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ULTRASTRUCTURE OF ALVEOLAR MACROPHAGES OF THE LUNGS DURING PERORAL ADMINISTRATION OF NITROSODIMETHYLAMINE

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The alveolar macrophages of the lungs (AML) constitute a barrier system of the body, maintaining its resistance to unfavorable environmental factors and, in particular, the action of inhaled substances. Their role in reactions of the body to toxic chemicals assimilated via the gastrointestinal tract has received less study. Experiments on rats have shown that during peroral administration of nitrosodimethylamine (NDMA) the functional state not only of the intracellular organelles of the liver — the target organ [3], but also of AML is disturbed [2, 5]. The number of AML is reduced, their viability is impaired, their surface architectonics and their ability to adhere and to spread out in a layer are modified. The study of AML in recent times has increasingly involved the use of lavage (flushing out AML from the air passages of the lungs), both in experiments on animals [6, 7, 10] and in the practive of pulmonology, for diagnostic and therapeutic purposes [1, 8, 9]. A mass of cytological data on the cell composition and morphology of cells flushed out of the human lungs, mainly of patients, and from the lungs of experimental animals of different species, is accumulating.

The aim of this investigation was to study changes in the ultrastructure of AML obtained by lavage from intact rats and rats receiving NDMA by gastric tube.

EXPERIMENTAL METHOD

AML were obtained by flushing out the lungs of two groups of noninbred male albino rats — one intact, the other receiving a single dose of NDMA by gastric tube in a concentration of 30 mg/kg body weight 3, 12, and 24 h beforehand. Under pentobarbital anesthesia 5 ml of physiological saline was injected by means of a syringe through a tracheostomy into the lungs. The liquid was aspirated from the lungs 15 min later and a second injection of the same volume of physiological saline was given. The procedure was repeated six times. The washings, the final volume of which was 25-30 ml, were centrifuged for 10 min at 1500 rpm. The residue was dehydrated in alcohols of increasing concentration and embedded in polyethylene capsules in a mixture of Epon and Araldite. Ultrathin sections were cut on an LKB-III Ultrotome, stained with lead and uranium salts, and examined in the Hitachi H-300 microscope (Japan).

EXPERIMENTAL RESULTS

Great polymorphism of the AML was discovered in the intact animals. The cells differed in size (their diameter varied from 6 to 25 μ), the abundance of their cytoplasmic processes and intercellular organelles, the electron density of their cytoplasm, and the configuration

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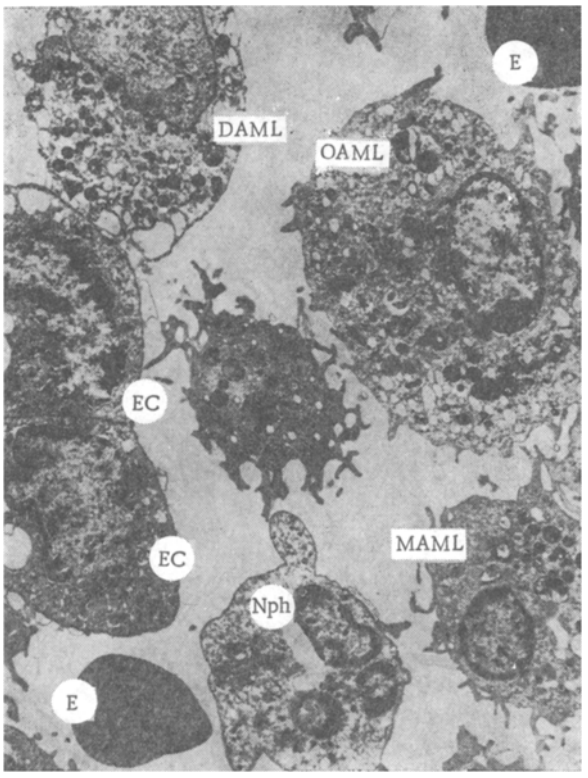

Fig. 1. Cells of washings from lungs of control rat. MAML) Mature AML; OAML) old AML; DAML) dead, disintegrated macrophage; EC) epithelial cells; Nph) neutrophil; E) erythrocytes. 6000 ×.

of their nucleus. However, among this diversity of cells, at least three morphological types of AML could be distinguished on the basis of their age features: young AML of monocytoid type, mature AML, numerically the largest group of cells, and old AML, showing some degree of destructive changes. Besides AML, cells of the ciliated epithelium, lymphocytes, neutrophils, and erythrocytes were frequently seen in the sections (Fig. 1). Erythrocytes could have entered the washings from the tracheotomy wound. Ultrastructural changes in AML could be clearly distinguished in the experimental animals as early as 3 h after a single intragastric dose of NDMA. By this time the number of ribosomes not bound to the membrane, per unit area of cytoplasm, was approximately doubled. The number of tubules of rough endoplasmic reticulum and the number of lysosomes were increased. Hypertrophy of the nucleolar apparatus and dilation of the nuclear pores could be seen in the nucleus. All these changes related to mature AML. Changes in the young and old AML could not be reliably associated with the action of NDMA, because the ultrastructural features of these cell forms in the control and experiment were more difficult to compare.

The length of the tubules of the rough endoplasmic reticulum showed a sharp increase 12 h after administration of NDMA. The rough endoplasmic reticulum showed up with particularly strong contast in the cytoplasm on account of accumulation of homogeneous electron-dense material in its dilated tubules. The electron density of the mitochondria was increased, but at the same time, these organelles were smaller. Marked hypertrophy of the Golgi complex was observed, on account of an increase in the number of membrane structures, in the form of flat cisterns. Parallel with this, profound destructive changes in AML increased in the nucleus and cytoplasm. Polysomes, for instance, broke down to monosomes. Many lipid droplets appeared, and without fusing together they could fill nearly all the cytoplasm, giving it the appearance of a "foam" cell. Often patterns of destruction of these "foam" cells could be seen. Many AML showed signs of autophagy and autolysis of areas of the cytoplasm. Often processes of autophagy and lipid infiltration of the cytoplasm were observed in the same cell, possibly indirectly confirmed the interconnection between these processes [9]. However, depite the presence of intracellular destructive processes, the AML exhibited phagocytic activity in the form of seizure of extracellular objects (surfactant, cell debris, etc.) by their cytoplasmic processes.

Profound changes in AML ultrastructure, leading to complete destruction of the cells, were found 24 h after administration of NDMA. Cisterns of the Golgi complex were swollen and the lysosomal apparatus exhibited even stronger autolytic activity. This was shown by an increase in the number of electron-dense horseshoe-shaped, circular, or multilocular

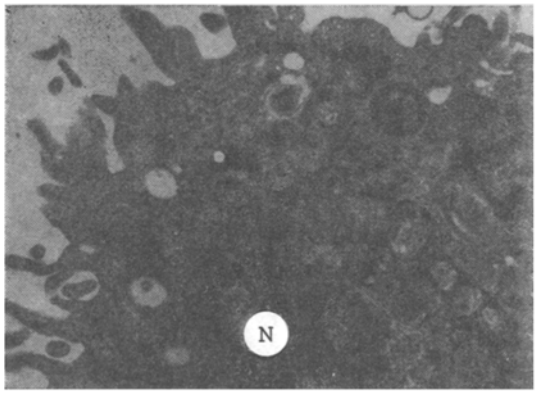

Fig. 2

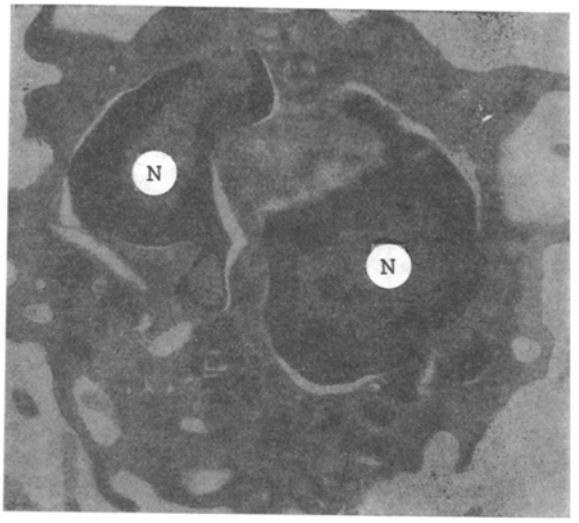

Fig. 3

Fig. 2. AML 24 h after administration of NDMA. Dark type of cell destruction. Nucleus (N) shows evidence of focal lysis of heterochromatin. Autophagic vacuoles of different types, occupying areas of cytoplasm (arrows) are indicated. 18,000 ×.

Fig. 3. AML of monocytoid type 24 h after administration of NDMA. Dark type of cell destruction with fragmentation of nucleus (N). 17,000 ×.

structures, including areas of cytoplasm (Fig. 2). The distribution of heterochromatin in the nucleus became insular in character with a tendency for it to become detached from the nuclear membrane. In the majority of AML the electron density of the nucleus and cytoplasm was increased, a characteristic feature of the dark type of cell destruction. Not only mature cells, but also young AML of monocycled type underwent the dark type of destruction. However, the process of autolysis in these cells was poorly developed, probably due to the insufficiently well developed lysosomal apparatus. The process of destruction of these cells was accompanied by the appearance of long translucent tubules, apparently separating the cytoplasm into discrete fragments. Another characteristic feature of destruction of the young AML was fragmentation of the nucleus (Fig. 3). Phagocytic activity of the AML was virtually nonexistent by this time after administration of NDMA.

The investigations thus showed that the cell fraction flushed out of the lungs by lavage is heterogeneous in composition. This is in agreement with data in the literature [1]. The AML themselves are a very polymorphic cell population, and this may make it difficult to assess active factors without a close study of control material. This is particularly true of the study of the mechanisms of the effect of low-intensity factors on AML in the early stages of exposure, for the changes arising under those circumstances in the cell ultrastructures cannot always be easily distinguished from changes connected with differentiation, physiological aging, and differences in the level of function of the cells. High doses of a toxic compound, during the action of which changes in ultrastructures of AML follow a clearly detectable time course from the stage of functional stress of the cell after 3 h to functional exhaustion and necrosis after 24 h, were used in the present investigation. The ultrastructural characteristics of the changes in AML thus obtained can be used to evaluate the cytotoxic action of various toxic chemical factors present in the environment, in the first place as a "reference point" for assessment of minor exposures, and second, for visual demonstration of the specificity and (or) nonspecificity of mechanisms of responses of AML to the action of chemical environmental factors. This latter aspect is particularly important in connection with the recognition of the study of mechanisms of responses of the body to the action of factors of varied nature as a fundamental problem in environmental hygiene [4].

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