Transmission and Scanning Electron Microscopic Findings in the Tracheobronchial Tree of Horses with Chronic Obstructive Pulmonary Disease

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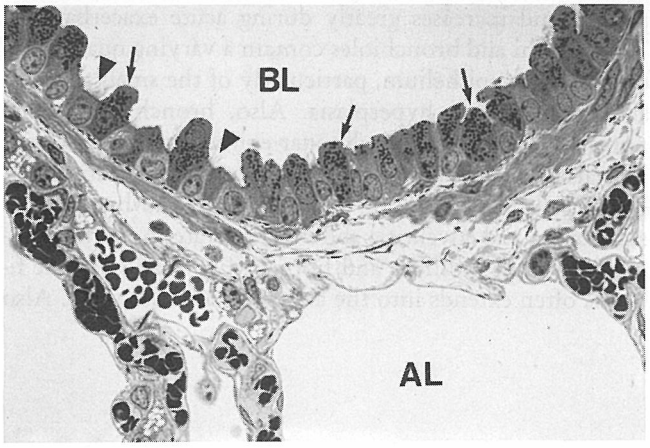

Fig. 1: The bronchiolar epithelium of a healthy horse is composed of ciliated cells (arrowheads) and mainly Clara cells (arrows) which contain numerous granules. BL = Bronchiolar lumen; AL = Alveolar lumen. Semi-thin section, toluidine blue. 560x

Introduction

Chronic obstructive pulmonary disease (COPD) in horses is characterized by a number of clinical signs. However, many aspects of etiology, pathogenesis, diagnosis and therapy need further clarification (*Damman-Tamke,* 1982). Based on clinical findings and histomorphological changes, COPD may be defined as a disease with dyskrinia, bronchospasm, obstruction of the airways as a result of inflammation and alveolar emphysema (*Schoon* and *Deegen*, 1983). In horses having had a thorough clinical examination, electron microscopic investigations are rare which is in contrast to the situation in man (*Gillespie* and *Tyler,* 1967 a, b; *Tyler* et *al.,* 1971; *Nicholls,* 1978).

Material and Methods

In 13 horses, a thorough clinical study was performed and tissue samples were obtained for electron microscopy. The range of clinical diagnosis was from mild acute to severe chronic obstructive bronchitis. After intratracheal instillation of glutaraldehyde (5%) tissue samples for light and electron microscopic studies were obtained from trachea, the main, lobar, segmental and small bronchi with a diameter of 2 mm. For evaluation of bronchioles and alveoli, four different sites of pulmonary parenchyma were studied. Tissue samples were prepared by conventional methods for transmission and scanning electron microscopy.

Results and Discussion

In healthy horses there are several epithelial cell types which are similar to pulmonary epithelial cells of other species (*Breeze* and *Wheeldon,* 1977; *Jeffery* and *Corrin,* 1984). In the respiratory tract there is a dominance of ciliated cells. Scanning electron microscopy reveals that these cells form a uniform ciliary surface from trachea to the small bronchi. In addition to ciliated cells, goblet cells can be found in the superficial epithelium of the respiratory tract. They are responsible for the synthesis and secretion of mucous substances into the pulmonary airways. In control animals, goblet cells are localized mainly in the bronchi and decrease in direction to the bronchioles. In the transition zone to the bronchioles there are only single goblet cells. The single-layer epithelium of the bronchioles is composed of ciliated cells and Clara cells which contain numerous granules. In the small peribronchiolar region a few smooth muscle cells can be observed (Fig. 1).

In horses with chronic obstructive pulmonary disease it appears that the starting point for pathological alterations in the pulmonary parenchyma involves epithelial changes at the level of the bronchioles. Clara cells have to be regarded as an important target cell. In the early stages of chronic bronchitis, the typical granules in Clara cells are lacking. Peribronchiolar inflammatory cells appear and encroach upon the bronchiolar epithelium and lumen (Fig. 2). With transmission electron microscopy, degeneration and necrosis are evident in some Clara cells and intercellular spaces are widened. Similar morphological features were observed in the early stages of experimentally induced equine bronchiolitis (*Turk* et *al.,* 1983; *Breeze* et *al.,* 1984).

Pathological differentiation of the epithelium in the bronchioles progresses to goblet cell metaplasia. In horses with

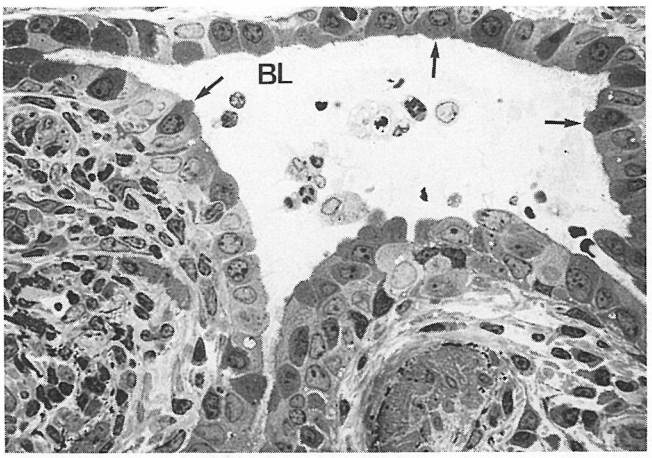

Fig. 2: Clara cells without granules (arrows) occur in the early stages of chronic obstructive bronchiolitis. Airway obstruction is caused by peribronchiolar and intraluminal inflammatory cells and secretions. BL = Bronchiolar lumen. Semi-thin section, toluidine blue. 560x

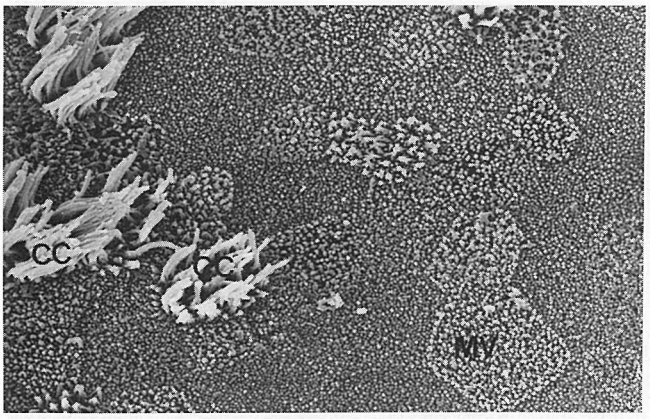

Fig. 3: Scanning electron microscopy of the tracheal epithelium in a horse with severe COPD. Numerous non-ciliated cells are covered by microvilli (MV) whereas only a few ciliated cells (CC) appear. 6000x

severe chronic bronchiolitis the normally one-layer bronchiolar epithelium is hyperplastic and is transformed superficially into PAS-positive goblet cells. Clara cells are no longer visible and a different kind of mucin is produced. The lumen of bronchioles often contains numerous neutrophilic granulocytes which may be located intraepithelially. There may also be fibrosis within the peribronchiolar inflammatory infiltrates, which causes further obstruction of the bronchiole. In the tracheal and bronchial epithelium of affected horses there is a loss of ciliated cells, the extent of which depends on the course of the disease. Use of scanning electron microscopy demonstrates that loss of ciliated cells starts focally in the small bronchi and progresses rostrally to finally reach the trachea. In severe chronic bronchitis very few ciliated cells can be identified. Frequently there are only remnants of cilia. Cells showing superficial microvilli predominate (Fig. 3). Transmission electron microscopy reveals that these cells represent undifferentiated cells which are in close proximity to degenerated ciliated cells in the superficial epithelium (Fig. 4). The cells present indicate that there is continuous replacement of cells, which progresses rapidly, not allowing complete cell differentiation. Their ultrastructure is similar to intermediate cells which are conventionally regarded as differentiating cells of the airways (*Breeze* and *Wheeldon*, 1977; *McDowell* et *al.*, 1983).

Due to a precipitous cell proliferation and insufficient differentiation, increased ciliary defects occur in some affected horses. It appears that before ciliated cells degenerate, parts

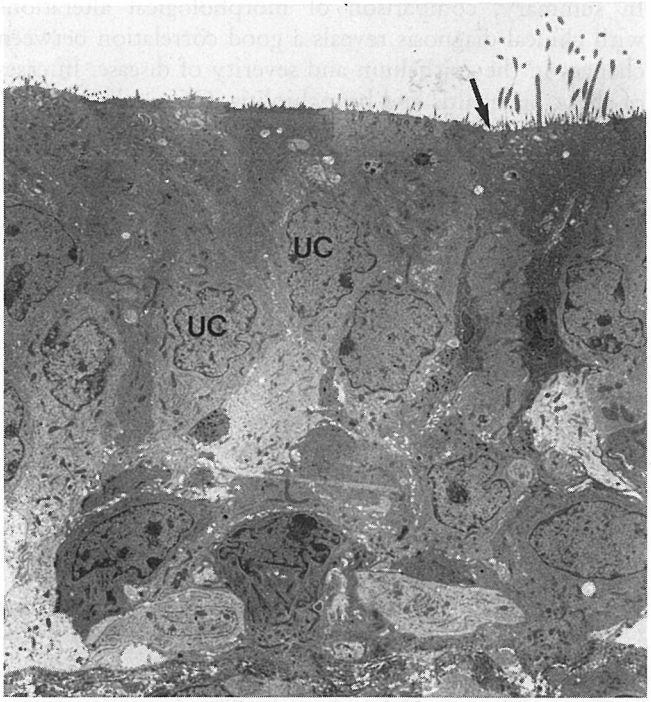

Fig. 4: Tracheal epithelium of a horse with severe COPD. Undifferentiated cells (UC) with apical microvilli in close contact to a ciliated cell (arrow) which reveals first signs of degeneration. 5000x

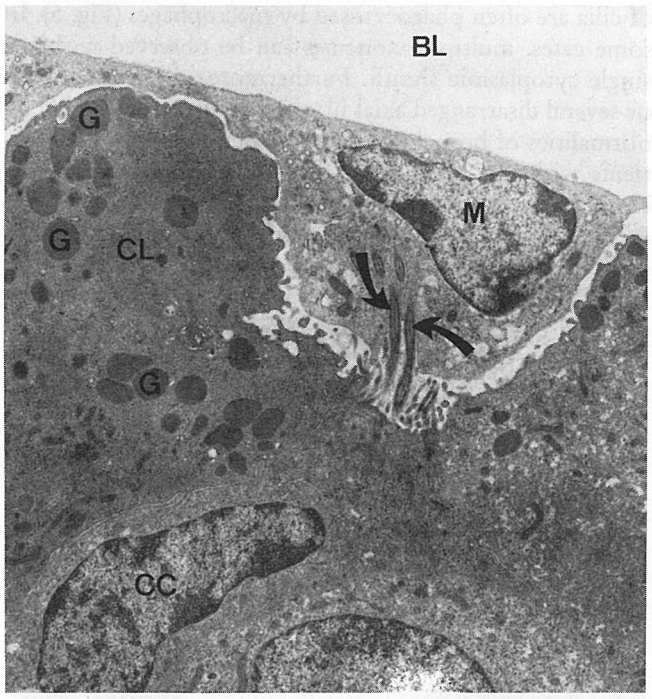

Fig. 5: Terminal bronchiolar epithelium showing Clara cells (Cl) with intracytoplasmic granules (G) and ciliated cells (CC) Phagocytosis of cilia (arrows) by a macrophage (M). 15.750x

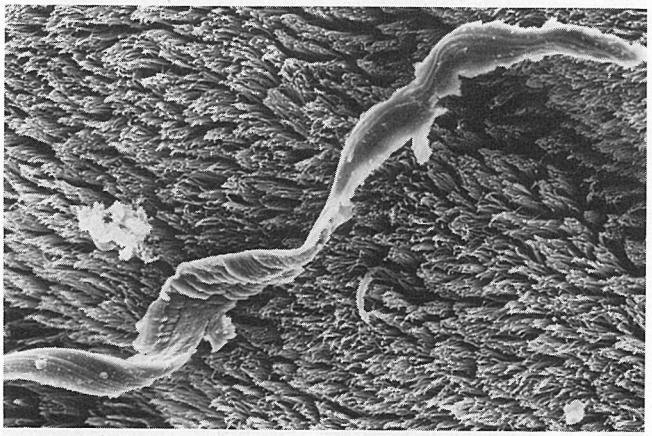

Fig. 6: Ciliary surface of the bronchial epithelium with a Curschmann spiral. 2500x

of cilia are often phagocytosed by macrophages (Fig. 5). In some cases, multiple axonemes can be observed within a single cytoplasmic sheath. Furthermore, cilia contain one or several disarranged axial filament complexes. Similar abnormalities of bronchial cilia were observed in human patients with the „immotile cilia syndrome", chronic bronchitis or neoplastic lung diseases (*McDowell* et *al.*, 1976; *Lungarella* et *al.*, 1983; *Fischer* et *al.*, 1984). Occasionally in conditions like chronic bronchitis and bronchial asthma in man (*Butler*, 1976), Curschmann's spirals are detected on the cilia of the airway surface (Fig. 6). In severe COPD there is marked epithelial hyperplasia of the upper airway epithelium, especially in middle-sized bronchi. Furthermore, the intercellular spaces are markedly widened (Fig. 7). This may morphologically correspond to unspecific hyperreactivity of the bronchial mucosa, in which an increased mucosal permeability together with stimulation of so-called „irritant receptors" induces bronchospasms (*Derksen* et *al.*, 1984; *Klein*, 1984). But the role of an increased mucosal permeability for the pathogenesis of hyperreactivity needs further investigation (*Hogg*, 1981, 1983). A further suggestion of hyperreactivity is the increase in mast cells in and on the respiratory epithelium (Fig. 8). According to our morphometric studies mast cells increased in chronically diseased horses not only in the peribronchiolar region but also in the bronchial epithelium. Mast cells in different phases of degranulation also occur in the alveolar septa and alveolar lumen (*Iregui*, 1985).

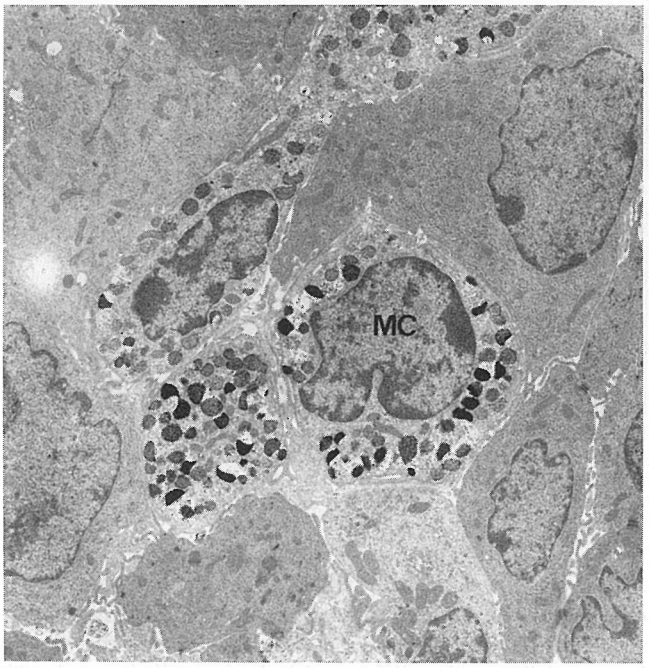

Fig. 8: Intraepithelial accumulation of mast cells (MC) in the main bronchus. In chronically diseased horses the mast cells are increased in and on the airway epithelium. 10.000x

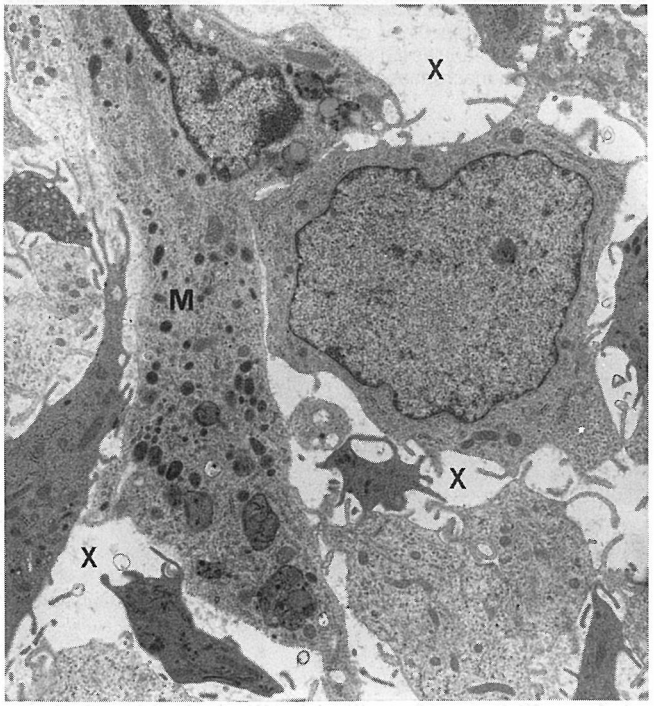

Fig. 7: Intercellular spaces (X) between bronchial epithelial cells are widened in horses with severe COPD. M = intraepithelial macrophage. 12.500x

In summary, comparison of morphological alterations with clinical diagnosis reveals a good correlation between changes in the epithelium and severity of disease. In cases of slight bronchitis and bronchiolitis, Clara cells are especially involved and are partially transformed into mucus-producing goblet cells. In severe chronic obstructive pulmonary disease, the number of goblet cells increases and Clara cells and ciliated cells degenerate, resulting in a marked disturbance of mucociliary clearance mechanisms. An increase in mast cells, together with widening of the intercellular spaces, suggests that hyperreactivity and allergic reactions may play a role in the pathogenesis of COPD in horses.

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