

The contribution of type II pneumocytes and alveolar macrophages to fibroplasia processes in the course of enzymatic lung injury

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Summary. The aim of the paper was to evaluate mutual relations in the system of alveolar macrophage (AM) - type II pneumocyte (PII) - interstitium of alveolar septa, in the course of experimental lung emphysema in rats subjected to BCG vaccine effect.

Administration of BCG vaccine resulted in the cumulation of AM within pulmonary alveoli. These cells exhibited morphological features of increased activity. Intratracheal papain injection induced intralobular emphysema changes, partly generalized, in the animal lungs. The emphysematous changes, with domination of interalveolar septum atrophy, were accompanied by focal accumulation of collagen and elastin. Fibroplasia processes were strongly pronounced in BCG- and papain-treated animals. The areas of connective tissue fibres cumulation revealed indistinctness of the boundary line between PII and the interstitium in some places. Anchorage of collagen fibres and microfibrillary structures were observed in the cytoplasm of PII.

The morphological examinations of AM - fibroblasts co-cultures as well as the evaluation of the uptake of ³H-thymidine did not show any significant differences between respective co-cultures of fibroblasts and AM isolated both from the lungs of control and experimental animals (treated with BCG or papain, and BCG+papain).

However, a significant growth was noted in ³H-thymidine uptake between fibroblast cultures realized with or without cells isolated from the lungs.

The results obtained suggest the possibility of active participation of PII and AM in fibroplasia processes in the course of lung rebuilding after papain administration and in pathological states of the pulmonary tissue, particularly when they are accompanied by increased activity of alveolar macrophages. They also support the inflammatory-repair hypothesis in the development of emphysematous changes.

Key words: Emphysema, Fibrosis, Type II pneumocyte

Introduction

Emphysema is defined as «permanent enlargement of the air-spaces distal to the terminal bronchiole», associated with destruction of their walls and without obvious fibrosis (Snider et al., 1985). The dominant hypothesis to explain the pathogenesis of the pulmonary emphysema relates it to perturbation in the balance of protease and antiprotease system, especially, in the balance of neutrophil and macrophage elastases and alpha-1-antitrypsin, which results in lung injury (Janoff, 1985). According to the inflammatory-repair hypothesis, emphysema is a consequence of inflammation and subsequent repair process (Snider et al., 1988). Although animal models of emphysema have provided insight into pathological mechanisms and clinical impact (Escobar et al., 1995), little is known of specific cellular or underlying molecular responses. As mentioned previously, lung tissue is very complex and is comprised of at least 40 cell types (Foster and Curtiss, 1990). Attempts to investigate specific cell responses to lung injury are very difficult.

Our earlier studies revealed quantitative changes in type II alveolar epithelial cells during the rebuilding of the pulmonary tissue after intratracheal infusion of a proteolytic enzyme - papain (Sulkowski et al., 1994a,b). In the present study we made a detailed TEM analysis of the septa which separate emphysematous spaces, with special attention being paid to the boundary line between the interstitium and type II pneumocyte. We also studied the effect of alveolar macrophages on fibroblast growth *in vitro*. We decided to use the co-culture system to more closely reconstruct the *in vivo* conditions (Hibbs et al., 1983).

Material and methods

Experimental animals and design of the study

The experiment was carried out on female Wistar rats of 180-220 g body weight. The animals were maintained in a well sunlit room, at 18-20° C on standard

granulated diet. They were divided into four experimental groups. Group I (control) animals were given 2 ml phosphate-buffered saline (PBS) solution intraperitoneally (i.p.), twice at a 14-day interval. At 7 days following the second PBS administration, these animals were given 1 ml PBS solution intratracheally. The rats of group II were given two doses (2 ml each) of BCG vaccine intraperitoneally and, seven days after the second dose, 1 ml PBS solution intratracheally. BCG vaccine (Serum and Vaccine Plant, Lublin, Poland) was administered twice (a single dose, 4×10^8 microorganisms in 2 ml of PBS solution) at a 14-day interval, which is the best found for macrophage system activation (Carswell et al., 1975; Sun et al., 1990; Tasaka et al., 1995). In group III, the rats were twice given 2 ml PBS solution (i.p.) and 7 days later 1 ml papain solution, intratracheally (Merck, Germany), at a dose of 20 mg/kg body weight. Group IV animals were given 2 ml BCG vaccine (i.p.) twice and once 1 ml papain solution, intratracheally. All experimental animals were sacrificed by intraperitoneal administration of 100 mg sodium pentobarbital after 7, 14 and 28 days of papain (or PBS) treatment.

Quantitative studies of type II alveolar epithelial cells were performed based on sections stained for alkaline phosphatase according to Beckstead et al. (1981) and modified by Edelson et al. (1988). Very thin specimens of the lungs were fixed for 24 h in Carnoy's fluid at 4 °C. The routine paraffin method was used to prepare sections for histological and morphometric analyses.

Tissue preparation for TEM

The lungs of 4 animals of each experimental group subjected to TEM observation prior to opening the thorax were perfused with Ringer's fluid and buffered 1.5% in glutaraldehyde. Then, a fine catheter was inserted in the abdominal part of the inferior vena cava to reach the right ventricle of the heart and transport these fluids at a pressure of 30 cm H₂O. At the same time the abdominal aorta was cut in order to allow easier back-flow of perfusive fluids from the lungs. The whole procedure lasted 10 min. Later, small blocks of 1 mm³ were cut out of the lungs and refixed in cold 2.5% glutaraldehyde solution and osmium tetroxide. After dehydration in alcohol-acetone series and embedding in epon, they were sectioned and contrasted with lead citrate and uranyl acetate and examined in an Opton 900 PC electron transmission microscope.

Isolation AM from the lungs

To obtain cells from the lungs, the multiple broncho-alveolar lavage (BAL) method of Myrvik, also applied in our earlier studies (Sulkowski et al., 1994a, 1996), was used. The lungs of 8 animals of each group were perfused with a 0.9% NaCl solution at 40 °C prior to removal from the thorax. Then, a cannula was inserted

into the pulmonary artery, the left atrium was incised and PBS solution administered at 20 cm H₂O pressure until complete pallor of the pleura was observed. To obtain cells from the bronchi and alveoli, PBS was administered intratracheally in 5 doses of 5 ml each. Next, the lungs were massaged gently and washings collected into dishes coated with silicone. All fluid portions obtained in the lung lavage were mixed and centrifuged at 600 g for 5 min, at 40 °C. The cells were resuspended in Parker fluid (medium 199, produced by Serum and Vaccine Plant, Lublin). After lavage, the cell vitality was estimated by means of 1% aqueous solution of trypan blue and total number of cells isolated from the lungs was counted in a Thoma counting chamber. Cytological preparations were made from the fluid concentrated to 1×10^6 cells per 1 ml of the solution. Cell composition was evaluated in smears stained with the hematoxylin-eosin (H-E) method, following the AS-D naphthol esterase cytochemical reaction according to Leder. All the work connected with cell isolation from the lungs was done in sterile conditions in a laminar airflow chamber.

Preparation of fibroblast cultures

The skin sections (2x3 mm) were placed in 1 ml of Collagenase-Dispase enzyme solution (Boehringer, Germany), in the Eagle medium (Serum and Vaccine Plant, Lublin). After the 24-hour incubation the sections were mechanically minced and centrifuged. The supernatant was removed. The culture was established in Falcon flasks (25 cm² in area) using the Eagle medium supplemented with: 20% calf fetal serum (Grand Island Biological Company), 3% glutamine (Flow UK) and 5% solution of basic amino acids (Ne AA, Grand Island Biological Company) as well as 120 U penicillin and 20 nmoles/100ml streptomycin (Polfa). The culture was done in a Haereus incubator at atmosphere of 5% CO₂ and 95% air, at 95% humidity and temperature 37 °C. The medium was changed every third day. When the whole bottom of the flask was lined the culture was passed to a number of smaller flasks, 0.5 ml of cell suspension to each of them.

AM - fibroblast co-cultures

Co-cultures of macrophages with fibroblasts were performed by the methods described by Hibbs et al. (1983) using contact - inhibited fibroblast cultures supplemented with 2 ml suspension of freshly BAL-isolated macrophages (1×10^6 /ml). On the same day as when the fibroblast culture was mixed with AM, the medium was replaced by a serum-free medium. The co-culture was performed for 24 hours in the same conditions. After 12 hours, each fibroblast-macrophage co-culture was supplied with 2.5 μCi ³H-thymidine. The medium was removed from the culture and the culture was rinsed with PBS after the next 12 hours. The uptake of ³H-thymidine was measured with the use of a 1209

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Rack-beta liquid scintillation counter (LKB Wallac Company). Similar determination was done for a pure fibroblast culture (without AM) as well as for the culture of AM. Co-cultures of AM with fibroblasts were only performed in groups after 7 days following papain treatment (28th day of experiment). As earlier studies had demonstrated, in this period of time the maximum inflow of macrophages to the pulmonary tissue could be observed (Chyczewski and Sulkowski, 1988; Sulkowski et al., 1994c).

Statistical analyses

All values are presented as means from five assays \pm standard deviation (SD). Student's t-test was used for the statistical evaluation and the p-value <0.05 was considered significant.

Cell preparation for SEM

Some of the cultures and BAL-isolated AM realized were designed for scanning electron microscope (SEM) analysis. Both pure fibroblast cultures and co-cultures with macrophages were performed in the same conditions without ^3H -thymidine. After fixation in glutaraldehyde (1% in phosphate buffer pH 7.2, for 30 minutes) the smears were dehydrated in a graded series of ethanol up to absolute, critical point dried in liquid CO_2 mounted on stubs, coated with gold and examined in a JEOL scanning electron microscope.

Results

BAL-isolated cells

In all experimental (II-III) and control (I) groups of

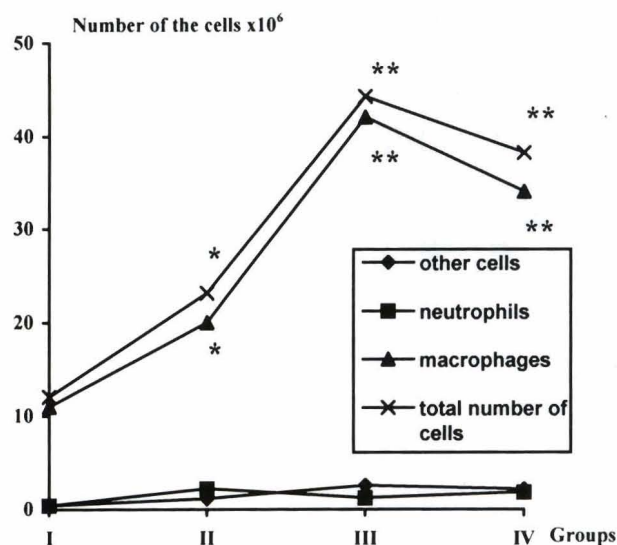


Fig. 1. Cell content of BAL 7 days following intratracheal papain or PBS administration. *: $p<0.01$; **: $p<0.005$.

animals, macrophages were the predominant population of cells isolated from the lungs. They constituted up to 94% of the total cell number and demonstrated a positive esterase reaction. A detailed analysis of cell composition of BAL is presented in Fig. 1.

AM - fibroblast co-cultures

No significant morphological differences were found (in SEM) between fibroblast and BAL-isolated cell co-cultures from the respective experimental groups. Slight differences were noted only between the fibroblast culture and co-culture of fibroblasts with macrophages, especially in groups II and IV. They referred to the production of more processes of the cytoplasmic membrane in fibroblasts - AM co-cultures in groups II and IV, which may be a cause of the more dense arrangement of fibroblasts in the culture.

The evaluation of ^3H -thymidine-uptake (dpm) revealed no significant difference between the respective experimental groups (Fig. 2). The increase observed in groups II and IV was not statistically significant. However, significant differences were found between fibroblast cultures done with and without AM ($p<0.001$). Simultaneous cultures of AM demonstrated a trace ^3H -thymidine uptake. Therefore, this radioactivity (dpm) was treated as the background for the other experimental groups.

Quantitative studies of type II pneumocytes in situ

A statistically significant increase in the number of type II pneumocytes was found in the lungs of rats in groups III and IV in all the intervals studied. Worth special mention was an increase in the number of PII in the lungs of BCG-treated rats (group II), although statistically significant differences were not found in all

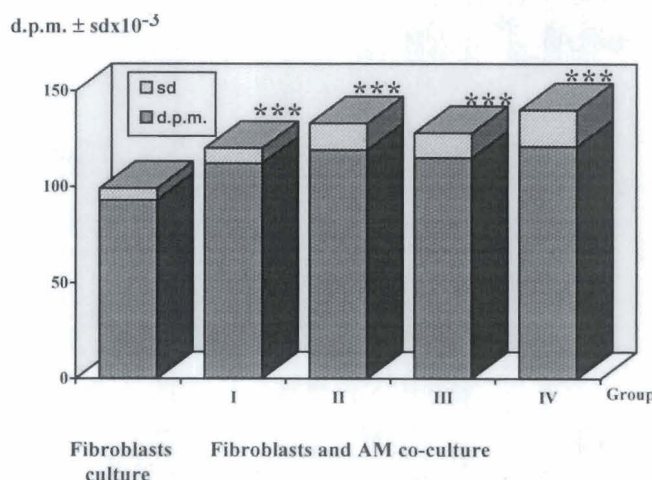


Fig. 2. The effect of macrophages isolated by BAL 7 days following intratracheal papain or PBS administration on fibroblast culture radioactivity (d.p.m.). ***: $p<0.001$; statistically significant difference between fibroblasts cultures and fibroblast-AM co-culture.

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subgroups. Summary results of the studies presenting absolute numbers of cells with positive reaction to alkaline phosphatase in 1mm² pulmonary tissue are

given in Fig. 3. Fig. 4 presents a histological picture of the pulmonary tissue stained for alkaline phosphatase according to Beckstead. Alkaline phosphatase-positive

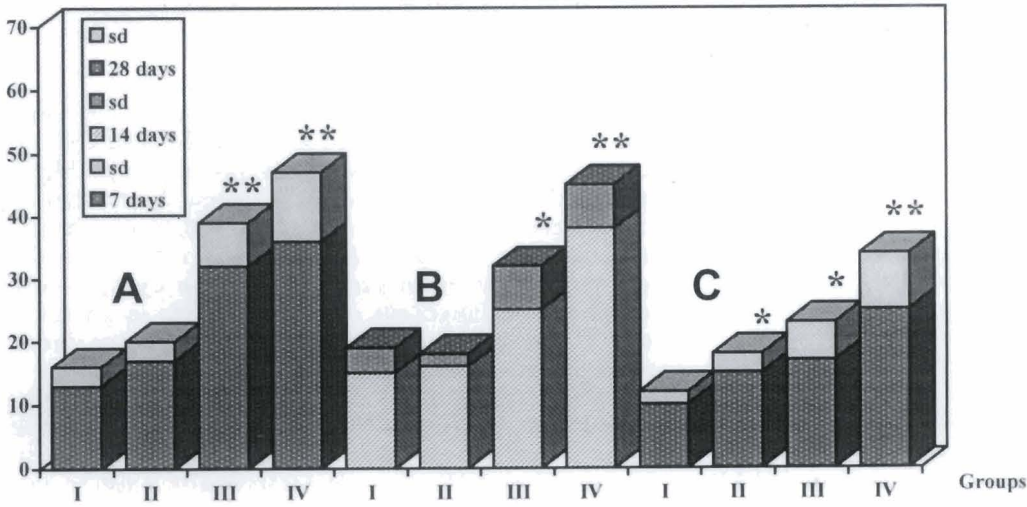


Fig. 3. The total number of phosphatase-positive cells in 1mm² of lung tissue: 7 (A), 14 (B) and 28 (C) days after intratracheal administration of papain or PBS solutions. *: p<0.01; **: p<0.005

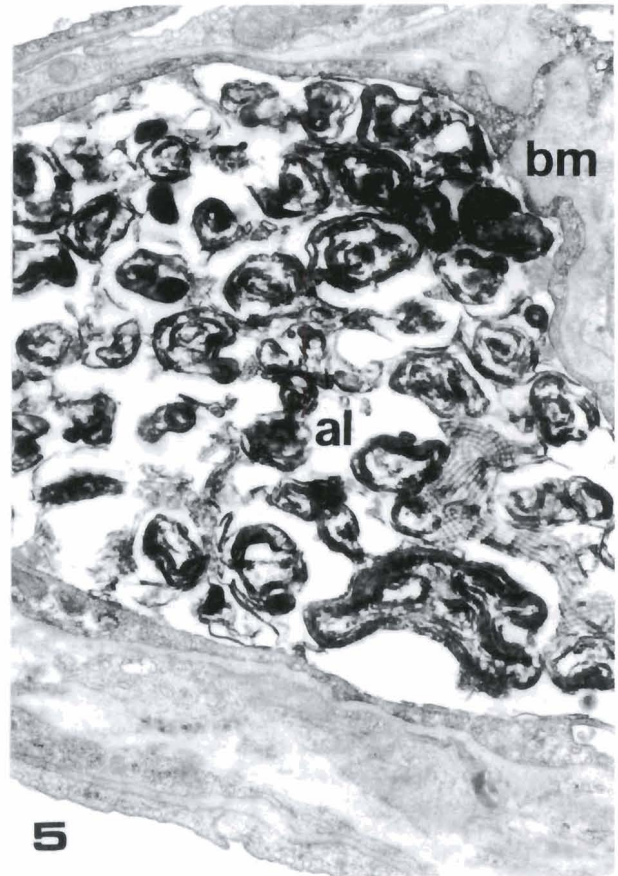
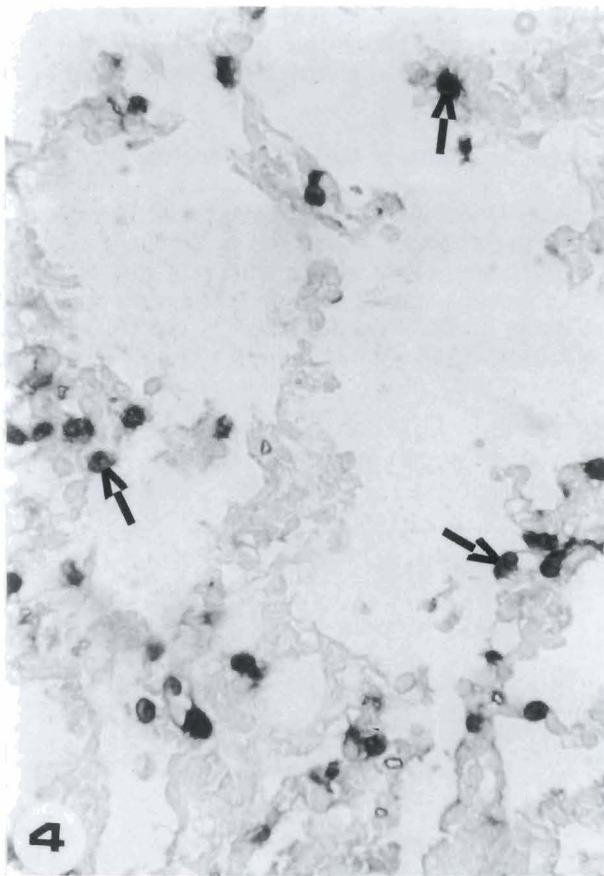


Fig. 4. Alkaline phosphatase-positive cells in staining according to Beckstead method (arrow) show close connection with alveolar septal walls. x 180

Fig. 5. Fragments of the atelectatically altered lung parenchyma. In the lumen of an alveolus (al), numerous lamellar structures and wide irregular basement membranes (bm) within thickened walls are seen. Group III (after 7 days following intratracheal papain administration). TEM. x 7,000

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cells in this method stain blue.

Transmission electron microscopy

Ultrastructural picture in TEM of the respiratory part of the lungs in control animals (group I - PBS+PBS) did not show any abnormalities. In the lungs of group II animals (BCG+PBS) an increased number of AM were found. Their distribution in the pulmonary tissue was irregular.

The ultrastructural pictures of the septa limiting abnormal air spaces in groups III and IV were similar. Therefore, these groups are described jointly. It should be stressed here that the changes described below occurred focally and were found only in those fragments of lung parenchyma where fibroplasia processes dominated. At the same time, the degree of intensity of the changes in the group IV (BCG+papain) was considerably higher, when compared with the group III.

Seven days after intratracheal papain administration, thickening of basement membranes was observed in groups III-IV: they were often irregular and showed tortuosity (Fig. 5). Focally, especially within atelectatic lung parenchyma, accumulation of lamellar bodies was

observed (Fig. 5) and an increased number of type II cells was found. These areas were seen to interweave with the fragments of lung parenchyma with largely increased amounts of connective tissue fibres, especially of collagen (Fig. 6). The sites of connective tissue fibre cumulation in the interstitium of the septa were frequently adjacent to the areas of the increased number of type II cells (P II). Basement parts of these cells were usually well developed and penetrated into the connective tissue fibres. In the vicinity of PII, numerous fibroblast processes were observed (Fig. 6). These sites frequently had an indistinct boundary line between the cellular membrane of the PII basement part and the adjacent fibres of the connective tissue.

After the fourteen and twenty-eight days that followed papain administration, the ultrastructural picture of the connective tissue in group III did not differ significantly from the one found in the previous interval. Large conglomerates of collagen were more frequently found. Microfibrillary structures, relatively common in group IV (Figs. 8-10), were a new and rare element in group III (Fig.7). They seemed to penetrate into the cytoplasm of PII (Fig. 8) and were linked with collagen fibres. In these areas, the cellular membrane of PII was

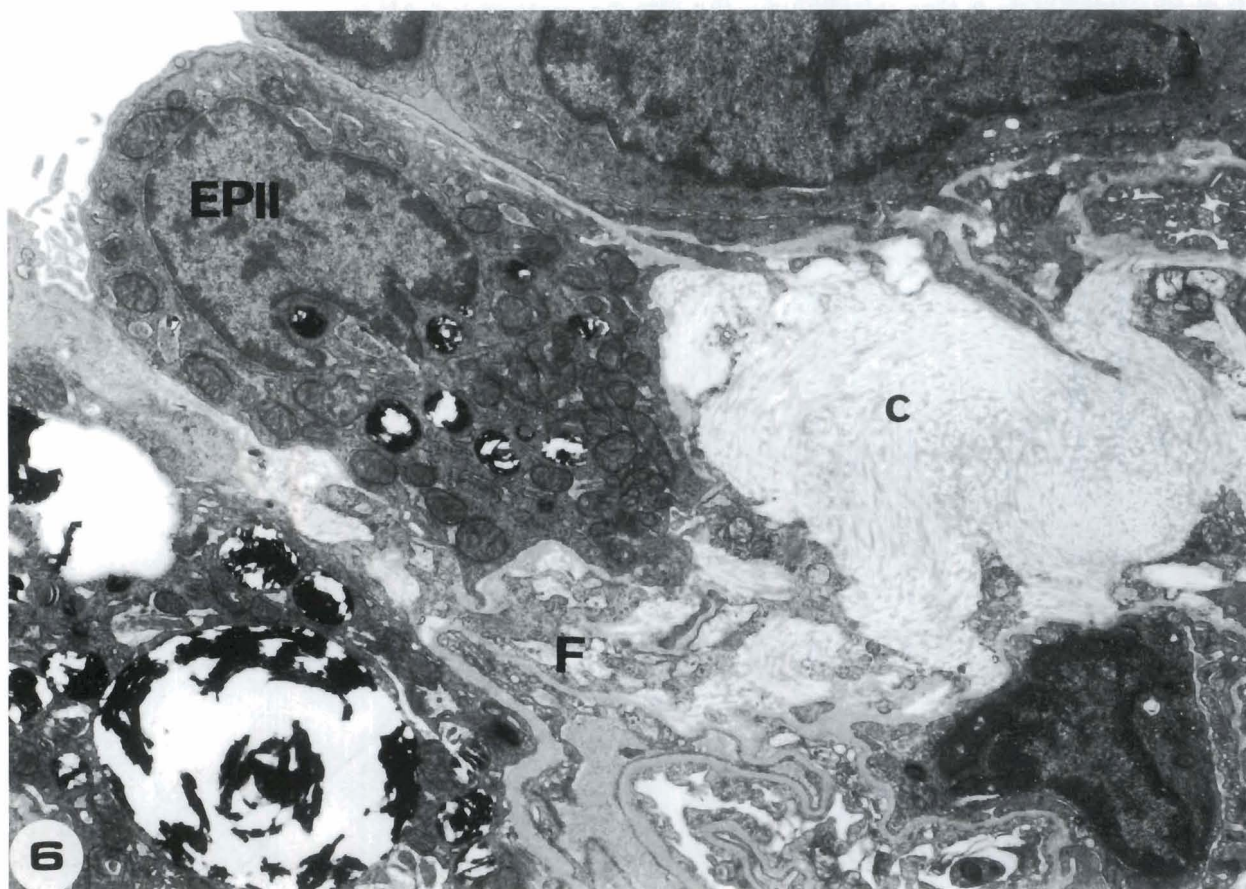


Fig. 6. Numerous fibroblast (F) processes and collagen (c) cumulation in the vicinity of a type II pneumocyte (EPII) - with well-developed basement part. Group III (7 days after papain). TEM. x 3,000

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completely obscure, while the cytoplasm showed well-developed rough endoplasmic reticulum and numerous free ribosomes. Thinner and more thickly arranged fibrils of similar structure (Fig. 7) were also observed in the cytoplasm of type II cells, especially in the parabasement parts. In the vicinity of these changes in type II pneumocytes, in the interstitium of the alveolar septa, numerous cellular cytoplasmic processes were observed, possibly belonging to fibroblasts. They were found among the connective tissue fibres, usually close to PII (Figs. 9, 10). In some cases, however, these processes could constitute fragments of well-developed PII (Fig. 10). In group IV, AM with morphological features of increased activity were also seen in the vicinity of collagen cumulation areas (Fig. 11). These cells had well-developed cytoplasmic membrane to form the microvilli. Similar AM were also frequently observed in group II. Some of them, however, particularly in group III, had poorly developed cellular cytoplasmic membrane, although they usually showed a large number of secondary lysosomes with lamellar structures or phospholipid-like substances.

Scanning electron microscope

We found a correlation between ultrastructural pictures of AM in TEM and SEM. SEM examinations showed most of BAL-isolated AM in groups II and IV as having well-developed cellular membrane, which formed numerous lamellar processes and blebs (Fig. 12A,C). These cells usually formed numerous phylopodia or veil-like pseudopodia. Group III had domination of cells with poorly-developed cytoplasmic membrane, although even they flattened relatively well on a glass substrate and/or formed numerous phylopodia (Fig. 12B). Quantitative relations between respective AM populations, which could be isolated in the analysed intervals, underwent small changes. The percentage of cells with morphological features of increased activity in groups II-IV was the highest after seven days following intratracheal papain or PBS administration.

Discussion

A variety of pulmonary diseases, such as interstitial lung diseases, tuberculosis, silicosis as well as trauma

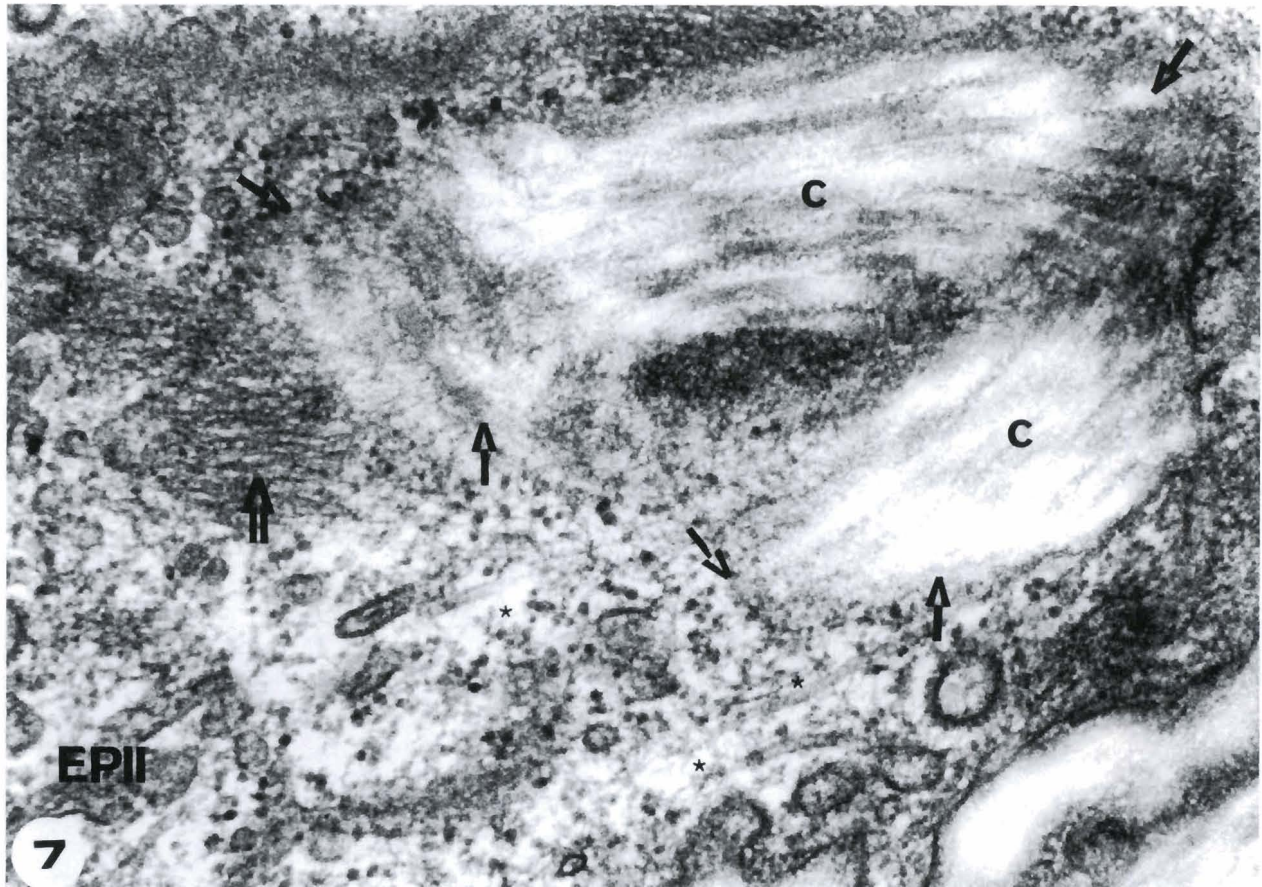


Fig. 7. Collagen (c) focus is surrounded by type II cell (EPII) cytoplasm. The boundary line (arrow) between collagen and PII is completely indistinct. The cytoplasm of PII shows numerous ribosomes and densely arranged microfibrillary structures (double arrow), which may constitute elements of cellular cytoskeleton. Thicker fibrillary structures are also visible (*). Group III (14 days after papain). TEM. x 30,000

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may result in lung injury and fibrosis. Macrophages are the most common inflammatory cells found in the lung after injury. The close proximity of fibroblasts and macrophages may be important in limitation of fibrosis after lung injury (Sibille and Reynolds, 1990). It has been demonstrated that stimulation of alveolar macrophages by BCG vaccine may enhance the process of lung fibrosis induced by bleomycin (Clark and Greenberg, 1987; Chyczewska et al., 1993). However, the results of another study indicate that the macrophage-derived suppressive factor may play a part in the limiting of fibrotic response to bleomycin-induced lung injury (Clark and Greenberg, 1987). Probably the effect of macrophages on fibroblasts is dependent in part on the balance of factors that are concurrently produced by AM and include a factor that suppresses and stimulates fibroblast proliferations and collagen production (Kelley, 1990). We are not aware of any previous study of the alveolar macrophage effect on fibroblast growth in experimental lung emphysema.

Our earlier studies (Sulkowski et al., 1994b,c) have demonstrated that on the seventh day following intratracheal papain injection, alveolar macrophages were the dominant population of cells isolated from

these lungs. At the same time the highest accumulation of these cells in the lungs and an intensive process of pulmonary tissue rebuilding were observed. Therefore, we have considered the seventh day of the development of emphysematous changes to be the optimal time for analysis of the effect of isolated AM on fibroblast cultures.

Differences observed in the experiments, between fibroblast cultures containing AM and without them especially those referring to the uptake of ^3H -thymidine, seem to indicate a stimulating effect of AM on fibroblasts, especially after the macrophage system activation with BCG vaccine; however, no significant differences were found between the groups I-IV. The number of macrophages isolated from emphysematous lungs of BCG-stimulated animals considerably exceeded the number of cells isolated from healthy lungs. Thus, the stimulating effect of macrophages on fibroblasts *in vivo* could be proportionally higher. This may result in intensified fibroplasia, observed in our morphological analysis. The confirmation of these suggestions could be very important for the studies of lung emphysema morphogenesis. Fibrotic processes seem to play a more important role in the pathogenesis of at least some forms

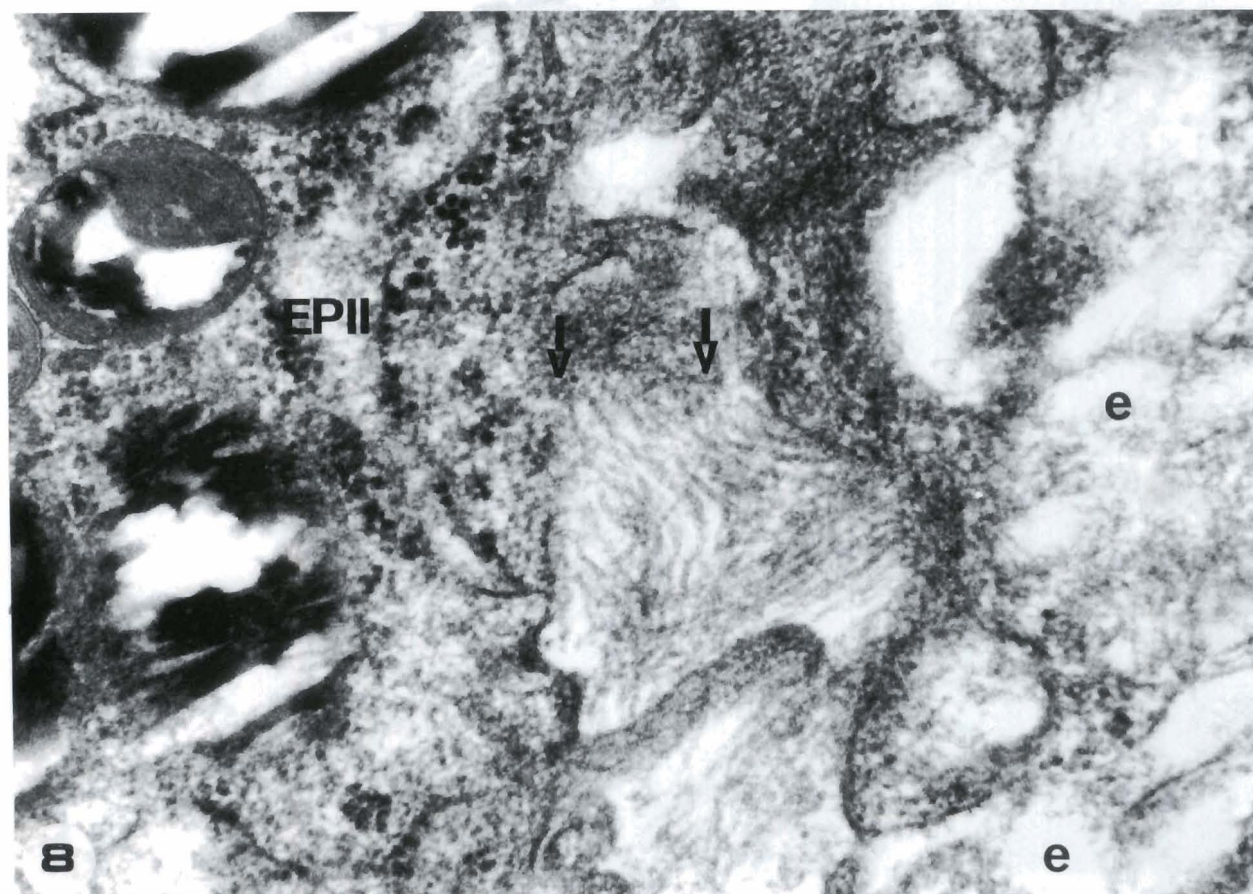


Fig. 8. Microfibrillary structures (arrow) seem to penetrate into the cytoplasm of type II cells (EPII) and to link with collagen or elastin (e) fibres. Numerous ribosomes are seen in EPII cytoplasm. Group IV (14 days after papain). TEM. x 30,000

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of emphysema (Kim et al., 1991; Nagai and Thurlbeck 1991) than it has been commonly considered up till now.

Macrophages, especially in an active form, can release many cytokines (Kelley, 1990). The tumour necrosis factor- α (TNF- α), interleukine-1 (IL-1), fibroblast growth factor (FGFs) and platelet-derived growth factor (PDGF) are known to be stimulators of cell proliferation. TNF- α and IL-1, acting separately, stimulate the increase in fibroblasts in the lungs, while their joint action inhibits cell divisions (Kelley, 1990). It has also been demonstrated that local macrophage proliferation is an important process in interstitial lung diseases (Pforte et al., 1993).

Our observations in TEM also suggest the possibility of AM contribution to the stimulation of fibroplasia processes. This refers mainly to the experimental group IV, where the greatest collagen cumulation was observed, especially in the region of rebuilt lung parenchyma as well as the cumulation of alveolar macrophages, frequently demonstrating morphological traits of increased activity. Also, ultrastructural pictures of alveolar macrophages in group II confirm a stimulating effect of BCG vaccine upon these cells.

A stimulatory effect of macrophages on fibroplasia processes in the course of experimental lung emphysema seems possible. However, the present results of the ultrastructural examinations do not allow us to exclude an active role of type II alveolar epithelial cells in the control and/or in the production of connective tissue elements. We cannot exactly describe the type of microfibrillary structures observed in our studies nor define the sites of their synthesis. Yet, the ultrastructural pictures observed may suggest that these structures are also produced in type II cells. It has been proved by other authors (Adamson et al., 1988), that severe injury and retarded repair of epithelium disturbs normal epithelial-fibroblast interaction and is sufficient to promote the fibrotic process. Less severe injury involving the endothelium only is not associated with fibrosis.

Studies carried out in the last years have revealed that type II cells isolated from adult animals synthesize a variety of extracellular matrix components, including fibronectin (Sage et al., 1983), type IV collagen (Crouch et al., 1987; Federspiel et al., 1991), and thrombospondin (Sage et al., 1983); fetal type II cells produce

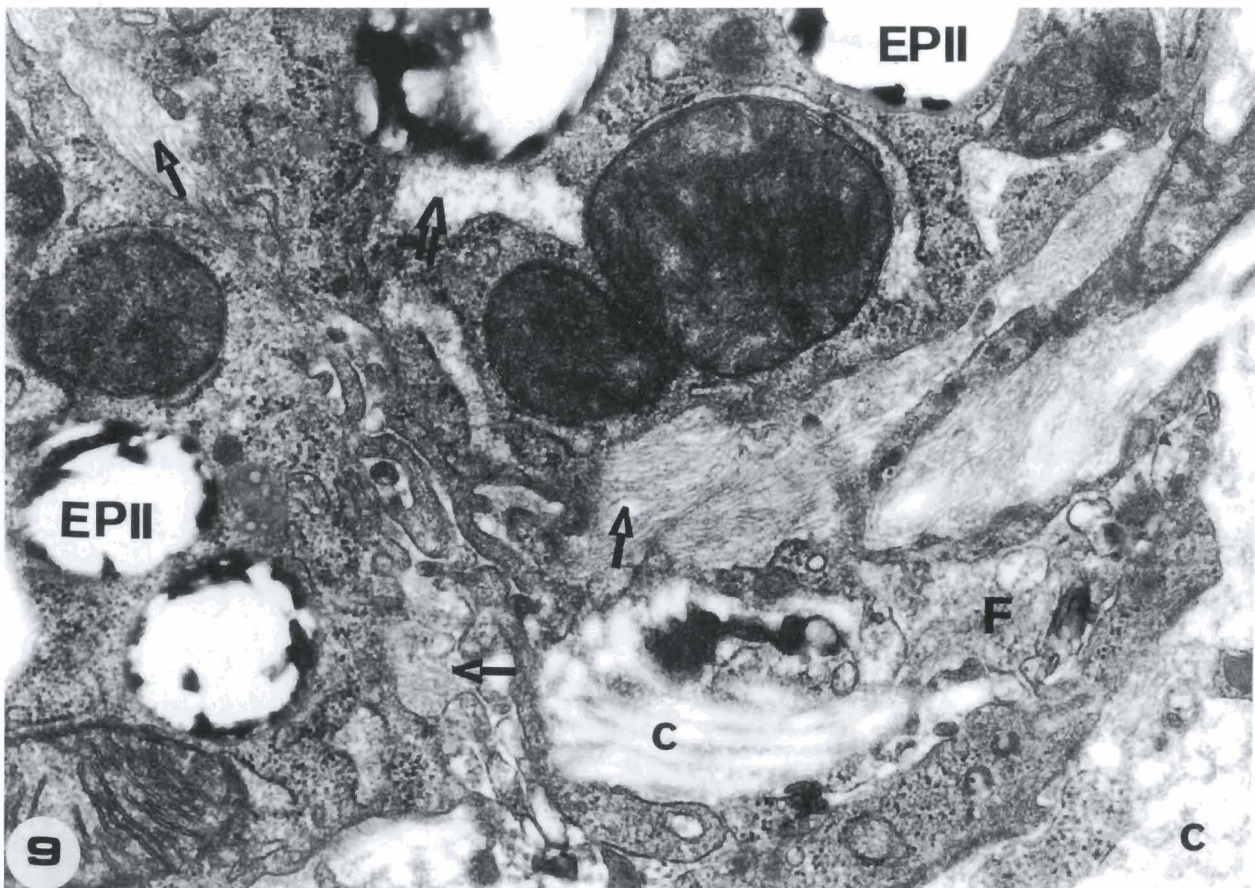


Fig. 9. Fragments of type II cells of the alveolar epithelium with numerous ribosomes and well-developed basement membranes. In their close vicinity, microfibrillary structures (arrow), fibroblast (F) processes and collagen (c) fibres. Within the epithelial cell (EPII) material (double arrow) of electron density similar to the collagen found in the interstitium can be seen. Group IV (14 days after papain). TEM. x 12,000

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proteoglycans and hyaluronic acid (Dunsmore and Rannels, 1995). This spectrum of matrix components is generally consistent with the reported patterns of integrin matrix receptor immunostaining both *in vivo* and *in vitro*.

Degradation of the extracellular matrix has been well studied in various systems, but information regarding matrix turnover by the pulmonary epithelium is limited (Lwebuga-Mukasa, 1991; Van de Lest et al., 1995). Turnover of matrix proteins by adult lung epithelial cells was suggested during culture of type II cells on the laminin-rich EHS basement membrane gel as well as after intratracheal injection of porcine pancreatic elastase (Van de Lest et al., 1995). The results of the studies mentioned above (Van de Lest et al., 1995) indicate that proteoglycans are target molecules for elastase, and may be involved in the pathogenesis of emphysema. The fibrillary structures observed by us in the ultrastructural pictures may constitute compounds of that type. Proteoglycans play a significant role in the formation of connective tissue stroma constituting a framework for the extracellular matrix. They also take part in the formation of collagen and elastic fibres.

The results of the present ultrastructural studies suggesting an increase in the number of type II pneumocytes in groups III and IV and their active participation in the processes of lung parenchyma rebuilding are confirmed by the studies on the activity of alkaline phosphatase in the pulmonary tissue. After 7, 14 and 28 days following intratracheal papain infusion, a statistically significant increase was observed in the number of alkaline phosphatase-positive cells in these groups. At the same time, neutrophils, which are also phosphatase-positive, were not found to accumulate in the lungs. It should be mentioned that Clara cells are also phosphatase-positive, but due to their localization they can be easily differentiated from type II pneumocytes.

The hitherto studies have concentrated on disorders in the protease-antiprotease system (Janoff, 1985). Only a few reports have suggested that fibroplasia processes may play a significant role in the development of emphysema (Kim et al., 1991; Nagai and Thurlbeck, 1991). Simultaneous biochemical studies concerned with collagen and elastin content in the pulmonary tissue have provided inconsistent results (Snider and Sherter, 1977;

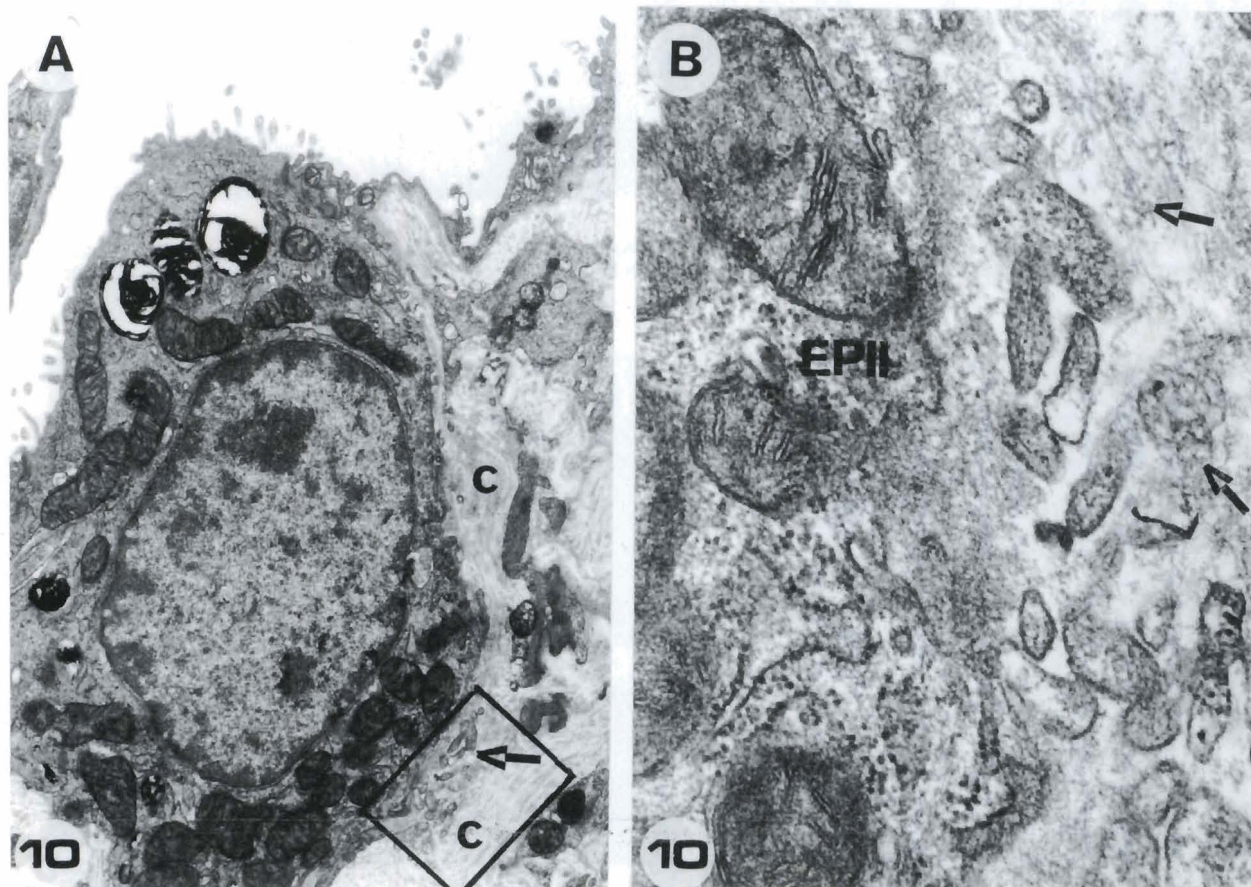


Fig. 10. **A.** Fragment of the rebuilt lung parenchyma with considerable collagen (c) cumulation. Numerous fine processes (arrow), which could belong to a fibroblast or EPII, are observed in the vicinity of a type II epithelial cell (EPII). x 4,400. **B.** Magnification of Fig. 10A fragment. Microfibrillary structures (arrow) in the vicinity of EPII and the processes described above. Group IV (14 days after papain). TEM. x 30,000

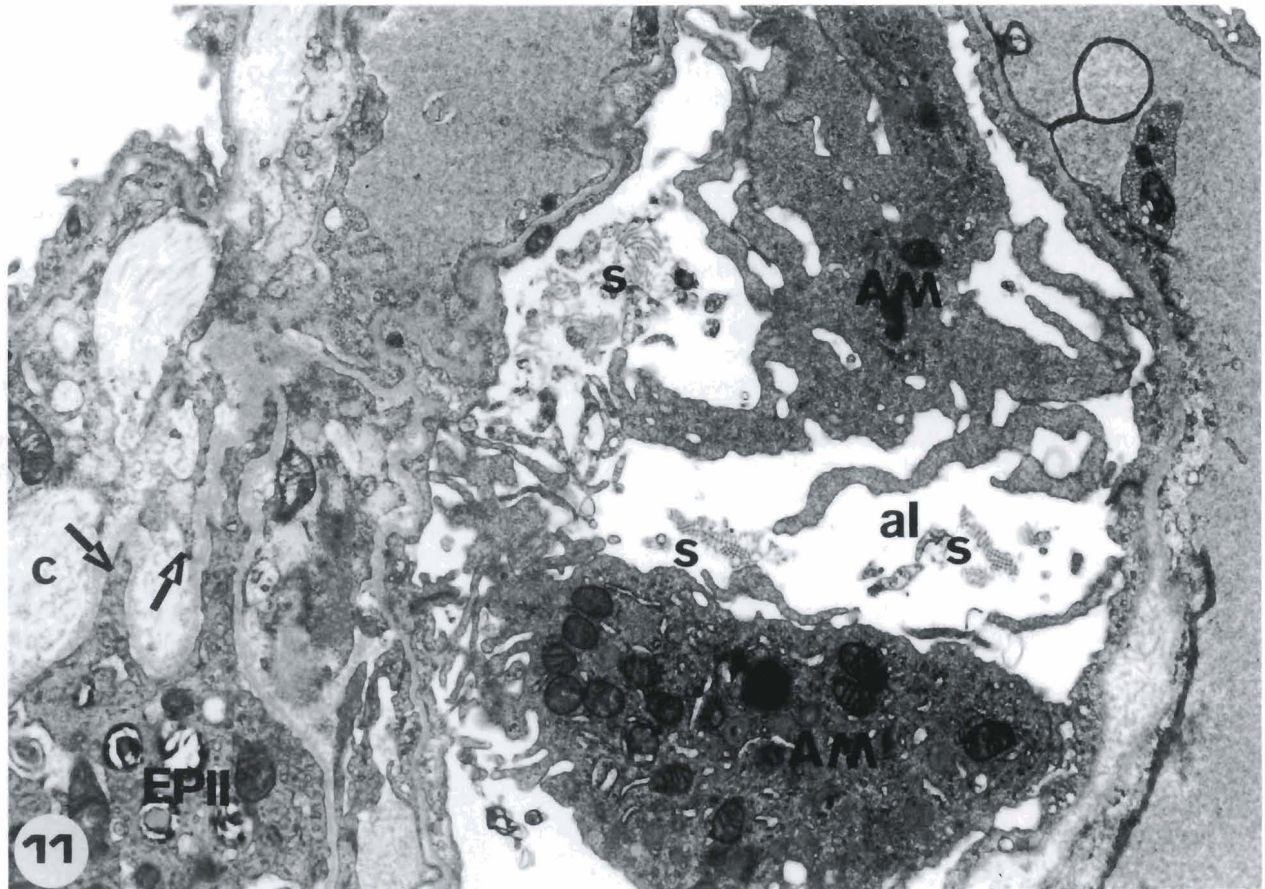
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Fig. 11. Fragment of the rebuilt lung parenchyma. Macrophages (AM) and surfactant (s) elements are visible in the lumen of a partly collapsed alveolus (al). A type II epithelial cell (EPII) penetrates in between collagen fibres (c) with long processes (arrows). Group IV (28 days after papain). TEM. x 4,400

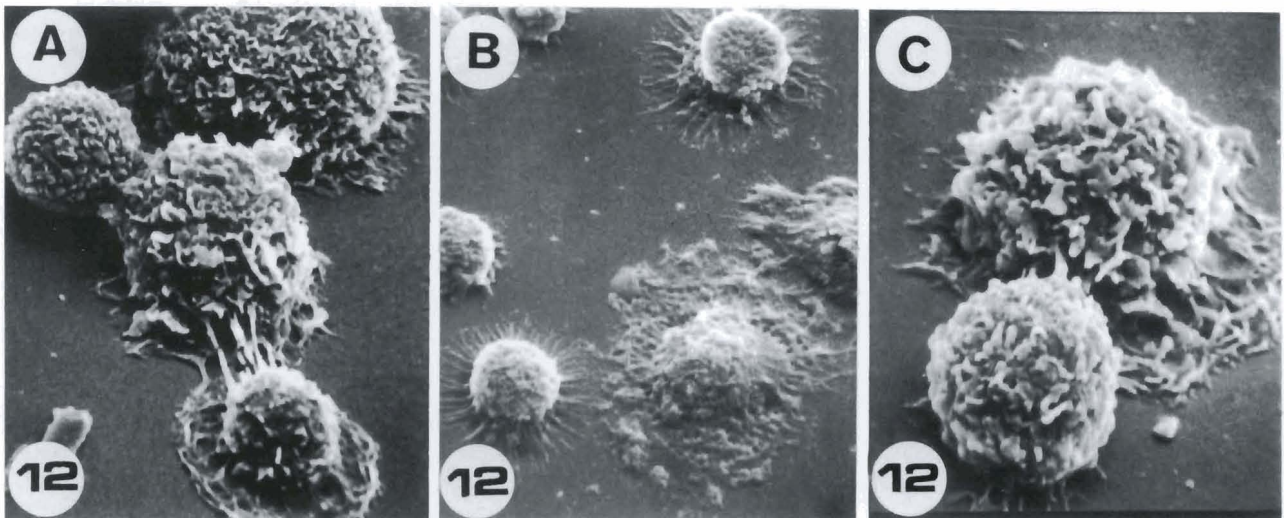


Fig. 12. A. A group of macrophages, differing in size, with well-developed cytoplasmic membranes in the form of lamellar processes. The cells form numerous phylopodia towards the base and neighbouring cells. One of them produces veil-like pseudopodium. Group II (BCG+PBS). x 3,000. B. Macrophages have relatively poorly developed surface with numerous follicular projections of the cytoplasmic membrane. Group III (PBS+papain). x 2,000. C. Well-developed cytoplasmic membrane of macrophages produces numerous lamellar projections. Group IV (BCG+papain). SEM. x 4,000

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Kobler et al., 1982; Cardoso et al., 1993). The present studies, however, seem to indicate that local collagen cumulation may play a significant role in emphysema-related pulmonary tissue rebuilding. In the present study no quantitative measurements were taken of collagen in the lungs. Its quantitative evaluation of the whole lungs seems useless taking into consideration its amount and distribution in the pulmonary tissue. It may even cause false results, which was probably the case in the past. Results of the analysis in TEM show that only focal collagen accumulation (more seldom elastin) occurs, frequently in the vicinity of emphysematous spaces. At the same time, lung parenchyma loss is observed, including connective tissue fibres. Total collagen content in the lungs may, thus, alter. Moreover, biochemical analysis of the whole lungs also determines collagen contained in the bronchi and vessels. Its mass is considerably higher than collagen amount in the respiratory part of the lungs only, which obscures potential differences.

In conclusion, we think that the results obtained in this study indicate possible participation of type II cells in fibroplasia processes in the course of lung parenchyma rebuilding after papain administration, and probably in other pathological conditions of the pulmonary tissue accompanied by increased activity of alveolar macrophages. They also support the inflammatory-repair hypothesis in the development of emphysematous changes.

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