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AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN BRONCHOALVEOLAR-LAVAGE MACROPHAGES IN CHRONIC INFLAMMATORY LUNG DISEASE

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KEY WORDS: chronic inflammation of the lungs; bronchoalveolar-lavage; proliferation of alveolar macrophages; autoradiography.

Alveolar macrophages account for about 90% of the total number of inflammatory and immunoeffector cells of respiratory tissue and they play an important role in the development of many pathological situations, a fact that largely determines interest in the study of their structural and metabolic characteristics [1-3, 7, 13, 15]. The results of a study of material obtained by bronchoalveolar-lavage has shown the great mobility of these cells, on the basis of which a number of diagnostic and prognostic criteria have been developed. The dynamic character of the macrophages is expressed not only by the ease with which they penetrate into the lumen of the alveoli and from it into the air passages, but also by the considerable and rapid increase in the number of cells of this population in various experimental situations and pathological processes, and as is now well known, in smokers [13].

Experiments on animals have shown that the pulmonary macrophage population can be replenished quantitatively through proliferation of these cells in situ [8, 10, 11, 14, 16]. Attempts have been made to determine whether these cells can proliferate in human respiratory tissue [9, 12].

The aim of this investigation was to study the proliferative activity of human alveolar macrophages in chronic inflammatory diseases of the lungs by the use of a radioisotope method [4, 6].

EXPERIMENTAL METHOD

Bronchial washings were obtained from 23 patients (14 men and nine women) between the ages of 26 and 61 years. Fifteen patients had chronic bronchitis, four had a chronic lung abscess, and four had fibrocavernous tuberculosis of the lungs. Bronchoalveolar lavage was carried out during bronchoscopy, when the patients were anesthetized and artificially ventilated. In the presence of destructive inflammatory changes segments of lung tissue collateral relative to the focus of destruction were investigated, whereas in patients with chronic bronchitis, various segments were studied. A No. 7 cardiac catheter was passed through a Friedel's bronchoscope as far as the origin of the subsegmental bronchus and sterile physiological saline, heated to 37°C, was injected in two portions, each of 50 ml. The fluid was aspirated 5-10

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TABLE 1. Differential Cell Count of Bronchoalveolar Washings and H-Thymidine Incorporation in Alveolar Macrophages in Chronic Inflammatory Diseases of the Lungs

Character of inflammatory disease in the lungs	Cell composition of washings, %							AM in washings % (mean value)	Labeling index of AM, %	Labeled forms of AM, %	
	AM	N	L	E	BE	AE	UC			young	mature
Chronic bronchitis:											
phase of exacerbation	48—70	15—42	1—6	1—8	3—6	1—2	2—5	59	0—0,2	89	11
phase of remission	56—83	6—18	2—7	0—6	2—4	1—3	3—6	74	0—0,15	100	0
Chronic destructive-inflammatory disease of the lung	22—60	28—68	2—8	0—3	3—7	3—5	2—7	48	1,31—2,9	72	28
Chronic lung abscess	25—54	30—65	1—5	0—5	1—5	4—6	2—4	39	1,25—3,33	86	14

Legend. AM) Alveolar macrophages; N) neutrophils; L) lymphocytes; E) eosinophils; BE) bronchial epithelium; AE) alveolar epithelium; UC) unidentified cells.

sec after injection of each portion into plastic receivers. The total volume of fluid returned was 35-45 ml. The fluid aspirated after lavage was centrifuged for 10 min at 600 g, the supernatant was decanted, and the cell residue was transferred to flasks with nutrient medium containing ^{3}H-thymidine in a concentration of 100 μCi/ml (specific radioactivity 24 Ci/mmole). The experimental and control (not containing the isotope) flasks were incubated at 37°C for 2 h, after which the cell residue was washed off in phosphate buffer, pH 7.4, fixed for 3 h in 4% paraformaldehyde, postfixed in 1% OsO_4, dehydrated for 1.5 h, and embedded in a mixture of Epon and Araldite. Semithin sections were cut and coated with type M photographic emulsion (in a dilution of 1:3). After an exposure of 6 days the sections were stained with 1% toluidine blue solution. The labeling index of the alveolar macrophages was calculated after analysis of at least 700 cells of this population in each observation. Ultrathin sections were cut from blocks embedded in the epoxide resins on an LKB Ultrotome, stained with uranylacetate and lead citrate, and examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

The light-optical study of the cell composition of the washings revealed macrophages, neutrophils, eosinophils, lymphocytes, and, sometimes, solitary alveolocytes and cells of the bronchial epithelium. Endopulmonary differential cell counts (Table 1) on patients with chronic bronchitis showed mainly alveolar macrophages (Fig. 1a), whereas in chronic destructive inflammatory lesions many of the lavage cells were neutrophils (Fig. 2a). Lymphocytes were present in very small numbers in all cases.

The ultrastructural investigation of alveolar macrophages obtained by bronchoalveolar lavage showed that they were quantitatively heterogeneous. Among the macrophages were found cells of various forms, described by the writers previously [5]. Alveolar macrophages of the third or fourth form were found most frequently (Fig. 3). These cells are characterized by a large size, the presence of numerous cytoplasmic outgrowths, a paddle-shaped nucleus, containing mainly decondensed chromatin, a hyperplastic laminar complex, round mitochondria with a moderately dense matrix, free ribosomes, profiles of the rough endoplasmic reticulum, and many primary and secondary lysosomes, located both close to the nucleus and at the periphery of the cell. Endosomes with remnants of phagocytosed material, lipid inclusions, and fibrillary structures also were found in the cytoplasm of these macrophages. The qualitative composition of the alveolar macrophage population in the washings depended on the phase of the inflammatory process in the lungs [5].

Analysis of sections from samples incubated with the radioactive precursor for DNA synthesis showed that the label was present in single cells of macrophagal type. Among them were macrophages whose structure closely resembled that of the blood monocytes, less frequently there were cells classed as mature alveolar macrophages (Table 1).

In semithin sections the alveolar macrophages of monocyte type (immature macrophages), which incorporated ^{3}H-thymidine, were round in shape, lacked the distinct halo of cytoplasmic outgrowths, and did not contain any appreciable amount of phagocytosed material in their cytoplasm (Fig. 1b; Fig. 2b). Mature alveolar macrophages incorporating ^{3}H-thymidine differed from cells of monocyte type in having numerous thin cytoplasmic outgrowths on the while of their

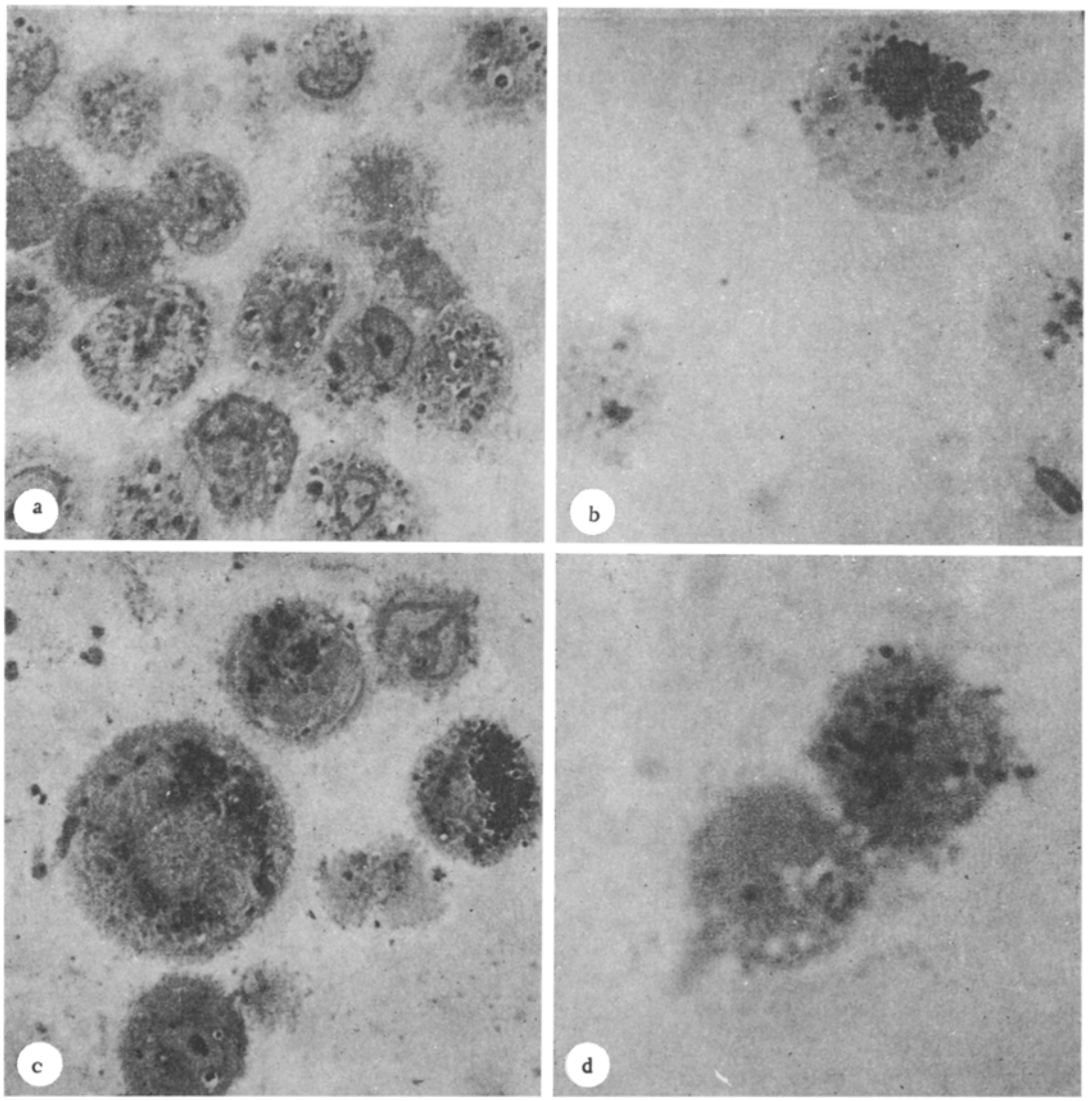

Fig. 1. Autoradiographic investigation of DNA synthesis in alveolar macrophages of washings from patients with chronic bronchitis: a) cells from washings, mainly alveolar macrophages; b) high density of ^{3}H-thymidine label in alveolar macrophage of monocyte type; c, d) DNA synthesis in mature alveolar macrophages. Here and in Fig. 2: semithin sections stained with toluidine blue; incubation of samples with ^{3}H-thymidine; 1000×.

cell surface and in containing various numbers of small, dense inclusions of phagocytosed material in their cytoplasm (Fig. 1c, d; Fig. 2c).

Accumulation of ^{3}H-thymidine in nuclei of the alveolar macrophages was as a rule intensive. In most cases it was impossible to count the total number of silver grains in the autoradiographs because of the high labeling density (Fig. 1b, c; Fig. 2b, c).

Mature alveolar macrophages of large size, round or oval in shape, and containing numerous small and large phagocytosed particles and optically empty vacuoles, and sometimes residual bodies in their cytoplasm, did not incorporate ^{3}H-thymidine (Fig. 2d). In a few cases mitotic figures could be seen in alveolar macrophages which did not contain the label.

Among other cell populations obtained from the respiratory portion of the lungs by bronchoalveolar lavage (polymorphonuclear leukocytes, lymphocytes) no labeled cells were found.

Macrophages incorporating ^{3}H-thymidine were more numerous in chronic lung abscess and fibrocavernous tuberculosis of the lungs (Table 1). The labeling index in these cases varied from 1.25 to 3.33%. In chronic bronchitis the number of macrophages which incorporated the

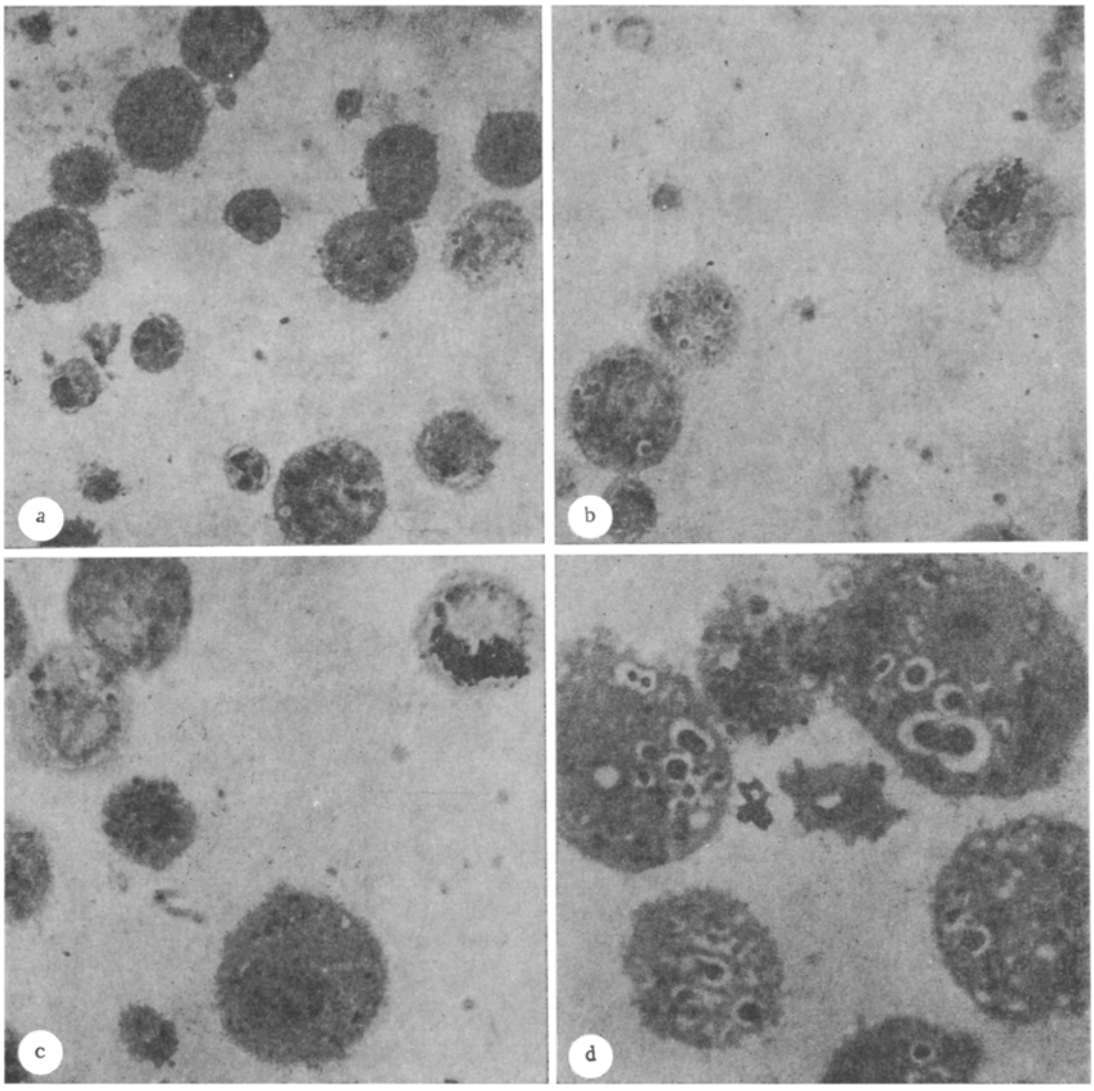

Fig. 2. Autoradiographic investigation of DNA synthesis in alveolar macrophages from washings taken from patients with chronic destructive inflammatory lesion in the lungs. a) Alveolar macrophages and neutrophils in washings; b) alveolar macrophage of monocyte type, synthesizing DNA activity; c) mature alveolar macrophage with average number of phagosomes, incorporating ^{3}H-thymidine; d) absence of label with ^{3}H-thymidine in majority of alveolar macrophages containing numerous phagosomes and residual bodies.

precursor for DNA synthesis was much smaller. The labeling index in these cases did not exceed 0.2%. The highest value of the labeling index of the alveolar macrophages in these observations was about 10 times higher than its value in healthy nonsmokers and 2.5-6 times higher than in smokers [9, 12].

The results are evidence that a few macrophages, located in the respiratory tissue of the lung, accumulate ^{3}H-thymidine. This accumulation is probably prereplication of DNA synthesis (the result of DNA synthesis), and not its repair. This interpretation is supported by the high intensity of labeling accompanied by a low labeling index, by the mitotic figures sometimes found in the macrophages, and also by the results of blocking experiments with cytosine arabinoside or with hydroxyurea [12].

The concept of the bone marrow origin of mononuclear phagocytes is now generally accepted. The kinetics of this process includes monoblasts, promonocytes, monocytes, and macrophages; maturation of monocytes into macrophages is completed, without cell division, and is accompanied by a more than threefold increase in the volume of the cell and by loss of peroxidase-positive cytoplasmic granules [11].

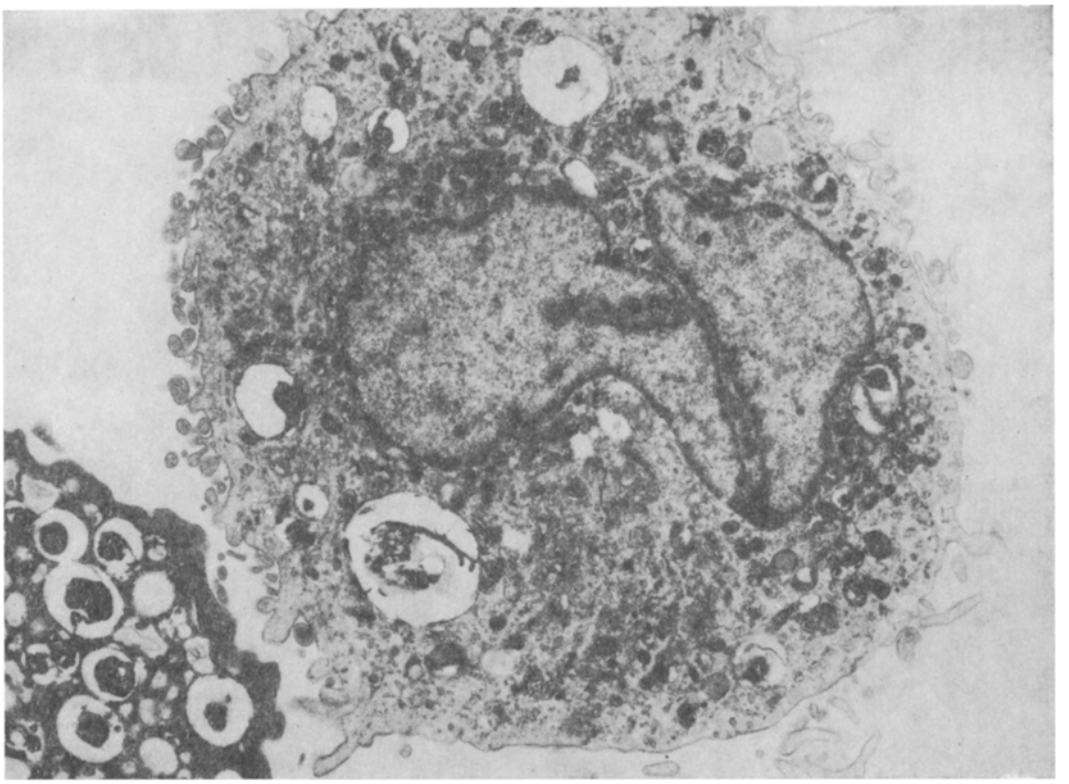

Fig. 3. Ultrastructure of alveolar macrophage from washings (10,000×).

On the basis of data in the literature and the results of this investigation the existence of at least two ways of replenishing the pulmonary macrophage population in man can be postulated. The most important of them is migration of precursor cells from the bone marrow, the other is replication in situ. The contribution of each of these pathways to the total increase in the cell population is evidently determined by the concrete situation.

According to data obtained on normal mice [11], of the total number of circulating monocytes 56% are transformed into Kupffer cells and 15% into pulmonary and 8% into peritoneal macrophages. In acute inflammation caused by intravenous injection of BCG, the number of circulating monocytes doubled in the first 48 h, and their influx into the lungs during this period increased eightfold. This is evidence of the marked lability of the mononuclear phagocyte system and the range of adaptive changes with redistribution of reserves within the population in favor of the target organ.

The second mechanism, namely proliferation in situ, probably is activated during long-term "irritation," in the presence of a definite degree of decompensation of the first (emergency) path, and as the present investigation showed, it is an important stage in the course of chronic pathological processes in the lungs.

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INTERMEMBRANE JUNCTION FORMATION DURING MYELINATION

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KEY WORDS: membrane; intercellular junctions; myelination.

The generally accepted hypothesis, due to Geren [8], explaining the formation of the myelin sheath by continuous spiral rotation of the lemmocyte around the axon, and its variants [5, 14], has recently been supplemented by new data to show that an important role in myelin formation is played by cooperation between the neuron and glial cell [9, 11-14]. The formation of the main and intermediate dense lines of the myelin sheath is accompanied by fusion of the inner and outer layers of the lemmocyte membrane. The question arises whether this process resembles that of the formation of intermembrane junctions. The important role of intercellular junctions in ontogenetic development and function of organisms is well known [2, 6]. Intercellular (glio- and axo-glial) junctions also play an important role in activity of the nervous system [4, 10].

The aim of this investigation was to study membrane junctions of a peripheral myelinated nerve fiber in the course of its myelination.

EXPERIMENTAL METHOD

The tibial nerves of 10 rats aged 5-10 days were used as the test object. Material was fixed in 2.5% glutaraldehyde solution in phosphate buffer and postfixed in 1% OsO_4. After dehydration of the nerves in alcohols they were stained with a saturated solution of uranyl acetate and embedded in Araldite. Longitudinal ultrathin sections were examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

In all preparations of developing nerve fibers studied an unusually large number of Ranvier nodes was observed at different stages of their formation (Fig. 1). Investigation of the Ranvier nodes revealed virtually all types of intercellular junctions: continuous, septate, gap, tight, and desmosome-like. Long processes of the lemmocyte (terminal loops filled with glioplasm), of unequal thickness, could be seen in the paranodal region. The intervals between these processes (intercellular gaps) were inconstant in size, and where neighboring processes widened the gap was narrowed (Fig. 2). In these narrow areas aggregation of the intercellular contrast substance was usually observed. If the gap was filled comparatively uniformly with the material, the picture resembled a continuous junction. If the aggregates in the gap were discretely arranged, in the form of separate masses, the picture corresponded to that of a septate junction. These septate and continuous junctions could be both glio-glial and axo-gilal (Figs. 1 and 2). In some cases considerable narrowing of the intercellular gap was observed or even complete fusion of adjacent membranes. These regions corresponded in structure to gap and tight junctions. Simultaneously with aggregation of electron-dense material was observed in these same places in the inner surface of the glial membrane of the loops (Fig. 2). Aggregation of the juxtamembranous electron-dense material on both sides of

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