Effect of Cytokines on the Generation of Active Oxygen Species by Pulmonary and Peripheral Blood Phagocytes

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The effect of naturally occurring cytokines on the chemiluminescence response of neutrophils from bronchoalveolar lavage and peripheral blood was studied in patients with chronic bronchitis. It was found that the response of neutrophils from bronchoalveolar lavage differs from that of peripheral blood neutrophils. The cytokines prestimulated the peripheral blood neutrophils for chemiluminescence.

Key Words: *cytokines; chemiluminescence; chronic bronchitis*

Bronchoalveolar neutrophils and macrophages act as effector cells. Impairments of the phagocytic component of immunity manifest themselves in reduced activity of alveolar macrophages, decrease in their content in bronchial mucus, increased content of functionally defective neutrophils, high activity of elastase and collagenase, and the presence of active oxygen forms in bronchoalveolar lavage [5,7]. The protective and damaging activities of pulmonary neutrophils have not been studied in sufficient detail. In the present study we used a model of spontaneous and stimulated chemiluminescence to determine the role of cytokines in the regulation of functional activity of pulmonary and peripheral blood neutrophils in chronic nonspecific pulmonary diseases.

MATERIALS AND METHODS

Neutrophils were obtained from 20 patients (8 men and 12 women) aged 23-65 years. The patients suffered from chronic bronchitis. Eight men had obtrusive bronchitis, four out them had lingering pneumonia and two had bronchoectasia. Aggravation of chronic bronchitis was diagnosed in all the patients.

Functional activity of neutrophils was assessed by measuring luminol-dependent chemiluminescence (LDCL) in an L1251 luminometer (LKB-Wallac). Neutrophil suspension was prepared by incubating heparinized (20 U/ml) venous blood (3 ml) with 3% gelatine solution at 37°C for 15 min. The resultant cell suspension was twice washed with medium 199.

Cellular composition was determined by the Zadorozhnyi-Dozmorov staining. The neutrophil count was adjusted to 2×10^6 cells/ml. Bronchoalveolar lavage was obtained by the conventional method. Cell suspension was prepared by filtration of the lavage through a capron filter and centrifugation in medium 199 15 min at 400*g* and for 10 min at 200*g*. The viability and counts of neutrophils and macrophages were determined using Zadorozhnyi-Dozmorov stain. The neutrophil content of the suspension was adjusted to 2×10^6 cells/ml. Since the amplitude and the rate of neutrophilic CL response development are greater than those in macrophages [2], we neglected the contribution of macrophages to the chemiluminescence of the suspension. The chemiluminescence of other cells (lymphocytes and

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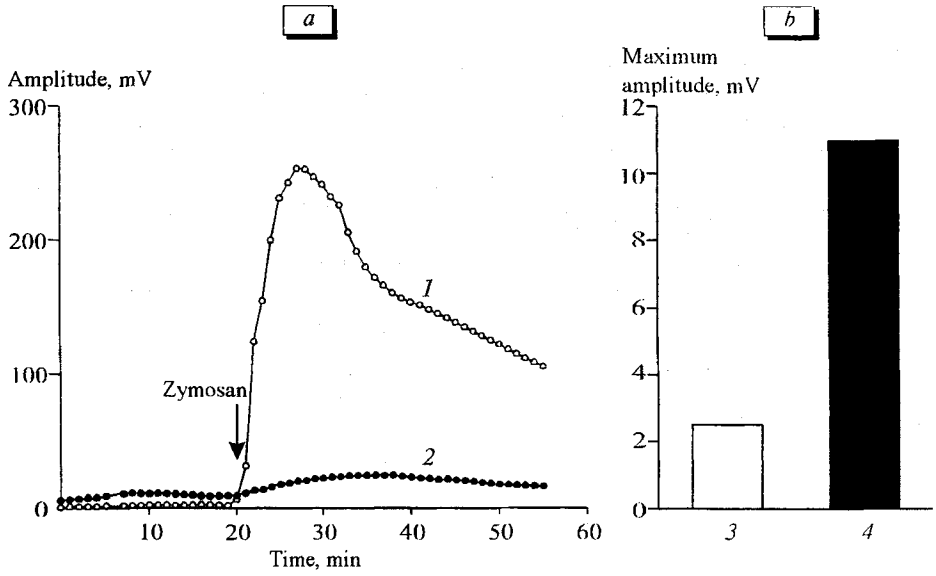

Fig. 1. Time course of the chemiluminescence response of peripheral blood *(1)* and bronchoalveolar neutrophils *(2)* in the patient M. *(a)* and amplitude of spontaneous chemiluminescence *(b)* of peripheral blood *(3)* and bronchoalveolar neutrophils *(4)* in the same patient.

epithelial cells in the lavage, lymphocytes and eosinophils in peripheral blood), whose counts were very low, was also very low. We have assumed that only neutrophils are responsible for the LDCL of the resultant cell suspensions. The neutrophil suspension (100 μl) was diluted with 300 μl medium 199, after which 100 μl luminol was added to a final concentration of 5×10^{-5} M in the cuvette. The luminescence was recorded for 45 min, and spontaneous LDCL was calculated. Then 100 μl zymosan opsonized with pooled serum (20 mg/ml) was added to the suspension, and induced LDCL was determined.

A complex of porcine cytokines was obtained as described [4]. The preparation exhibited the interleukin-1, -2, -6, growth transforming factor-β, and migration-inhibiting factor activities.

Statistical significance of results was evaluated by Student's *t* test at $p<0.05$.

RESULTS

In healthy subjects, neutrophils are not present in the bronchoalveolar space or their number is very small [7,8]. In the studied patients, the mean neutrophil content in bronchoalveolar lavage was 59% (37-81%), far surpassing the content of other cells.

In all the patients, the maximum spontaneous LDCL of neutrophils from bronchoalveolar lavage was not lower and in some of them was much higher than that of peripheral blood neutrophils (Fig. 1). The level of spontaneous CL, which indirectly reflects the initial intensity of intracellular metabolism [3], was higher in bronchoalveolar than in peripheral blood neutrophils: 11.2±1.21 *vs.* 4.16± 1.04 mV ($p<0.05$).

The maximum level of LDCL induced by opsonized zymosan in the suspension of bronchoalveolar neutrophils was reached much later than that in peripheral blood neutrophils. The maximum amplitude of induced LDCL was much lower (up to 10-fold) than that of peripheral blood neutrophils: 61±12.7 and 170.9±24.7 mV, respectively ($p<0.05$). Thus, the index of LDCL stimulation was much lower in bronchoalveolar neutrophils in comparison with that in peripheral blood neutrophils.

The complex of naturally occurring cytokines (CNOC) acts as a priming factor and stimulates generation of active oxygen radicals during oxidative burst, i.e., markedly increases the response induced by another stimulus (latex or zymosan) without any effect on the amplitude of spontaneous LDCL [1,6]. The maximum amplitude of LDCL did not change at low and decreased at higher concentrations of

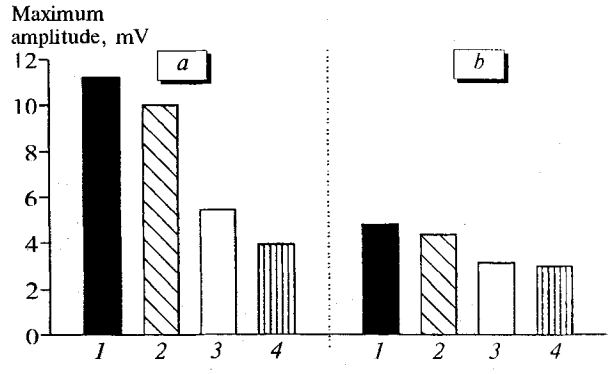

Fig. 2. Effect of naturally occurring cytokines on spontaneous luminol-dependent chemiluminescence of bronchoalveolar *(a)* and peripheral blood neutrophils *(b)* of patients with chronic nonspecific pulmonary diseases. Here and in Fig. 3: *1)* control chemiluminescence of neutrophils without cytokines, *2)* with cytokines in a dose of 3 μg/ml, *3)* 10 μg/ml *4)* 30 μg/ml.

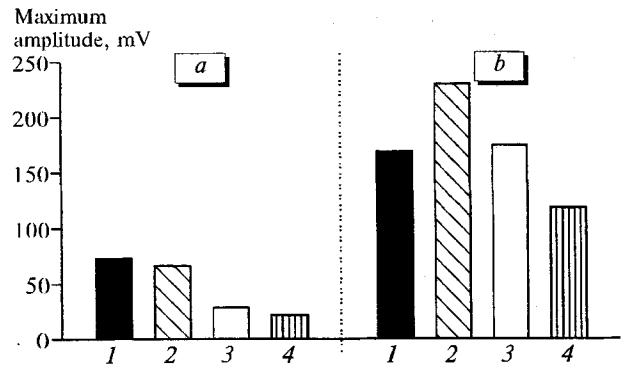

Fig. 3. Effect of naturally occurring cytokines on induced luminol-dependent chemiluminescence of bronchoalveolar *(a)* and peripheral blood neutrophils *(b)*.

CNOC, the decrease being greater in neutrophils from peripheral blood than from bronchoalveolar lavage in comparison with that in control suspensions incubated without cytokines. At 3 μg/ml (the total protein concentration) CNOC had a weak effect on induced LDCL of bronchoalveolar neutrophils and suppressed it after being added to higher concentrations (Fig. 2). In the suspension of peripheral blood neutrophils, the amplitude of induced LDCL increased considerably under the effect of CNOC (Fig. 3). Taking into account the fact that the neutrophil suspension was not washed after incubation with CNOC, it can be suggested that the suppression of LDCL by high CNOC concentrations is due to antioxidizing activity of CNOC, implying that cytokines act as a free radical "trap".

Is was shown that the intensity of spontaneous LDCL is related to the level of the initial intracellular metabolism [3]. Increased generation of active oxygen species in the absence of stimulus points to increased intensity of metabolic processes in cells. In neutrophils this probably reflects their readiness to react with a pathogenic agent. A long-term increase in the production of active oxygen radicals, which may occur in chronic nonspecific pulmonary diseases, may lead to exhaustion of intracellular resources and the loss of the cell ability to react adequately to a stimulus. *In vitro,* however, an increase in spontaneous LDCL probably reflects the ability of neutrophils to generate considerable amounts of free radicals even in the absence of pathogenic agents. This may be associated with the damaging effect of these cells on the lungs.

From our findings it can be concluded that 1) in chronic nonspecific lung diseases the generation of the active oxygen radicals by bronchoalveolar neutrophils is changed in comparison with that of peripheral blood neutrophils and 2) naturally occurring cytokines stimulate the production of active oxygen forms by peripheral blood neutrophils in patients with chronic nonspecific pulmonary diseases.

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