ULTRASTRUCTURAL CHANGES IN THE ALVEOLAR EPITHELIUM IN RESPONSE TO MYCOBACTERIAL INFECTION*

Koomi Kanai, Eiko Kondo** and Tomoyoshi Yasuda***

The First Department of Bacteriology, **Department of Tuberculosis, and ***Laboratory of Technology, National Institute of Health, Kamiosaki, Tokyo 141

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SUMMARY: Electron microscopic examination of the lung of mice infected with a virulent strain of M. bovis (Ravenel) revealed marked alterations in the alveolar epithelial cells, particularly Type 2 cells (granular pneumocytes), in addition to the development of interstitial and intra-alveolar granuloma. Unlike the feature in uninfected mice, more than one Type 2 epithelial cells were often found adjacent to one another within a single alveolus. Some of these cells showed mitotic figures. Their characteristic lamellar inclusions were morphologically altered.

INTRODUCTION

Mice are the experimental animal of the most frequent use for tuberculosis research. Intravenous or air-borne infection causes disseminated granulomata, most prominently in the lungs. However, little is known about the changes in the fine structure of the lung tissue responding to infection and about ultrastructural morphology of infecting mycobacteria. Only a limited amount of information can be obtained from the papers of Brieger and Glauert (1954) and Merckx, Brown and Karlson (1964).

This situation, as well as our interest in the pathogenesis of tuberculous infection, led us to an attempt to see the cellular response to infection in the mouse lungs and the intracellular habitat of tubercle bacilli at an ultrastructural level. This paper is to describe the response of the alveolar epithelium to tuberculous infection.

MATERIALS AND METHODS

Infection: Mice were infected intravenously with 0.5 mg of a kanamycin-resistant strain of Mycobacterium bovis Ravenel strain originating from TCT...
401 (Trudeau Mycobacterial Culture Collection) grown on Sauton synthetic liquid medium for 2 weeks. Bacterial suspensions were prepared as described previously (Kondo and Kanai, 1976).

Experimental animals: Albino male mice of ddY strain, weighing around 20 g, were used.

Electron microscopy: Lung tissue specimens were prepared from normal mice and those infected 3 to 4 weeks previously. The mice were killed by iv injection of a 1:1 mixture of 2.5% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 (Karnovsky, 1965). The lungs were removed and minced into small cubes to be prefixed with the above mixture at 37 C for 2 hr. After postfixation in cold 1% OsO4 in the same buffer, the tissue was stained with 0.5% uranyl acetate in acetate-veronal buffer, pH 5.0. They were then dehydrated in graded ethanol and embedded in Epon 812. Although it is known that glutaraldehyde employed here can kill tubercle bacilli in specimens within 30 min (Armstrong and Hart, 1971), the procedure upto this step was carried out in a hood placed in a bacteriology laboratory.

Ultrathin sections of lung tissues were cut on an LKB ultratome 8800 and stained with uranyl acetate (Watson, 1958) followed by lead acetate (Venable and Coggeshall, 1965). The sections were observed with a Hitachi H-500 electron microscope at 75 kv.

Results

In Normal Mice

Figures 1 and 2 show pictures of the typical alveolar septum in normal mice. It consists of the inner alveolar lining, pulmonary capillaries and the interstitial connective tissue space.

In the central part of Fig. 1, there is a pulmonary capillary between two alveoli (ALV). In its lumen (CL), a polymorphonuclear leukocyte (PNL) with many electron-dense lysosomal granules and red blood cells (RC) are present. In the upper right, there is another capillary in which the nucleus of an endothelial cell (EC) is seen. Between these two capillaries, a Type 1 epithelial cell (EP1) (squamous alveolar cell) is resting on a thin basal lamina. This large cell contains scanty cytoplasmic structure in marked contrast to Type 2 cell (EP2) as seen in Fig. 2. In the lower left of Fig. 1, a mononuclear phagocyte (MP) is seen on the alveolar surface which appears ingesting a filamentous material by surrounding it with pseudopods (pc).

In Fig. 2, a Type 2 epithelial cell (granular pneumocyte) with a large rounded nucleus is present in their usual location, the corner of alveoli, showing its cuboidal shape. The cytoplasm contains many well-developed mitochondria and lamellated inclusions (LI) as the most characteristic appearance. Relatively uniform microvilli are seen along the free border facing to alveolus. This epithelial cell is resting on the basal lamina (BL). In the right upper, there
is a fibroblast (FB) with cytoplasmic strands and lipoidal droplets (LD) in the interstitial connective tissue space. In this area, we see collagenous fibrils and the elastic fibers in amorphous appearance. In the upper left, there are another location of cross- or longitudinally-sectioned collagenous fibrils (CF) and the elastic fibers (EF).

Figure 3 shows a fibroblast with a deeply lobed nucleus. There are some osmophilic lamellar inclusions in the cytoplasm. However, their appearances differ from those we see in Type 2 cells.

In Infected Mice

In the infected mice, granulomatous lesions were so extensive as to fill up most of alveolar sacs. Cell accumulation was remarkable in the interstitial space. The fine structure of these lesions will be described in the succeeding paper. On the other hand, such changes were observed also in the alveolar epithelial cells, though the bacilli were not found there.
Fig. 2. Type 2 epithelial cell and other components of the alveolar septum in the normal mouse. EP2, Type 2 epithelial cell; ALV, alveolar space; CL, capillary lumen; BL, basal lamina; LI, lamellar inclusion; FB, fibroblast; LD, lipoidal droplet; CF, collagenous fibrils; EF, elastic fibers; PNL, polymorphonuclear leukocytes; RC, red blood cell.

In Fig. 4, there are two alveolar spaces, one in the upper right and the other in the lower. The upper one is filled with the exudate in which a polymorphonuclear leukocyte is seen. The surface of the lower alveolar space is overlaid with a small amount of the exudate.

In the center, there are two Type 2 epithelial cells. They are separated at least in this plane of section by the cytoplasmic strand of a fibroblast locating in the upper left. The cells found in the lower left can not be identified but tentatively designated as intermediate epithelial cells (IC). The presence of the above various cells, adjacent to one another making a mutual close contact, was an unusual feature not found in normal mice. The shape of lamellar inclusions in Type 2 cells is not so typical but rather abnormal.

In Fig. 5, we find two Type 2 cells recognized by the presence of lamellar inclusions. Between them, a cell is located adjacent to the capillary (lower right). This cell, though lacking in lamellar bodies, does not appear to be Type 1 cell because of the presence of abundant mitochondria. It might be corresponding to the intermediate epithelial cell proposed by Adamson and Bowden (1974) as the transformation from Type 2 to Type 1 for alveolar epithelial regeneration. If it is so, it follows that Fig. 5 shows the side-by-side
accumulation of Type 2 cells and their decendant. Such accumulation of Type 2 cells within a single alveolus is so common in infected mice and has never been observed in normal mice. A similar situation has been reported by Faulkner and Esterly (1971) in rabbits injected intravenously with complete Freund's adjuvant.

In agreement with the above statement, we found mitotic figures in Type 2 cells (Figs. 5 and 6) as indicated by the absence of the nuclear membrane and the condensed chromosomes (NC).

Figure 7A-F show examples of altered features of lamellar bodies in infected mice in comparison with those in normal mice.

Figure 8 shows two macrophages in the alveolar space filled with exudate. They are ingesting many lamellar inclusions and tubular myelin figures. In the left one, two cross-sectioned mycobacteria are seen. These macrophages are partly in close contact showing interdigitation of their surface membrane, but, in their free border, microvilli are extending free into the exudate. On the other hand, the small but uniform microvilli of EP2 are separated from the exudate and also from the surface of the right macrophage by a clear electron-transparent space suggesting the presence of hydrophobic substance(s) around the microvilli.
DISCUSSION

The present study demonstrated clearly that marked changes are brought about in the alveolar epithelium, particularly in Type 2 cells, by severe tuberculous infection. It was unusual that more than one Type 2 cell were often adjacent to one another in a single alveolus, some of which showed mitotic figures. Their characteristic lamellar inclusions were frequently of abnormal shape.

Cytodynamics of pulmonary alveolar cells in the mouse have been studied by Bowden (1968), Adamson and Bowden (1974) and Aronson, Johns and Pietra (1976) by various techniques including autoradiography after tritiated thymidine and mitotic arrest with colchicine. Adamson and Bowden (1974) stated that the changing pattern of nuclear labeling and the presence of cellular forms intermediate between Type 2 and Type 1 after exposure to a high concentration of oxygen suggest replacement of damaged Type 1 cells by proliferation of Type 2 cells which would be subsequently transformed into the squamous type 1 cell. Aronson et al. (1976) found the nuclear labeling only in Type 2 cells and never in Type 1 cells.
The proliferation of Type 2 cells was also observed by Faulkner and Esterly (1971) in the rabbits injected intravenously with complete (mycobacteria-containing) Freunds' adjuvant. Type 2 cells became numerous so that they were often found adjacent to one another within a single alveolus. These observations are in good agreement with those in our present study. We could not find any direct morphological association of infecting bacilli with pulmonary alveolar cells, but the infection-induced changes in Type 2 cells were remarkable. The fact that we encountered occasionally mitotic figures of Type 2 cells would be a strong evidence to prove that this type of cells can proliferate in response to mycobacterial infection. However, we could not see to what extent the damage of Type 1 cells was present as a possible prelude to the proliferation of Type 2 cells.

The hypothesis that Type 2 cells are the major source of pulmonary surfactant and their lamellar bodies are involved in secretion and storage of phospholipids of the surfactant lipoprotein complex are now gaining an increasing amount of evidences through cytochemical and biochemical approaches (Askin and Kuhn, 1971; Kikkawa et al., 1975; Engle, Sanders and Longmore, 1976). The changes in lamellar inclusions as we observed in the mycobacteria-infected mouse lungs are striking. However, we do not know whether these
changes are specific to mycobacterial infection or of a nonspecific response to inflammatory event in general in the lung.

Finally, Fig. 8 is interesting because of the same finding of Nichols (1976) that alveolar macrophages ingest lamellar inclusions of Type 2 cell origin and tubular myelin which are the constituents of the intraalveolar lining layer. The features of tubular myelin figures as observed here are exactly the same as those of the materials separated from lavages of rat lungs by other authors (Katyal, Esters and Lombardi, 1977).

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Fig. 6. Type 2 epithelial cell in infected mice. A cell showing mitotic figure may be an intermediate type of epithelial cell (IC). EP2, Type 2 epithelial cell; NC, nuclear chromatin; ALV, alveolar space; CL, capillary lumen; FB, fibroblast.

Fig. 7A–F. Comparison of the morphology of lamellar inclusions between normal and infected mice in the same magnification. A, B, C are from normal mice, and D, E, and F are from infected mice. (see p. 323)
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Fig. 8. Infected alveolar macrophages ingesting lamellar inclusions and tubular myelin figures. Two macrophages (AM) are adjacent with interdigititation (ID) of their surface membranes in the alveolus (ALV) filled with exudate (Exu). EP2, Type 2 epithelial cell; Tmf, tubular myelin figure; LI, lamellar inclusion; MB, mycobacteria; small single and double arrows show microvilli of macrophages and EP2, respectively.

REFERENCES


