CORRELATION OF ULTRASTRUCTURE OF THE AIR–BLOOD BARRIER AND SURFACTANT ACTIVITY

A. K. Zagorul'ko, A. A. Birkun,
G. V. Kobozev, and L. G. Safronova

UDC 616.24-002.2-092.9-092:612.215.8

KEY WORDS: air–blood barrier; surfactant; inflammation of the lungs.

There are as yet only solitary reports in the literature of changes in ultrastructure of the air–blood barrier (ABB) in the course of nonspecific inflammatory conditions of the lungs. Nevertheless, some publications contain evidence that in nonspecific inflammation of the lungs the lung surfactant system (LSS) undergoes considerable changes [1, 3, 6, 8], and the disturbances of its surface activity, according to the investigators cited, play an important role in the pathogenesis of diseases of this respiratory organ [2, 5].

The aim of this investigation was to study changes in the ultrastructure of ABB and to compare them with parameters of surface activity of LSS in the course of nonspecific experimental inflammation of the lungs.

EXPERIMENTAL METHOD

The lungs from 35 guinea pigs, in which inflammation was induced by the method in [4], served as the material for study. However, unlike in the original method, the sterile thread was passed beyond the bifurcation of the trachea into one of the main bronchi, so that an electron-microscopic investigation could be conducted and the state of the surface activity of LSS studied both on the side of the lesion and also in the contralateral lung. The animals were killed by decapitation under thiopental anesthesia 3 and 14 days, and 1, 2, and 4 months (five animals in each group) after introduction of the sterile thread. The control group consisted of five healthy guinea pigs, also killed by cacapitation. Pieces of lung for electron-microscopic study were fixed soon after decapitation in a 2.5% solution of glutaraldehyde in phosphate buffer (pH 7.2-7.4) and then postfixed in 1% OsO_4 solution. After dehydration in alcohols of increasing concentration and in absolute acetone the material was embedded in a mixture of epoxide resins: Epon-812–DDSA–MNA. Ultrathin sections were cut on an LKB Ultrotome, stained with uranyl acetate and lead citrate, and examined in the UÉMV-100K electron microscope. To study the surface-active properties of LSS and of 10% saline extracts of the lungs, the surface-active fraction [7] was isolated and its surface tension (ST) determined on Wilhelmy scales. The content of phospholipids of LSS and also in the animals' blood serum also was determined [9]. Phospholipids were separated into fractions by thin-layer chromatography on Silufol UV-254 plates (Czechoslovakia).

EXPERIMENTAL RESULTS

Histological investigations of the lung in whose main bronchus the thread was situated revealed signs of catarrhal bronchitis and bronchiolitis, interstitial and intra-alveolar edema, and serous, in places serofibrinous pneumonia as early as 3 days after its insertion. Later, in the affected lung, while the changes in the bronchi progressed with the formation of bronchiectases, areas of atelectasis and dystelectasis, emphysema, and pneumofibrosis could be identified. The latter appeared in the form of carnification and interstitial and peribronchial fibrosis.

The electron-microscopic investigations are evidence that 3 days after introduction of the thread, changes affecting all components of ABB developed in the affected lung. Marked congestion was observed in the pulmonary capillaries. The endothelial cells appeared swollen,

Department of Pathological Anatomy, Crimean Medical Institute, Simferopol'. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 617-621, November, 1988. Original article submitted February 20, 1988.

0007-4888/88/0011-1637$12.50

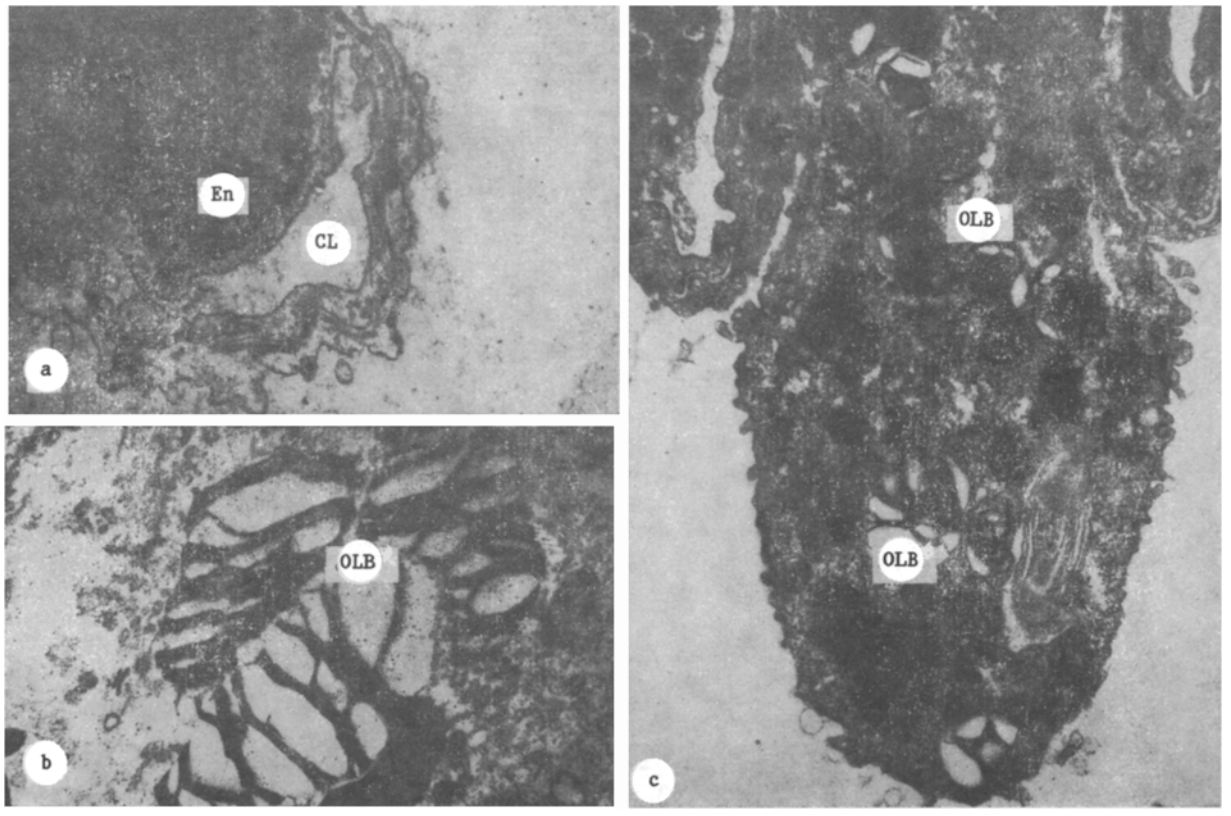

Fig. 1. Ultrastructural changes in ABB of affected lung 3 days after insertion of thread. a) Capillary lumen (CL) constricted by projecting endothelial cell (En); increased number of micropinocytotic vesicles in cytoplasmic processes of endothelium. 32,000 ×; b) vacuolation of OLB with appearance of translucent intervals of different sizes between layers. 40,000 ×; c) increase in number of OLB in cytoplasm of a type II alveolocyte; small immature OLB can be seen. 26,000 ×.

their volume being increased mainly on account of the central part, so that the lumen of the vessels became constricted (Fig. 1a). The number of vesicles in the cytoplasmic processes increased, and they frequently joined together to form large vacuoles. The basement membrane preserved its normal structure. Signs of edema and vacuolation also predominated in cells of the alveolar epithelium. In the cytoplasmic processes of the type I alveolocytes, besides an increase in the number of microvesicles, focal translucencies of the cytoplasm also were observed. The hyperhydration led to swelling of the mitochondria, which increased in volume and became round in shape; these changes were accompanied by shortening and swelling of their cristae. Besides changes in the mitochondria, in the type II alveolocytes dilatation of the tubules of the rough endoplasmic reticulum was observed due to the accumulation of edema fluid in them. Signs of vacuolation were reflected in the structure of the osmiophilic lamellar bodies (OLB), in which loosening of the lamellae took place, with widening of the translucent spaces between them (Fig. 1b). Meanwhile some changes in the ultrastructure of ABB were evidence of the active functioning of its components. Condensation of the nuclear chromatin, which was predominantly located at the periphery of the karyoplasm, a change in the configuration of nuclei of the endothelial and epithelial cells, which became lobular in shape, hypertrophy of the Golgi complex, and an increase in the number of mitochondria could be regarded as signs of enhanced functional activity of some of the cells composing the ABB. It is a highly significant fact that in some type II alveolocytes the number of OLB increased to 15-20 per cell (in the control they numbered 6-8 per cell). Besides mature forms small immature OLB also were observed (Fig. 1c). These changes were evidently aimed at maintaining the surface activity of LSS when reduced due to increased permeability of ABB. Reduction of the surface-active properties of LSS during this period is shown by the increase in ST_{min} of the surface-active fraction of the lung extracts to 17.0 ± 1.1 mN/m (12.4 ± 0.3 mN/m in the control; $p < 0.001$), with a simultaneous decrease in the phosphatidylcholine fraction, which has the strongest surface-active properties, in the composition of phospholipids (down to 23.9 ± 2.1% compared with 34.7 ± 2.9% in the control; $p < 0.001$).

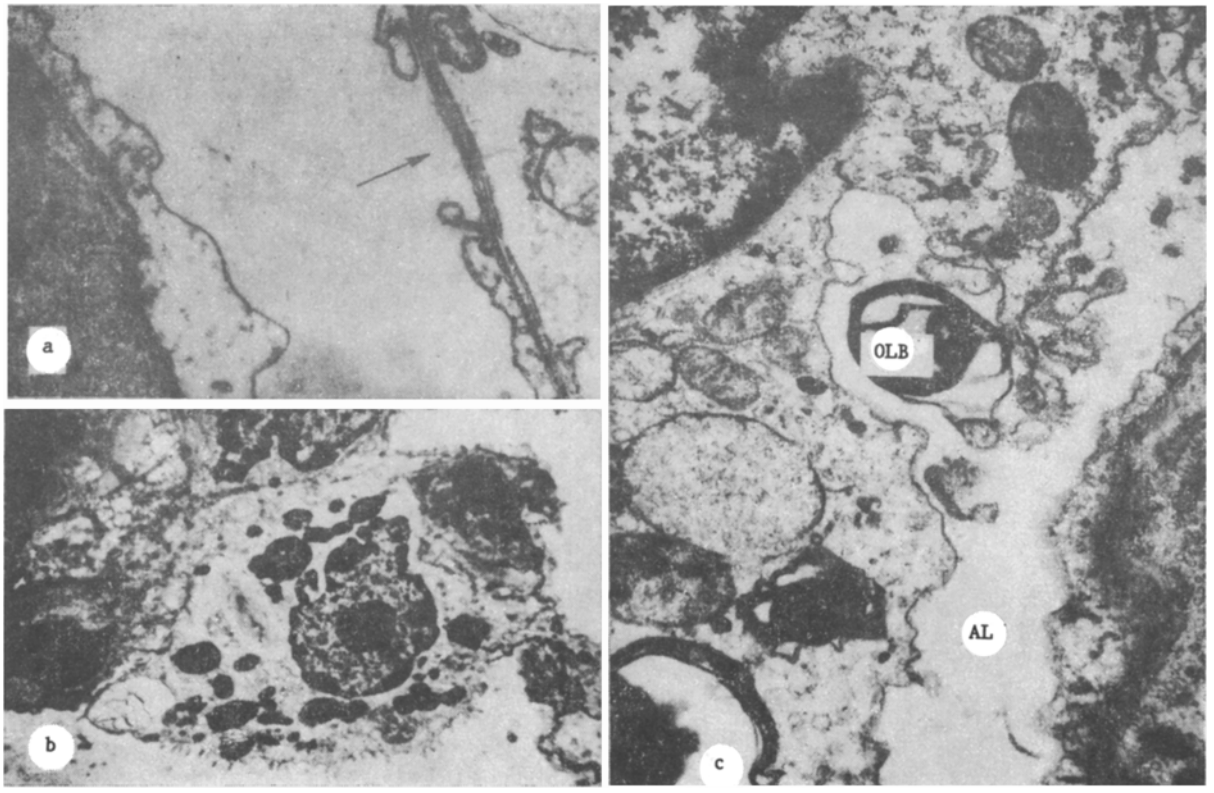

Fig. 2. Ultrastructural changes in ABB of affected lung 1 month after introduction of thread. a) Focal translucencies and ruptures of plasmalemma (arrow) of cytoplasmic processes of type I alveolocyte. 40,000 ×; b) homogeneous OLB in cytoplasm of type II alveolocyte. 16,000 ×; c) secretion of OLB by type II alveolocyte into alveolar lumen (AL). 36,000 ×.

Electron-microscopic investigation of fragments of the contralateral lung also indicated on increased permeability of ABB, although this was much less marked in degree; however, it was also reflected in the state of surface activity of LSS: ST_{min} was 16.1 ± 0.8 mN/m ($p < 0.01$) and the phosphatidylcholine concentration in fibrolipids of LSS was 23.1 ± 1.8% ($p < 0.001$).

Pronounced evidence of edema dominated the picture 14 days after the beginning of the experiment in the ultrastructure of ABB of the affected lung. The state of the surface activity of LSS during this period was characterized by high values of ST_{min} (20.7 ± 0.5 mN/m) and a low phospholipid level (0.023 ± 0.007 mmole/liter compared with 0.046 ± 0.007 mmole/liter in the control; $p < 0.05$), phosphatidylcholine accounting for only 22.9 ± 4.2% of the total. In the intact lung, however, despite less marked signs of edema, the surface-active properties of LSS also were characterized by high values of ST_{min} (22.2 ± 0.4 mN/m) and by a low phospholipid level (0.025 ± 0.007 mmole/liter). This state of affairs suggests that in the initial period of inflammation the entire system of LSS as a whole is involved in the response to damaging factors, and not merely that part of it which belongs to the affected lung.

Changes connected with disturbance of permeability of the vascular wall continued to progress in the components of ABB 1 month after insertion of the thread. Destructive processes developed in cells of the alveolar epithelium, involving primarily the type I alveolocytes, in whose cytoplasmic processes ruptures of the plasmalemma (Fig. 2a) and destruction of single remaining mitochondria were observed. Type II alveolocytes on the whole preserved their structure. Signs of edema in these cells extended mainly to OLB and mitochondria. However, it must be noted that besides vacuolated OLB and mitochondria, small homogeneous osmiophilic bodies and mitochondria with a dense matrix began to appear in the cytoplasm of the type II alveolocytes (Fig. 2b). Tubules of the rough endoplasmic reticulum in most cells were dilated and the Golgi complex hypertrophied. The number of microvilli on the apical surface of the cells was increased. The cells actively secreted osmiophilic material into the alveolar lumen (Fig. 1c), where numerous undeveloped OLB and fragmented osmiophilic lamellae were found. Meanwhile dystrophic changes were found in some type II alveolocytes. The number of OLB was

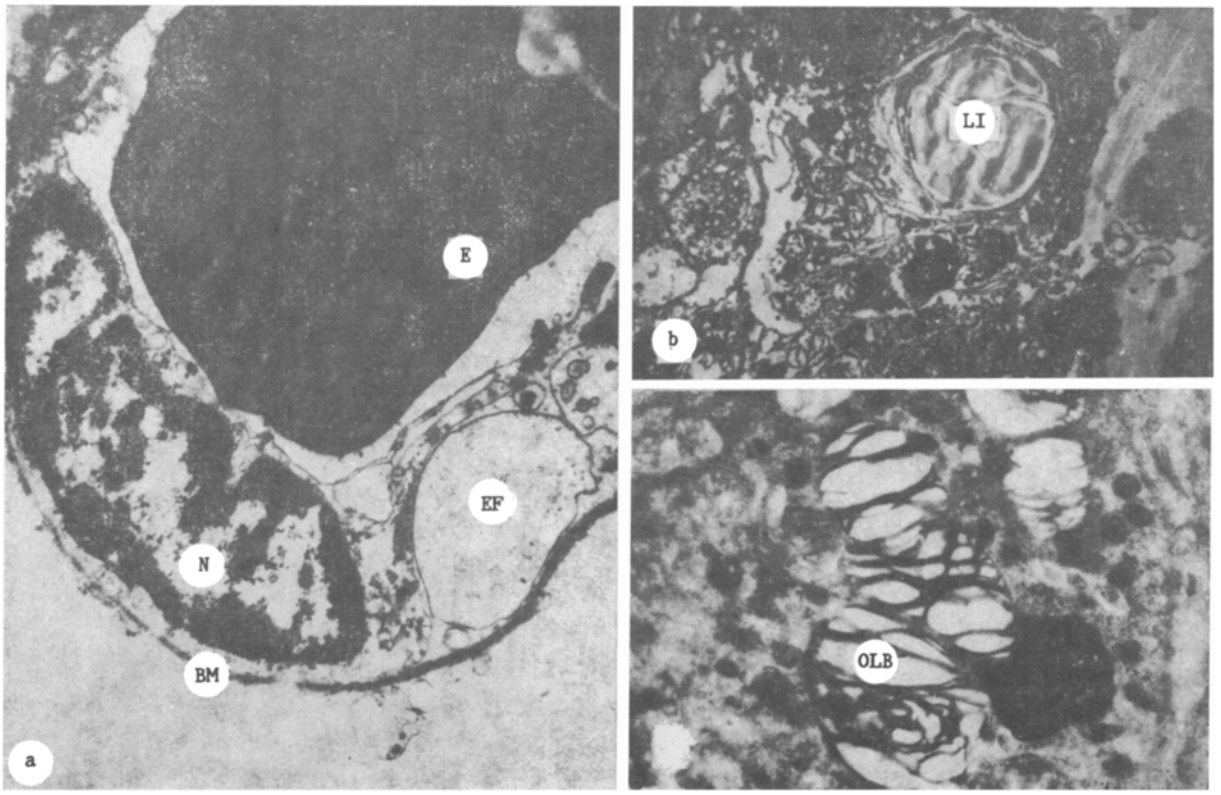

Fig. 3. Ultrastructural changes in ABB of affected lung 2 months after introduction of thread. a) Accumulation of edema fluid under endothelium with detachment of cell from basement membrane: E) erythrocyte in vessel lumen; N) nucleus of endothelial cell; EF) edema fluid beneath endothelium; BM) basement membrane. 32,000 ×; b) homogeneous lipid-like inclusions (LI) in cytoplasm of type II alveolocyte. 18,000 ×; c) both well formed, mature and small, immature, mainly homogeneous OLB can be seen in the cytoplasm of a type II alveolocyte. 40,000 ×.

sharply reduced in these cells and OLB with normal structure were almost absent: the overwhelming majority of mitochondria had a pale matrix and shortened, disoriented cristae. Many vacuoles of different sizes could be seen in the cytoplasm.

The surface active properties of LSS at this time were characterized by high values of ST_{min} (25.9 ± 0.4 mN/m) and a low phospholipid level (0.027 ± 0.010 mmole/liter); the fraction of phosphatidylcholine in their composition was sharply reduced (18.0 ± 3.1%). Reduction of the surface activity of LSS was evidently due, first, to the development of dystrophic and destructive processes in some of the type II alveolocytes, and second, to disturbance of the supply of phospholipids essential for LSS synthesis from the blood stream through the damaged ultrastructures of ABB. In the intact lung only indistinct signs of edema were observed on electron microscopy. Most of the type II alveolocytes responsible for LSS formation had a normal structure, but signs of increased functional activity were observed in some of them.

In the next period of the experiment (2 months) changes leading to disturbance of function of the alveolar–capillary barrier continued to progress in the ultrastructure of ABB of the affected lung. In the endothelial cells ruptures of the plasmalemma were observed in some places. Edema fluid also accumulated under the endothelium, causing detachment of the cells from the basement membrane (Fig. 3a), which appeared thinner than normal and interrupted. The intermembranous space was greatly widened and edematous, and depolymerization and loosening of the fibrous structures were found in it. During this period phosphatidylcholine accounted for only 14.3 ± 2.7% of the phospholipids of LSS, and the fraction of phosphatidylethanolamine, which is inactive from the surfactant point of view, was increased from 32.5 ± 6.4% in the control to 37.7 ± 10.4% ($p < 0.05$), although on the whole the phospholipid content in the composition of LSS was increased to 0.037 ± 0.003 mmole/liter. The increase in the phospholipid content taking place under these circumstances in the animals' blood was accompanied by a tendency toward normalization of the surfactant properties of LSS in the contralateral lung,

most of the type II alveolocytes of which were in a state of enhanced functional activity and contained from 8 to 12 well developed OLB in their cytoplasm. ST_{min} in the intact lung was reduced to 15.1 ± 0.3 mN/m, whereas the level of phospholipids with a high phosphatidylcholine content (30.7 ± 6.4%) was increased up to 0.042 ± 0.008 mmole/liter.

This tendency still remained 4 months after the beginning of the experiment. Values of ST_{min} of the surface-active fraction of the contralateral lung approximated to the control level (11.9 ± 0.9 mN/m). Ultrastructural changes on the side of the lesion during this period were characterized by polymorphism. Besides the changes described above, large homogeneous lipid-like inclusions with average electron density appeared in the cytoplasm of the type II alveolocytes (Fig. 3b), evidence of a switch by these cells to synthesis of neutral lipids. In other areas of the lung increased activity of the components of ABB was observed and was expressed, in particular, by intensification of micropinocytosis in the endothelial cells and the preserved type I alveolocytes, and by intensification of the formation and maturation of OLB in the type II alveolocytes (Fig. 3c). This polymorphism is reflected in the state of the surface activity of LSS. The surface-active fraction as before possessed high ST (19.2 ± 0.4 mN/m), and the phosphatidylcholine fraction accounted for only 19.3 ± 3.6% of the total phospholipids of LSS, the content of which rose to 0.038 ± 0.002 mmole/liter. The low level of surface activity of LSS, in whose composition the phosphatidylcholine fraction was reduced, could be a factor with an unfavorable influence on the subsequent course of the disease.

It can thus be concluded from the results that changes whose initial phase is edema of the ultrastructure of its components develop in ABB of the affected lung in a model of chronic inflammation. Later, dystrophic and destructive changes arise in some areas of the lung and lead to death of the components of ABB and to the development of an alveolar–capillary block, whereas in other areas, on the contrary, processes of a compensatory and adaptive character increase in intensity, and are primarily aimed at making good the deficit of LSS thus produced. Under these circumstances in the initial periods of the experiment (3 days to 1 month) reduction of the surface activity of LSS was observed both on the side of the lesion and also in the intact lung, evidence that the LSS reacts as a whole to unfavorable influences. In the later stages (2-4 months) the surface-active properties of LSS in the contralateral lung returned to normal, but on the side of the lesion the level of surface activity of LSS was determined, on the one hand, by the extent of spread and the severity of the dystrophic and destructive changes and, on the other hand, by the level of functional activity of the residual type II alveolocytes, responsible for formation of the LSS.

LITERATURE CITED

1. L. Kh. Adil'gireeva, V. P. Verbolovich, E. P. Petrenko, and P. L. Teplova, "Morphochemical characteristics of surfactant in chronic nonspecific lung diseases," Abstract Lodged with the All-Union Institute of Scientific and Technical Information, No. 4365-84, dated June 27, 1984.
2. T. A. Gevondyan, P. A. Kazaryan, A. G. Allaverdyan, and P. S. Simavoryan, Morphological Aspects of Defensive Mechanisms in Tuberculosis and Nonspecific Pathology of the Lungs [in Russian], Moscow (1985), pp. 99-102.
3. T. V. Kotenko, N. N. Zybina, G. K. Kiryanina, and O. V. Zubtsovskaya, Lung Surfactants under Normal and Pathological Conditions [in Russian], Kiev (1983), pp. 134-137.
4. E. N. Nesterov, "Problems in the structural restoration of the lungs and of cor pulmonale in chronic lung diseases," Author's abstract of dissertation for the degree of Doctor of Medical Sciences, Simferopol' (1970).
5. E. N. Nesterov, G. V. Kobozev, L. G. Safronova, and V. A. Sheleg, Vrach. Delo, No. 10, 49 (1983).
6. O. I. Shpak, L. V. Rolik, V. P. Kuprash, et al., Bronchological Methods in the Diagnosis of Lung Diseases [in Russian], Leningrad (1984), pp. 56-59.
7. M. Abrams and F. Taylor, J. Appl. Physiol., 21, 718 (1966).
8. A. Baskerville, A. Dowlett, and M. Baskerville, Lab. Anim., 16, 351 (1982).
9. D. Fiske and V. Subbarow, J. Biol. Chem., 226, 486 (1957).