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Detection of *Chlamydophila pneumoniae* in Mouse Respiratory Ciliated Epithelium Using Targeted Sections of the Lung Tissue

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Chlamiophila pneumoniae were detected in targeted sections of mouse lung tissue by means of transmission electron microscopy and immunofluorescent staining. Incorporation of microorganisms into the axonemal matrix of cilia was observed 24 h after infection. The ciliary axoneme was characterized by pronounced swelling. At the late stages *Chlamiophila pneumoniae* were present in cytoplasmic vacuoles. Structural abnormalities and dysfunction of mucociliary clearance followed by incorporation of *Chlamiophila pneumoniae* into the cytoplasm of epitheliocytes were revealed in the early stage of infection. The proposed method allows studying the very early events of *Chlamiophila pneumoniae* infection.

Key Words: *targeted lung sections; Krumdieck microtome; cilia; Chlamydophila pneumoniae*

Mucociliary clearance is a complex physiological mechanism. Cilia in epitheliocytes play an important role in elimination of infectious agents (*e.g.*, viruses and bacteria) [3,5]. Functional disturbances in cilia are associated with various factors [4,7]. Inflammation resulting from viral or bacterial infection is followed by damage to cilia and reduction in beating rate. *Chlamiophila pneumoniae* are gram-negative bacteria causing various lung diseases. *Ch. pneumoniae* can *in vitro* infect epitheliocytes [1,10,11]. C. L. Krumdieck *et al.* [6] proposed a method of preparation and maintenance of viable tissue cultures. Cultured lung tissues can be studied under different conditions using targeted sections (TS). In thin sections of the lung tissue, vibration of ciliated cells is preserved. Therefore, this method allows studying functional state of cells [2,8]. The effect of bacterial infection on the ciliary apparatus of bronchi in thin TS remains unknown.

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In the present work the mechanisms of mucociliary clearance and the effect of bacterial infection were studied in thin TS of lungs from laboratory mice *in vitro* infected with *Ch. pneumoniae*.

MATERIALS AND METHODS

Experiments were performed on 3-4-month-old BALB/c and apoprotein E-deficient female mice obtained from the Medical School University (Lubbock, Dr. H. Kothe). The animals were euthanized. The lungs were filled with warm 0.75% agarose (37°C, Sigma), which was delivered through a cannula implanted into the trachea. Lungs samples were cooled on ice, the lobes were isolated, and segments (2×2 cm) were excised.

TS (200 μ) were prepared on a Krumdiek microtome (Alabama Research and Development) and placed in a serum-free medium for cell culturing (Promo Cell). Tissue samples were placed in 24 wells and incubated for no more than 96 h. The medium was refreshed at 30-min intervals over the first 2 h and at

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1-h intervals over the next 2 h to wash out agarose from the air passages. The sections were incubated in 24-well plates at 37°C. TS were examined under an ITM inverted microscope (Olympus).

Sections were infected with *Ch. pneumoniae* CWL (solution of microorganisms in dilutions of 1:1 and 1:2). TS were removed from the medium, fixed in glutaraldehyde, and embedded into Epon 1, 2, 4, 8, 16, 24, 48, 72, and 96 h after the start of the experiments. Semithin sections for orientation were obtained using a Reichert OmU3 microtome. Ultrathin sections were prepared from the selected samples and examined under a Zeiss EM10 electron microscope.

Ch. pneumoniae were detected using antiserum against *Chlamydia* spp. lipopolysaccharide (BioDesign). Antibodies were used in different concentrations to achieve optimum results. The best results were obtained at serum dilution of 1:100 (1:200 for *Ch. pneumoniae* antiserum). Sections were incubated with primary (2 h) and secondary antibodies labeled with trimethylrhodamine B isothiocyanate (1:50, 2 h). Stained sections were examined under a Leica confocal laser microscope and Olympus immunofluorescence microscope.

Non-infected sections obtained in the same period served as the control.

RESULTS

The lung tissue preserved integrity for at least 72 h after the start of culturing. After 24-h incubation we observed focal lysis of mitochondrial crypts and smoothening of the granular endoplasmic reticulum. Culturing was accompanied by thickening of interalveolar membranes and development of degenerative changes in type 2 alveolar cells. The loss of cilia was observed after 48-72-h incubation. Cilia of epitheliocytes retained their normal arrangement (9+2). The outer and

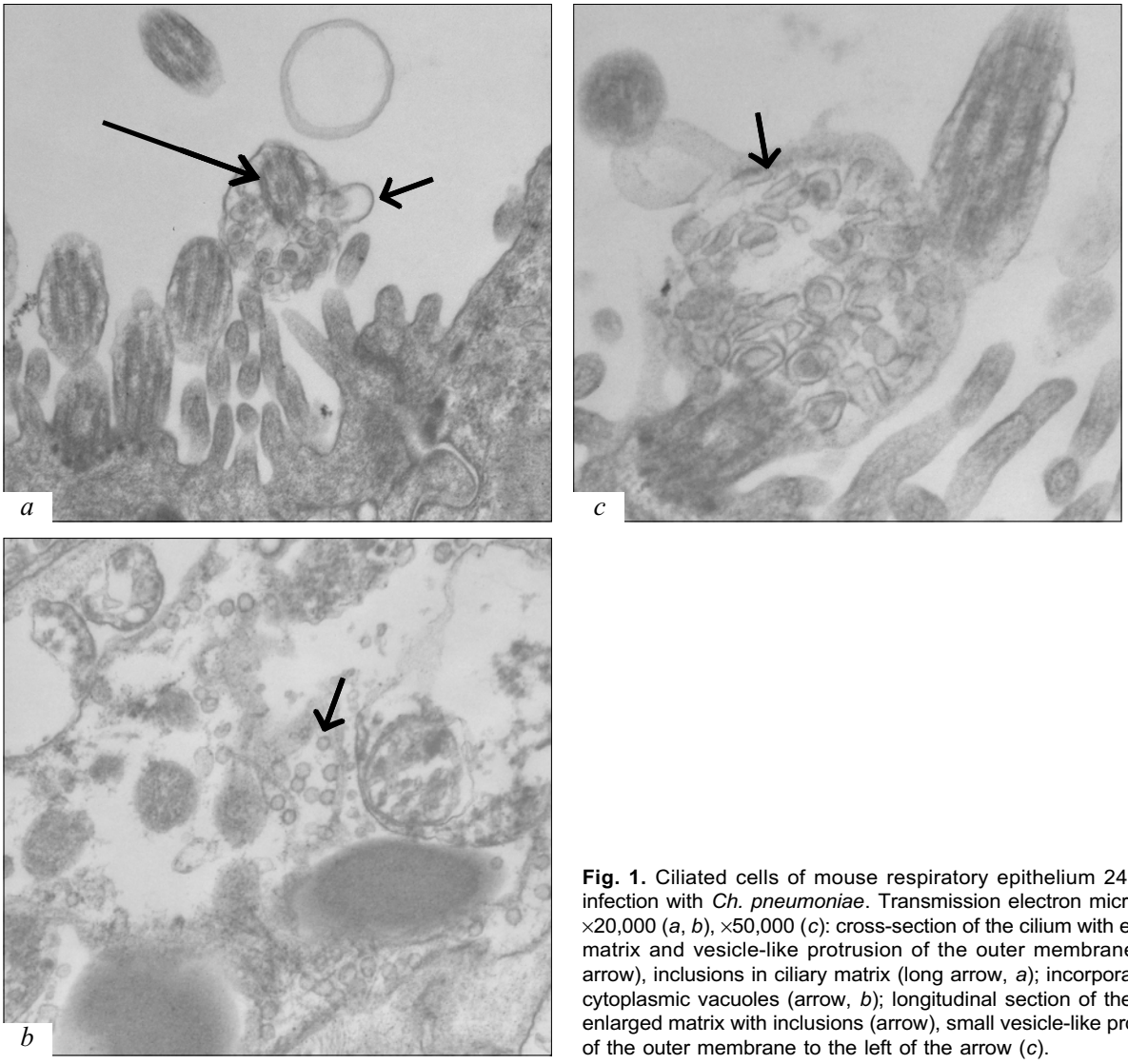

Fig. 1. Ciliated cells of mouse respiratory epithelium 24 h after infection with *Ch. pneumoniae*. Transmission electron microscopy; ×20,000 (*a*, *b*), ×50,000 (*c*): cross-section of the cilium with enlarged matrix and vesicle-like protrusion of the outer membrane (short arrow), inclusions in ciliary matrix (long arrow, *a*); incorporations in cytoplasmic vacuoles (arrow, *b*); longitudinal section of the cilium, enlarged matrix with inclusions (arrow), small vesicle-like protrusion of the outer membrane to the left of the arrow (*c*).

inner denein projections and radial spokes remained unchanged. The tissue underwent autolysis after 72-96-h incubation.

Similar changes were observed in samples of infected tissues. After 24-h incubation the axonemal matrix in individual cilia from apoprotein E-deficient mice contained rounded electronically dense particles. The ciliary matrix was partially enlarged in damaged regions (Fig. 1). The outer membrane of cilia was intact. No changes were found in the structure of outer and inner denein projections. Radial spokes and central tubules retained their normal position. The basal apparatus of cilia had no lesions associated with incorporations. We did not reveal lesions of cilia adjacent to damaged structures. Incorporations disappeared at later stages of infection. Reticular bodies were detected in perinuclear vacuoles of the cytoplasm.

Immunofluorescence assay showed that *Ch. pneumoniae* appeared as green incorporations in the cytoplasm of epitheliocytes near to the cell nucleus.

Ch. pneumoniae are gram-negative bacteria detected during various diseases, including atherosclerosis and pulmonary emphysema [10]. These microorganisms are characterized by specific cycle of development [9]. Elementary bodies are incorporated into cells by endocytosis. In the cytoplasm elementary bodies are transformed into reticular bodies, which can be detected in perinuclear vacuoles. Changes in the lungs at the early stage of infection are poorly known. The method of TS allows us to study these processes. Our results show that *Ch. pneumoniae* are detected in the ciliary matrix in certain periods of infection. Changes in infected cilia probably impair their functions and modulate vibration of adjacent cilia, which affects the intensity of mucociliary clearance. The loss of this protective barrier allows *Ch. pneumoniae* to reach the cell membrane and penetrate into the cell.

Early incorporations of microorganisms into cilia can play a role in the pathogenesis of chlamydial infection [2,8].

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