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ORIGINAL ARTICLE

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Ultrastructural study of the destructive and repair processes in pulmonary inflammation and following endobronchial laser therapy

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Abstract Examination of 127 biopsy specimens from 45 patients with inflammatory lung diseases showed changes consistent with increased permeability of the capillary endothelial cells as an initial stage in the development of the inflammatory reaction. Associated interstitial oedema, deformation of the interalveolar septa, and structural disorganization of alveolar epithelium cells occur, and local microcirculatory problems result in tissue hypoxia and fibrosis. The ultimate morphological picture is determined largely by the intensity of repair. Laser biostimulation minimizes the inflammation and stabilizes fibroplastic process.

Key words Inflammation of the lung · Biopsy · Ultrastructure · Laser therapy

Introduction

The development of the inflammatory reaction is accompanied by considerable structural change in the respiratory tissue of the lung [1–4, 7]. There is a balance between destructive and repair processes, which depends in part on the pattern of inflammation and in part on therapy.

Materials and methods

One hundred and twenty-seven biopsy specimens of respiratory tissue taken from 45 patients with acute and chronic inflammatory lung disease (acute, necrotizing and chronic abscess of the lung) were studied. In 21 cases (55 biopsy specimens of the respiratory tissue) a bronchoscopy was carried out with scattered irradiation of the bronchi using an LG-75 helium-neon laser continuously emitting light with a wavelength L=632.8 nm; the output was 3 mW. The number of sessions depended on the therapeutic effect and varied from two to six, with an exposure of 3–5 min. The laser dose was determined experimentally [6]. This pattern has been shown to be optimal for mild biostimulation without damage to the components of the bronchial wall.

The control group consisted of 24 patients with similar lung diseases treated by traditional anti-inflammatory measures, without laser therapy. Biopsy specimens were obtained from both groups; one fragment from the wall of the cavity of an abscess, one fragment from a perifocal zone and one fragment from an area without visible signs of inflammation.

Most of the biopsy specimens were fixed in a 10% solution of neutral formalin. That part of the biopsy specimen intended for electron microscopy was fixed in a 4% solution of paraformaldehyde and a 1% solution of OsO_4 and embedded in a mixture of Epon and Araldite.

In each case, paraffin, semithin and ultrathin sections had been cut. The paraffin sections were stained with haematoxylin and eosin together with Perls' reaction, by Van Gieson's method with elastic fibres stained with Weigert's resorcinol-fuchsin. Semithin sections were stained with Azure II, while the ultrathin sections were stained with urane acetate and lead citrate.

Results

Examination of abscesses in the lung in the group of patients treated by traditional methods revealed cavities filled with pus and necrotic tissue fragments (Fig. 1a) with an unpleasant smell. In 11 cases the abscess was at the centre of an area of inflammation and purulent infiltration. Encysted chronic abscesses were observed in 13 cases. Abscesses varied in shape and size.

Differing structural manifestations of the inflammatory reaction were found adjacent to and distant from the central abscess, allowing us to divide the specimens into four relatively stable groups reflecting consecutive stages of the inflammatory reaction [4]:

1. Tissue fragments without changes in their structural organization;
2. Tissue fragments with reactive endothelial cells, but without interstitial manifestation of the inflammatory reaction;

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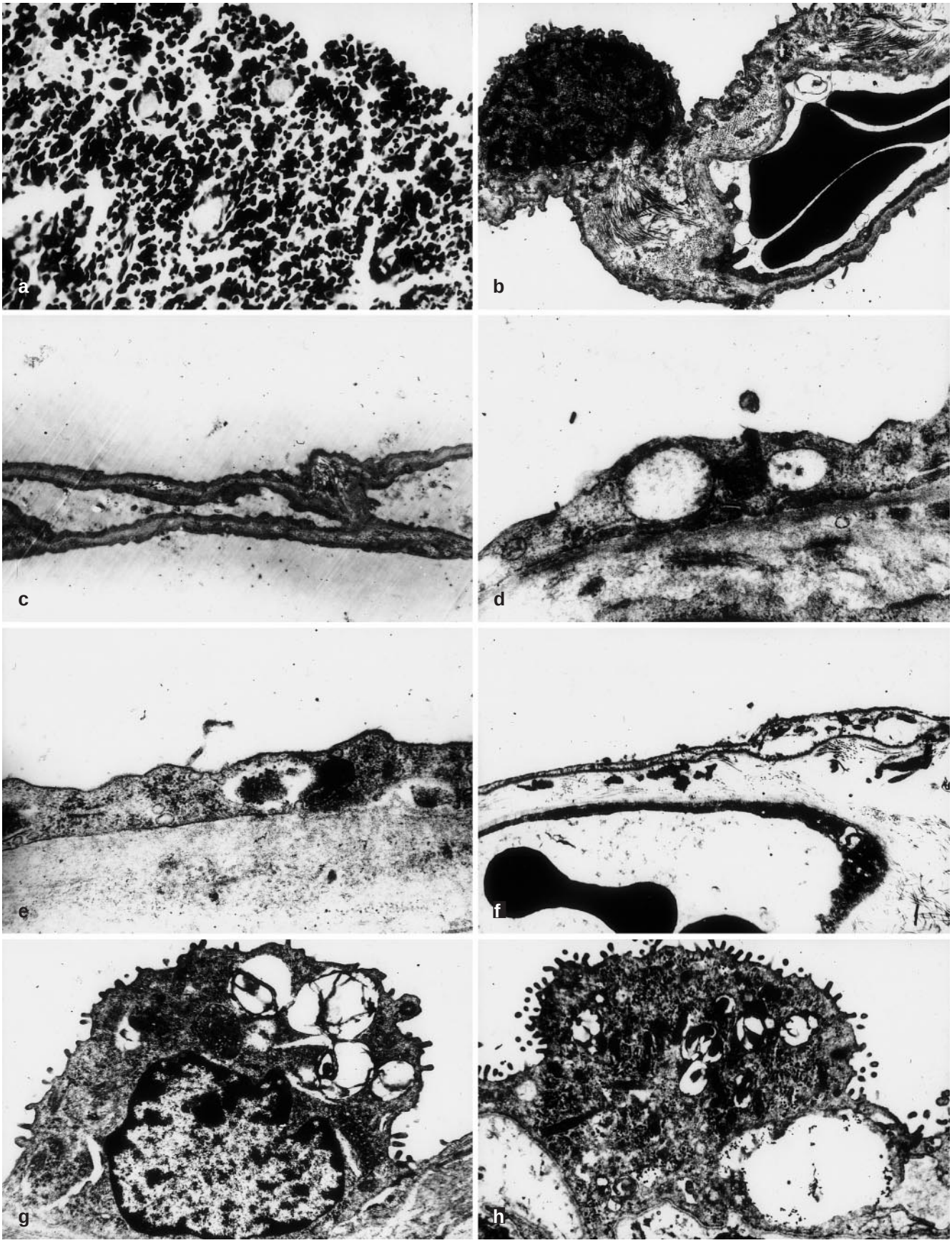
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3. Tissue fragments with reactivity of endothelial cells in combination with manifestation of inflammation in the interstitial tissue;
4. Tissue fragments with fibrotic processes in the interstitial tissue predominating.

The author is aware of the limitations of the subjectivity of this classification. However, it is useful to describe the processes of destruction and repair that were seen. The first group was small (Fig. 1b). The second and the third groups made up the majority.

Ultrastructural disorganization of the capillaries in the interalveolar septae (IAS) was one of the most frequent findings suggesting an influence of the abscess on surrounding tissue. Slight changes in endothelial cells were not usually accompanied by changes in the structural organization of the IAS. The second group of the biopsies showed this change. In a few epithelial cells, swelling of the mitochondrial matrix and vacuolation of the cytoplasmic reticulum was seen.

Lesions in the endothelial cells were more often combined with structural disorganization of the IAS. Increased interstitial oedema with infiltration by monocytes, lymphocytes and neutrophils was seen and was accompanied by depolymerization of ground substance and loosening and dissociation of connective tissue fibres. Basal membranes retained their structure, but sometimes appeared loosened and thinned. Thinned IAS were seen near foci of atelectasis (Fig. 1c).

Observed ultrastructural disorganization of alveolar type I cells was often connected with changes in endothelial cells. They were characterized by vacuolation of the cytoplasmic reticulum of their flattened parts (Fig. 1d), by swelling of the mitochondria with lesions in their internal arrangement and by local clarifications of cytoplasmic matrix with partial degradation (Fig. 1e). Accumulation of intracellular fluid was evident (Fig. 1f). Occasional destruction of the thin parts of the epithelial layer and uncovering of the basal membrane and connective tissue of the alveolar walls were observed.

Increased volume of the mitochondria in alveolar type II cells, with clarification of their matrix and total destruction of their cristae, and dilatation of the cytoplasmic reticulum with vacuolation were observed frequently. The ultrastructure of osmophilic lamellar bodies was also altered: some simply increased in size, but changes in structure were also noted. Division into layers with wide light spaces, thinning, and destruction with the decrease of osmophilia was seen in many forms; sometimes lamellar bodies transformed into large vacuoles containing a homogeneous substance of slight electron density (Fig. 1g, h) and isolated segregated fragments of osmophilic lamellae.

In some cells union of vacuolated lamellar bodies, with signs of degradation of the cytoplasmic matrix, was observed (Fig. 2a). Destruction of osmophilic lamellae with transformation into a homogeneous substance typical of neutral lipids was seen (Fig. 2b).

There was marked variability in the lesion in the IAS. In the capillaries endothelial cells with an increased volume of cytoplasm containing numerous mitochondria, elements of granulated cytoplasmic reticulum and lamellar Golgi complexes, and multiple cytoplasmic processes on basal and luminal surfaces were observed. Thinning of the peripheral parts of endothelial cells was noted in some capillaries.

Hypertrophied alveolar type II cells and transitional forms from alveolar type II cell to alveolar type I cell were frequently found. In some alveolar type II cells the formation of unusual long thin cytoplasmic processes covering naked areas of basal membrane (like those of alveolar type I cells) was observed (Fig. 2c). Moreover, local proliferation of alveolar type II cells restoring the integrity of epithelial cover and replacing damaged alveolar type I cells was noted (Fig. 2d).

In the alveolar type I cells manifestations of intracellular hypertrophy and hyperplasia (development of elements of granulated cytoplasmic reticulum and lamellar Golgi complexes, increase of number of lysosomal structures and intracellular organelles in the perinuclear area) were observed frequently (Fig. 2e).

The fourth group of biopsy specimens accounted for approximately one third of the samples studied and was distinguished by high variability. Generally, massive scars with developing perivascular and peribronchial fibrosis formed in the lung.

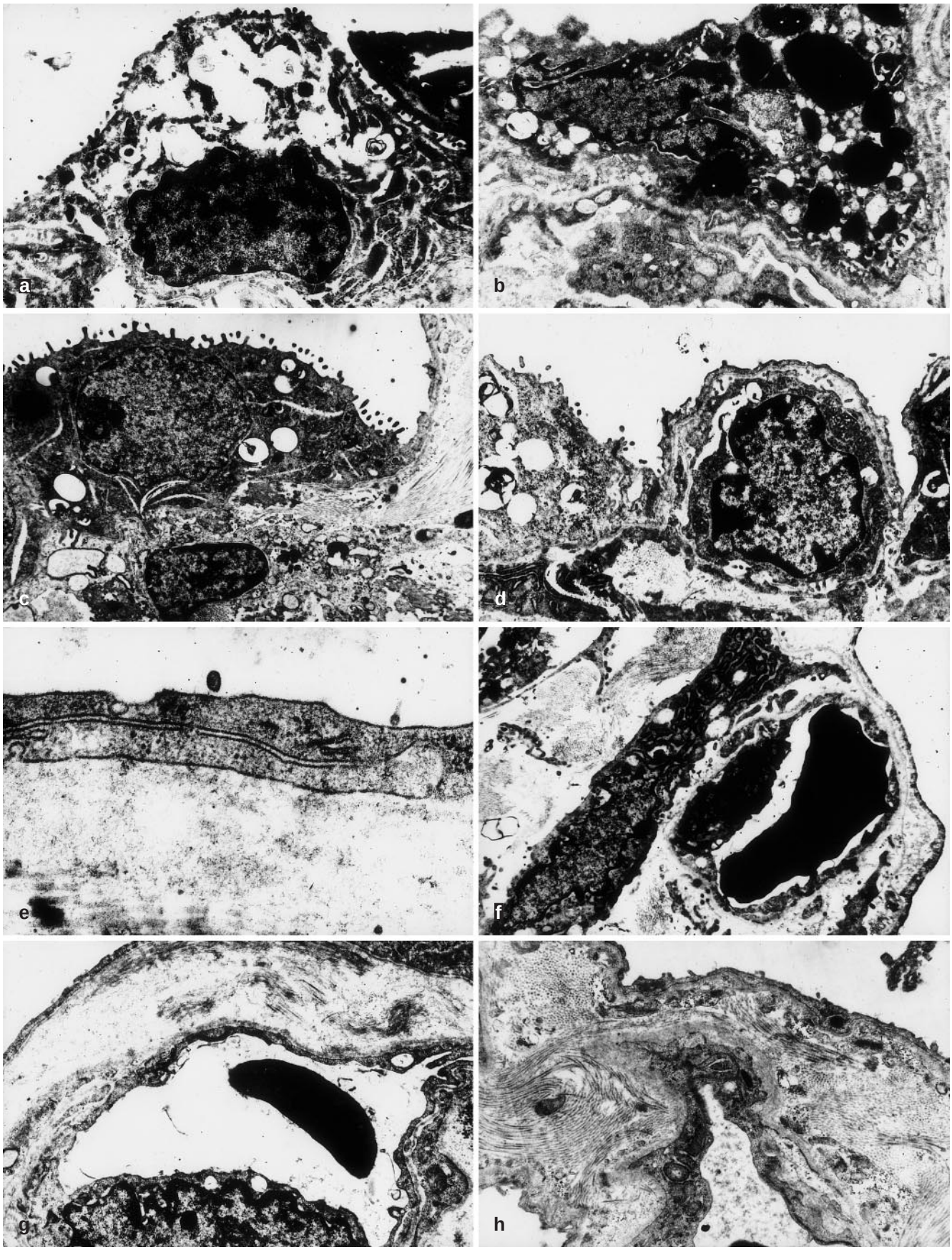
In the interstitial tissue of the IAS formation of fibrous connective tissue by fibroblasts was seen. The ultrastructure suggested a high functional activity for fibroblasts (Fig. 2f). Collagen fibrils were placed loosely, forming longitudinal-transversal bundles with no definite orientation (Fig. 2g). In other cases they were irregular and were placed longitudinally relative to basal membranes (Fig. 2h). Fibrocytes transforming from active fibroblasts were found among large bundles of collagen fibres.

The formation of fibrous connective tissue produces thickening of the blood-air barrier (Fig. 3a) and destruction of the blood capillaries. IAS with complete replacement of interstitial structures by rugged fibrous connective tissue were also observed (Fig. 3b). These septa appeared as thin connective tissue structures delimited on both sides by alveolar epithelium.

The majority of the capillaries within connective tissue had ultrastructural appearances consistent with heightened functional activity of endothelial cells.

Proliferation of the alveolar type II cells, which often completely covered compressed alveolar lumina, was noted in the areas of pulmonary fibrosis (Fig. 3c). Young

Fig. 1 **a** Fragment of the wall of an abscess. Paraffin section. Haematoxylin and eosin, ×240 **b** Normal ultrastructural organization of the IAS. ×3000 **c** Thinning of the IAS. ×2000 **d** Vacuolation of cytoplasmic reticulum in the flattened part of the alveolar type I cell. ×8000 **e** Local degradation of cytoplasmic matrix of the alveolar type I cell. ×8000 **f** Intraepithelial oedema in the flattened part of the alveolar type I cell. ×3000 **g** Dissociation and thinning of osmophilic lamellae of the alveolar type II cell. ×4000 **h** Large vacuole in the cytoplasm of the alveolar type II cell. ×4000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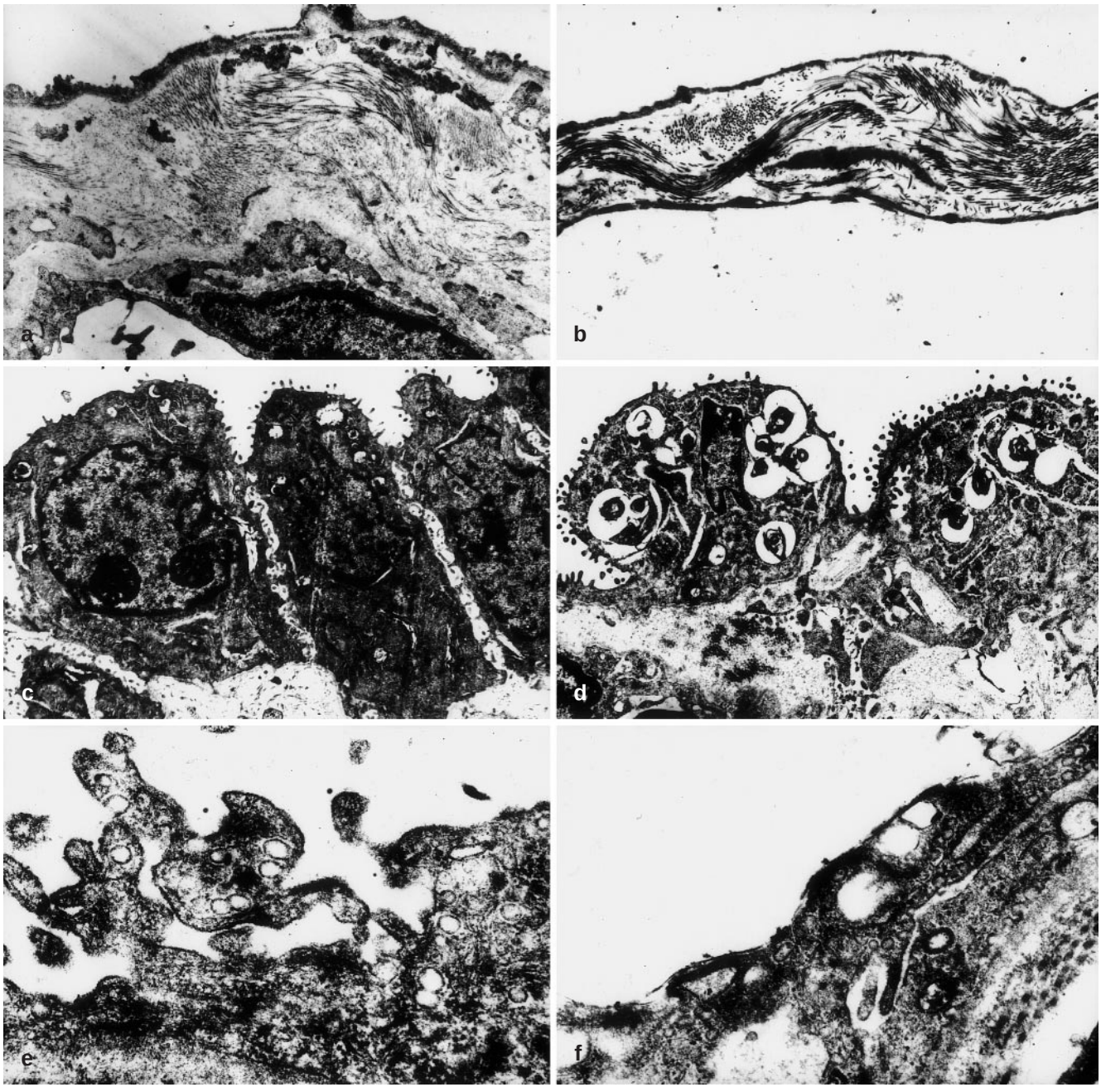

Fig. 3 **a** Thickening of the blood-air barrier. ×3000 **b** Connective tissue structure with bilateral epithelialization. ×3000 **c** Metaplasia of the alveolar epithelium. ×3000 **d** Hypertrophy of the proliferating alveolar type II cells. ×3000 **e** Forming cytoplasmic processes of the alveolar type I cell. ×8000 **f** Double epithelial covering. ×8000

◀ **Fig. 2** **a** Vacuolation and junction of osmophilic bodies in the alveolar type II cell. ×4000 **b** Homogeneous electron-dense inclusions in the cytoplasm of the alveolar type II cell. ×4000 **c** Flattening of the alveolar type II cell. ×4000 **d** Proliferation of the alveolar type II cells. ×3000 **e** Elements of the cytoplasmic reticulum in the flattened part of the alveolar type I cell. ×8000 **f** Activated fibroblast. ×3000 **g** Interstitial oedema and formation of thin bundles of the collagen fibres. ×4000 **h** Longitudinal (relative to basal membranes) orientation of the hypertrophied bundles of the collagen fibres. ×4000

proliferating cells were cuboidal and contained rare small structures of osmophilic lamellar bodies. In the mature cells intensive formation of these bodies was observed (Fig. 3d). They were seen on alveolar surfaces and within alveolar macrophages.

Processes projecting deeply into alveolar lumina were typical for the alveolar type I cells (Fig. 3e). Occasional superposition of peripheral parts of cells with the formation of a double epithelial cover was also observed (Fig. 3f).

In patients receiving laser therapy, lesions differed significantly from those described above. Much less inflammatory reactions and “cleaner” abscess cavities were the most marked distinctions. Most abscesses were small.

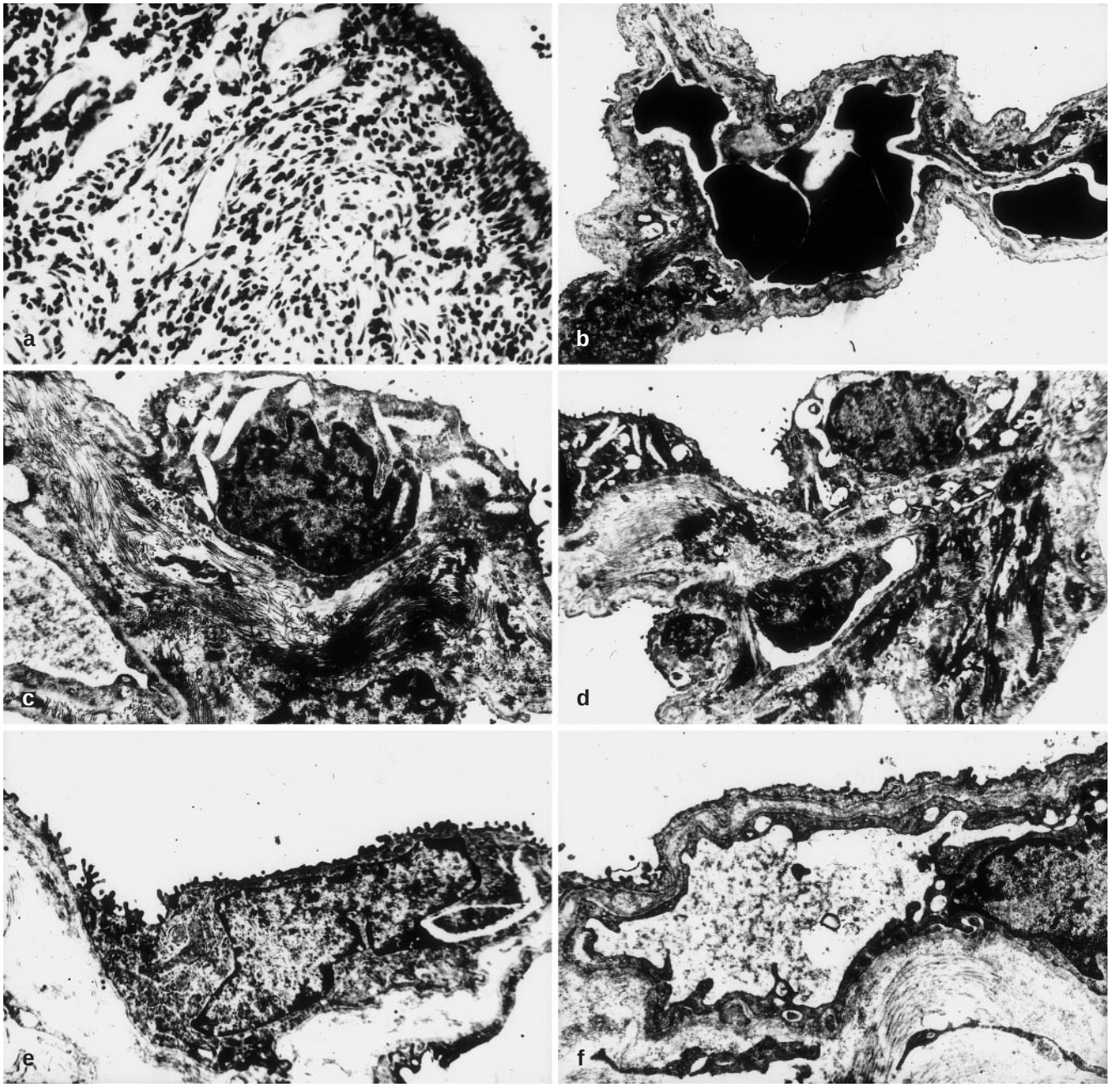

Fig. 4 **a** Epithelialization of the wall of an abscess. Paraffin section. Haematoxylin and eosin, ×240 **b** Normal ultrastructural organization of the IAS. ×3000 **c** Regular orientation of the bundles of collagen fibres. ×3000 **d** Depolymerization and dissociation of some bundles of collagen fibres. ×3000 **e** Transitional form of the alveolar epithelium cell. ×3000 **f** Heightened permeability of the blood capillary endothelial cells. ×3000

There was little evidence of marked inflammation. Epithelialization of internal surfaces of abscess cavities followed laser therapy in 11 cases of 13 (Fig. 4a). The epithelium was stratified ciliated or sometimes stratified squamous. The presence of basal cells, a small number of goblet glandular cells, and variability of height were typical of stratified ciliated epithelium. In some areas epithelium was transitional and consisted of young, actively proliferating basal and interstitial cells. Cicatricial formations of rugged fibrous connective tissue without signs of perifocal inflammation were found in abscess zones in 8 instances.

Ultrastructural studies showed significant features. There was a lack of destructive change, and the majority of septa appeared normal (Fig. 4b).

Fibrosis was persistent in the septa that were affected, but the fibrosed interstitial tissue of the IAS showed regular orientation of collagen fibres (Fig. 4c). Depolymerization and break-up of connective tissue structures were observed (Fig. 4d).

Ultrastructural variations in blood-air barrier cells included hypertrophy of the alveolar type I cells with invaginations of nucleolemma and multiple intracellular organelles in cytoplasm. Flattened transitional forms with small number of osmophilic lamellar bodies against a background abundance of other intracellular organelles were more typical for alveolar type II cells (Fig. 4e). No signs of active proliferation of these cells were observed.

Sometimes, signs of interstitial oedema and local alteration of some cells were seen (Fig. 4f).

Discussion

The initial changes produced by abscesses in nearby tissue and in the development of the inflammatory process in the lung depend on a increased permeability of the blood capillary endothelial cells of the IAS. The progressive increase in permeability results in the structural changes to the IAS, and local lesions in the microcirculation are accompanied by tissue hypoxia and activation of fibroblasts.

The changes described encompass considerable individual variation and are closely connected with compensatory-adaptive and repair processes.

In the early stages of inflammation the structure of the blood-air barrier cells alters markedly. However, endothelial and epithelial cells with active function and hypertrophied cells providing normal level of respiration are also observed together with damaged cells.

The development of the inflammatory reaction is accompanied by cell proliferation, with hypertrophy of alveolar type II cells providing surfactant and proliferation providing for the restoration of epithelial integrity.

Destructive processes progress and prevail over repair where there is chronic inflammation. Functional loading on blood-air barrier cells increases, and local hypoxia develops. Local lesions in the microcirculation and tissue hypoxia promote the development of fibrotic processes in the respiratory tissue [8].

In the areas of pulmonary fibrosis active proliferation of the alveolar type II cells is observed, accompanied by the covering of the deformed alveolar lumina. Thus, fibrosis combines with hyperplasia of alveolar type II cells and metaplasia of the alveolar epithelium. The process is compensatory: it replaces alveolar type I cells with cells that are more resistant to injury but does not promote normalization of respiration.

Replacement of the capillary system by scarring disrupts the IAS. The direction of therapy should be toward elimination of lesions in the microcirculatory bed and their consequences. Laser therapy answers the criteria required [4].

The local biostimulating influence of laser irradiation promotes restructuring of the bronchial mucosa [4, 5]. This results in restoration of the drainage and clearing function of the bronchial system and the effective evacuation of purulent masses from abscesses. Elimination of influence of pathogenic and toxic factors on surrounding respiratory tissue creates the conditions for reversal of the inflammatory reaction.

The morphological picture of the lung differs appreciably in our groups of patients. Marked manifestations of acute inflammatory reaction are typical of patients receiving traditional treatment, while cicatricial formations or epithelialization of cavities with slightly expressed perifocal inflammatory reaction and an absence of signs of active abscesses are typical of patients receiving laser therapy in addition.

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