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Scanning Electron Microscopic Observations on the Intracellular Structures of the Ciliated Tracheal Epithelium—Especially on the Morphological Differences between Conventional Rats and Specific Pathogen-Free Rats

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The three-dimensional architecture of the intracellular structures of the tracheal ciliated epithelium was studied by scanning electron microscopy, paying particular attention to the morphological difference between conventional rats and specific pathogen-free (SPF) rats. The surface of the trachea was densely covered with cilia in conventional rats, but less in SPF rats. The smooth endoplasmic reticulum (ER) formed a three-dimensional tubular meshwork under the basal bodies in both rats. In conventional rats, the Golgi apparatus was highly developed and many Golgi vesicles were attached to the lateral margin of the Golgi cisternae. In addition, the rough ER spread around the nucleus. In the SPF rats, however, the Golgi appatatus was not so highly developed and the rough ER was scarcely visible. The development of the Golgi apparatus and rough ER observed in the conventional rats indicate the active protein synthesis for the formation of the cilia which plays an important role in antimicrobial defense mechanisms.

Key words: ciliated epithelium; intracellular structure; scanning electron microscopy; specific pathogen-free rat; trachea

Although laboratory animals are frequently exposed to a variety of microorganisms, little attention has been paid in morphological studies to the conditions in which the animals were kept. The surface of the trachea is densely covered with ciliated cells which play an important role in eliminating foreign bodies by ciliary movement, when rats are kept under standard laboratory conditions (Alexander et al., 1975). In contrast, less ciliated cells are observed when rats are housed in germ free conditions (Jeffery and Reid, 1975). It is then easily speculated that the ultrastructures of ciliated cells are different between animals raised in conventional conditions and pathogen-free ones.

The smooth endoplasmic reticulum (ER), distributed all over the ciliated cells, shows enzymatic activity such as acethylcholinesterase (Graf and Stockinger, 1966), glucose-6-phosphatase (Kanamura, 1975; Hume and Burchell, 1996) and peroxidase (Kataoka, 1971; Watanabe, 1980; Christensen et al., 1981; Aoki et al., 1986; Sakai et al., 1989; Kinbara et al., 1992). In particular, acetylcholinesterase in the ER beneath the basal body is closely associated with the ciliary movement. Additionally, the endogenous peroxidase activity has not been localized in tracheal mucosal epithelial cells of specific pathogen-free (SPF) rats, but it became localized in the epithelial cells after natural infection with *Micoplasma pulmonis* (Kinbara et al., 1992).

In this study, we studied the three-dimensional architecture of the intracellular structures

Abbreviations: DMSO, dimethyl sulfoxide; ER, endoplasmic reticulum; SEM, scanning electron microscopy; SPF, specific pathogen-free; TEM, transmission electron microscopy

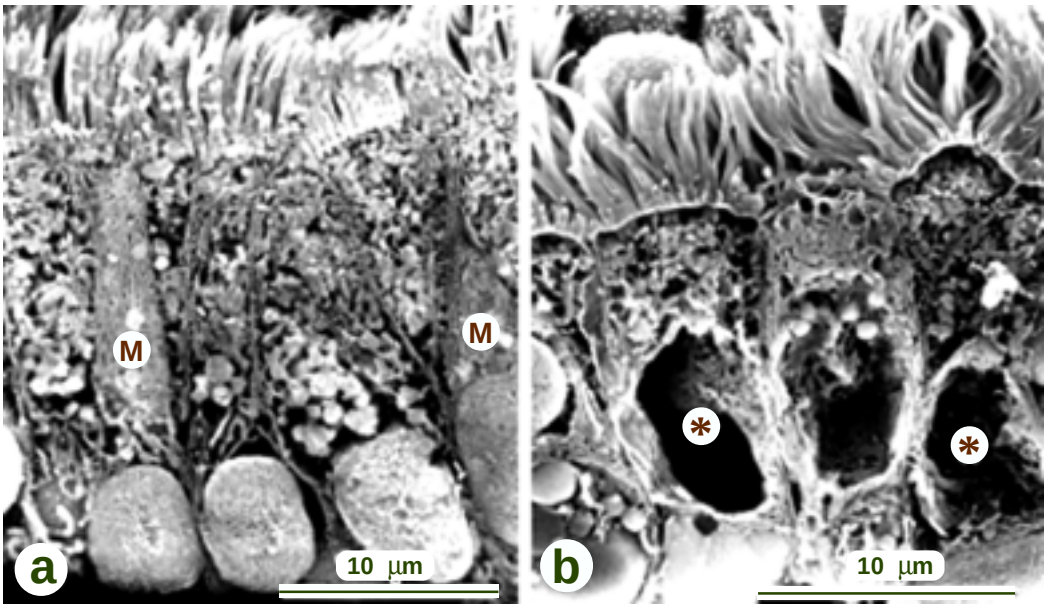

Fig. 1. Longitudinally fractured surfaces of the tracheal epithelial cells of a conventional rat (**a**) and a specific-pathogen free (SPF) rat (**b**). **a**: The surface of the epithelium is uniformly covered with cilia. Mucous cells (M) are often visible between the ciliated cells. × 2,400. **b**: Ciliated cells and non-ciliated cells are visible. The apical surface of the non-ciliated cells are covered with short microvilli. Mucous cells are sometimes seen. Asterisks show the perinuclear intracellular structures exposed by the removal of the nucleus during the specimen preparation procedure. × 2,800.

by scanning electron microscopy (SEM), paying particular attention to the morphological differences between conventional rats and SPF ones.

Materials and methods

Ten female adult Wistar rats conventionally raised on standard laboratory chow and water ad libitum were used as the control animals. Ten female adult Wistar SPF rats, purchased from Japan SLC, Ind. (Shizuoka, Japan), were used as the experimental animals. The SPF rats were selected from a carefully monitored stock declared free of the following specific pathogens: Sendai virus, Sialodacryoadenitis virus, Hanta virus, *Psedomonas aeruginosa, Salmonella* spp., *Pasteurella pneumotropica, Bordetella bronchiseptica, Streptococcus pneumoniae, Corynebacterium kutscheri, Mycoplasma* spp., Tyzzer's organism, *Syphacia* spp., *Giardia* spp., *Spironucleus* spp., *Trichomonas* spp. and *Entamoeba* spp.

SEM specimens were fundamentally prepared by the A-O-D-O method (Tanaka and Mitsushima, 1984). After the animals were anaesthetized with an intraperitoneal injection of Nembutal (50 mg/kg body weight), they were fixed by perfusion with a mixture of 0.5% glutaraldehyde and 0.5% formaldehyde (1/10 mol/L phosphate buffer, pH 7.4). The tracheas were then removed from the animals and excised into small pieces. After rinsing with the buffer, they were immersed in 25% and 50% dimethyl sulfoxide (DMSO) for 30 min each. The specimens were frozen on a metal plate chilled with liquid nitrogen and fractured into two pieces using a razor blade and a hammer. The fractured pieces were then placed in 50% DMSO solution and thawed at room temperature. After they were completely rinsed in the buffer, they were placed in 0.1% osmium tetroxide solution (1/10 mol/L phosphate buffer,

pH 7.4) and left standing for 3 days or more at 20˚C. After rinsing with the buffer, the specimens were postfixed in 1% osmium tetroxide for 1 to 2 h. They were then rinsed and treated with 1% tannic acid and 1% osmium tetroxide for conductive staining (Murakami, 1974). The specimens were dehydrated through a graded ethanol series, replaced with t-butyl alcohol and finally dried in a freeze-drier (ES-2020, Hitachi Ltd., Tokyo, Japan) (Inoué and Osatake, 1988). The dried specimens were coated with platinum of about 2 nm by an ion coater with a rotating stage (VX-10R, EIKO Engineering Co. Ltd., Mito, Japan) and observed with a field emission SEM (S-4500, Hitachi Ltd.) operated at 7 to 15 kV.

Results

The surface of the trachea was covered with three types of epithelial cells: ciliated, non-ciliated and mucous cells (Fig. 1).

Cytoplasmic matrices which prohibit the visualization of intracellular structures were completely removed by the osmic maceration procedure of the A-O-D-O method; consequently, both the outer and intracellular structures of ciliated cells could be demonstrated in three dimensions by SEM.

Common ultrastructural findings of both conventional and SPF rats

Basal bodies, extensions of the interior of the cilia, were lined beneath the apical plasma membrane (Fig. 2). The smooth ER formed a three-dimensional tubular meshwork under the basal bodies comprised of tubules of about 70 to 80 nm in diameter. The nucleus, located in the basal portion of the ciliated cells, was sometimes removed during the specimen preparation procedure, thus the perinuclear structures could be often observed. The Golgi apparatus, which was composed of vesicles, vacuoles and parallel-arranged cisternae, was located in the

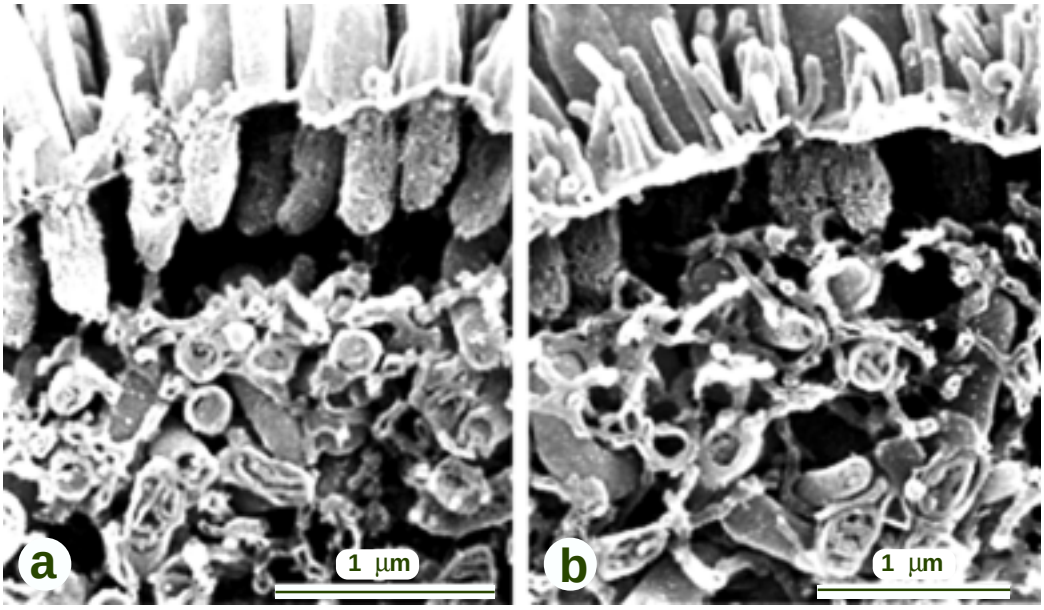

Fig. 2. Fractured surfaces of the apical portion of tracheal ciliated cells of a conventional rat (**a**) and SPF rat (**b**). Basal bodies are lined beneath the apical cell membrane. The tubular smooth endoplasmic reticulum forms a three-dimensional meshwork, partly trapping the mitochondria. Microvilli are more numerous in the SPF rat than in the conventional rat. **a**: × 36,000. **b**: × 26,000.