ULTRASTRUCTURAL FEATURES OF THE CAPILLARIES, ALVEOLAR EPITHELIUM, AND MACROPHAGES OF THE LUNGS IN GERM-FREE RATS

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The ultrastructural features of certain components of the lung tissues were studied in germ-free and ordinary rats. The lumen of the capillaries in the lungs of the germ-free rats was smaller than in ordinary animals, and the grossly dilated microvessels found in the lungs of ordinary rats were totally absent. Many mitochondria of the alveolar epithelial cells in the lungs of the germ-free animals differed from mitochondria of ordinary rats in their larger size, and the arrangement of the thickened cristae. A study of the alveolar macrophages in the germ-free rats showed that primary lysosomes were more numerous than secondary. The ultrastructural features distinguishing the lung tissue of germ-free animals thus revealed were associated with components involved at the subcellular level in the response of the host to microbial action.

KEY WORDS: germ-free rats; alveolar macrophages; ultrastructure of the lungs; capillary lumen.

Investigations at the cell level have shown significant structural and functional differences between germ-free and ordinary animals, particularly in organs and systems that under normal conditions are exposed to microbial action [1, 3, 5, 9, 10]. However, few investigations have so far been made of the ultrastructure of the tissues of germ-free animals [4, 8, 11]. An investigation was accordingly carried out to study the ultrastructure of the alveolar epithelium, the capillaries, and the macrophages of the lungs in germ-free rats, a subject that has so far remained almost unstudied.

EXPERIMENTAL METHOD

Germ-free and ordinary inbred AG-49 Wistar rats aged 10 months were used. The animals were obtained from the Central Institute of Laboratory Animals (Hannover, West Germany) and kept in Trexler isolators. The animals were fed and the microbiological control was set up in the usual way [12, 13]. Healthy animals of the same line kept under the same conditions were used in the control experiments. Pieces of lung tissue for electron-microscopic study were taken from the hilar zone immediately after the animals had been anesthetized with ether, they were fixed in the cold by Palade's method, dehydrated in alcohol and acetone, and embedded in Araldite. Sections were cut on the LKB ultratome, stained with salts of heavy metals, and examined in the IEM 7A electron microscope.

EXPERIMENTAL RESULTS

A study of the ultrastructure of the alveolar capillaries of the lungs from an ordinary rat showed that most of them varied in the size of their lumen and the number of blood cells that they contained. Cor-

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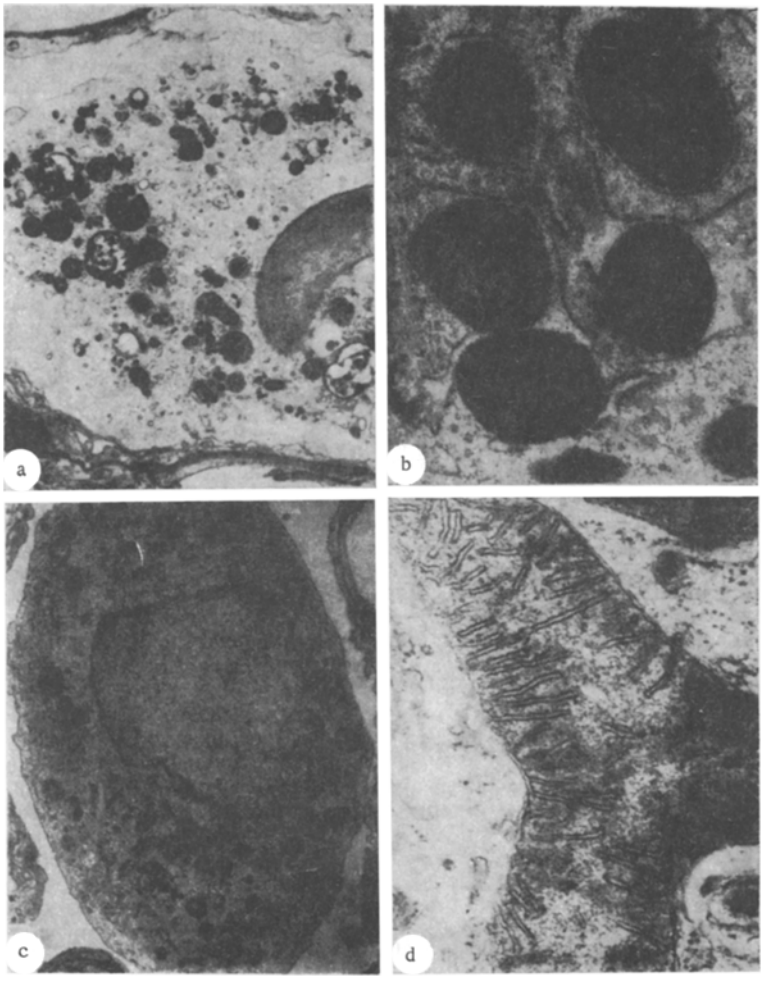

Fig. 1. Ultrastructural features of macrophages and mitochondria of alveolar epithelial cells from the lungs of an ordinary (a, b) and a germ-free (c, d) rat: a) phagosomes in the cytoplasm of a pulmonary macrophage (12,750×); b) macrophage in alveolar space of the lung of a germ-free rat, pseudopodia hardly visible on its surface, and in the cytoplasm primary lysosomes are much more numerous than phagosomes (15,000×); c) mitochondria in a large alveolar cell from the lung of an ordinary rat (34,275×); d) large atypical mitochondrion from a large alveolar cell in the lung of a germ-free rat with cristae 1.5-2 times thicker than in the control (75,000×).

responding to these features, the capillaries could be divided conventionally into three groups. The most numerous group consisted of capillaries with a lumen 6-8 μ in diameter. They usually contained 1 or 2 blood cells. The second group of capillaries had an unusually large lumen for the normal animal (11-12 μ in diameter) and they contained more blood cells, chiefly leukocytes. Many capillaries contained from 7 to 11 red and white blood cells. The latter were the more numerous and they consisted of neutrophilic granulocytes, lymphocytes, and eosinophils. Finally, the third group consisted of alveolar capillaries with a lumen not exceeding 6 μ in diameter and, as a rule, containing no formed blood cells.

The study of the ultrastructure of the lungs of the germ-free rats showed that, by contrast with the lungs of ordinary rats, only 2 groups of alveolar capillaries could be identified. Alveolar capillaries with a lumen ranging from 3 to 7 μ in diameter formed a large group. Blood cells were less frequently seen in the lumen of these capillaries than in the lumen of the pulmonary capillaries of an ordinary rat. Half-closed capillaries with a lumen 1.7μ in diameter and slit-like capillaries (lumen 0.7μ, Fig. 1a) can be classed in the second group. Hardly any alveolar capillaries with a large lumen and many leukocytes in it were thus found in the lungs of the germ-free rats.

Alveolar macrophages in ordinary rats were found to the number of 1 or 2 per alveolar space, usually close to the air–blood barrier. On the surface of most macrophages many pseudopodia, folds, and invaginations were observed, and their cytoplasm contained many primary lysosomes, a well-developed Golgi apparatus, and phagosomes. The number of primary lysosomes, calculated per area of section, varied from 26 to 30 and the number of secondary lysosomes or phagosomes varied from 4 to 9. The primary lysosomal granules varied in size from 0.2 to 0.8 μ, and the secondary varied from 1 to 1.6 μ (Fig. 1a).

Fewer macrophages were found in the alveoli of the lungs of the germ-free rats than of the ordinary rats, and their surface contained far fewer folds and cytoplasmic outgrowths. These cells contained approximately 1.6 times more primary lysosomes (from 40 to 47) and about half the number of phagosomes (from 1 to 6) compared with macrophages from the lungs of ordinary animals. The lysosomes varied in size from 0.1 to 0.6 μ. The diameters of the phagosomes (from 1 to 6) were 0.5-0.6 μ (Fig. 1b). The alveolar macrophages from the lungs of the germ-free rats were thus distinguished by their lower functional activity than phagocytic cells of the same type in the lungs of ordinary rats.

Mitochondria were few in number in the small alveolar cells of the lungs of the ordinary rats, and in size they did not exceed 0.5-0.8 μ longitudinally and 0.3 μ transversely. Transverse cristae could be seen in the moderately dense mitochondrial matrix. Mitochondria were fairly numerous in the large alveolar cells, and in shape they were oval or rod-like. The length of the oval mitochondria did not exceed 0.5-0.8 μ and that of the rod-shaped mitochondria varied from 0.8 to 2.5 μ (Fig. 1c).

The mitochondria of most cells of the alveolar epithelium of the lungs from the germ-free rats were somewhat larger. Regularly alternating rounded depressions and projections were often seen on the surface of many of them, by contrast with the mitochondria of ordinary rats. Sometimes mitochondria with a diameter up to 1.3 μ could be seen in the small alveolar cells; this is 3-5 times greater than the diameter of the ordinary mitochondrion (0.3-0.5 μ). In addition, an atypical arrangement of the thickened cristae was found in these mitochondria. The rod-shaped mitochondria in the large alveolar cells attained a length of 3 μ, about 1.5-2 times longer than the mitochondria of the large alveolar cells of ordinary rats (Fig. 1d).

Comparative investigation of the lungs of germ-free and ordinary animals revealed grossly dilated alveolar capillaries, packed with leukocytes, in the latter. The diameter of the capillaries in the lumen should not exceed 6-7 μ [2, 7]. Accordingly this state of the capillaries can be described as parapathological. The microbial flora evidently plays an important role in the appearance of such capillaries, for this category of microvessels is not seen in the lungs of germ-free rats. These findings are in agreement with the observations of Kenworthy [6], who found a mild "physiological" inflammation caused by constant interaction between the intestinal tissues and the surrounding microflora in the intestine of ordinary animals. In germ-free rats, moreover, most of the alveolar capillaries had a lumen of smaller diameter than the pulmonary capillaries of ordinary animals. These observations are in agreement with those of Gordon [4], who observed a lower stroke volume of the heart and slower blood flow in the organs of germ-free rats. The study of the ultrastructure of the alveolar macrophages in germ-free rats showed that primary lysosomes are more numerous than secondary. These results agree with those described in the literature [8]. Meanwhile, the morphological features of increased functional activity found in the alveolar macrophages of the lungs of ordinary rats confirm the role of the saprophytic microbial flora in the changes observed in a certain proportion of the alveolar capillaries. Differences in the ultrastructure of the lung tissue of germ-free and ordinary animals revealed by these experiments are connected with components involved at the subcellular level in the response of the host to microbial action.

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