# MITOTIC ACTIVITY OF ALVEOLAR MACROPHAGES IN NONSPECIFIC DIFFUSE LUNG PATHOLOGY

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Alveolar macrophages (AM) not only protect the body against infection and tumor cells, but also remove various substances from the lung [1-3, 6]. In the modern view stem cells of bone marrow origin are the source of AM [10]. The sequence of differentiation of the cells, namely monoblast—promonocyte—monocyte—macrophage, likewise is no longer in dispute [9]. The macrophage is the most mature form of this cell line. Two types of macrophages are distinguished in the lung: histiocytes, or "fixed" cells of lung tissue structures, and "wandering" AM, located on the surface of the epithelial lining of the respiratory tract [5]. The problem of whether differentiated "wandering" AM can divide by mitosis is still a matter for discussion [5, 6, 9, 12]. It has been shown that human AM obtained from bronchoalveolar washings (BAW) can incorporate $^3$H-thymidine, i.e., they can take part in premitotic DNA synthesis [4, 11]. However, the scale of mitotic activity of these cells in native BAW from human lung has not been discovered.

The aim of the present investigation was to determine the level of mitotic activity of "wandering" AM in BAW from patients with nonspecific diffuse lung pathology and to discover the degree of differentiation of cells found in mitosis.

## EXPERIMENTAL METHOD

The cell composition of BAW from 30 patients aged from 22 to 60 years (16 men and 14 women) was investigated. The number of smokers among the men was 13, and among the women, one. Bronchial asthma (infectious-allergic form, moderately severe course) was diagnosed in eight patients, chronic bronchitis with a bronchospastic component , in the exacerbation phase, in 11 patients, nonspecific disseminated lung disease (sarcoidosis in three, exogenous allergic fibrosing alveolitis in three, diffuse interstitial fibrosis of the lungs based on scleroderma in three, microlithiasis in one, and pneumonia in one) in 11 patients. BAW were obtained under local anesthesia by the transnasal method, by means of an Olympus single-channel fibroscope (Japan) at the same time of day (10-11 a.m.). The number of cells in 1 ml of BAW was determined in a Goryaev counting chamber and their viability was studied by staining with 1% trypan blue. Cytologic preparations of the cell monolayer were made from the cell suspension of BAW by an original method, and stained by the Pappenheim—Kryukov method. The first portion of BAW was not used for cytologic study. The endopulmonary cytogram, i.e., the relative percentages of all the different cells, excluding bronchial epithelial cells and erythrocytes, was compiled on the basis of examination of 1000 cells. In each case the number of mitoses was counted in 4000 mononuclear viable AM and the mitotic index (MI) was expressed in promille. In 10 cases the cell suspension of BAW was fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4). The cells were postfixed in 1% $OsO_4$ solution. After dehydration the material was embedded in Epon-Araldite and examined in the JEM-100C electron microscope.

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TABLE 1. Mitotic Activity and Viability of AM from BAW from Patients with Lung Pathology

| Diagnosis | No. of cells in 1 ml BAW ($\times 10^6$) | No. of viable AM, % | MI, ‰ |
|---|---|---|---|
| Bronchial asthma, infectious-allergic form (8) | 0,17±0,38 | 63,7±0,004 | 0,38±0,24* |
| Chronic bronchitis with bronchospastic component (11) | 0,27±0,16 | 75,0±0,003 | 0,64±0,16* |
| Nonspecific disseminated lung disease of uncertain etiology (11) | 0,39±0,08 | 86,4±3,1 | 0,86±0,22* |
| For all patients (30) | 0,28±0,03 | 72,0±3,14 | 0,65±0,03 |

Legend. Asterisk indicates that differences between parameters are not significant ($p > 0.05$). Here and in Table 2, the number of patients shown between parenthesis.

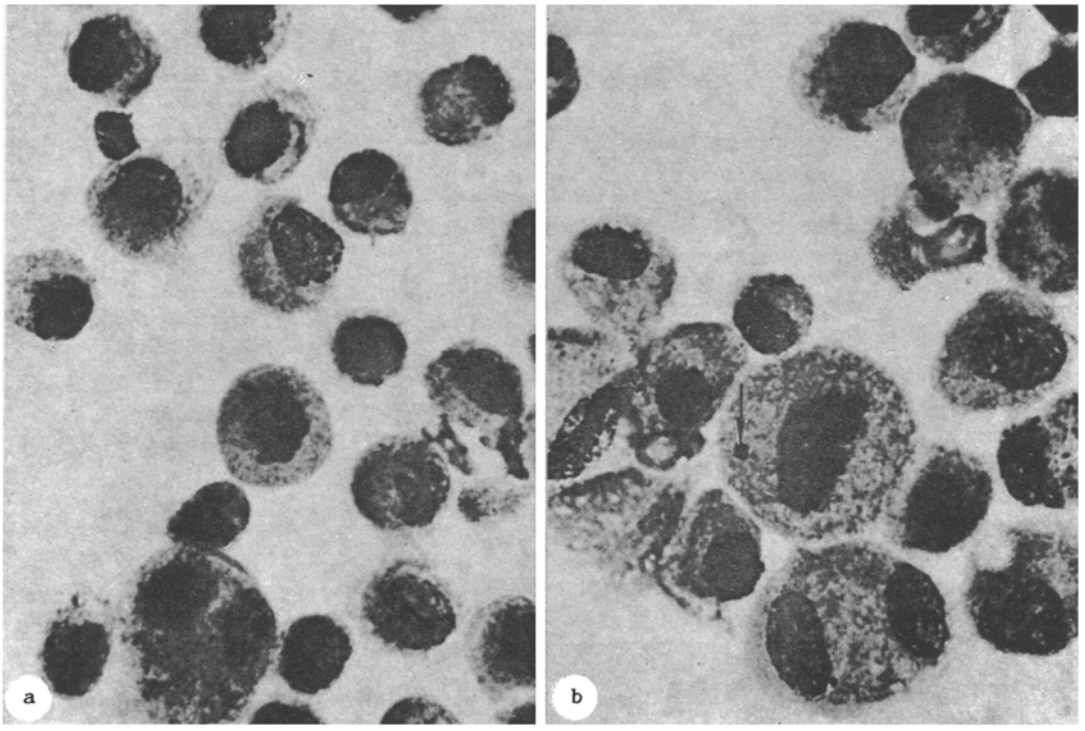


Fig. 1. Mitoses in AM from BAW of patients with lung pathology. a) Metaphase (patient with microlithiasis). 1000×; b) metaphase (patient with exogenous allergic alveolitis): phagocytosed particle can be seen (arrow) in cell cytoplasm. 1000×. Staining by Pappenheim–Kryukov method.

TABLE 2. Endopulmonary Cytogram of Patients with Lung Pathology

| Diagnosis | AM—monocytes | Lymphocytes | Neutrophilic leukocytes | Eosinophilic leukocytes |
|---|---|---|---|---|
| | % | | | |
| Bronchial asthma, infectious-allergic form (8) | 70,13±10,9 | 4,7±1,51 | 7,37±4,52 | 10,34±4,06 |
| Chronic bronchitis w/bronchospastic component (11) | 93,7±1,16 | 3,36±0,81 | 1,9±0,86 | 0,99±0,62 |

Legend. Because of the considerable heterogeneity of the cell composition of BAW from patients with nonspecific disseminated disease of the lungs, it was decided that mean values of relative percentages of cells in BAW would serve no useful purpose.

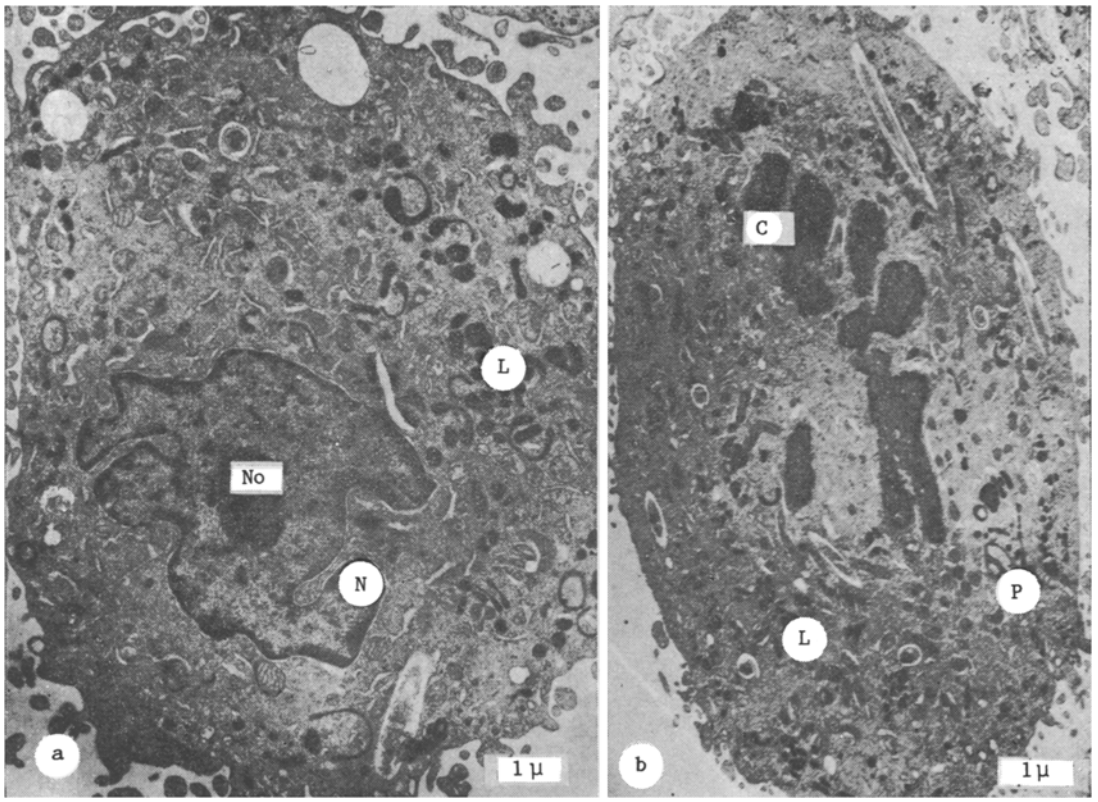


Fig. 2. Ultrastructural organization of AM of a patient with microlithiasis. a) AM in interphase. 9500×; b) AM in state of mitosis. Metaphase: lysosomes and phagosomes can be seen in the cytoplasm. 7000×. N) Nucleus; No) nucleolus; L) lysosomes; P) phagosome, C) chromosomes.

## EXPERIMENTAL RESULTS

The number of cells in 1 ml of BAW varied in different patients, with an average value of $(0.28 \pm 0.03) \cdot 10^6$ (Table 1). In some long-term smokers suffering from chronic bronchitis asthma, this figure reached $0.6 \cdot 10^6$ cells/ml BAW. Light-optical and electron-microscopy of the preparations revealed monocytes, alveolar macrophages, lymphocytes, neutrophilic, basophilic, and eosinophilic leukocytes and, in individual cases, solitary mast cells, epithelioid cells, type II alveolocytes, bronchial epithelial cells, and multinuclear AM of the foreign body giant cell type.

AM predominated in the endopulmonary cytograms of the majority of patients (Table 2, Figs. 1 and 2). In seven of eight patients with bronchial asthma, however, a high eosinophilia was observed (from 5.8 to 33.4%). In the BAW of patients with chronic bronchitis with a bronchospastic component either eosinophils were absent (two cases) or there was a mild (up to 1%) eosinophilia (eight cases); only in one case did the BAW reveal a high eosinophilia (7.1%). Neutrophilic leukocytes constituted a high proportion of the endopulmonary cytogram only in patients with chronic inflammatory conditions in the flare-up stage. In two patients with sarcoidosis the number of lymphocytes was increased (25 and 35.1% respectively).

Mitosis in AM from BAW were found in 24 of 30 cases (Fig. 1). All phases of mitosis were seen, from prophase to telophase. Mitotic activity of AM showed individual variations and varied from 0.25 to 2.75%. The largest number of mitoses (0.86 ± 0.22%) was found in a group of patients with nonspecific disseminated lung disease. In chronic bronchitis and bronchial asthma, mitotic activity of AM was rather lower (0.64 and 0.38 %; Table 1). However, differences between the average values for each group of patients were not significant ($p > 0.05$). It was accordingly decided that the mean value of MI could be determined for AM of all the patients, namely 0.65 ± 0.03%. The peak value of MI (2.75%), which we found in one case of microlithiasis, was about one-tenth of the peak value of the labeling index of human AM, obtained from BAW, in culture [4, 11].

Electron-microscopic investigation of AM in the state of mitosis showed that they have features of highly differentiated cells. Cytoplasmic outgrowths were preserved on the surface of proliferating AM and many lysosome-like structures and solitary phagosomes were observed in their cytoplasm (Fig. 2). It was thus shown convincingly that mature forms of AM, colonizing the internal medium of the human lungs under conditions of lung pathology, are capable of mitotic division. Because of these results it is possible to share the views of those workers [11] who consider that the human AM population is replenished in two ways: by an inflow of monocytes from the blood stream into the lungs and by proliferation of local "wandering" AM. In the human lungs, AM evidently constitute a slowly proliferating cell population capable of self-maintenance.

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