Reactivity of Airway Phagocytes during the Development of Acute Pneumonia under Conditions of Stimulation of Mononuclear Phagocyte System with Zymosan

O. P. Makarova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Suppl. 1, pp. 35-38, 2008
Original article submitted July 29, 2008

Pneumonia was induced in (CBA×C57Bl)F_1 mice under conditions of stimulation of the mononuclear phagocyte system with zymosan. The number of neutrophils in airways increased after 3 days; by day 14, the number of cells in the bronchoalveolar lavage fluid further increased due to migration of macrophages. After zymosan prestimulation, the number and functional activity of neutrophils during the early period of inflammation (3 days) did not change, but the increase in phagocytic activity of macrophages was inhibited by 20%. By day 14, the effect of prestimulation manifested in 4.5-fold decreased capacity of neutrophils and macrophages to reduce NBT.

Key Words: *mononuclear phagocyte system; pneumonia; bronchoalveolar lavage fluid; neutrophils; macrophages*

Mononuclear phagocytes participate in initiation of the inflammatory process by producing specific (IL-8, macrophage inflammatory protein-2, cytokine-induced neutrophils chemoattractants) and unspecific chemoattractants (c5a complement component, leukotriene B_4) for neutrophils and via a cytokine network (IL-1, TNF-α, and IL-6) produce local and systemic effects on the inflammatory focus [1]. Changes in the function of the mononuclear phagocyte system (MPS) can be a promising approach to the regulation of the inflammatory process. Zymosan, a polysaccharide complex from *S. cerevisiae* yeast cell walls, acts as MPS inductor. Previous studies showed that activation of macrophages *in vivo* improves lung resistance to *P. carinii* and *L. monocytogenes* infections [5.7]. However, MPS stimulators sometimes produce negative side effects related with phagocyte hyperactivation.

Research Center of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** opmak@soramn.ru. O. P. Makarova

Here we studied the reactions of airway phagocytes after stimulation with zymosan and during the development of acute pneumonia after lung damage under conditions of MPS stimulation with zymosan.

MATERIALS AND METHODS

The experiments were carried out on 2-2.5-month-old female (CBA×C57Bl)F_1 mice weighing 18-20 g. The animals were maintained under standard vivarium conditions and on standard ration with free access to water. All tests were performed during morning hours.

In series I (20 mice), the effects of zymosan on functional state of MPS and alveolar macrophages were evaluated. Experimental mice received intraperitoneal injection of zymosan suspension (Biolar) in a dose of 100 mg/kg in 0.25 ml 0.85% NaCl (group 1); controls (group 2) received the same volume of 0.85% NaCl. The tests were performed on day 7 after zymosan administration.

In series II we used 52 mice. Acute pneumonia was induced by intratracheal application of 0.1 ml

0007-4888/08/1466-0719

0.4% $AgNO_3$ on day 7 after zymosan injection (experimental mice, group 3) or on day 7 after 0.85% NaCl injection (controls, group 4). Intact mice served as an additional control. The tests were performed on days 3, 14, and 28 after lung damage.

Samples of lung tissue were fixed in 10% neutral formalin, dehydrated, and embedded in paraffin blocks; 4-5-μ sections were stained with hematoxylin and eosin and examined under a light microscope.

Cells of the bronchoalveolar lavage fluid (BALF) were obtained as described earlier [4]. The BALF cells were counted in a Goryaev chamber and their relative number per 1 g wet lung tissue was determined, percent ratio of poly- and mononuclear phagocytes was evaluated on cytological preparations stained after Romanovskii—Giemsa (×1000).

The absorption capacity of MPS was evaluated by the rate of blood clearance from gelatin-coated colloid charcoal particles Gunter Wagner c11/1431a (0.8-1.2 μ) [6]. Functional activity of AM was evaluated by the production of reactive oxygen species (NBT test) and absorption of 0.9-μ methacrylate granules (Research Institute of Macromolecular Chemistry, Czech Academy of Sicence) or killed *S. aureus* bacteria (Khar'kov Bacterial Preparation Plant) [4]. Methacrylate granules and bacteria were not opsonized before addition to the incubation medium (medium 199 supplemented with 20% calf serum).

The data were processed statistically using nonparametric Mann—Whitney test.

RESULTS

Examination of BALF obtained from mice on day 7 after zymosan injection showed that the count of AM was not increased, but AM from group 1 animals more actively (1.5-fold) absorbed bacteria and

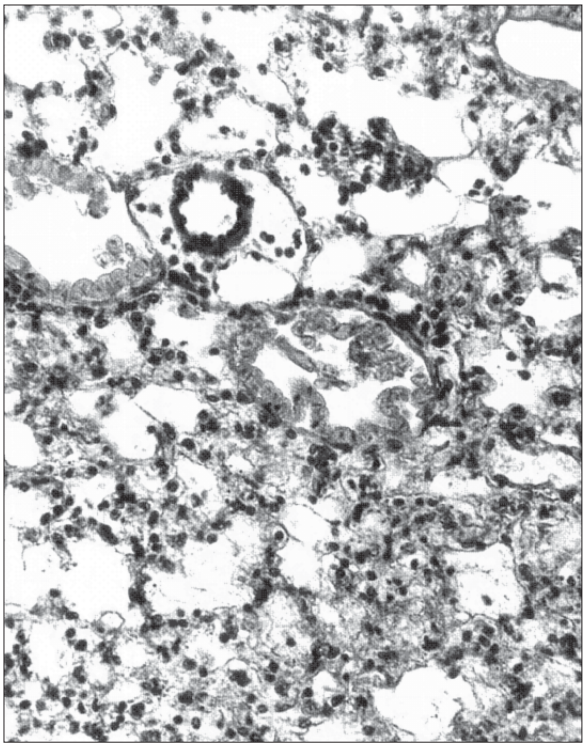

Fig. 1. Initiation of acute inflammation in the lungs. Necrobiosis and desquamation of bronchial epithelium. Hematoxylin and eosin staining, ×400.

2-fold more rapidly reduced NBT compared to the control (Table 1). In group 1 mice, MPS more rapidly (by 30%) cleared the blood from colloid charcoal particles than in group 2 mice. The increase in functional activity of AM was associated with an increase in neutrophil count in BALF. This suggests that reactivity of airway phagocytes during the development of acute pneumonia under conditions of MPS stimulation with zymosan will differ from the normal.

Application of 0.4% $AgNO_3$ induced damage to bronchial epitheliocytes due to coagulation of

TABLE 1. Phagocyte Count in BALF and Functional Activity of Macrophages on Day 7 after Zymosan Injection (*M*±*m*)

Parameter		Control (*n*=5)	Experiment (*n*=5)
BALF cells, $\times 10^6$/g lung tissue			
	total cell count	1.30±0.16	1.56±0.16
	AM	1.26±0.14	1.41±0.17
	neutrophils	0.05±0.02	0.14±0.02**
AM functions			
	NBT test, %	10.67±1.21	22.6±4.7*
	phagocytosis, *S. aureus*., %	50.50±4.09	74.40±4.35**
MPS functions			
	blood clearance	0.080±0.001	0.100±0.001*

Note. *p<0.05, **p<0.01 compared to the control.

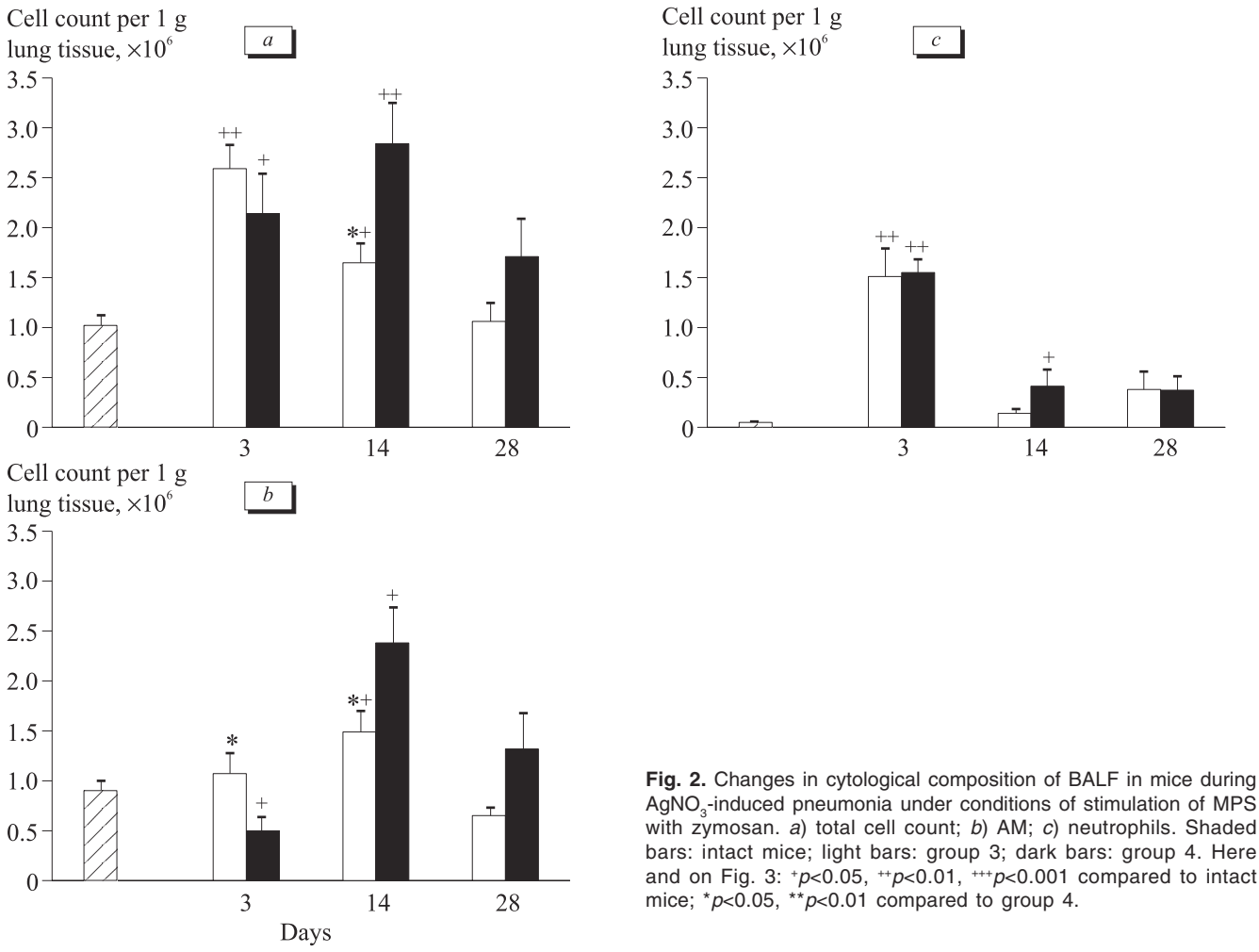

Fig. 2. Changes in cytological composition of BALF in mice during $AgNO_3$-induced pneumonia under conditions of stimulation of MPS with zymosan. *a*) total cell count; *b*) AM; *c*) neutrophils. Shaded bars: intact mice; light bars: group 3; dark bars: group 4. Here and on Fig. 3: $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ compared to intact mice; $^{*}p<0.05$, $^{**}p<0.01$ compared to group 4.

membrane proteins, desquamation of the epithelium, and initiation of inflammation in the lungs (Fig. 1). On day 3 of acute pneumonia, the total cell count in BALF from mice of groups 4 and 3 increased by 2.1 and 2.8 times, respectively, but in group 3 mice this parameter more rapidly returned to normal (Fig. 2). On days 14 and 28, the number of cells obtained from the lungs of group 3 mice was lower than that in group 4 mice by 1.7 and 1.6 times, respectively. The dynamics of neutrophils count in BALF in the experimental and control groups was similar, but the dynamics of AM count in BALF differed. On day 3, the number of AM in BALF of group 4 mice was 1.8-fold lower than in intact mice, while in group 3 animals, AM count did not decrease. By day 14, the content of AM in BALF from mice of groups 3 and 4 increased by 1.4 and 4.8 times, respectively, compared to day 3. By day 28, this parameter in group 3 decreased by 2.3 times compared to that on day 14, while in group 4 it decreased by only 1.8 times.

Prestimulation of MPS with zymosan modulated not only the dynamics of AM count in BALF after lung damage, but also the pattern of changes in functional activity of phagocytes (Fig. 3). By day 14, the NBT-reducing capacity of neutrophils in group 4 mice increased by 1.6 times compared to day 3, while in animals of group 3 this parameter decreased by 3.6 times. Activity of neutrophils in this group was also 4.5-fold below the control. There were no significant intergroup differences in the dynamics of phagocytic activity of neutrophils. In group 3 mice, changes in NBT-reducing capacity were observed in both neutrophils and AM. Thus, in group 4 mice on day 14 after induction of inflammation, activity of AM in NBT test increased by 3.2 times compared to the corresponding parameter on day 3 and 2-fold surpassed the normal, while in group 3 animals, activity of AM in NBT test did not increase (Fig. 3). In group 3 mice, NBT-reducing capacity of AM was lower by 2.6 times than in group 4 mice. On day 3 after lung damage, the number of phagocytizing AM increased both in control and experimental animals. However, in group 3 mice the percent of phagocytizing AM was lower than in controls by 1.2 times. By

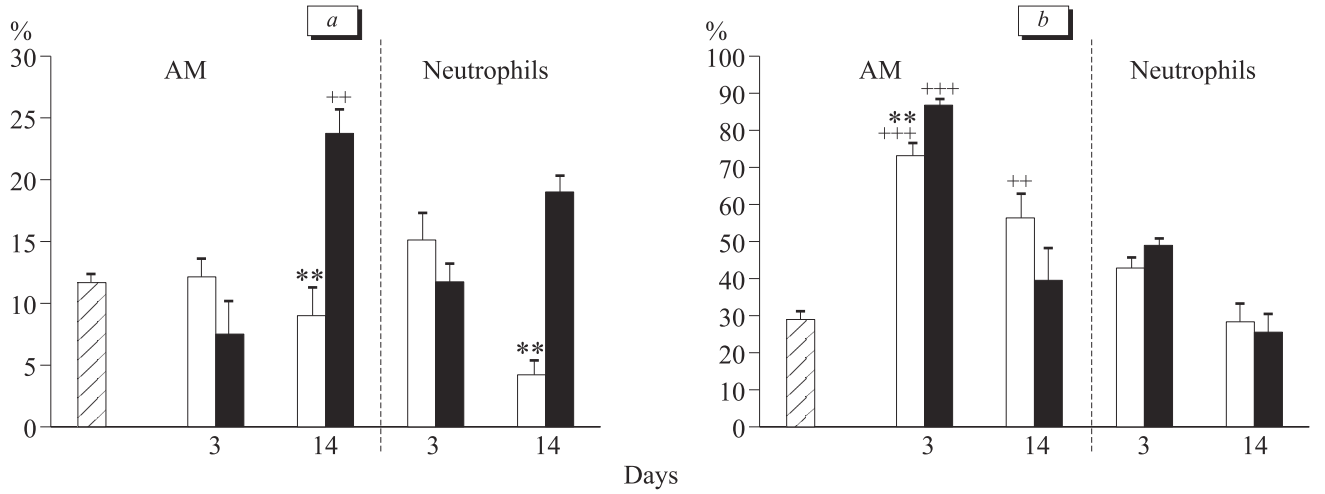

Fig. 3. Changes in functional activity of airway phagocytes in mice during $AgNO_3$-induced pneumonia under conditions of stimulation of MPS with zymosan. *a*) NBT test; *b*) phagocytosis of methacrylate granules.

day 14, the percent of phagocytizing AM decreased compared to day 3; in group 4 it decreased by 2.2 times, while in the experimental group this parameter decreased by only 1.3 times.

Thus, functional activity of not only resident macrophages, but also free macrophages in the lungs increased after stimulation of MPS with zymosan. These findings agree with modern concept of MPS reactions to stimulation [2,3]. However, reactivity of airway phagocytes during the development of acute pneumonia under conditions of MPS stimulation with zymosan decreased, which can be explained by their deactivation related to increased sensitivity to the inhibiting effects of glucocorticoids [8].

REFERENCES

1. I. G. Akmaev, *Uspekhi Fiziol. Nauk*, **34**, No. 4, 4-15 (2003).
2. G. K. Zakenfeld, *Immunological mechanism of the Effects of Saccharomyces cerevisiae Yeast Polysaccharides* [in Russian], Riga (1990).
3. D. N. Mayanskii, *Chronic Inflammation* [in Russian], Moscow (1991).
4. L. N. Shishkina, D. N. Mayanskii, and M. V. Bogomolova, *Byull. Eksp. Biol. Med*., **100**, No. 10, 454-457 (1985).
5. J. M. Antonini, J. R. Roberts, H. M. Yang, *et al., Lung,* **178**, No. 6, 341-350 (2000).
6. G. Biozzi, B. Benacerraf, and B. N. Halpern, *Int. J. Exp. Pathol*., **34**, No. 4, 441-457 (1953).
7. K. M. Empey, M. Hollifield, and B. A. Garvy, *Infect. Immun*.,**75**, No. 7, 3382-3393 (2007).
8. J. Ma, T. Chen, J. Mandelin, *et al., Cell. Mol. Life Sci.*, **60**, No. 11, 2334-2346 (2003).