MORPHOLOGY AND PATHOMORPHOLOGY

In Vitro Effects of Pulmonary Surfactant on Macrophage Morphology and Function

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The effects of pulmonary surfactant on the morphology and functioning of young macrophages were studied on the model of monocyte/macrophage differentiation *in vitro* and on macrophages of the bronchial alveolar lavage fluid. Surfactant is not a differentiation inductor, but it stimulated the maturation and phagocytic activity of young macrophages. The stimulatory effect of surfactant on phagocytic activity of macrophages persisted even after its removal from the culture medium.

Key Words: *tuberculosis; surfactant BL; macrophages; differentiation*

Pulmonary surfactant (PS) is a membrane complex of phospholipids (80-90%), proteins (8-10%), and carbohydrates (2-4%) on the inner lining of alveoli maintaining the respiratory biomechanics and presumably involved in reactions of congenital and acquired immunity [2,3,7,11]. During development of an infectious inflammation in the lungs, for example, in tuberculosis, functional activity of macrophages is maintained at a sufficiently high level for as long as the level of PS in the alveoli remains high [2]. Disorders in its production by type 2 alveolocytes lead to accumulation of young immature mononuclears with underdeveloped lysosomal system without signs of phagocytosis. On the other hand, drug stimulation of endogenous production of pulmonary phospholipoproteins or inhalations of native PS by experimental animals lead to normalization of macrophage reactions of the lungs [6]. Studies of the direct effects of PS on mononuclear phagocyte differentiation and/or maturation are practically important, because functional inertness of these cells promotes long-term extracellular persistence of *Mycobacterium tuberculosis* and is responsible for inefficiency of etiotropic therapy [1].

We studied the effects PS on the morphology and functions of monocytes/macrophages. The tasks of the study were as follows: 1) to evaluate the possibility of induction of monocytic cell differentiation by PS; 2) to detect structural characteristics of macrophageal cell population in the presence of PS; 3) to evaluate the effects of PS on phagocytic activity, lysosomal compartment status, and phagosome formation in macrophages; and 4) to evaluate the prolonged effect of PS on phagocytic activity of macrophages.

MATERIALS AND METHODS

Continuous cultures of human acute monocytic leukemia *THP-1* cells and bronchoalveolar lavage fluid (BAL) macrophages obtained from patients with active disseminated pulmonary tuberculosis were used in the study. Monocytes were cultured on slides in 2-ml Petri dishes in RPMI-1640 with 10% FCS at 5% CO_2. Differentiation of monocytes into macrophages

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in vitro was induced by adding phorbol ether (PE) in a concentration of 10^{-7} M (Sigma).

The effects of PS on macrophage differentiation were studied using a natural surfactant, Surfactant-BL (SBL; Biosurf), which was added to the culture medium in a concentration of 1 mg/ml for 24 h, after which it was removed from the culture medium and the cells were incubated for 3 days from the beginning of treatment. Untreated *THP-1* culture and *THP-1* culture treated with PE without SBL served as controls. The morphology of monocytes adhering to the glass was studied using standard hematoxylin and eosin staining. The cells were counted in 100 visual fields selected at random in 2 independent experiments.

For evaluation of phagocytic activity of macrophages, FITC-labeled latex beads (FluoSchere) were added to the culture medium for 2 h. The cells were then fixed for 20 min in 10% paraformaldehyde in phosphate buffer (pH 7.2-7.4), poststained with DAPI nuclear stain, and examined under a SPE Leica confocal microscope.

Macrophages from BAL were examined under an electron microscope after 45-min exposure with SBL (1 mg/ml). After exposure, the tubes with cells were centrifuged, the supernatant was discarded, and cell precipitate was fixed (60 min) in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2-7.4), postfixed (40 min) in 1% OsO_4, dehydrated in ascending alcohols, and embedded in epoxy resin. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM-100B electron microscope.

For evaluation of phagocytic activity, the cell population was divided into 2 subgroups: cells without latex particles or containing just solitary particles (subgroup 1) and cells with the cytoplasm filled with latex beads (subgroup 2). The cells were counted in 100 fields of view selected at random.

Statistical differences in the data were calculated by variability analysis after evaluation of Student's coefficient. The differences were considered significant at $p<0.05$.

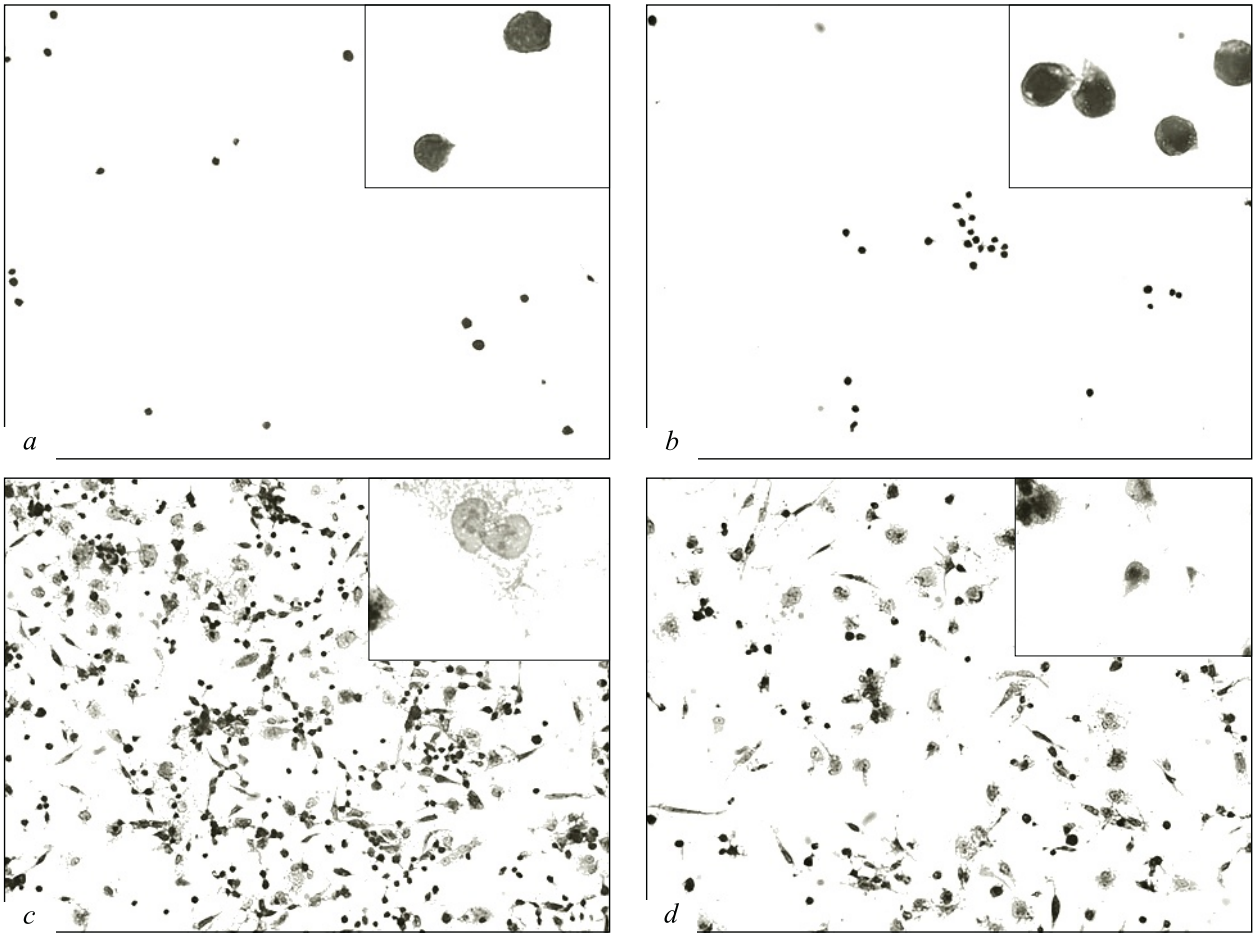

Fig. 1. Effects of SBL on *THP-1* culture (3 days of culturing, hematoxylin and eosin staining, ×2000). *a*) control: few adhesive cells; *b*) addition of 1 mg/ml SBL, few adhesive cells; *c*) differentiation into macrophages after addition of 10^{-7} M PE; *d*) differentiation into macrophages after addition of PE (10^{-7} M) and SBL (1 mg/ml), increase in the counts of cells with processes and multinuclear cells.

TABLE 1. Effects of SBL on Phenotype of Macrophage Cells from *THP-1* Human Monocytes (*M*±*m*)

Group	Monocyte-like cells, %	Flattened nonpolar cells, %	Flattened polar cells, %		Multinucleated cells, %
			total	>80 μ long	
THP-1+PE (control)	31.3±3.7	43.1±0.1	25.6±3.9	8.6±3.9	2.7±0.7
THP-1+PE+SBL (1 mg/ml)	19.8±0.2*	48.2±3.1*	32.1±3.4	20±0.1*	5.4±0.9*

Note. *$p<0.05$ in comparison with the control.

RESULTS

After PE induction of *THP-1* cells the overwhelming majority of suspended monocytes adhered to the substrate as early as after 24 h. By day 3, monocyte-like cells and flattened macrophages with processes were seen on slides; multinuclear cells appeared. If SBL was added to the culture medium simultaneously with PE, the count of monocyte-like cells was 1.5 times lower, while that of flattened polar cells was 1.3 times higher (Table 1); many of these cells had long processes (Fig. 1, *d*). The number of bi- and trinucleated cells increased 2-fold. It should be noted that without PE the SBL exhibited no appreciable effect on the adhesion of *THP-1* cells: just solitary cells adhered to the substrate (Fig. 1, *b*), their little number makes quantitative analysis impossible.

Hence, PS does not induce monocyte differentiation into macrophages, but it directly influences the modification of the morphological status of "young" macrophages by stimulating their flattening and formation of processes.

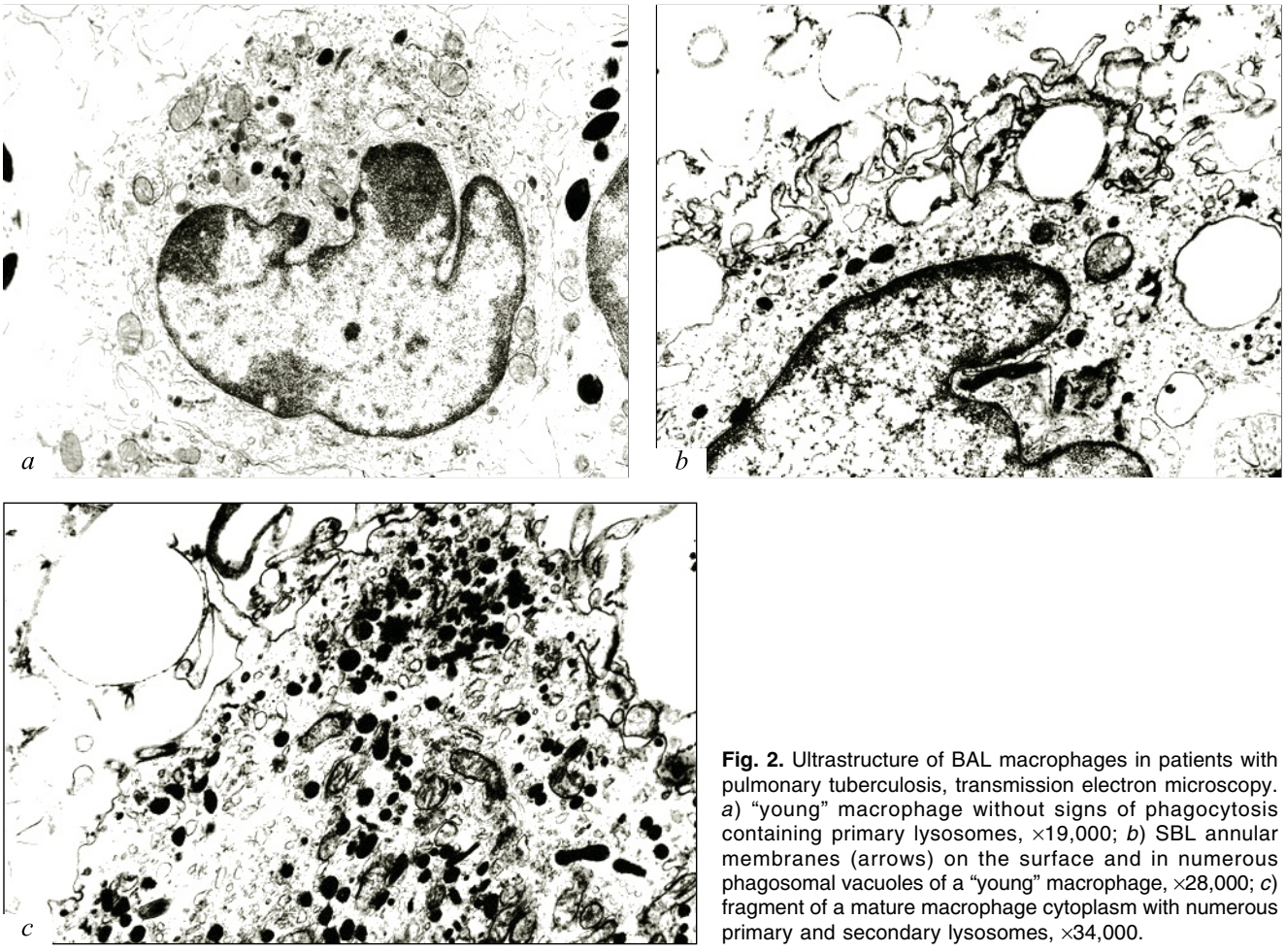

Fig. 2. Ultrastructure of BAL macrophages in patients with pulmonary tuberculosis, transmission electron microscopy. *a*) "young" macrophage without signs of phagocytosis containing primary lysosomes, ×19,000; *b*) SBL annular membranes (arrows) on the surface and in numerous phagosomal vacuoles of a "young" macrophage, ×28,000; *c*) fragment of a mature macrophage cytoplasm with numerous primary and secondary lysosomes, ×34,000.

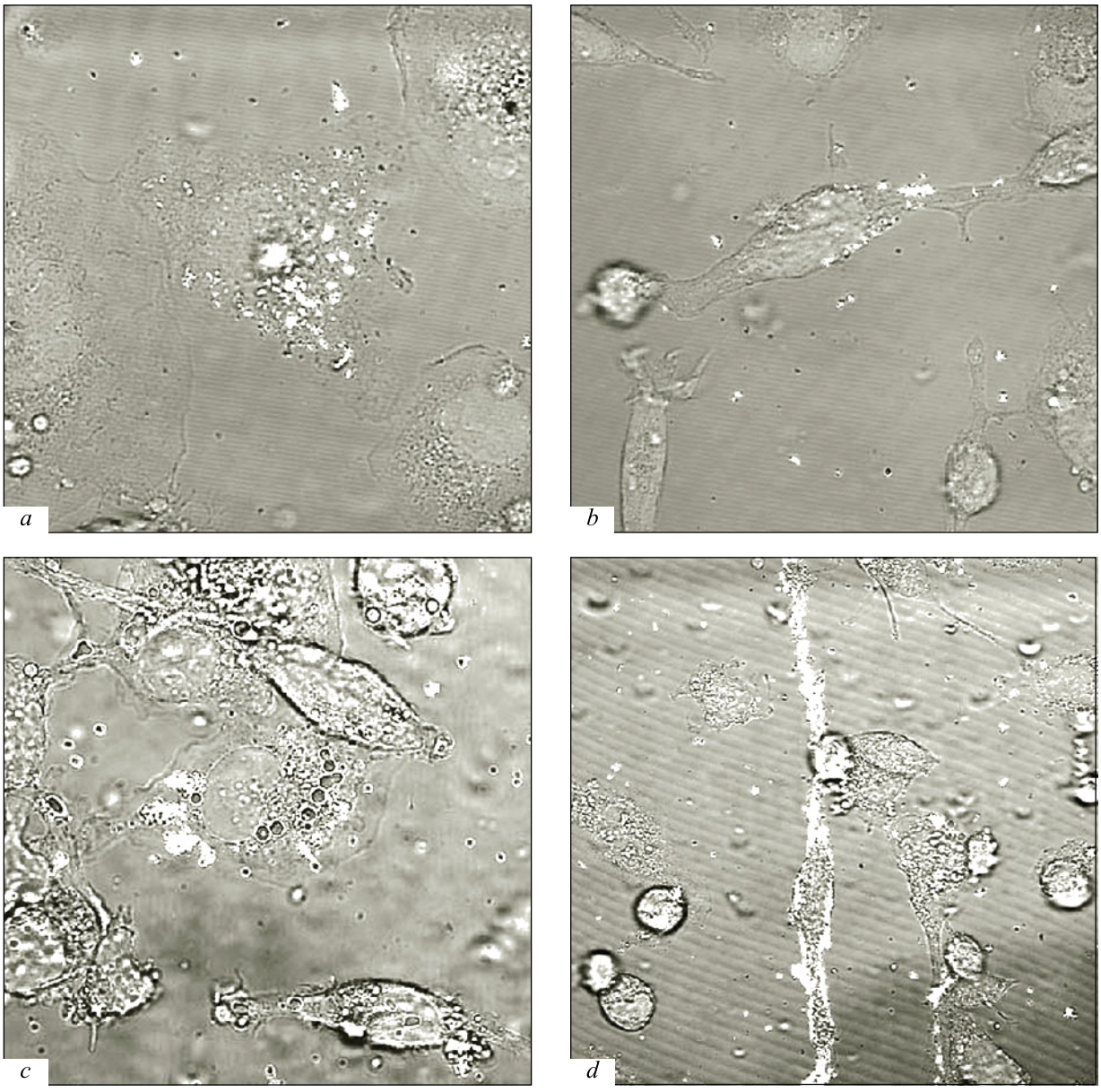

Fig. 3. *THP-1* culture treated with 10^{-7} M PE (*a*, *b*) and 1 mg/ml SBL (*c*, *d*). *a*, *b*) diffuse distribution of latex beads in the cytoplasm; *c*, *d*) uneven accumulation and distribution of latex beads in the cells. FluoSphere, DAPI staining, superposition with phase contrast, ×100.

For evaluation of the effects of SBL on maturation of "young" macrophages, we carried out electron microscopic study of cells from BAL of patients with active tuberculosis, where the content of these cells did not exceed 40% [5] and they have no ultrastructural signs of phagocytic activity (Fig. 2, *a*). They have some plasmalemmal microprocesses and well-developed elements of the Golgi system, where primary lysosomes are concentrated; no phagocytic vacuoles are detected.

Addition of SBL to BAL fluid caused:

- reduction of the level of non-phagocytic macrophages to 25%;
- activation of the cell surface, appearance of numerous microprocesses and pseudopodias, directed to the adjacent annular membranes of SBL, appearance of these membranes in phagosomal vacuoles (Fig. 2, *b*);
- stimulation of the lysosomal system in young and mature phagocytes: apearance of large (0.2 μ) phagolysosomes in the cytoplasm, which was not characteristic of the control group (Fig. 2, *c*).

In order to evaluate the persisting stimulatory effect of SBL on macrophages, we studied the phago-

cytosis of cells derived from *THP-1* 48 h after SBL stimulation. The level of cells with cytoplasm filled with latex increased to 14±0.6% (*vs.* 8.6±0.5% in the control).

In the control cells, latex beads were distributed mainly diffusely in the entire cytoplasm or formed clusters near the plasma membrane (Fig. 3, *a*, *b*). This was characteristic of large flattened cells and of small monocyte-like cells. After SBL stimulation of cells, latex particles usually formed groups and aggregations located in the perinuclear region and at the cytoplasm periphery or intensely filled the entire cytoplasm (Fig. 3, *c*, *d*). Phagocytic cells with processes were characterized by intense filling of the cytoplasm by latex beads along the entire length of processes (Fig. 3, *d*). This experiment showed that high phagocytic activity of macrophage cells persisted even 48 h after cell stimulation with SBL.

Hence, we have shown on the model of macrophages from *THP-1* human monocyte culture and on the BAL material from patients with pulmonary tuberculosis that PS being not the inductor of macrophage differentiation directly influences maturation and phagocytic activity of macrophage cells. Other authors also observed the effects of various PS preparations on macrophage differentiation on the models of blood monocytes [9] and normal animal alveolar macrophages [10].

Importantly, the stimulatory effect of PS on phagocytic activity of macrophage cells persisted even after SBL removal from the medium. The preparation stimulates phagocytosis in macrophages and the appearance of more numerous lysosomes in the macrophage cytoplasm and (which is particularly important) of phagolysosomes, that is, promotes more complete phagocytosis. This is confirmed by good results of PS use in phthisiological practice: addition of SBL to protocols of etiotropic therapy led to normalization of the lung macrophage formula [5] and to reduction of the bacterium isolation from patients with active tuberculous process [6]. Stimulation of phagosome fusion with lysosomes can be due to the presence of SP-D protein in SBL (this protein stimulates phagolysosome formation in human macrophage cells infected with *M. tuberculosis* [8]). Another apoprotein, SP-A, stimulates absorption of PS phospholipids by alveolar macrophages [7].

We think that the stimulatory effect of SBL on the function of the macrophage cells can be also due to improvement of the bioenergetics of cells utilizing PS material as the main substrate for oxidative phosphorylation [4] and as a membrane intermediate product for rapid enlargement of the plasmalemma surface and for formation of the lysosomal system.

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