BIOPHYSICS AND BIOCHEMISTRY

Interaction of Alveolar Macrophages from Pulmonary Patients and Healthy Subjects with Liposomes

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Formation of primary metabolites of oxidative burst in alveolar macrophages from healthy subjects and patients with chronic lung diseases is studied using the method of luminol-dependent chemiluminescence. Incubation with liposomes composed from phosphatidylcholine and a phosphatidylcholine—fatty acid esters mixture induces similar activation of oxidative burst in alveolar macrophages from healthy subjects and patients with lung diseases.

Key Words: *human alveolar macrophages; liposomes; oxidative burst; luminol chemiluminescence*

The idea of using liposomes as a vehicle for drug delivery to various organs and tissues proposed 20 years ago is now extensively studied in experiments [5]. However, pharmacological effects of liposomes remain obscure. Anti-inflammatory, antiexudative, and healing effects of liposome suspensions have been demonstrated on operative wounds and gunshot injuries in the lungs and skin of experimental animals [1]. Inhalation of liposomal aerosol [2] seems to be a very promising method for treating patients with bronchial asthma.

Liposomes injected into tissues are phagocytized by reticuloendothelial cells, primarily Kupffer cells, spleen macrophages, and, to a lesser extent, by lung macrophages [6]. Interaction of liposomes of various composition with transformed macrophage strains [4], bone marrow [3], and animal alveolar macrophages [7] is now intensely investigated. However, the information on interactions with cells of the human mononuclear phagocyte system is scarce [8].

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In the present study we evaluated the formation of primary oxidative burst metabolites in alveolar macrophages (AM) from healthy subjects and patients with chronic lung diseases incubated with liposomes composed from phosphatidylcholine (PC) alone or in combination with fatty acid esters (FAE) enriched with arachidonic acid.

MATERIALS AND METHODS

Bronchoscopy was carried out under local anesthesia (lidocaine) using a standard method.

Bronchial lavage was filtered through cotton wool and centrifuged for 10 min at 400*g* and 4°C. The pellet was suspended in 4 ml Hanks' solution, layered on Isopaque-Ficoll (1.076 g/ml), and centrifuged for 20 min at 1500 rpm and 22°C. The top macrophage-containing layer (0.5-0.7 ml) was centrifuged at 150 rpm and room temperature for 5-10 min. The pellet was suspended in 2 ml Hanks' solution, layered on a Percoll gradient (1.123, 1.077, and 1.043 g/ml), and centrifuged at 400*g* for 10 min. The top layer was collected, centrifuged, and suspended

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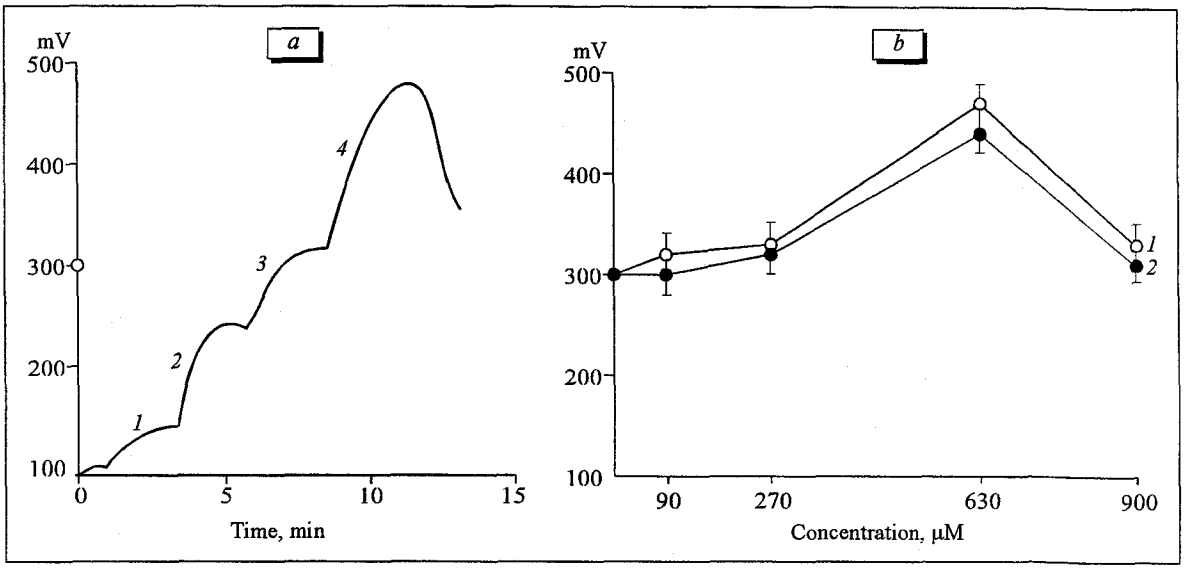

Fig. 1. Intensity of chemiluminescence in the presence of alveolar macrophages (AM) and different activators (*a*) and in human AM incubated with different concentrations of liposomes (*b*). *a*: *1*) AM; *2*) phorbol myristate; *3*) ionophore A23187, and *4*) liposomes. *b*: *1*) liposomes consisting of phosphatidylcholine and *2*) phosphatidylcholine+fatty acid esters (2%, w/w).

in 0.5 ml Hanks' solution. Macrophages were counted in a Goryaev chamber. The obtained fraction contained 90-95% macrophages, trypan blue staining revealed no more than 10% dead cells.

The formation of oxidative burst metabolites was assessed by luminol chemiluminescence at excitation and emission wavelengths of 350 and 450 nm in a chemiluminometer.

For preparation of liposome suspension, aqueous dispersions of PC and PC+FAE mixture (7.5 mg/ml) were sonicated for 5-7 min at a frequency 22 kHz and a power of 20 W with cooling (UZDN-21 device). The suspension was centrifuged at 5000*g* for 5 min to separate large lamellae and mechanical inclusions.

Macrophages (10^6 cells) were incubated in a cell containing 1 ml Hanks' solution for 3 min at 37°C, then 5 μl luminol was added (final concentration of 5×10^{-5} M). After measuring the baseline luminescence, different activators were added to the cell: phorbol myristate (PM, 10^{-7} M) or liposomes of various composition in a volume of 10-100 μl.

RESULTS

Functional activity of AM from patients with different chronic obstructive lung diseases was studied by the method of luminol-dependent chemiluminescence. In the absence of activators (control) AM induced a weak luminol luminescence (300-400 mV). Addition of PC liposomes enhanced luminescence, the effect was proportional to PC concentration, but above PC concentration of 500-600 μM the intensity of luminescence decreased (Fig. 1).

Similar changes were induced by liposomes consisting of PC and 2% arachidonic acid-enriched FAE. According to modern views, arachidonic acid is a second messenger activating metabolic processes. Bearing in mind that free arachidonic acid is highly oxidizable, we used arachidonic esters. We assumed that free arachidonic acid is released intracellularly due to the action of cell hydrolases. However, this assumption was not justified, and the effect of FAE-containing liposomes on AM did not differ from that induced by PC liposomes in the whole concentration range (Fig. 1).

The effect of liposomes of different compositions on the formation of primary activated oxygen metabolites was studied on AM isolated from bronchial lavage samples of 25 patients with chronic obstructive lung disease. It should be noted that division of patients into groups according their diagnosis (asthma, acute pneumonia, and chronic bronchitis) is a matter of convention, since, first, many patients had combined pathology and, second, the group of patients with chronic bronchitis included patients with mucoviscidosis and tuberculosis. The control group consisted of 2 convalescent patients without acute pulmonary and bronchial processes judging from patient's condition and bronchoscopy.

In 80% experiments, liposomes enhanced luminol-dependent chemiluminescence in AM from patients with all forms of chronic obstructive lung diseases by 20-50%, the same was observed in the control group (Fig. 2). However, a 4-10-fold activation of chemiluminescence was noted in 4 patients with different lung diseases: tuberculosis, bronchial asthma,

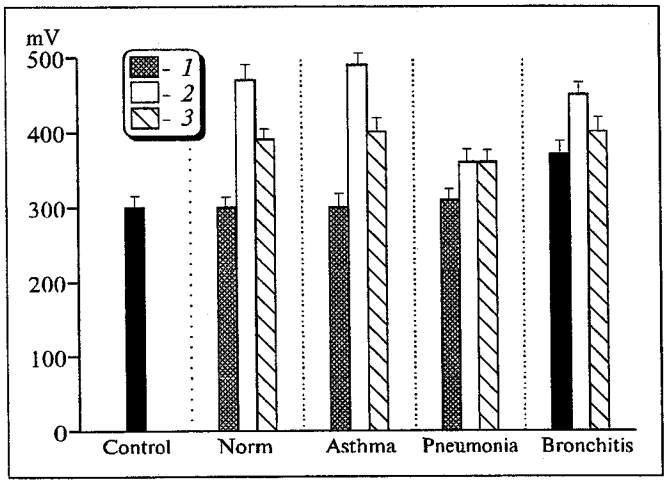

Fig. 2. Intensity of luminol chemiluminescence in alveolar macrophages from patients with different diseases in the presence of activators: phorbol myristate (*1*), liposomes consisting of phosphatidylcholine (*2*), and liposomes consisting of phosphatidylcholine+fatty acid esters (*3*).

and chronic obstructive bronchitis. When calculating the mean values presented in Fig. 2, these values were excluded. The erythrocyte sedimentation rate (ESR) in these patients varied from 20 to 58. The high degree of inflammation is apparently a necessary but not sufficient condition for AM hyperreactivity, since in some patients with high ESR such an extreme activation was not observed.

In the present study we evaluated the effect of PM (10^{-6}-10^{-8} M) on the production of primary oxygen metabolites in AM. It was found that effective concentration of PM is 10^{-7} M. The stimulating effect of PM on AM was noted only in patients with chronic obstructive bronchitis (Fig. 2). Moreover, AM were hyperreactive only in some patients in this group. In other groups of patients and in the control group PM had no effect on AM. Increased production of primary oxygen metabolites by AM in response to PM and liposomes was observed in different patients, AM hyperreactivity to PM being characteristic of patients with low ESR.

For understanding this phenomenon it should be noted that PM and liposomes principally modulate the same component of metabolic pathways, protein kinase C, but via different mechanisms. Liposomes activate protein kinase C through a multistep mechanism involving phosphatidylinositols and 1,2-diacylglycerol formation, while PM is a direct activator of protein kinase C.

These data are consistent with the finding [8] that PM has a greater effect on the formation of superoxide radical in AM from patients with cirrhosis of the liver in comparison with healthy subjects. In our experiments activation of oxidative burst was noted only in AM from patients with chronic bronchitis. This difference is probably due to a number of factors, for instance, different sensitivity of experimental approaches and different methods of AM isolation and, consequently, different functional activity. Nevertheless, the more pronounced effect of PM in patients with various diseases implies structural and functional changes in AM membranes.

Our findings suggest that liposomes composed from PC alone and with PC—FAE activate the formation of primary metabolites of oxidative burst in human AM. The above-mentioned pharmacological effect of liposomes can be attributed to changes in the functional state of AM, an important component of organism's defense system. No differences were found in the effect of liposomes on AM from healthy individuals and patients with chronic lung diseases.

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