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ULTRASTRUCTURAL CHANGES IN THE LUNGS DURING EXPERIMENTAL INFECTION BY *Mycoplasma pneumoniae*

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The important role of *Mycoplasma pneumoniae* in the etiology of acute respiratory diseases and pneumonias in children and adults has now been explained [4, 8, 11]. Morphological investigations have demonstrated the particular features of *M. pneumoniae* infection in man and in experimental infection of animals [1, 2, 5-7, 10, 14]. Meanwhile, very few investigations have so far been devoted to the analysis of fine structural changes in the trachea and lungs in mycoplasmosis. Some of them were undertaken on organ cultures of the trachea of Syrian hamsters [1, 9, 13]. It has been shown that *M. pneumoniae* causes metabolic and ultrastructural changes in cells of the ciliated epithelium, leading to ciliostasis and death of the cells. It has also been found that the ATP and RNA content is reduced in tracheal explants exposed to the action of *M. pneumoniae* cells or their membranes [12]. Electron-microscopic studies of the lungs of Syrian hamsters infected intranasally with a culture of *M. pneumoniae* have shown adhesion of mycoplasmas to the surface of the cells of the ciliated epithelium of the bronchi and their ingestion by alveolar macrophages [3, 10, 15].

The object of this investigation was to make a dynamic study of interaction between mycoplasmas and lung cells in experimental aerosol infection (i.e., under conditions close to those of natural infection), in an inhalation chamber, with a culture of *M. pneumoniae*.

EXPERIMENTAL METHOD

The experimental material consisted of lungs of 36 female Syrian hamsters weighing 60-80 g, infected in an inhalation chamber with a culture of *M. pneumoniae* (titer 10^8-10^9 CFU/ml), in a dose of 0.1 ml per animal

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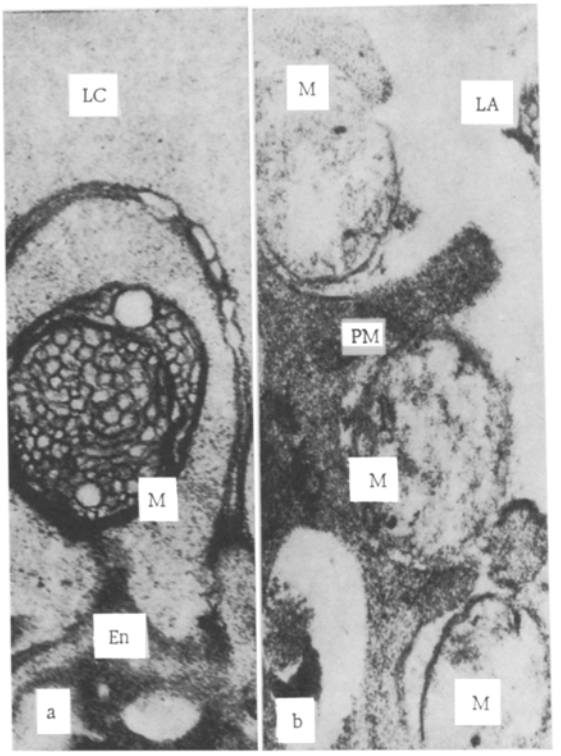

Fig. 1

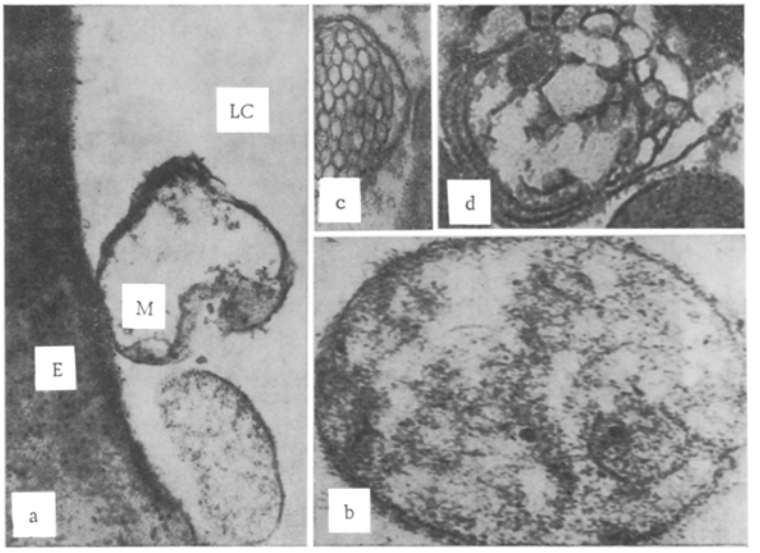

Fig. 2

Fig. 1. Phagocytosis of mycoplasmas by outgrowth of endothelium (a) and processes of macrophage (b). M) Mycoplasma; PM) processes of macrophage; LA) lumen of alveolus; LC) lumen of capillary; E) erythrocyte; En) endothelium. Magnification: a) 20,000, b) 25,000×.

Fig. 2. Changes in ultrastructure of mycoplasmas during interaction with the host (a, c, d) and mycoplasma with ordinary structure (b). Legend as in Fig. 1. Magnification: a) 15,000, b) 25,000, c) 15,000, d) 50,000×.

for 30 min.* The experimental animals were killed under pentobarbital anesthesia at intervals: from 1 h to 14 days after infection. Pieces of lungs for electron-microscopic investigation were fixed in 2.5% glutaraldehyde solution and postfixed in 2% OsO_4. After dehydration the material was embedded in a mixture of Epon and Araldite. Sections were cut on the LKB Ultramikrotome and examined in the JEM-100B electron microscope. Material for light microscopy was fixed in Carnoy's fluid and, after dehydration, it was embedded in paraffin wax. The paraffin sections were stained with hematoxylin-eosin and by the PAS reaction.

EXPERIMENTAL RESULTS

Analysis of the material showed that in Syrian hamsters infected with mycoplasmas the pathological process in the lungs began with a marked vascular reaction. Under the light microscope, 1 h after introduction of the culture of M. pneumoniae, perivascular edema and swelling of the endothelium of the blood vessels were observed in the lungs of the experimental animals; later destruction of the endothelium took place, the vessel wall swelled until it became fibrinoid, and PAS-positive material accumulated in it. The blood vessels were congested and signs of intravascular hemolysis and thrombus formation were observed in them. Electron-microscopic investigation revealed swelling of the capillary endothelium with an increase in the number of pinocytotic vesicles in it.

The localization of the mycoplasmas in the blood vessels was very characteristic, for they were found in the immediate neighborhood of erythrocytes and leukocytes (Fig. 2a). The discovery of free-lying mycoplasmas in the lumen of a blood vessel confirms the possibility of blood-borne dissemination. Phagocytosis of

*The experiments were conducted jointly with workers at the Laboratory of Epidemiological Immunology (Head, Professor V. I. Vasil'eva), N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

mycoplasmas by endothelial cells on the viropexis principle was discovered, when the microorganism was ingested by endothelial processes (Fig. 1a).

The reaction of the interstitial tissue of the lungs developed very quickly. On the 2nd day after infection with M. pneumoniae a picture of interstitial and sero-desquamative pneumonia with a hemorrhagic component was diagnosed. Numerous, mainly peribronchial and perivascular, foci of infiltration with lymphoid cells and plasmablasts, foci of infiltration of the granuloma type composed of histiocytes, localized thickenings of the alveolar septa, desquamation of alveolocytes, considerable hemorrhages, and groups of alveoli whose lumen was filled with serous fluid and desquamated alveolocytes, were observed. In the PAS reaction, PAS-positive granules of M. pneumoniae antigen were detected in the desquamated alveolocytes, in the alveolar septa, and extracellularly in the lumen of the alveoli.

Type II alveolocytes were characterized by swelling of the mitochondria and destruction of osmiophilic bodies. Surfactant material and remnants of destroyed cells were observed in the lumen of the alveoli.

The number of free lung macrophages was considerably increased. These macrophages had many processes by means of which they ingested mycoplasmas (Fig. 1b); phagosomes with remnants of destroyed mycoplasmas were visible in some of them.

Changes in the mycoplasmas during interaction with the host, observed in this investigation, were particularly interesting. Besides microorganisms with normal structure (Fig. 2b), mycoplasmas with structural changes also were observed (Fig. 2a, c, d): They became translucent, vacuolar structures were formed in them, and these gradually filled the whole cavity of the microorganism. The membranes of the mycoplasmas also underwent vacuolar degeneration. Later the changes could proceed in two directions: coarsening of the walls of the vesicles and membranes with the formation of lamellar bodies, or destruction of the membrane, with fragmentation of the mycoplasma.

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