ELECTRON-MICROSCOPIC AND AUTORADIOGRAPHIC STUDY OF CHANGES IN BRONCHOALVEOLAR WASHINGS IN CHRONIC INFLAMMATION OF THE LUNGS TREATED BY HELIUM–NEON LASER

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The morphological manifestations of the clinical effect of laser irradiation have received little study. The role of the cell membrane structures in the formation of the response to laser irradiation, reflected in stimulation of energy metabolism and biosynthetic processes has been demonstrated experimentally [2]. The biostimulating effect of laser therapy has been related to induction of regeneration [1, 4, 13, 14]. If laser therapy is included in the combination treatment of chronic inflammatory diseases of the lungs, the structural changes arising in the air passages can be studied with the aid of bronchial biopsy [8]. However, no morphological investigations of respiratory tissues have been described in the literature.

In the investigation described below bronchoalveolar lavage (BAL) was carried out in order to study structural and metabolic reactions of the respiratory compartment in the presence of chronic inflammatory disease of the lungs treated with helium–neon laser radiation.

EXPERIMENTAL METHOD

Bronchoalveolar washings (BAW) (136) from 45 male patients aged from 25 to 62 years with chronic inflammatory diseases of the lungs, were studied. Besides the traditional combination of therapeutic measures, 35 patients also were treated by laser therapy. During therapeutic bronchoscopy, the drainage bronchi were irradiated by scattered radiation of an LG-75 helium–neon laser, emitting light continuously with a wavelength λ = 632.8 nm and with an output power of 3 mW, along the light guide. The number of sessions depended on the therapeutic effect and varied from 2 to 6, with an exposure of 3-5 min. The washings were obtained before each session of laser therapy. Material was taken from the affected lung segments, and in nine cases from neighboring, unaffected segments. The control group consisted of 10 patients with chronic inflammatory diseases of the lungs, receiving traditional anti-inflammatory treatment without laser therapy. BALB was performed on these patients also before and after the course of treatment. After bronchoscopy and BAL [3, 5] the total number of cells in 1 ml of washings (cell count) was determined, and a differential cell count of the washings was undertaken on films stained by Pappenheim's method. Some of the residue in all cases was fixed with 4% paraformaldehyde solution and postfixed with 1% osmium tetroxide solution, after which the standard treatment was carried out for embedding in epoxide resins. Semithin sections were stained with azure II and Schiff's reagent; ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-100B electron microscope. In 10 cases unfixed specimens were incubated for 1.5 h at 37°C in medium 199 containing 200 μCi/ml of ^{3}H-uridine with specific radioactivity of 29 Ci/mmole (12 specimens) or with 100 μCi/ml of ^{3}H-thymidine with specific radioactivity of 40 Ci/mmole (eight specimens). Later the incubated material was fixed and embedded in epoxide resins. An autoradiographic analysis was carried out on semithin sections exposed with type M photographic emulsion for 5 days.

EXPERIMENTAL RESULTS

Before laser irradiation, the BAW contained representatives of different cell populations, but in every case neutrophils predominated (Table 1). They accounted for 94.8 ± 1.3%

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TABLE 1. Cell Count and Differential Count in BAW from Patients with Chronic Inflammatory Diseases of the Lungs under Treatment by Helium–Neon Laser

Inflammatory disease	Parameter	Before laser treatment	After laser treatment, days		
			15	35	over 50
Chronic abscess	Total cell count, $\times 10^6$/liter	934,1±147,2	616,0±80,1	343,0±68,3	264,5±47,2
	Neutrophils, %	94,8±1,3	90,4±2,9	87,8±3,3	77,7±4,9
	Macrophages, %	2,8±1,2	6,8±2,9	9,0±2,9	19,7±5,0
	Lymphocytes, %	2,2±0,4	1,8±0,4	1,3±0,6	2,0±0,6
	Eosinophils, %	0,2±0,1	0,9±0,2	1,8±0,9	0,7±0,3
Chronic pneumonia	Total cell count, $\times 10^6$/liter	631,3±86,0	621,3±96,8	519,2±95,6	524,0±94,3
	Neutrophils, %	95,1±0,8	91,3±1,8	89,3±3,1	84,7±2,9
	Macrophages, %	2,4±0,6	6,0±1,8	8,0±2,4	12,0±2,6
	Lymphocytes, %	1,8±0,3	2,0±0,3	2,0±0,9	2,0±0,6
	Eosinophils, %	0,8±0,2	0,9±0,2	0,8±0,3	1,3±0,7
Gangrene of the lung	Total cell count, $\times 10^6$/liter	1095,2±128,1	555,0±59,9	368,0±110,7	345,7±60,0
	Neutrophils, %	96,2±0,6	93,2±0,6	90,5±1,3	80,0±2,0
	Macrophages, %	1,8±0,8	4,0±0,8	6,3±1,7	13,0±2,0
	Lymphocytes, %	1,8±0,3	2,2±0,7	3,0±0,4	4,5±2,0
	Eosinophils, %	0,2±0,1	0,6±0,2	0,3±0,2	2,5±0,2

in patients with chronic abscesses, 95.1 ± 0.8% with chronic pneumonia, and 96.2 ± 0.6% in patients with chronic abscesses complicated by gangrene. The number of macrophages was sharply reduced in the cases studied (not less than 90% in the differential count of the healthy subjects), and the number of lymphocytes also was reduced; quite often alveolar epithelial cells were found. The cell count was 5-10 times higher than normally. The number of neutrophils in the washings from unaffected lung segments was smaller (82.8 ± 2.2%), and the cell count was 351.5 × 10^6/liter, or 1.5-2 times above the normal value.

During laser therapy the number of neutrophils was reduced in the BAW in all cases, and the relative percentage of macrophages was increased, more especially (by 10 times) in the patients with chronic abscesses. The greatest decrease in the cell count also was observed in patients with chronic abscesses.

Ultrastructural analysis showed marked heterogeneity of the population of lavage macrophages. We distinguished five main structural-metabolic forms of these cells. The 1st form consisted of cells with small cytoplasmic outgrowths, a bean-shaped nucleus, and a small number of cytoplasmic organelles. The structure of these cells corresponded to the transitional form between monocytes and macrophages. The 2nd form was distinguished by ultrastructural features characteristic of cells with active protein synthesis. Macrophages of the 3rd form were characterized by the development of a lysosomal compartment: macrophages of the 4th form were actively phagocytic cells. Cells of the 5th form showed signs of degeneration. Metabolic activity (as shown by incorporation of ^{3}H-uridine) was highest in young and activated forms of macrophages (1st, 2nd, and 3rd forms).

Before laser therapy macrophages of the 4th and 5th forms predominated (Fig. 1a) in BAW and cells of the 1st and 2nd forms were hardly ever seen. In the early stage after the 1st laser treatment macrophages belonging to the 1st and 2nd structural-metabolic forms appeared (Fig. 1c), and after 30-35 days, all five cell forms described were present in the macrophage population of the washings.

The autoradiographic investigation of cells of BAW showed an increase in metabolic activity of the alveolar macrophages during laser treatment (Fig. 2a, b). The labeling index with ^{3}H-uridine was 53.9 ± 4.4% for macrophages of BAW of the affected segments, and 56.6 ± 9.1% for macrophages of BAW from unaffected segments, before treatment. These figures rose after 20-25 days to 75.0 ± 5.6 and 79.8 ± 6.5% respectively in the affected and unaffected segments. The labeling index with ^{3}H-thymidine rose after 7-10 days from 0.4 to 1.6%, but later it fell to regain its initial value (Fig. 2c, d). Hence it can be concluded that the process of proliferation of the macrophages is induced by laser radiation, and their structural and functional heterogeneity reflects the successive stages of development from precursor cells to cells with a fully developed lysosomal cycle with subsequent phagocytosis.

Such a broad spectrum of phenotypic expression of macrophages corresponds to the concept of a series of interconnected compartments, participating in the formation, transport, and adaptive response of macrophages [11]. Experimental studies have demonstrated a significant increase in mitotic activity of the interstitial and alveolar macrophages following injection of carbon particles into animals, evidence that under "loading" conditions, all three compartments

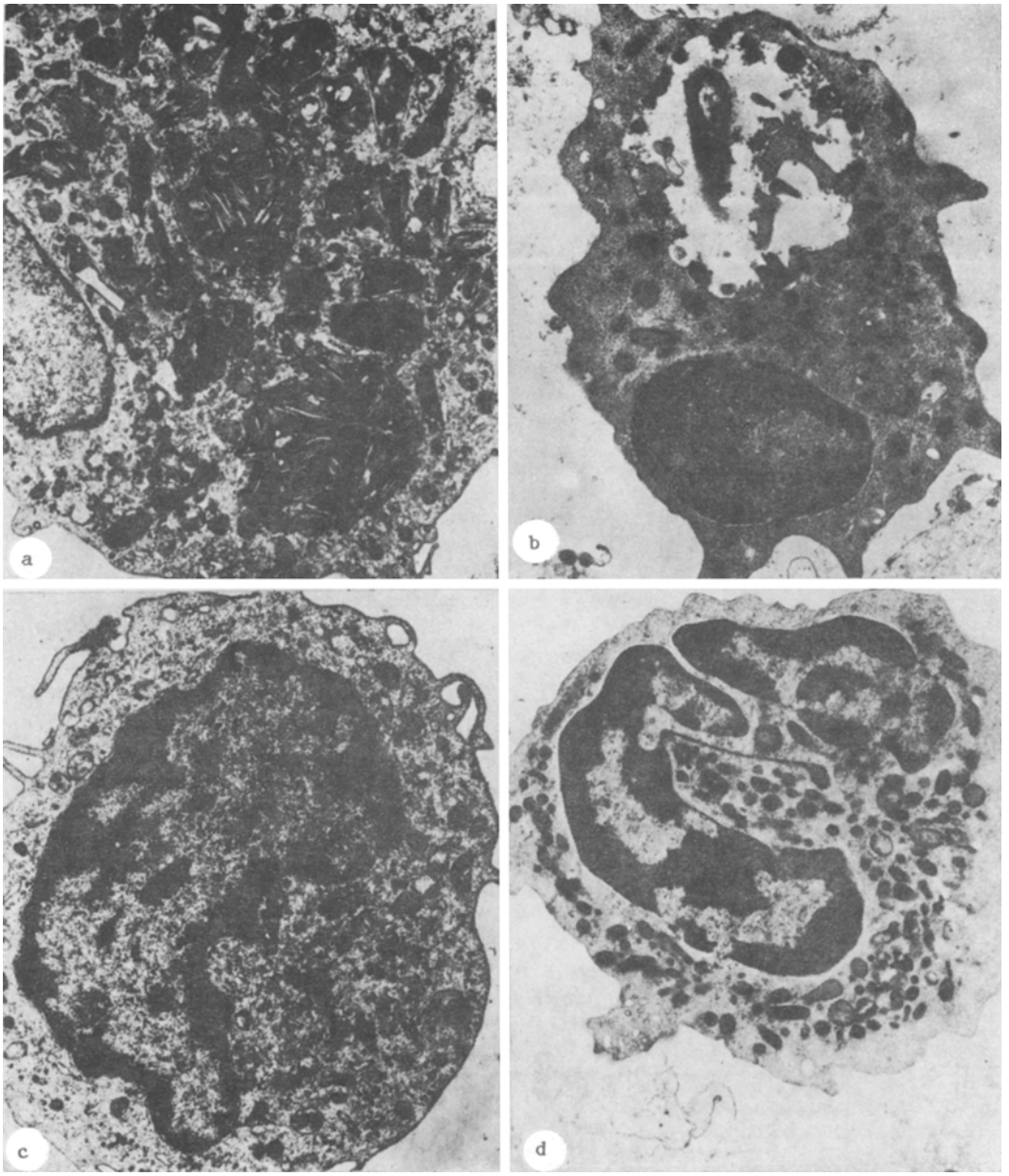

Fig. 1. Ultrastructure of macrophages and neutrophils of BAW in chronic inflammation of the lungs treated by endobronchial helium–neon laser therapy. a) Macrophage with abundant polymorphic phagosomes, many containing needle-shaped structures. 3300×; b) neutrophil containing a few specific granules and a large, fragmented phagosome. 8300×; d) alveolar macrophage of monocyte-like type with bean-shaped nucleus. 8300×; d) neutrophil with typical nucleus and abundant polymorphic granules. 6600×. a, b) Before and c, d) after laser therapy.

(hematogenous, interstitial, and alveolar) make their own contribution to the increase in number of macrophages [10, 12, 15].

Neutrophils in the BAW likewise were heterogeneous. Ultrastructural analysis showed that before laser treatment degenerative forms of the cells predominated. After the first session of laser therapy, many cells containing a segmented nucleus and a large quantity of heterochromatin, together with large numbers of specific azurophilic granules in their cytoplasm, appeared (Fig. 1b, d). There were also many phagocytic cells, but degenerative forms were less frequent.

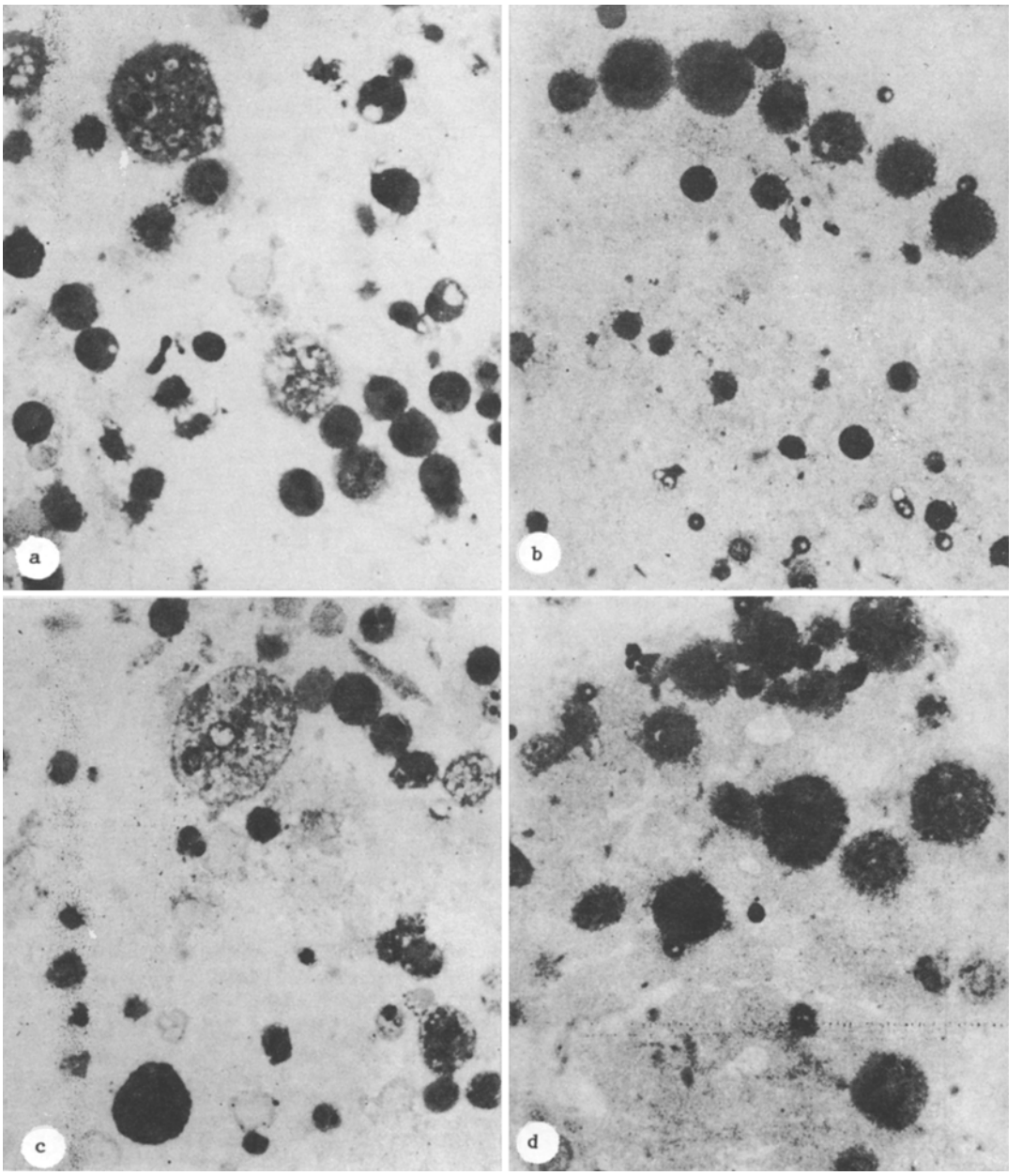

Fig. 2. RNA and DNA synthesis in cells of BAW in chronic inflammation of the lungs, with endobronchial helium–neon laser therapy. a) Incorporation of ^{3}H-uridine into macrophages and neutrophils; b) many macrophages contain label with ^{3}H-uridine; c) absence of label with ^{3}H-thymidine in cells of BAW; d) DNA synthesis in a macrophage. a, b) Before and c, d) after laser treatment. Semithin sections, stained with azure II. 1000×.

No such significant changes, either quantitative or qualitative, were found in the lymphocyte population, but it will be recalled that laser irradiation led to the appearance of activated forms of these cells. Alveolocytes also were found extremely rarely.

A trend of this kind was not found in the BAW of patients not receiving laser therapy. The decrease in the cell count was very small, the relative percentage of neutrophils was unchanged, and the number of macrophages was actually reduced.

Changes in the respiratory tissue during laser treatment were judged on the basis of data for BAW, paying due regard to the fact that this picture differs significantly from that observed in situ. However, considering the marked clinical effect and the data of bronchologic (roentgenologic) investigations, the results of BAL can be interpreted as reflecting positive

changes in the trend of the cell populations in the respiratory zones of the lungs. The exudative–necrotic component of the inflammatory reaction was reduced under these circumstances, as shown by a decrease in the number of neutrophils (especially degenerating forms, in the washings and disappearance of the alveolar epithelium from them also. Compensatory reactions were intensified, meanwhile, as reflected in activation of the mononuclear phagocyte system and also in quantitative and qualitative changes in the lymphocytes and neutrophils.

Comparison of the structural and metabolic changes in the air passages [7, 8] and the respiratory zones of the lungs in patients undergoing endobronchial laser therapy leads to the conclusion that the clinical effect of laser therapy of chronic inflammatory diseases of the lungs is based on stimulation of regeneration. An important property of regenerative processes is the structural and functional integrity of the newly formed tissue and restoration of its organ-specificity [9]. During laser treatment, regenerative processes in our observations took place synchronously in the air passages and the respiratory zones of the lungs, and taken as a whole this leads to some kind of balance between functions of interconnected and interdependent tissue components of the respiratory organs [6].

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