MORPHOLOGICAL CHANGES IN LUNG ALVEOLAR MACROPHAGES AFTER EXPOSURE TO ALCOHOL AND NITROSODIMETHYLAMINE

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The alveolar macrophages of the lungs (AML), obtained by lavage, are widely used as an experimental model with which to study general problems of cell biology and to assess the biological effects of various substances polluting the environment [1, 3, 4]. The morphological and functional characteristics studied with the aid of scanning electron microscopy (SEM), namely the surface architectonics, shape, and adhesive properties, reflect the phagocytic potential of AML and the cytotoxic properties of various active agents, and they can be used to analyze the course of a pathological process and to determine the resistance of the body to unfavorable factors [4, 5]. Besides AML, epithelial cells, lymphocytes, neutrophils, and other cells present in the alveoli, bronchi, and trachea are flushed out in the bronchoalveolar washings. We know that AML are spherical in shape and have a highly crimped surface, i.e., a surface consisting of cytoplasmic outgrowths shaped like frills [2, 6]. The presence of frills on the surface is a marker feature by which cells of the mononuclear phagocyte system (MPS) can be identified. Changes in the surface architectonics of AML in various pathological processes and under the influence of xenobiotics have been studied quite inadequately and they call for further investigation. It was shown previously that 24 h after peroral administration of a toxic dose of nitrosodimethylamine (NDMA) sharp changes take place in the surface architectonics of AML [2]. These changes make it difficult to identify the AML and they call into question the justification for regarding certain cells with a modified surface as belonging to MPS.

A comparative electron-microscopic investigation of the ultrastructure of AML with their surface architectonics under transmission and scanning conditions can shed light on the mechanism of transformation of the surface of the AML under the influence of toxic agents and can help with the drawing up of criteria for their identification. As active agents in the present investigation two substances were used: NDMA, a widely distributed toxic environmental factor, and alcohol, a harmful social factor.

EXPERIMENTAL METHOD

AML for study were taken by lavage from the lungs of noninbred male albino rats. Lavage was carried out under pentobarbitol anesthesia. Three groups of animals were used: 1) intact animals, 2 and 3) animals studied 12 and 24 h after a single intragastric injection, in one case of NDMA in a concentration of 30 mg/kg, and in the other case of 50% ethanol in a dose of 2 ml/100 g body weight. Electron-microscopic preparations were obtained by the use of a cell suspension and cells after short-term culture for 30 min at 37°C. For scanning electron microscopy the preparations were dried by two methods: in air, and by taking through the critical point. The preparations were studied and photographed by means of the H-3010 scanning system (Hitachi, Japan), with accelerating voltage of 20 kV. For transmission microscopy the cells were embedded in a mixture of Epon and Araldite and ultrathin sections were studied in the H-300 microscope (Hitachi) with an accelerating voltage of 75 kV.

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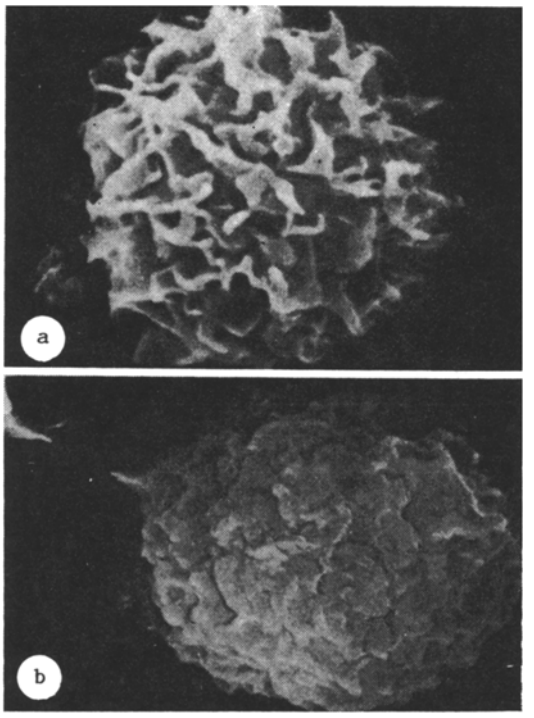

Fig. 1. Structure of AML on scanning electron microscopy (10,000×). a) AML with crimped surface from intact rat; b) AML with folded and nodular surface from rat 24 h after injection of alcohol. Slit-like spaces indicated by arrow.

EXPERIMENTAL RESULTS

On scanning electron microscopy the AML of intact animals had long cytoplasmic outgrowths or frills, stretching from the cell body at different angles to one another, to form curiously shaped depressions on the surface (Fig. 1a). The thickness of the frills was about 0.1-0.3 μ and their length 2-4 μ. During phagocytosis, the length of the frills holding the object could increase considerably. In short-term culture the AML adhered to the substrate (pieces of coverslips), and some of them spread out over the surface of the substrate. Spreading of the cell was accompanied by gradual smoothing of the frills until they disappeared completely.

In ultrathin sections under the transmission microscope, cytoplasmic outgrowths forming the surface architectonics of AML were twisted and ran in a direction opposite to the cell body. The cytoplasmic outgrowths of cells of the MPS are one of the chief instruments used to hold and ingest large extracellular objects, with the aid of actinomyosin and tubular systems, fragments of which could be seen under high power. On the basis of the study of ultrathin sections of a cell suspension washed from the lungs it is possible to distinguish the basic "ration" of AML: fragments of structures of the alveolar lining, namely surfactant, fragments of cells of desquamated ciliated epithelium of the bronchi, and other cells dying as a result of physiological aging, bacteria, and viruses, and unidentifiable solid particles inhaled during breathing (Fig. 2a).

In animals exposed to NDMA and alcohol, the relief of the AML was smoothed, so that the cells were reduced in size (Fig. 1b). Folds and nodules, varying in width from 0.4 to 1 μ, and measuring about 1 μ in height, projected above the surface of the AML, and sometimes slit-like spaces were visible between the folds. These cells appeared as early as 12 h after poisoning, and they formed the majority after 24 h. During culture the AML with a folded nodular surface could not spread out normally. They lost their plastic quality, became rigid, and during the partial spreading to the amount which these cells were capable of, they preserved their folded, nodular surface.

Profound destructive changes affecting the cytoplasmic organelles and nucleus were seen in AML in ultrathin sections. The thickness of the cytoplasmic outgrowths did not differ significantly from the control, but the number of their profiles above the cell body was at least doubled. Some observations in which the axes of the cytoplasmic outgrowths coincided

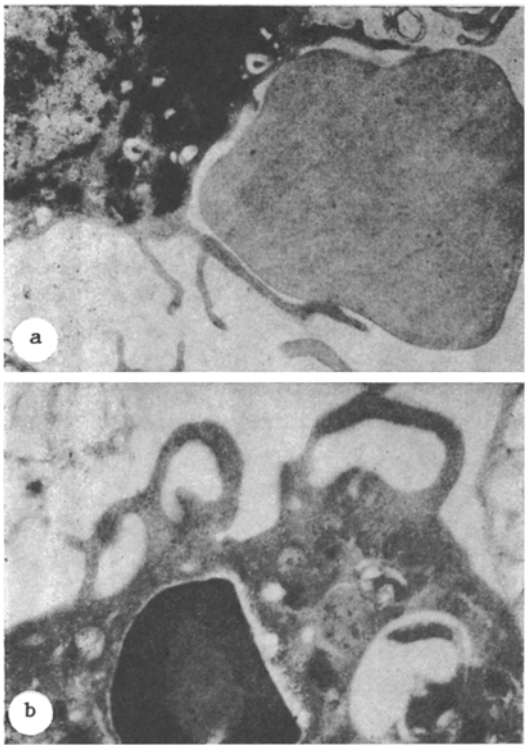

Fig. 2. Structure of AML on transmission electron microscopy. a) Erythrocyte seized by cytoplasmic outgrowths of AML of intact rat. 20,000×; b) Collapse, coiling, and fusion of cytoplasmic outgrowths with one another and with body of AML 24 h after injection of NDMA. 15,000×.

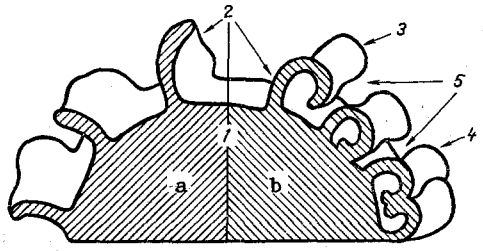

Fig. 3. Scheme of transformation of cell surface of AML after injection of NDMA and alcohol. a) Control, b) experiment. 1) Cell body, 2) cytoplasmic outgrowths, 3) fold, 4) nodule, 5) slit-like space.

with the direction of the ultrathin section suggested that after the action of NDMA and alcohol the cytoplasmic outgrowths collapsed, curved toward the cell body, whereupon the outgrowth merged by its distal part with the cell body or by its lateral wall with a neighboring outgrowth (Fig. 2b). Transformation of cytoplasmic outgrowths, taking place in consecutive stages analogous to those observed during phagocytosis, is illustrated schematically in Fig. 3. Slit-like spaces are formed by fusion of the cytoplasmic outgrowths with the cell body, but they are not found in cases when the outgrowths fused with one another. By contrast with phagocytosis, this is evidently a passive process, during which the actomyosin and tubular systems are damaged. Their structures cannot be made out in ultrathin sections of AML. Ultrastructural changes in AML were combined with changes in their functional state, as shown by the absence of phagocytic activity, which was clearly visible in the ultrathin sections from intact animals, and a change in their ability to spread out on the substrate. It must be pointed out that collapse of the frills and the formation of a folded, nodular surface of AML may take place also in control preparations if the drying conditions are infringed. However, in this case fusion of the cytoplasmic outgrowths with one another and with the cell body does not take place. Spread-out AML, whose cell surface is smooth, can be used as the control in this case. Moreover, in intact animals, AML are flushed out of the lungs only in small numbers, and instead of a crimped surface, it is covered with low folds or with folds and nodules. These cells are distinguished by their larger size – they

may reach 20-30 μ in diameter. In ultrathin sections, on the basis of all their ultrastructures taken together, these AML can be classed as old or dying cells, in which, besides changes in the other intracellular organelles, reduction of the cytoplasmic outgrowths is taking place by a decrease in their number and size.

On the basis of these results the formation of the folded-nodular surface of AML after intragastric injection of NDMA and alcohol can be explained by collapse, curving, and fusion of the cytoplasmic outgrowths or frills. This change probably reflects destabilization of the cell membrane and of the membranes of the intracellular structures, described by Merkur'eva, et al. (1983) on the basis of a biochemical study of the membrane-damaging effect of NDMA.

Transformation of the surface of AML, a response of the cell to the unfavorable action of chemical agents, can thus be used as a criterion for evaluation of the cytotoxic action of various biologically active environmental factors.

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IMMUNOCYTOCHEMICAL LOCALIZATION OF TRYPTOPHANYL-tRNA-SYNTHETASE IN A BOVINE KIDNEY CELL LINE AND IN SUBSTRAINS WITH ELEVATED ENZYME LEVELS

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Tryptophanyl-tRNA-synthetase (TRS) is an enzyme which catalyzes the addition of tryptophan to specific tRNA. The study of the intracellular localization of TRS is important in order to elucidate the topography of the structural and functional components of the protein-synthesizing system of the cell.

Previously [2, 9], in an immunomorphologic investigation using mono- and polyclonal antibodies, the writers demonstrated the distribution of TRS in the cytoplasm of a bovine kidney cell culture (line MDBK). Sublines of MDBK cells resistant to competitive TRS inhibitors – the tryptophan analogs tryptamine and tryptophanol [3] – have now been obtained. According to the results of biochemical analysis, the TRS content in these sublines is 10-50 times greater than that in the original culture [3]. However, morphological data on possible differences in the intracellular distribution of TRS, connected with the higher enzyme levels in these sublines, are not available.

One unexpected result of the biochemical investigation [3] was the discovery of TRS in the detergent-insoluble fraction of MDBK cells. We know that this fraction includes several structural cellular components: polysomes, fragments of membranes of the rough endoplasmic reticulum (RER), nucleus, cytofilaments, and lipid inclusions [7]. The immunocytochemical study of the cells after detergent treatment could give additional information on the connection between TRS and these intracellular structures.

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