Ultrastructure of Respiratory Tract Epithelium of Rats Experimentally Infected with the CAR Bacillus

Satoru MATSUSHITA
National Institute of Radiological Sciences, 4-9-1 Anagawa, Chiba 260, Japan
(Received 25 October 1990/Accepted 27 December 1990)


KEY WORDS: CAR bacillus, rat.

In recent years there have been several studies concerned with spontaneous cilia-associated respiratory (CAR) bacillus infection in laboratory animals [3, 6, 7, 15]. Experimental transmission was successfully performed using lung washings and lung homogenate from natural cases and chicken embryo-passaged CAR bacillus [3, 8, 13]. Although there have been histological studies [3, 7, 8, 13, 15], detailed electron microscopy of host cells colonized with CAR bacillus has not been performed.

Twenty-one 5-week-old male Wistar/Ms rats were derived from the breeding colony of this institute, which had been proved to be negative for Mycoplasma (M) pulmonis, Pasteurella pneumotropica, Bordetella (B) bronchiseptica, Corynebacterium (C) kutscheri, Salmonella spp., Escherichia coli 0115a, e:k(B), and Pseudomonas aeruginosa by culture [9], for M. pulmonis, Sendai virus, mouse hepatitis virus and Tyzzer’s organisms by complement fixation (CF) test [14], for B. bronchiseptica and C. kutscheri by agglutination [10], and for Giardia spp. and Hexamita spp. by microscopy [11]. Three rats each were housed in an autoclaved cage and provided autoclaved commercial pellets (MB-1: Funabashi Farm Co., Funabashi) and chlorinated (10–15 ppm) drinking water ad libitum. The cages were placed in a safety cabinet controlled at 24±2°C and 55±10% relative humidity with 12 hr light/dark cycles.

Stock (−80°C) lung homogenate containing 8.5×10^7/ml of CAR bacillus strain from a spontaneously affected Wistar/Ms rat [7], was used. For preparing the inocula [8] a piece of affected lung from the diseased rat was removed and homogenized with PBS, and the homogenate was intranasally inoculated into BALB/c mice, which were bred at this laboratory. Smear preparations of the lung homogenate from these infected mice as well as nontreated BALB/c mice were counted for the number of the CAR bacilli after the Fontana staining. Eighteen rats were intranasally inoculated with 8.5×10^6 of the bacilli in 0.1 ml PBS, and 3 rats each were killed with chloroform on days 4, 7, 14, 21, 28 and 56 postinoculation (p. i.). Three non-infected rats were also killed on day 56 p. i. At each day of sampling, one rat was microbiologically examined, and sera were collected from all rats for serology, revealing that all cases were free from the above mentioned respiratory pathogens.

The trachea was removed from all rats and the anterior half was cut into two pieces for scanning electron microscopy (SEM). The lungs were removed and cut into 1–4 mm³ for transmission electron microscopy (TEM). Tissues were fixed in 3.5% glutaraldehyde with 0.1 M sodium cacodylate (pH 7.4) for 2 hrs at 4°C, postfixed in 1% osmium tetroxide for 1 hr at 4°C. After dehydration the trachea sections for SEM were dried using the critical point method [4] with liquid carbon dioxide, coated with gold (thickness of 150 Å) using the ion sputtering method [4]. After embedding in Epon 812, 1 μm sections of the lung were stained with 1% toluidine blue and observed by light microscopy. Selected 90 nm-sections were made, stained with uranyl acetate and lead citrate, and examined by TEM. Identification of epithelial cell types was based on the proposed criteria [5].

On day 7 p. i., a few filamentous rods were observed among cilia of some trachea-lining cells. These rods resembled cilia in shape while somewhat longer. Some filamentous rods of various length were observed also on nonciliated cells (Fig. 1). On day 14 p. i., there were “moss-grown” foci consisting of numerous filamentous rods being indistinguishable from cilia which had no more wavy arrangement on the surface of the trachea. The “moss-grown” foci were gradually enlarged until day 28 p. i.

Fig. 1. Some filamentous rods projecting from the nonciliated cell surface of the tracheal epithelium. Day 14 p. i. Bar=1 μm.

Fig. 2. Mucous cells colonized with numerous bacilli in a distal bronchiole. Day 21 p. i. Bar=1 μm.
i., and then they were decreased in size on day 56 p. i. and the nonciliated area consisting of widened cells being 50 μm in maximum in width was developed.

By TEM the CAR bacilli were found on the surface of both ciliated and nonciliated mucous-goblet cells (Fig. 2) as well as in the lumina of some bronchi and bronchioles on day 14 p. i., while more frequently on ciliated cells. Colonization of the bacilli with submucosal lymphoid cell infiltration gradually spread to the periphery of the bronchial trees and the severity increased with time until day 56 p. i. In the mildly infected bronchi and bronchioles, the number of ciliated cells was similar to that of non-infected controls. However the number of Clara cells decreased and that of the mucous-goblet cells increased.

In moderately and severely infected bronchi and bronchioles, most epithelial cells were increased in height up to three times the normal. There were numerous goblet cells having abundant rough endoplasmic reticulum (ER) and granules as well as tall ciliated cells having abundant organella. Clara cells could not be detected in such area. Most ciliated cells parasitized with numerous bacilli lost cilia and had irregularly scattered basal bodies in the apical cytoplasm and abundant elongated microvilli. In some severely infected bronchi and bronchioles showing severe lymphoid cell infiltration, there were flat unidentified cells up to 30 μm in width, which had an irregularly outlined round to oval nucleus with an obvious nucleolus, electron-lucent cytoplasm containing some rough and smooth ER and mitochondria, and microvilli. They had no cilia, basal bodies and tonofilaments. In the severely infected bronchioles, some necrotized epithelial cells were shown to have a few CAR bacilli in the cytoplasm. A few bacilli were observed in the lysosomes of neutrophils and macrophages within the bronchial and bronchiolar lumina.

The ends of the CAR bacillus attached to the host cells, sometimes of bulbous form were 0.15–0.18 μm in width, being thinner than the rest part of bacterial body (0.24–0.35 μm in width). By higher magnification, fibrillar structures were observed between the bacillus and the host cell membrane (Fig. 3). The length of bacilli varied from 3.5 to more than 9 μm. The bacterial cytoplasm contained a central nuclear region of tiny fibrils with ribosomes and peripheral electron-dense matrix. It was configured by a three-layers; electron-dense cell wall, lucent inter space and dense cell membrane, approximately 12, 16 and 9 nm thick, respectively.

The present findings strongly support the previously reported histology [8]. Moreover, both SEM and TEM observations showed that the CAR bacillus colonized not only ciliated cells but also nonciliated ones, while this has never been noted in the previous papers [3, 15]. Although CAR bacilli seemed to prefer colonizing ciliated cells to nonciliated cells, the host cell cilia might not be essential for the bacterial parasitism.

SEM observation revealed that the “moss-grown” foci on the tracheal epithelium consisted of filamentous rods. Although the CAR bacillus is morphologically indistinguishable from the host cell cilia, the extensive colonization on the epithelium showed similar characteristics to that reported previously [8]. The early demonstration of CAR bacilli in the trachea on day 7 p. i. is in agreement with the previous paper [3]. The upper respiratory passage seems to be a preferable target of the bacillus [3], suggesting that the trachea is important in detecting early stage of the infection.

By TEM, cilia were lost and basal bodies were scattered. Such ciliary change has been reported to be common in chronic respiratory infection [1]. The epithelial layers were taller in the infected bronchi and bronchioles. The increase being attributable to hypertrophy of the ciliated and mucous-goblet cells might be due to activated mucociliary clearance of irritants [1]. Decreased number of Clara cells and increased number of the mucous-goblet cells suggested metaplasia of Clara cells to mucous cells [12]. Ulceration, squamousoid changes or thinning of the epithelial layer, which are often noted in chronic bronchitis [1], have been reported in severely affected airway epithelium [3, 8, 13, 15].

The fibrillar structures observed between the CAR bacillus and the surface of epithelial cells are similar to those reported in a natural case in rabbits [6], which might have played a significant role in adhesion and colonization of the bacillus to the host cell surface. These structures might be related to mucous substance, glycoalyx, or pili of the bacillus playing some role in virulence [2]. CAR bacilli are negatively stained by the Gram's method [3, 7, 15], having the triple-layered cell membrane resembles as other gram-negative bacteria [2].

REFERENCES