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Electron-microscopic examination of primary diffuse tracheobronchial amyloidosis

Role of fibroblasts in amyloid formation

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Summary. The author reports on the electron-microscopic examination of the diffuse tracheobronchial amyloidosis of a 51-year-old patient. The amyloid deposits were located in the lamina propria of the tracheal and bronchial mucosa. At the edge of the nodular deposits, condensation and radial arrangement of amyloid fibrils could be seen. Closely connected with the amyloid, active fibroblasts were present and their cytoplasm contained amyloid fibrils. The cell membrane of the fibroblasts was missing in part. Intracellular amyloid fibrils mingled with extracellular deposits. It can be assumed that active fibroblasts play an important role in local amyloid formation.

Key words: Tracheobronchial amyloidosis – Electron microscopy – Amyloid fibril – Fibroblast

Introduction

Isolated amyloidosis of the respiratory tract is a rare disease. It occurs in the lungs in diffuse or nodular forms (Kanada and Sharma 1979; Mäkinen et al. 1977; Schoen et al. 1980; Spencer 1977) and in the tracheal and bronchial mucosa (Attwood et al. 1972; Bejui-Thivolet et al 1982; Cook et al. 1973; Flemming et al. 1980; Michaels and Hyams 1979; Schraufnagel et al. 1980).

By 1980 a total of 42 cases of isolated tracheobronchial amyloidosis had been reported (Flemming et al. 1980). However, electron-microscopic studies have been described in only one report (Michaels and Hyams 1979).

In the present study, diffuse tracheobronchial amyloidosis in a 51-year-old male patient is reported. The chief aim of our investigations was to examine the cells in, and adjacent to, the amyloid deposits thereby gaining new data on the mechanism of amyloid formation.

Case report

K.F., 51-year-old male patient was admitted to the hospital on 21st of June, 1981 with a history of recurrent right sided pneumonia. For half a year he had had dyspnoea, and had lost 10 kg over 20 years. His chest x-ray was negative. Tracheo-bronchoscopic examination showed a knobby-surfaced mucosa suggestive of tumour. Histologically the surface of the mucosa was covered in part by ciliary columnar epithelium and partly by metaplastic stratified epithelium. The lamina propria contained nodular eosinophilic masses (Fig. 1) positive on staining with Sirius and Congo reds and showing green birefringence by polarisation microscopy. The diagnosis of tracheobronchial amyloidosis was made.

In view of the histological diagnosis, generalized amyloidosis was assumed to be present. Blood count, immunoelectrophoresis, sternal puncture, urinalysis showed normal values. X-ray of the bones and rectal biopsy were negative.

Owing to stenosis of the respiratory tract, he was operated on on the 3rd September 1981, when the amyloid masses narrowing the lumen of the trachea were removed and an endoprothesis was introduced.

Method of electron-microscopic examination

1 mm^3 Palade-buffered pieces of tracheal and bronchial mucosa obtained from several places were fixed in osmium tetroxide, dehydrated in a graded series of ethanols and embedded in Araldite. The sections were prepared by Reichert's ultramicrotome and photographed by the JEM CX electron microscope. For orientation, 0.5 micron semi-thin sections stained with toluidine blue were made. For control purposes, 3 specimens of normal tracheal and bronchial mucosae were examined using the same method.

Results

The nodularly arranged amyloid deposits consisted of fibrils of a thickness of 70 to 80 Å.

At the edge of amyloid deposits fibroblasts could be seen (Fig. 2). Their nucleus was oval, the nuclear membrane was serpiginous, the nuclear chromatin was marginally condensed. The cytoplasm of the cells contained well-developed rough endoplasmic reticula, free ribosome granules, mitochondria and Golgi zones. The cell membrane was missing in several areas and the cytoplasm of the cells was closely connected to the bundles of amyloid fibrils with the condensation and radial arrangement of fibres (Fig. 3). In several places the cytoplasm of the fibroblasts was eroded and only the residual nucleus could be recognized in the amyloid mass (Fig. 4).

The cytoplasm of the fibroblasts contained, in several places, amyloid fibrils surrounded by a single membrane (Fig. 5). In other places, free fibrils could be seen in the cytoplasm of the cells (Fig. 6). The continuity of the cell membrane was interrupted, the intracytoplasmic fibrils were mingled with extracellular amyloid masses (Fig. 7). The condensation was most obviously expressed at the edge of the nodular amyloid masses (Fig. 8).

The distribution of cellular elements around the amyloid deposits was as follows: fibroblast 90%, macrophage 8%, mast cell 2%. Plasma cells were absent from our material.

In the epithelial cells of the glands of the bronchial mucosa, accumulation of intermediate sized filaments (of a diameter of 40–60 Å) was found (Fig. 9a). These were arranged in bundles, around the nuclei or in other regions of the cytoplasm. The basal lamina was intact (Fig. 9b).

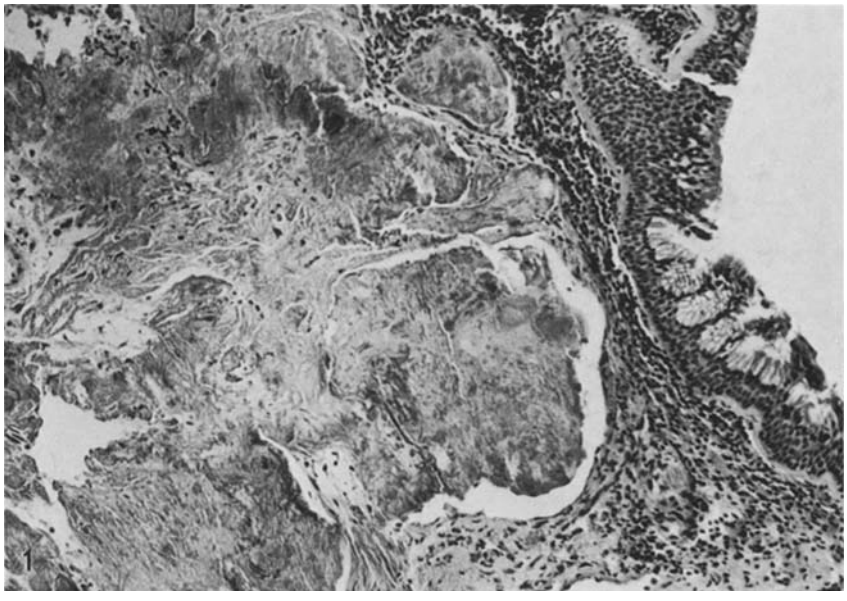

Fig. 1. The surface of the bronchial mucosa is covered by ciliary columnar epithelium with several layers of basal epithelial cells. The lamina propria contains nodular amyloid deposits (HE $\times 120$)

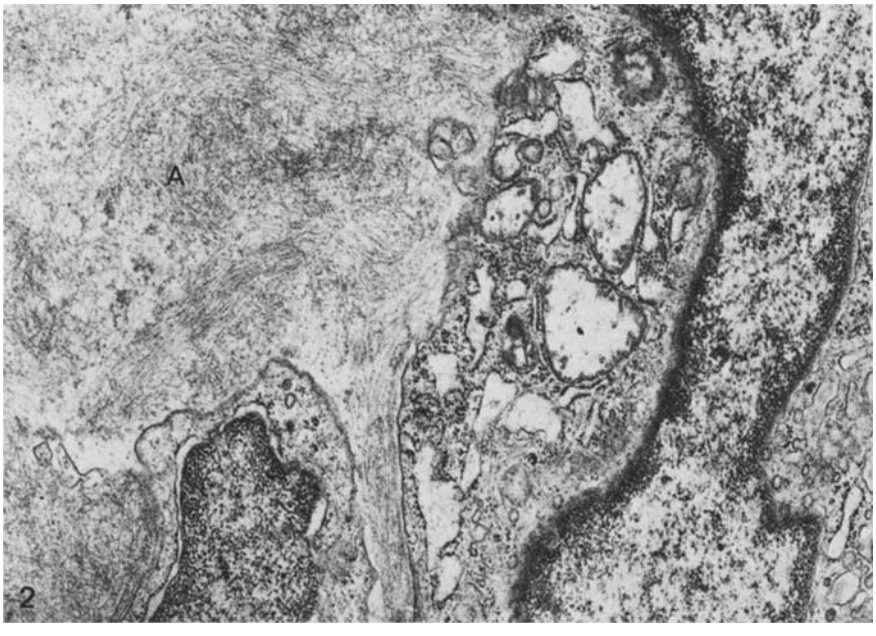

Fig. 2. *On left*, amyloid (*A*), *on right* of the Figure, detail of two fibroblasts can be seen. The nucleus of the cells is elongated, the cytoplasm contains rough endoplasmic reticulum, free ribosome granules and mitochondria ($\times 25{,}000$)

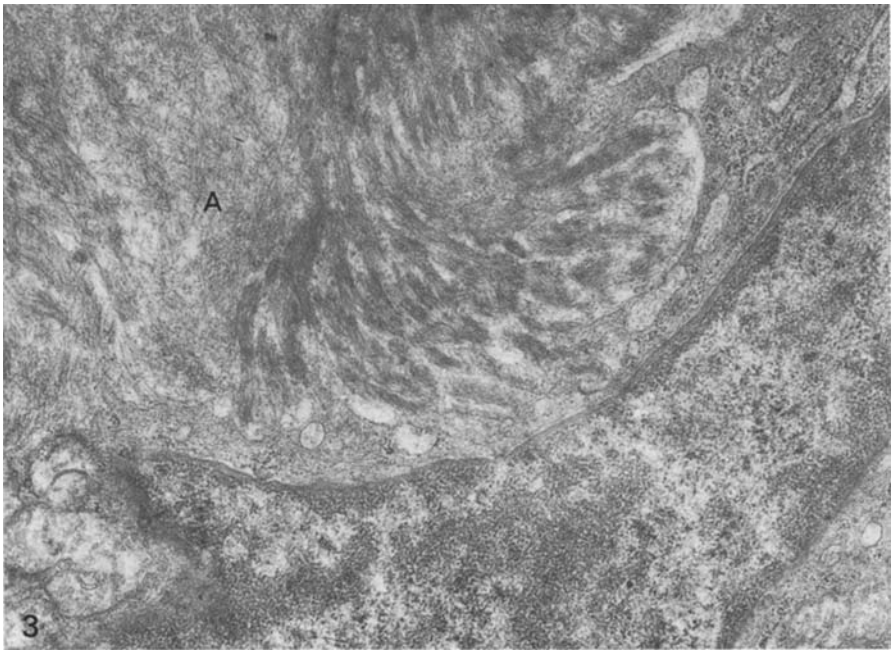

Fig. 3. Fibroblasts adjacent to an amyloid deposit (*A*). The radially arranged fibrils are closely connected to the cell membrane of the fibroblast (× 25,000)

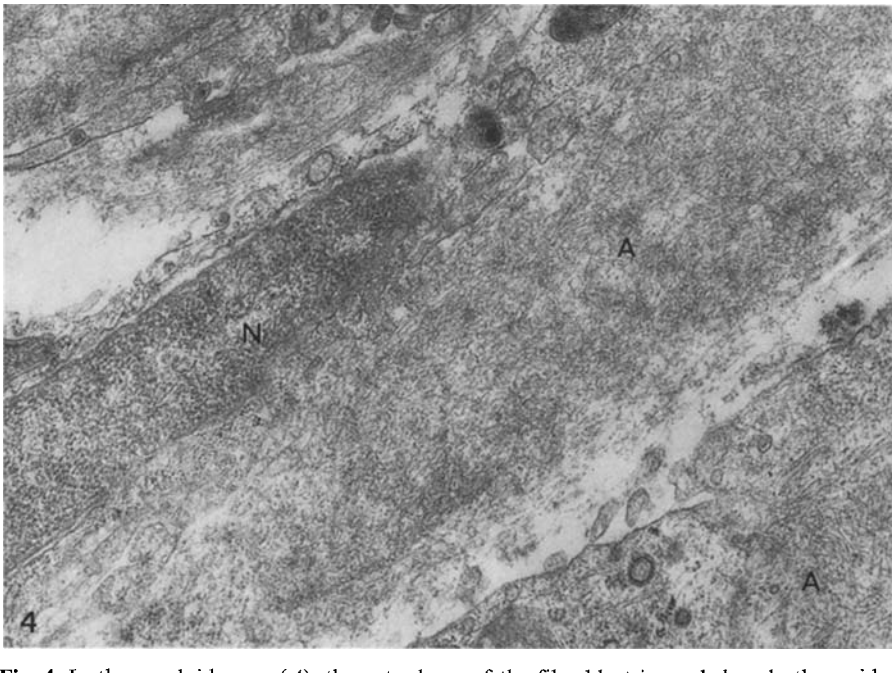

Fig. 4. In the amyloid mass (*A*), the cytoplasm of the fibroblast is eroded, only the residuum of the nucleus (*N*) can be recognized (× 32,500)

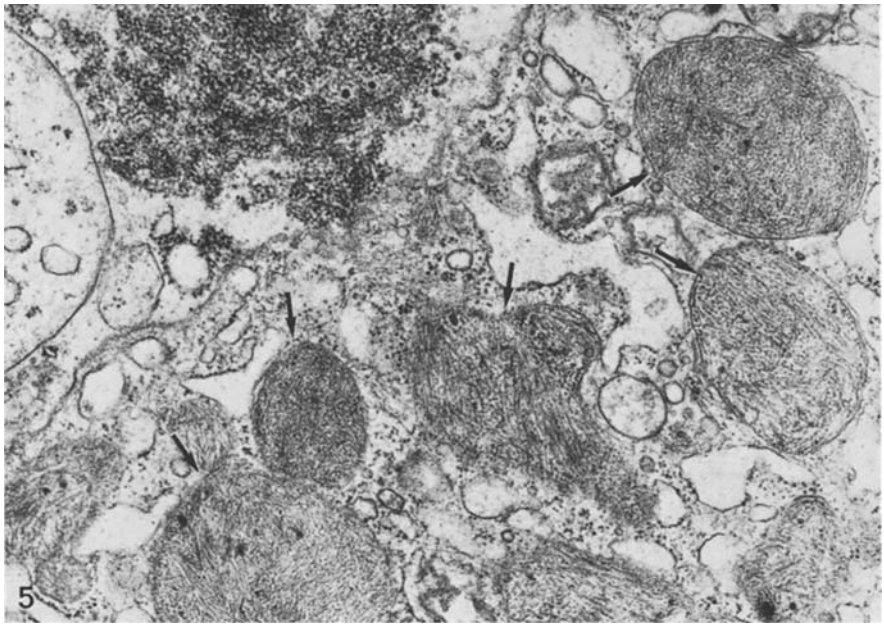

Fig. 5. Amyloid fibrils (*arrows*) surrounded by membrane in the cytoplasm of the fibroblast (× 32,500)

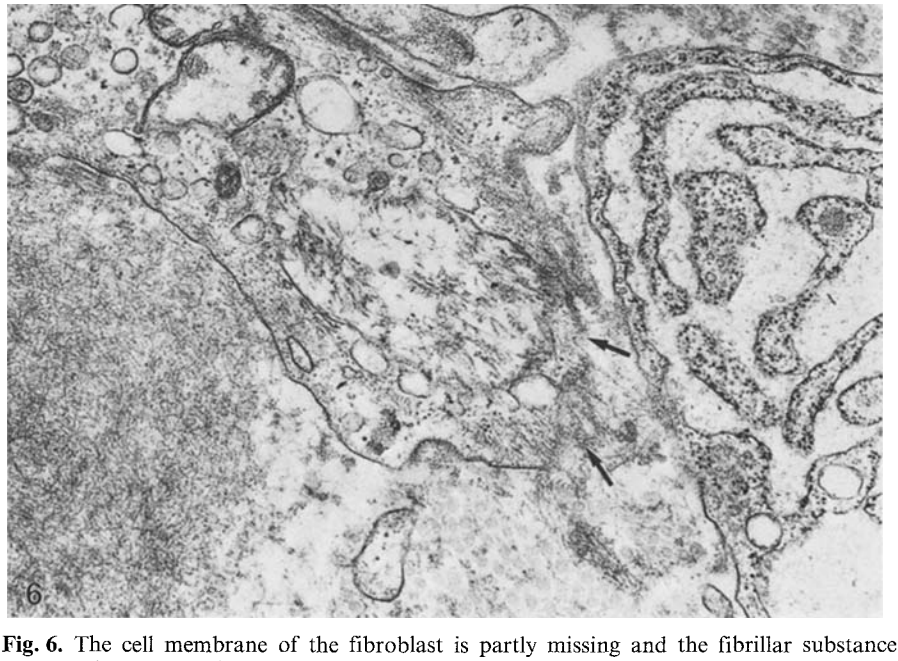

Fig. 6. The cell membrane of the fibroblast is partly missing and the fibrillar substance of the cytoplasm is mingling with extracellular amyloid (× 32,500)

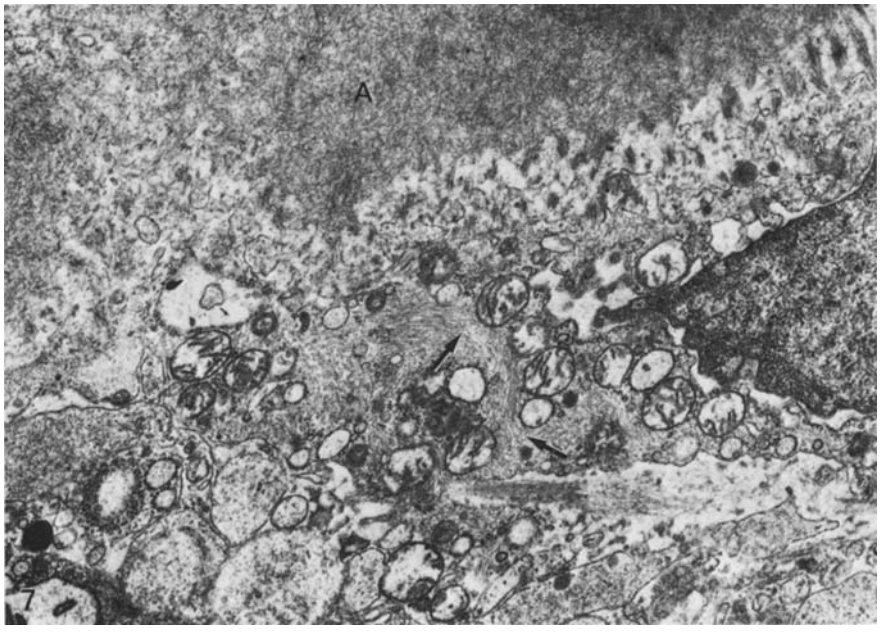

Fig. 7. The cell membrane of the fibroblast is missing. The cytoplasm contains fibril bundles (*arrows*), the extracellular amyloid is in close contact with the cytoplasm of the cell (× 16,500)

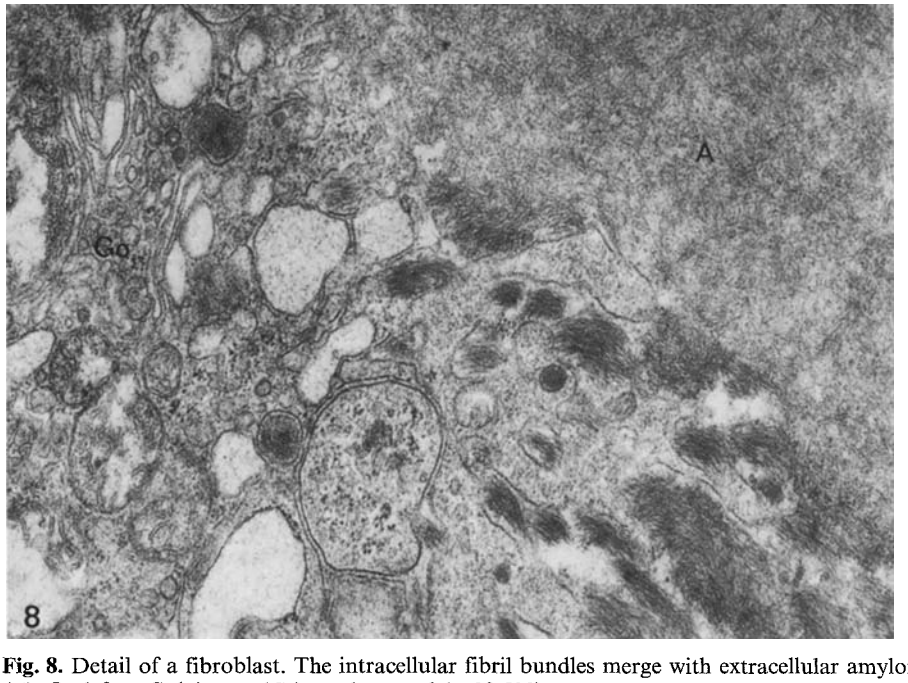

Fig. 8. Detail of a fibroblast. The intracellular fibril bundles merge with extracellular amyloid (*A*). On left, a Golgi zone (*Go*) can be noted (× 32,500)

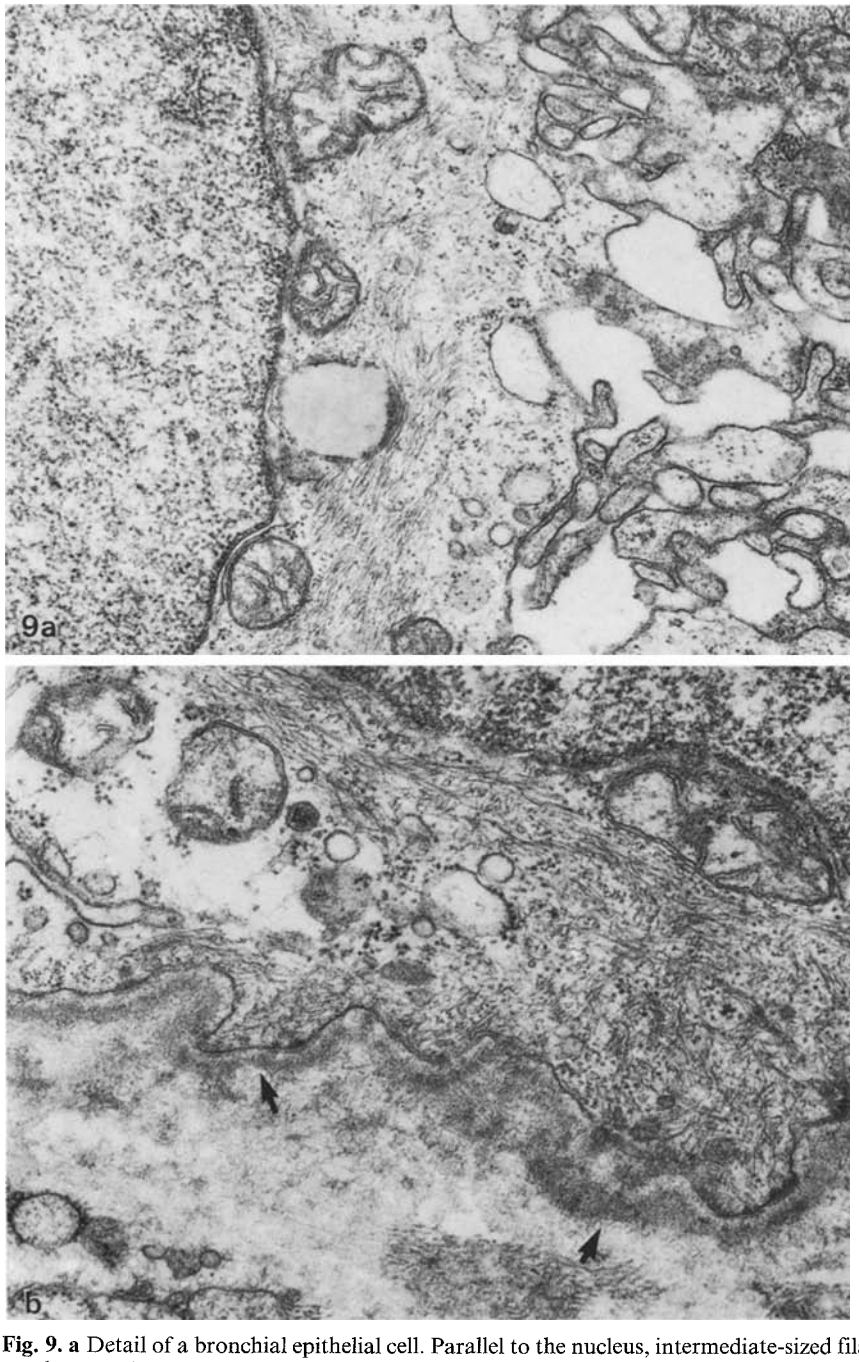

Fig. 9. a Detail of a bronchial epithelial cell. Parallel to the nucleus, intermediate-sized filaments can be seen (×20,750). **b** Basal region of an epithelial cell. There are intermediate filaments in the cytoplasm. The continuity of the basal lamina is preserved (*arrows*) (×15,000)

Discussion

Amyloidosis is a long-known and much studied disease. Despite the abundance of new data, the exact mechanism of amyloid formation is still not known (Cohen et al. 1978; Glenner 1980; Langer and Missmahl 1980; Leading Article 1979; Linke and Nathrath 1982; Romhányi 1972; Wuchter and Schirmeister 1978). Researchers in electron microscopy have long been concerned with the site of amyloid fibril formation (Machado et al. 1979; Zucker-Franklin and Franklin 1970). Gueft and Ghidoni (1967) observed, in mouse experiments, the intracytoplasmic appearance of amyloid fibrils in histiocytes. Ben-Ishay and Zlotnick (1968) demonstrated intracytoplasmic amyloid in the reticulo-endothelial cells of the bone marrow of a patient with plasmocytoma. Kjeldsberg et al. (1977) described amyloid in the plasma cells and histiocytes, Zucker-Franklin and Franklin (1970) in the monocytoid reticulo-endothelial cells of patients with plasma-cell myeloma. Shirahama and Cohen (1975) observed the appearance of amyloid in the lysosomes of macrophages. According to Nakagawa (1980) the cell membrane of cells adjacent to amyloid play an important role in the pathogenesis of the disease.

In our previous work we reported on an electron-microscopic study of isolated amyloidosis of the stomach (Balázs 1978). The distribution of the cellular element in the amyloid mass was found to be 30% plasma cells, 60% fibroblasts, a majority of these being myofibroblasts. In the present material plasma cells have not been found. Ninety per cent of cellular elements in and, adjacent to, the amyloid were fibroblasts. Myofibroblasts were encountered only sporadically.

In our present material the cytoplasm of active fibroblasts contained amyloid fibrils. In several places, amyloid deposits were surrounded by single membrane. Neither pinocytotic activity nor accumulation of lysosomes was observed in the fibroblasts. We therefore see no evidence to suggest that they are phagolysosomes. In other places, free fibrils were seen in the cytoplasm of the fibroblasts and the cell membrane was partly eroded with the fibrillar substance of the cytoplasm mingling with the extracellular amyloid. In the outermost regions of the amyloid deposits, the condensation and radial arrangement of fibres was seen. In these areas, there was no demarcation between radial amyloid fibrils and the cytoplasm of the fibroblasts. In some places only the bare residua of cell nuclei were found in the amyloid masses. The amyloid-producing capacity of "incorrectly-programmed" fibroblasts was also observed by Runne and Orfanos (1977) in local skin amyloidosis.

In the small number of macrophages lysosomal accumulation of amyloid was not seen; we could not confirm the data of Shirahama and Cohen (1975).

In the epithelial cells of the seromucinous glands of the bronchial mucosa, accumulation of microfilaments was seen. The accumulation of similar filaments was also observed in tumor and non-tumor cells (Bannasch et al. 1981; Bejui-Thivolet 1982; Gabbiani et al. 1981). Immunofluorescent

studies demonstrated that intermediate filaments in the epithelial cells of the bronchial mucosa are composed of keratin-polypeptides (Bejui-Thivolet et al. 1982). In our case, it can be assumed that the accumulation of intermediate-sized filaments is correlated with the leukoplakia observed by light microscopy.

As already mentioned, only one previous electron-microscopic study has been made on amyloidosis of the respiratory tract. In this, Michaelis and Hyams (1979) found the accumulation of a fibrillar substance in the epithelial cells of the glands of the bronchial mucosa. They observed the deterioration of the basal lamina of the glands and considered this fibrillar substance to be an amyloid precursor.

Our examinations have not confirmed the data of Michaelis and Hyams (1979). There is no evidence of participation of epithelial cells in local amyloid formation. We have found no relationship between the intermediate filaments accumulating in the epithelial cells and the amyloid deposits in the lamina propria.

Summing up our results, in our case the exclusive amyloid-producing activity of fibroblasts was established. No plasma cells were found. From these results it may be supposed that active fibroblasts play an important role in the local formation of amyloid deposits.

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