A New Myelin–like Laminated Body Found in Two Cases of Pulmonary Alveolar Proteinosis

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SATO, S. and TAKISHIMA, T. A New Myelin-like Laminated Body Found in Two Cases of Pulmonary Alveolar Proteinosis. Tohoku J. exp. Med., 1978, 126 (3), 257-266 — Electron microscopic observations were made on lung tissue, broncho-pulmonary lavage fluid and sputum in 2 cases of pulmonary alveolar proteinosis. Diagnosis was established by open lung biopsy in both cases. In Case 1 the electron microscopic samples were obtained from the surgical specimen. In Case 2 an electron microscopic observation was made only on the lavage fluid and sputum. The alveolar air spaces were full of amorphous or fine granular-appearing material intermingled with two kinds of laminated bodies (abbreviated to “Lamella A” and “Lamella B”). Lamella A, about 0.2~4 μm in diameter, presented a concentric laminated structure with approximately 300 Å periodicity and was found in the alveolar air spaces, lung washings and sputum. It corresponded to what has hitherto been reported in the same disease and was always located outside the cells. Lamella B showed a laminated structure of high electron density, approximately 50 Å in periodicity, usually looking like a concentric circle with a diameter of 0.5 μm or less. It was intensely stained with methanamine silver. It was mainly observed in mesenchymal cells of the alveolar septa and free macrophages in the alveolar air spaces, although it also occurred in the type I and type II alveolar epithelial cells. Furthermore, the Lamella B could be found in the alveolar air spaces outside the cells and in the bronchopulmonary lavage fluid. This body has not been demonstrated previously and, although its significance in this disease is still uncertain, the structural similarity of the Lamella B to the intra-alveolar material suggests a possible role of it in the production of intra-alveolar material. —— pulmonary alveolar proteinosis; laminated body; ultrastructure

Rosen et al. (1958) described a disease entity characterized by the accumulation of proteinaceous material within the alveolar air space, which they termed pulmonary alveolar proteinosis. The etiology of this disease is unknown, and since the original description, the origin of the intra-alveolar material has been discussed extensively. Rosen et al. (1958) proposed that this material accumulated as a result of the alteration and degeneration of the alveolar lining cells, but others believed that it was derived from passive transudation of plasma constituents (Taxay et al. 1960; Stansier and Bourgeois 1965). Some investigators considered that the material accumulated in the alveoli either from overproduction (Larson and Gordinier 1965; Nicholas et al. 1965) or, more likely, as a result of inadequate clearing (Heppleston et al. 1970; Corrin and King 1970). Recent electron

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microscopic observations (Kuhn et al. 1966; Yamaguchi et al. 1969; Okada et al. 1971; Heppleston and Young 1972; Basset et al. 1973) have indicated that the intra-alveolar material would be derived from the type II alveolar epithelial cells which increase in number in this disease and slough off into the alveoli. It has been also demonstrated (Kuhn et al. 1966; Yamaguchi et al. 1969; Okada et al. 1971; Heppleston and Young 1972; Basset et al. 1973; Costello et al. 1975) that the intra-alveolar material contains evenly spaced, lamellar bodies characterized by concentric lamellae. But their origin and nature are uncertain.

Recently we had the opportunity to study two patients afflicted with pulmonary alveolar proteinosis and to obtain tissue at the time of lung biopsy, lung washings and sputum for electron microscopic study. Our electron microscopic observations revealed that there was a new myelin-like laminated body which had not been noted previously either in alveolar lining or free alveolar cells or in alveolar air spaces. In this paper, the ultrastructural features of the new myelin-like laminated body are described.

**Cases**

*Case 1.* The patient was a 16-year-old male who was found to have a “butterfly” pattern of shadow around the hilus of the lung on chest radiographs taken at a school health examination in April 1972 (Fig. 1).

Diagnosed as pulmonary tuberculosis, he was admitted to Shonai Hospital, and remained there for 6 months, during which time he received antituberculosis drugs such as Streptomycin, PAS and INH. As the abnormalities on chest radiographs were not resolved, he was referred to Tohoku University Hospital in October 1972. Physical examinations on admission revealed no particular findings and he remained asymptomatic during the course of the disease. In November 1972, an open lung biopsy was performed and the diagnosis of pulmonary alveolar proteinosis was established histopathologically. A part of the lung tissue obtained was processed for electron microscopic observation. By February

![Fig. 1 (left). Chest radiograph taken in October 1972, in Case 1, showing a “butterfly” pattern concentrated about the hilus.](image1.png)

![Fig. 2 (right). Chest radiograph taken in October 1971, in Case 2, showing widely distributed densities in the bases of both lungs, more marked on the left.](image2.png)
1973, he had completely recovered without any treatment and was discharged.

Case 2. A 44-year-old man, who had been working as a carpenter for about 20 years, had been well until March 1970, when he had a spell of high fever and received treatment under the diagnosis of pneumonia. Four months later, he got well and was restored to employment. From July 1971, he had shortness of breath when he walked hurriedly or went up the stairs, and cough and bloody sputum developed, so he was admitted to Tome Public Hospital where a diagnosis of sarcoidosis was considered highly probable. In October 1971, he was referred to Tohoku University Hospital for more extensive examinations.

Chest radiographs revealed a fine, diffuse, soft nodular density extending from around the hilus of the lung to the periphery bilaterally showing the "butterfly" distribution (Fig. 2). On auscultation of the chest small-sized moist rales were heard over the left lower lung field and the percussion note over the anterior chest on the both sides was short.

The clinical diagnosis was uncertain but on April 4, 1971, an open lung biopsy established the diagnosis of pulmonary alveolar proteinosis. The lung tissue was examined by light microscopy, but not submitted for electron microscopy.

Because of the progressive nature of the condition, broncho-pulmonary lavage was started in June 1971, and electron microscopic observations were made on lung washings and sputum.

**MATERIALS AND METHODS**

Diagnosis of alveolar pulmonary proteinosis was established in both cases on the basis of the open lung biopsy and the use of a variety of staining techniques. Ultrastructural studies were made on lung tissue from Case 1, which was obtained from the right lower lobe, and lung washings and sputum from Case 2. The lavage fluid contained flecks and pieces of a gray-tan material which precipitated on the bottom of a bottle after standing at 4°C for 10 min.

The tissues and the precipitated material from lung washings and sputum were fixed for electron microscopy in 2% glutaraldehyde buffered at pH 7.4 with isotonic phosphate buffer at 4°C for 2 hr. They were washed in two successive changes of phosphate buffer over a one-hour period and postfixed in phosphate-buffered 1% osmium tetroxide (pH 7.4) at 4°C for 1 1/2 hr. The specimens were dehydrated in graded alcohols and embedded in Epon 812. Ultrathin sections were cut with a diamond knife on a Porter-Blum ultramicrotome MT-2 and stained with uranyl acetate and lead citrate, or with methenamine silver. Grids were examined with a JEOL 100C electron microscope at an accelerating voltage of 80 KV.

**RESULTS**

**Light microscopy (Cases 1 and 2)**

Sections stained with hematoxylin and eosin showed coarse granular material in the alveoli of the lungs from both patients (Figs. 3 and 4). The material gave an intensely positive reaction with the periodic acid-Schiff stain. The interstitial tissue appeared normal. However, in Case 2, slightly increased numbers of type II alveolar epithelial cells along the alveolar septa were found. Gomori methenamine-silver nitrate stain for the demonstration of *Pneumocystis carinii* was negative in both cases.

**Electron microscopy of the lung tissue (Case 1)**

The alveolar spaces were full of amorphous or fine granular materials
intermingled with both granular and laminated bodies (Fig. 5). Among the intra-alveolar materials two kinds of laminated bodies were found. The one (Fig. 6), about 0.2–4 µm in diameter, presented a concentric laminated structure showing a periodicity of approximately 80 Å and 220 Å with a total spacing of 300 Å, which was identical to that recorded by Basset et al. (1973) in a similar biopsy material. It was relatively low in electron density when stained with lead citrate and uranyl acetate and was not stained with methenamine silver. This type of laminated body hereinafter will be called “Lamella A”.

The other type of laminated body (Fig. 7) showed a myelin-like laminated structure which was highly electron dense on staining with lead citrate and uranyl acetate and showed a periodicity of approximately 50 Å. Profiles of these ring-like bodies were 0.5 µm or less in diameter. Sometimes they were compressed and irregular in shape. These laminated structures stained intensely with methenamine silver (Fig. 8). This type of myelin-like laminated body will be referred to as “Lamella B”. As far as the authors are aware Lamella B has not been previously demonstrated in this disease. Fig. 8 shows a free cell (possibly a macrophage) in the alveolar air space containing in its cytoplasm many particles of intra-alveolar material. Whereas Lamella A was found only in the alveolar air spaces outside cells, Lamella B was seen both in the cell and in the alveolar air space. In the cell, it was usually found in groups and sometimes appeared wrapped up in a limiting membrane. In the alveolar air space, it was mainly dispersed, but was sometimes found in a group. No limiting membrane was observed in the air space.

Not only was Lamella B observed in free cells of the macrophage type in the alveolar air space, but it was also seen in the types I and II alveolar epithelial cells.
A New Laminated Body in Pulmonary Alveolar Proteinosis

Fig. 5 (upper). Case 1. Lung biopsy. Intra-alveolar, extracellular materials containing two kinds of laminated bodies (A, B). Electron micrograph, stained with lead citrate and uranyl acetate, ×26,000.

Fig. 6 (lower left). Case 1. Lung biopsy. Lamella A found in the alveolar air space. Electron micrograph, stained with lead citrate and uranyl acetate, ×53,000.

Fig. 7 (lower right). Case 1. Lung biopsy. Lamella B found in the alveolar air space outside the cell. Electron micrograph, stained with lead citrate and uranyl acetate, ×140,000.

and in the mesenchymal cells in the alveolar septa (Figs. 9, 10 and 11). The incidence of Lamella B in these cells was high in free cells in the alveolar air space, moderate in the mesenchymal cells in the alveolar septa, low in the type I alveolar
Fig. 8 (upper). Case 1. Lung biopsy. A free cell in the alveolar air space and the intra-alveolar materials. Lamella B (zigzag) is seen both in the cell and in the alveolar air space outside the cell. Lamella A (→) is seen only outside the cell. Electron micrograph, stained with methenamine silver, × 10,000.

Fig. 9 (lower left). Case 1. Lung biopsy. Lamella B (zigzag) found in the mesenchymal cells in the alveolar septa. Electron micrograph, stained with lead citrate and uranyl acetate, × 15,000.

Fig. 10 (lower right) Case 1. Lung biopsy. Lamella B (zigzag) found in a type I alveolar epithelial cell. Electron micrograph, stained with lead citrate and uranyl acetate, × 13,000.
epithelial cells, and rare in type II alveolar epithelial cells.

*Electron microscopy of lung washings and sputum (Case 2)*

Both Lamella A and Lamella B were often encountered in the broncho-pulmonary lavage fluid of Case 2 (Figs. 12, and 13). The latter was of a high
electron density, sometimes was aggregated, and showed a 50 Å periodicity (Fig. 13). This morphology was in agreement with that found for Lamella B in Case 1. In sputum, Lamellae A were quite often present, but not any "Lamella B".

**DISCUSSION**

Ultrastructural studies of lung tissue in pulmonary alveolar proteinosis have demonstrated the presence of characteristic laminated bodies in amorphous or fine granular materials filling the alveolar air spaces (Kuhn et al. 1966; Yamaguchi et al. 1969; Okada et al. 1971; Heppleston and Young 1972; Basset et al. 1973; Costello et al. 1975). The periodicity of the lamellae reported in previous papers, though it shows a considerable variation (Table 1), agrees fairly well with that of Lamella A in the present study, i.e. approximately 300 Å. Considering the fact that these laminated bodies were observed only outside cells in the alveolar air spaces, lung washings and sputum, they seem to be similar in general character.

Another characteristic finding in the present study is the appearance of evenly spaced laminated bodies with approximately 50 Å periodicity, that is, Lamella B. Costello et al. (1975) reported that the central zone of the lamellar bodies with 300 Å periodicity contained in lung washings was often amorphous or showed laminations with 50 Å spacing. Although our Lamella B is similar in periodicity to this description, it differs entirely from the lamellar bodies of Costello et al. in general structure.

Myelin-like structures are often seen in cells, particularly during the course of degeneration. However, it may not be possible to find them in such a large number as noted in Case 1. The periodicity of Lamella B is a little different from that of the normal osmiophilic lamellar inclusion in type II alveolar epithelial cells, which was reported to be approximately 66 Å by means of transmission electron microscopy (Stratton 1975) and approximately 40 Å by means of a freeze-fracture replication method (Lauweryns and Gomber-Desmecht 1973). As to the formation of this material, lipids and proteins, when they coexist, probably assume such a specific configuration through chemical interaction. Lamella B is presumed to contain polysaccharide since it gives a positive reaction to the methenamine silver staining. For confirmation, glucosidase should be used to see if a negative reaction to the methenamine silver staining takes place. But we were not able to study on this point because of the limited availability of samples.

As to the presence of Lamella B in the cell, it may be interpreted in two ways; that is, (1) it results from abnormal metabolism of the cell and (2) it is derived from the ingestion of Lamella B from the alveolar air spaces. It is likely that if it results from abnormal metabolism, then it may be due to a reaction made by an abnormal substance or excessive accumulation of a substance entering the cell from outside. Electron microscopic pictures show only a static images of cell components at a point in time, so there is naturally a limit to using them in discussing a change in, or the transfer of, a substance in cells.

Type I or type II alveolar epithelial cells probably have no active digesting
Table 1. Periodicity of laminated bodies found in pulmonary alveolar proteinosis

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Year</th>
<th>Periodicity (Å)</th>
</tr>
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<tbody>
<tr>
<td>Kuhn et al.</td>
<td>1966</td>
<td>149</td>
</tr>
<tr>
<td>Yamaguchi et al.</td>
<td>1969</td>
<td>180-250</td>
</tr>
<tr>
<td>Heppleston et al.</td>
<td>1972</td>
<td>Approximately 200</td>
</tr>
<tr>
<td>Basset et al.</td>
<td>1973</td>
<td>&quot;</td>
</tr>
<tr>
<td>Costello et al.</td>
<td>1975</td>
<td>&quot;</td>
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<tr>
<td>Present study</td>
<td></td>
<td>Lamella A</td>
</tr>
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<td>approximately 300</td>
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<td>50</td>
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function, although this function in these cells is still a matter of controversy. It is unlikely, therefore, that a large number of Lamellae B observed in the cells are the result of digestion. In these cells, the possibility is rather high that abnormal substances are accumulated in the cytoplasm and then released into the alveolar air spaces as a result of abnormal metabolism of an unknown cause. Meanwhile, macrophages in the alveolar air spaces and mesenchymal cells in the alveolar septa primarily have an active digesting function; so, it is next to impossible to judge whether Lamella B found in these cells represents abnormal material resulting from abnormal metabolism or one which is digested and taken into the cell. The findings of macrophages and type II alveolar epithelial cells being degenerated and broken down in the alveolar air spaces suggest that materials present in the alveolar air space are, at least in part, due to the breaking down of these cells. Furthermore, the Lamellae B outside the cell tend to gather in groups in a manner similar to that occurring inside the cell.

In connection with the mechanism by which this disease is produced, Rosen et al. (1958) gave an explanation that the septal cell of the alveolar septum proliferates and consequently that this septal cell enters the alveolar air space and becomes necrotic to form a proteinaceous material. Previous electron microscopic observations showed the proliferation, degeneration and release of type II alveolar epithelial cells in this disease. These cells were considered accountable for the formation of a material retained in the alveolar air spaces. In the present study, we also encountered instances in which type II alveolar epithelial cells containing plenty of Lamellae B were degenerated and released, but such an encounter was not very frequent. Accordingly, it appears irrational to explain a large amount of material retained in the alveolar air space merely by the proliferation, degeneration and breaking down of type II alveolar epithelial cells.

The incidence of Lamella B contained in the cytoplasm is overwhelmingly high in the free cells such as macrophages in the alveolar air spaces and mesenchymal cells in the alveolar septa, followed by the type I and type II alveolar epithelial cells. Only type II alveolar epithelial cells have hitherto been incriminated in connection with the mechanism by which this disease is produced, but the other cells should also be considered to play a part.

It is not clear why Lamella B has not been found so far. It is therefore
important to examine other cases as for whether the present two cases are special ones in pulmonary alveolar proteinosis or it is merely because not enough attention has been directed to such material. Moreover, when we encounter such a case in the future, biochemical identification of this material will be needed in connection with the mechanism by which this disease is produced.

References


