lavage fluid samples taken on day 14 after lung damage indicated intensified macrophage activity. In the mice instilled with $AgNO_3$ immediately after being stressed, the recruitment of PML and macrophages to the lungs was markedly decreased, although the percentage of macrophages reducing nitro blue tetrazolium had significantly increased. That the lungs of stressed mice sustained less injury than those of unstressed animals was indicated by the finding that lactate dehydrogenase activity in the cell-free fraction of bronchoalveolar lavage fluid was less damaged in response to intratracheal instillation of the destructive agent.

Key Words: *bronchoalveolar lavage fluid; macrophages; polymorphonuclear leukocytes; strenuous physical exercise; lung damage*

We showed earlier that different classes of macrophages react to strenuous physical exercise in different or opposite ways [1]. For example, the function of Kupffer cells is inhibited, whereas both fixed and free pulmonary macrophages exhibit heightened phagocytic activity, accompanied by enhanced migration of polymorphonuclear leukocytes (PML) to the airways. Since the resistance of the lungs to damage is largely dependent upon pulmonary phagocytes [2], alterations in the reactivity of respiratory tract ph-

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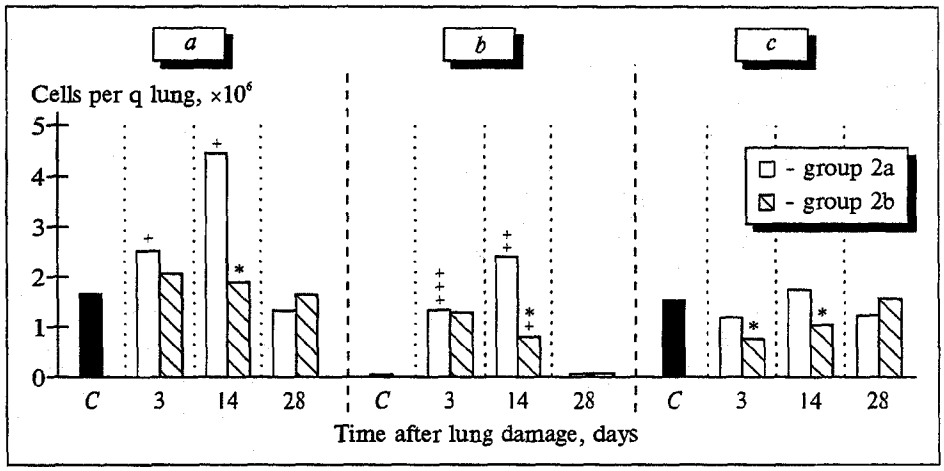

Fig. 1. Cellular composition of bronchoalveolar lavage fluid samples collected from mice with lung damage produced by $AgNO_3$ immediately after strenuous physical exercise. *a*) total number of cells; *b*) PML; *c*) alveolar macrophages. Here and in Fig. 2: $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ in comparison with intact controls; $^{*}p<0.05$ in comparison with group 2a.

agocytes that occur in stress and, in particular, during strenuous physical activity should have a profound effect on the susceptibility of respiratory organs to damage. In the present study on mice we investigated responses of phagocytes from pulmonary alveoli and interstitium to strenuous physical exercise, on the one hand, and to the damage inflicted on the airways immediately after such exercise, on the other.

MATERIALS AND METHODS

Female (CBA×C57Bl) F_1 mice (body weight 18-22 g) from the *Stolbovaya* Nursery of the Russian Academy of Medical Sciences were used. Mice of group 1 were subjected to strenuous physical exercise (swimming for 60 min in water heated to 32-34°C). In two other groups, 0.1 ml of a 0.4% $AgNO_3$ solution was instilled into the trachea of intact mice (group 2a) or of mice that had just completed one hour of swimming (group 2b). The same mice when intact served as controls.

On days 0, 3, 14, and 28, the lungs of all mice were flushed using a previously developed procedure [3], the collected bronchoalveolar lavage fluids were centrifuged at 250 g for 7 min at 4°C, and the cells were resuspended in 1 ml of medium 199 supplemented with 20% calf serum. The biocidal potential of alveolar phagocytes was evaluated by their ability to reduce nitro blue tetrazolium (NBT test) [4]. The ingesting activity of alveolar macrophages (AM) and PML was assessed by their ability to engulf metacrylate granules 0.9 μ in diameter (produced at the Institute of Macromolecular Chemistry, Academy of Sciences, Czechoslovakia) [4]. Cells in the lavage fluid samples were counted in Goryaev's chamber, and the percentage ratio of poly- to mononuclear phagocytes was calculated in cytological preparations stained by the Romanowsky-Giemsa method at a magnification of 1000 (immersion). In hematoxylin-eosin-stained lung sections, PML and macrophages laden with colloidal carbon were counted in 10 randomly chosen visual fields per interstitium (×1000, immersion). The ingestive function of the mononuclear phagocyte system was evaluated by the rate of blood clearance from colloidal carbon particles [6]. In the cell-free fraction of lavage fluid, the concentration of complement fragment C_{5a} was determined using an Amersham reagent kit. The degree of lung tissue destruction was assessed by measuring lactate dehydrogenase activity in the cell-free fraction of lavage fluid using a Boehringer Mannheim reagent kit.

Finally, proadhesive properties of blood plasma samples were evaluated from the balance between

Table 1. Cytological Composition of Bronchoalveolar Lavage Fluid Samples from Mice after Strenuous Physical Activity ($M\pm m$)

Parameter	Intact mice (n=6)	Group 1 (n=5)
Cell count in lavage fluid, ×10⁶/g lung		
Total number	1.65±0.25	2.28±0.48
AM	1.52±0.24	1.73±0.79
PML	0.04±0.02	0.11±0.003**
Alveolar macrophages		
NBT, %	9.7±0.6 (n=6)	31.7±3.5*** (n=7)
Phagocytosis, %	22.1±3.2 (n=5)	45.6±4.3* (n=5)
Interstitial macrophages		
Phagocytosis, %	21.0±5.9 (n=5)	49.4±3.6* (n=5)

Note. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ in comparison with the intact controls.

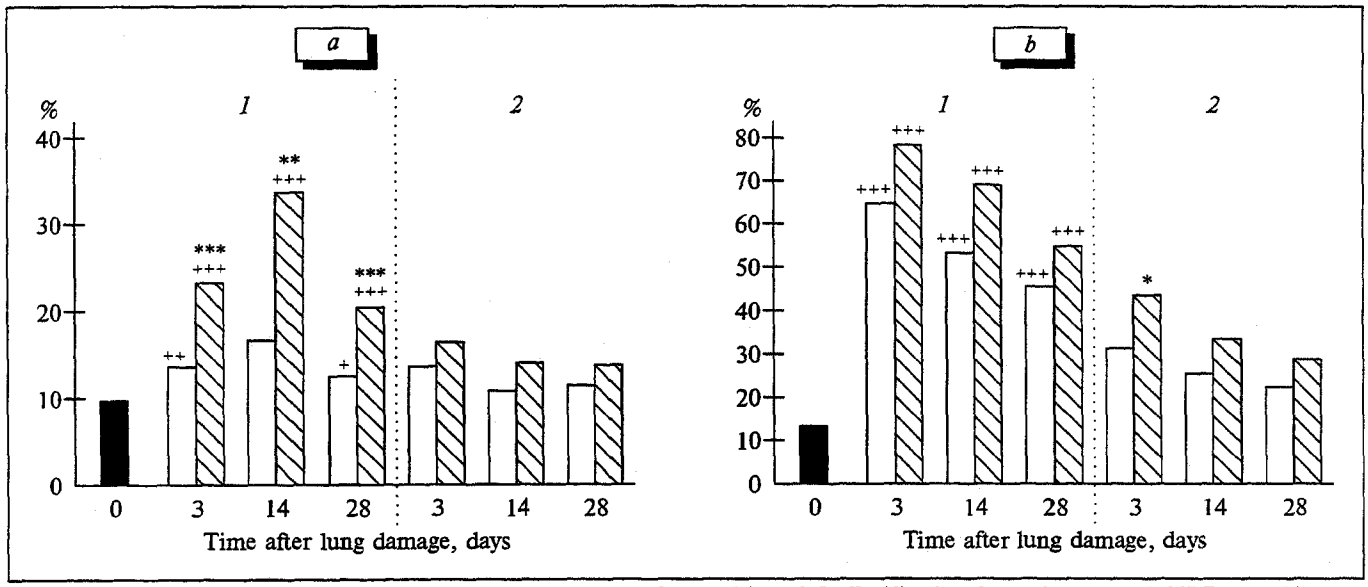

Fig. 2. Percentages of functionally active alveolar macrophages (*1*) and PML (*2*) after lung damage. *a*) NBT test; *b*) phagocytosis of metacrylate granules. $^{**}p<0.01$, $^{***}p<0.001$ in comparison with group 2a. Other designations of statistical significance as in Fig.1.

thromboxane A_2 and prostaglandin I_2, as estimated by measuring the concentrations of their stable metabolites thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ in a radioimmunoassay using a Reanal reagent kit.

The statistical significance of differences was estimated by Student's *t* test.

RESULTS

Group 1. Immediately after the strenuous physical exercise, the total number of cells in the bronchoalveolar lavage fluid was increased by a factor of 1.4 (Table 1). The absolute number of PML was increased 2.5 fold in the lavage fluid and 4-fold in the pulmonary interstitium (180.8±31.1 *vs.* 42.7± ±3.75 in the intact controls; $p<0.01$). The number of free AM in the lavage fluid was 1.2 times higher, while their biocidal activity had trebled (Table 1); moreover, these AM ingested twice as many colloidal carbon particles as did the AM of intact mice. Similar activities were displayed by interstitial macrophages. However, despite this, the ingesting capacity of the mononuclear phagocyte system as a whole was inhibited; thus, the rate of blood clearance from colloidal carbon immediately after the swimming session was decreased 1.8-fold (0.04±0.003 *vs.* 0.07±0.001 in the controls; $p<0.05$).

Leukocyte accumulation in the lung tissue was attended by an almost 1.4-fold rise of the C_{5a} concentration in the cell-free fraction of lavage fluid (20.5±1.0 ng/ml *vs.* 14.5±0.52 ng/ml in the controls; $p<0.05$). The plasma level of the proadhesive mediator thromboxane B_2 was 1.7 times higher ($p<0.01$) than in the controls, while that of its antagonist 6-ketoprostaglandin $F_{1\alpha}$ was 1.3 times lower (insignificant difference) (Table 2).

Group 2a. Three and 14 days after lung damage, the total number of cells in the bronchoalveolar lavage fluid exceeded that in the intact controls 1.5-fold and 2.7-fold, respectively, and then dropped to the control (baseline) level by day 28 (Fig. 1). PML had increased in number about 30-fold by day 3 and even more by day 14 (Fig. 1). The number of AM increased by half between days 3 and 14. It was only by day 28 that the ratio between poly- and mononuclear phagocytes returned to normal.

The functional characteristics of AM present in the lavage fluid also varied. On day 3 postdamage, for example, the number of AM reducing NBT was 1.4 times higher than in the lavage fluid from intact mice, while the number of AM engulfing

Table 2. Thromboxane B_2 and 6-Ketoprostaglandin $F_{1\alpha}$ Activity in Plasma from Intact Mice and Mice Subjected to Strenuous Physical Activity

Group of animals	Thromboxane B_2, pg/ml	6-Ketoprostaglandin $F_{1\alpha}$, pg/ml	Thromboxane B_2/6-ketoprostaglandin $F_{1\alpha}$
Intact mice	837.8±113.6 (n=9)	143.0±49.5 (n=5)	5.9
Group 1	1399.5±119.7* (n=8)	110.0±6.78 (n=6)	12.7

Note. *$p<0.01$ in comparison with the intact controls.

Table 3. Lactate Dehydrogenase Activity in the Cell–Free Fraction of Lavage Fluid from Mice with Damaged Lungs ($M \pm m$)

Group of animals	Examination time, days		
	3	14	28
Intact mice	30.0±6.58 ($n=5$)		
Group 2a	192.9±30.82*** ($n=7$)	93.3±16.8*** ($n=6$)	31.0±3.76 ($n=7$)
Group 2b	157.0±22.34*,*** ($n=10$)	51.0±5.57** ($n=11$)	25.0±2.11** ($n=10$)

Note. *$p<0.05$ in comparison with group 2a; **$p<0.01$, ***$p<0.001$ in comparison with the intact controls.

metacrylate granules was 4.9 times higher (Fig. 2). On day 14, both NBT-reducing and metacrylate-engulfing AM were more numerous than on day 3, because of enhanced macrophage recruitment into the alveoli. By day 28, their numbers had dropped but were still above baseline (Fig. 2).

On day 3 after damage, when PML were accumulating in the lungs, lactate dehydrogenase activity in the cell-free fraction of lavage fluid was 6 times above normal (Table 3). Later, enhanced macrophage migration to the airways was accompanied by depression of this activity, which had dropped nearly 2-fold by day 14.

Group 2b. On days 3 and 14 after lung damage, the number of cells washed out from the airways in this group was 1.2 and 2.4 times lower, respectively, than in group 2a (Fig. 1). On day 3, the lavage fluids of both groups contained nearly equal numbers of PML, whereas on day 14, when the recruitment of phagocytes was at its peak, PML in group 2b fluids were much less numerous, so that the PML/AM ratio in this group was only half that in group 2a (1.43±0.34 *vs.* 0.67±0.26; $p<0.05$). The number of AM in group 2b was 1.6 times lower than in group 2a on day 3 and 1.7 times lower on day 14, although their numbers tended to rise as the pathological process developed in the lungs. The engulfing activity of alveolar PML on day 3 was 1.4-fold higher than in group 2a, whereas the biocidal potential of these cells, as estimated by the NBT test, was the same in both groups (Fig. 2). As regards AM, they were at all times more active in reducing NBT than in group 2a.

In group 2b, lactate dehydrogenase activity in the cell-free fraction of lavage fluid on day 3 was lower than in group 2a, and it returned to normal more rapidly (Table 3).

Thus, as the results presented above show, strenuous physical exercise is followed by increased ingress of PML into the airways and by the accumulation in the lungs of macrophages with high capacities for engulfing inert particles and generating reactive oxygen metabolites (as indicated by the NBT test). The increase of PML numbers in the lungs after strenuous physical exercise such as prolonged swimming may be associated with C_{5a} accumulation in lung tissue, since macrophages themselves are involved in the production of this complement fragment [10]. Moreover, AM synthesize and release leukotriene B_4, platelet activation factor, and also interleukin 8, which selectively attracts neutrophils to the lungs [8-10]. An additional factor promoting leukocyte migration to the lungs may be increased adhesiveness of the pulmonary venular endothelium resulting from enhanced expression of special glycoproteins of the E-integrin type on its membranes [5]. Endothelial adhesiveness could also increase because of the shift we detected in the balance between thromboxane A_2 and prostaglandin I_2 in favor of the proadhesive mediator thromboxane A_2/B_2 [7]. The reactions of airway phagocytes to strenuous physical activity modify the lungs' susceptibility to damage, as is indicated by the suppressed increase in the activity of lactate dehydrogenase - an enzyme marker of destructive processes in the bronchoalveolar fluid from the lungs damaged immediately after swimming. Indirect evidence that the strenuous physical exercise made the lungs more resistant to damage is provided by the more rapid fall in the number of PML in the airways with a concomitant rise in the number of active macrophages there in response to the damage.

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