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In vitro study of the bronchial mucosa during *Pseudomonas aeruginosa* infection

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Abstract. The route of bacterial infection of the lower respiratory tract is generally one of descent subsequent to colonisation of the oral and oropharyngeal mucosa. The interaction between *Pseudomonas aeruginosa* (wild type) and the bronchial epithelium was studied in bronchial mucosal probes cultured in tissue culture medium. It was possible to demonstrate that, even after loss of the mucus layer, adherence between the bacteria and the bronchial epithelium does not take place if ciliary function remains intact. Only after mechanical destruction of the bronchial epithelium, in proximity to squamous metaplasia or after loss or malfunction of the cilia of the bronchial epithelial cells was adhesion between bacteria and bronchial epithelial cells or basement membrane demonstrated by electron microscopy. After loss of the cilia following adenovirus-infection, adhesion between *P. aeruginosa* and the bronchial epithelial cells was visible. These results indicate that ciliary function must be of crucial significance in bacterial epithelial colonisation.

Key words: *Pseudomonas aeruginosa* – Bacterial adherence – Bronchial mucosa – Electron microscopy

Introduction

Bacterial colonisation of the oral and oropharyngeal mucosa is considered to be the initial event in bacterial infection of the respiratory tract. Bacteria subsequently descend to the lower respiratory tract and bronchitis or pneumonia may develop. *Pseudomonas aeruginosa* is the most frequent pathogenic micro-organisms affecting patients with cystic fibrosis of the lungs. The specific mechanisms inclued in the colonisation of airways by *P. aeruginosa* in cystic fibrosis remain unclear. Erosion of the respiratory epithelium is a common finding in these patients (Baltimore et al. 1989). Cell surface sugars on epithelial cells spreading to cover a wound have been shown to differ from sugars found on normal epithelial cells (Gibson et al. 1983; Ball et al. 1989). Bacterial adhesion to host cells may arise from direct interaction between bacterial adhesions and host glycoconjugate receptors (Sharon 1987). Today morphological studies of the interactions between bacteria and bronchial mucosa in the initial stage of the disease in human beings have not been performed for technical reasons. In this report interactions between the bronchial epithelium and *P. aeruginosa* during the initial phase of infection were studied in vitro in human bronchial mucosa cultured in tissue culture medium.

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Materials and methods

Biopsy material obtained from main bronchi of 12 patients and samples of the bronchial wall from the lobar or main bronchi of 15 lung resection specimens were immediately placed in sterile tissue culture medium at 4° C containing four different antibiotics (penicillin, dihydrostreptomycin, neomycin sulphate and bacitracin) and a mycostatic (Fungizone) before removal from the operating room. Four hours later the specimens were brought into sterile tissue culture medium and incubated at 37° C. They were then inoculated with *P. aeruginosa*. Care was taken to place the lateral resection line of the bronchial wall flat on the floor of the petri dish, so that the ciliated epithelium could be observed by an inversion light microscope (Olympus IT-M2) at a 600-fold magnification. Each specimen measured 3×1 mm and was incubated in a 1 ml tissue culture medium with 10^4 *P. aeruginosa* (wild type). The incubation periods were of 1, 6, 12, 24 and 30 h duration. From each patient one probe from the beginning and the end of the experiment respectively served as controls. The function of the cilia of the ciliated epithelium was monitored constantly during the whole course of the experiment via a 3-chip colour video camera (Sony DXC 750P). The ciliary beat frequency was calculated by slow-motion playback. Following removal of the mucous layer and mechanical alteration of the epithelium (tweezers and scalpel) a further 15 probes taken from the 15 lung resection specimens and incubated with the bacteria under the same experimental conditions. Additional bronchial wall samples from the same lung resection specimens were first incubated with 1 ml adenovirus suspension (10^6 $TCID_{50}$ for 6 h in culture medium and subsequently exposed to the bacteria.

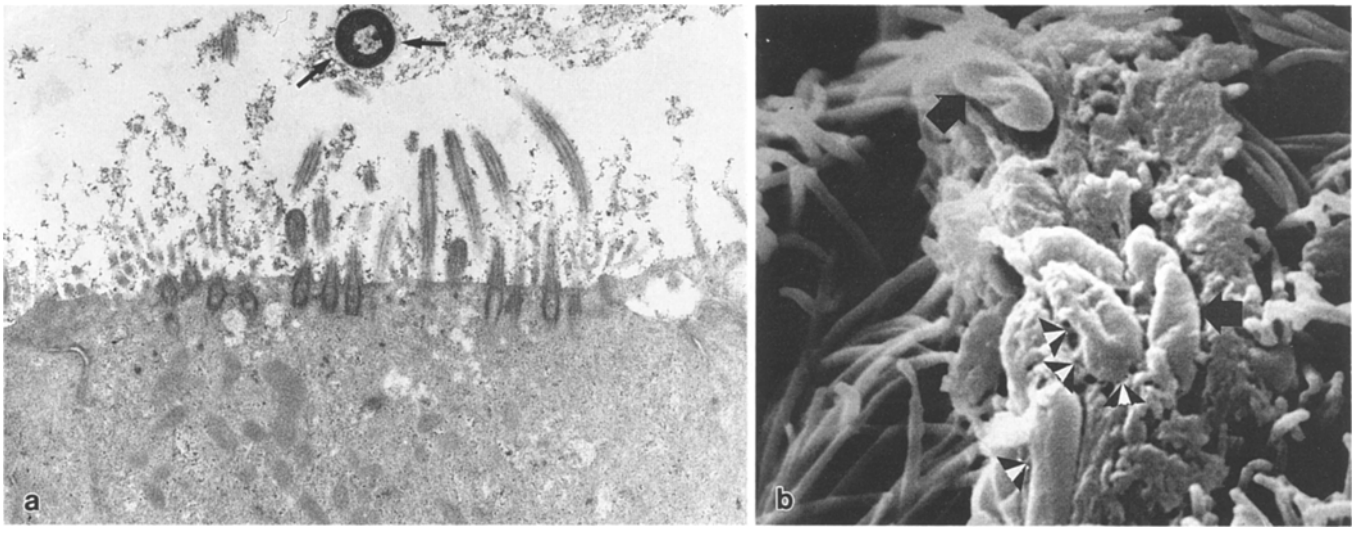

Fig. 1. a *Pseudomonas aeruginosa* lying in the mucous layer without contact with the cilia of the bronchial epithelium. Fine adhesions (*arrow*) to the mucus have developed. Transmission electron micrograph. ×14666. **b** Scanning electron microscopic demonstration of *P. aeruginosa* (*arrows*) in the mucous layer with development of fine adhesions (*arrowheads*). Cilia are visible at the end of the mucosa. Scanning electron micrograph. ×93351

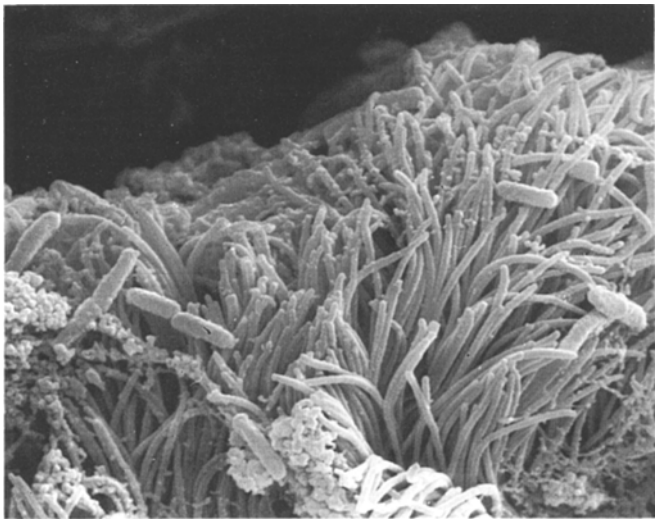

Fig. 2. Several *P. aeruginosa* between the cilia without development of adhesion. Scanning electron micrograph. ×52578

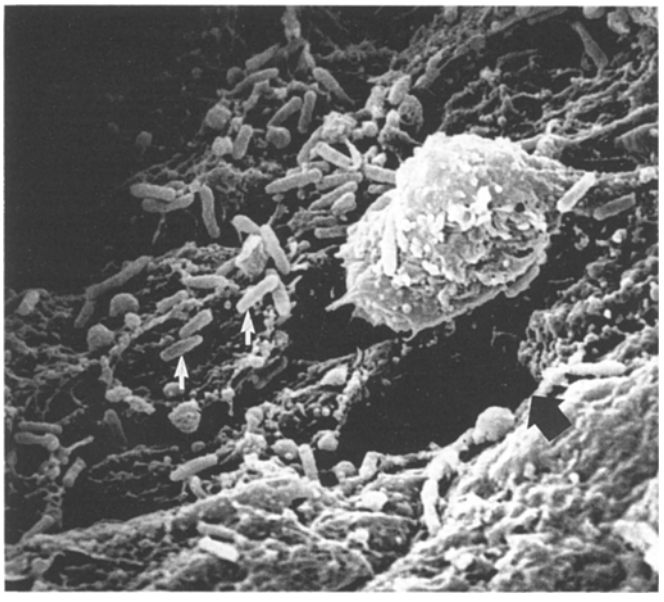

Fig. 3. Dense colonisation of a defect in the bronchial epithelium by *P. aeruginosa*. The basement membrane lies naked at the surface and is colonised by bacteria (*short arrows*). Adjacent to an opened capillary (*long arrow*) a remaining basal cell is also colonised by bacteria. Scanning electron micrograph. ×25000

After the respective incubation periods the specimens were fixed in 2.5% glutaraldehyde for 18 h and embedded in Epon 812 as usual for transmission electron microscopy (Zeiss EM 10). Alternatively some underwent critical point drying and sputtering with gold for scanning electron microscopy. All 15 lung resection specimens contained carcinoma. The 12 patients undergoing bronchoscopy did so because of radiological suspicion of a round lung nodule.

Results

By means of inversion light microscopy it can be seen that when the mucous layer is intact bacteria remain in the mucous layer and are transported via the ciliary beat. These findings are confirmed by the electron microscopical studies. The bacteria are evident only in the mucous layer (Fig. 1a, b), which, as compared to the controls, is thicker even after only a 12 h incubation period. Very fine adhesions between the bacteria and the mucous are apparent (Fig. 1a). Direct contacts between bacteria and bronchial mucosa are not visible.

After removal of the mucous layer, the bacteria reach the cilia of the surface epithelium, but do not display adhesion to the ciliary surface on light- or electron microscopy (Fig. 2). In light microscopy the bacteria are seen to be pushed away into the liquid culture medium by the ciliary beat.

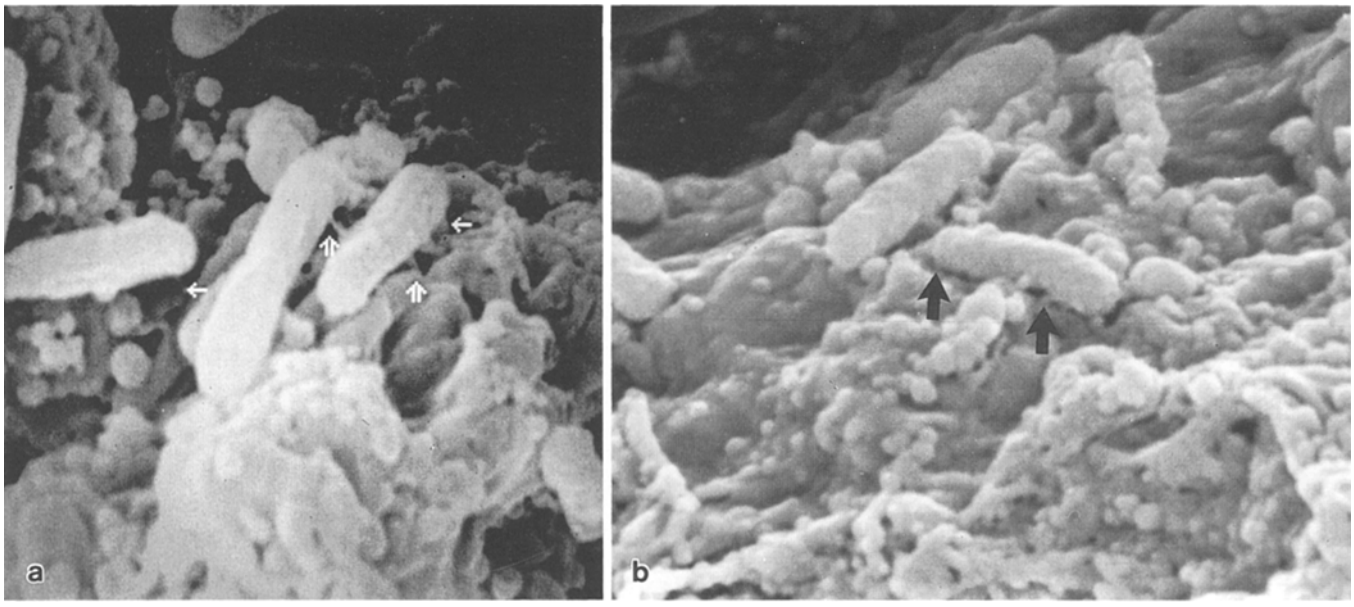

Fig. 4. a Colonisation of basal cell by *P. aeruginosa* by development of adhesions (*arrows*). Scanning electron micrograph. ×103333. **b** Development of adhesions between *P. aeruginosa* (*arrows*) in proximity to the basement membrane. Scanning electron micrograph. ×88188

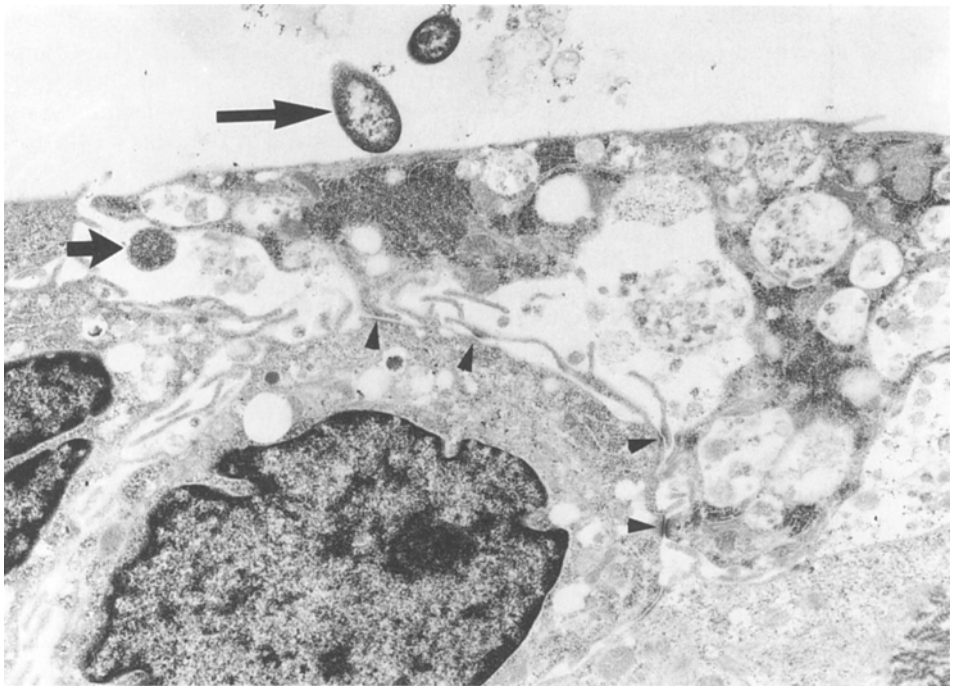

Fig. 5. Direct contact (*long arrow*) of *P. aeruginosa* at the surface of the cytoplasmic membrane of a bronchial epithelial cell following loss of its cilia. The intercellular space is distended with otherwise intact cell contact zones (*arrowheads*). A bacterium lies within the intercellular space (*short arrow*). Transmission electron micrograph. ×12600

After mechanical removal of the surface epithelium the defective zones are colonised by bacteria within 12 h (Fig. 3). At the edge of the damaged areas there is also direct contact between bacteria and the sides of the cytoplasmic zones of the epithelial cells. Fine adhesions between the epithelial cells and the bacteria have developed. At the base of the defect remaining basal cells are visible, which are also colonised by bacteria. There are adjacent zones of basement membrane lying naked. Fine adhesions between the bacteria have developed both here as well as in vicinity of the basal cells (Fig. 4a, b). Bacterial colonisation was also observed close to areas of squamous metaplasia.

In the bronchial wall specimens initially treated with a 6 h incubation with adenovirus and subsequent 24 h incubation with bacteria an increased number of cells with only few cilia and microvilli or even without cilia could be seen after 30 h (Fig. 5). In some, abortive forms of cilia had developed. Not until now was there direct contact between the bacteria and the surface epithelial cells.

In the bronchial wall samples from the lung resection

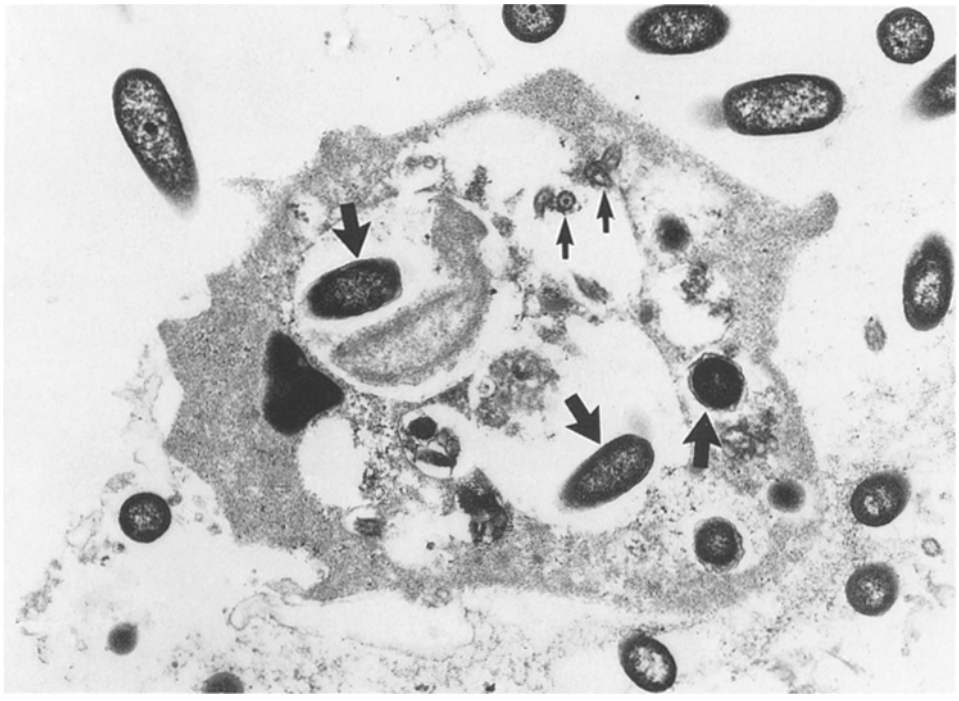

Fig. 6. *P. aeruginosa* phagocytosed by a polymorphonuclear granulocyte (*thick arrows*) with optically intact structure. In-between phagocytosed ciliary remains (*narrow arrows*). Transmission electron microscopy. ×15619

specimens emigration of polymorphonuclear granulocytes were evident in the bronchial epithelium and at the epithelial surface after 12 h. Slight intercellular oedema developed. Bacteriophagocytosis by the granulocytes could be seen (Fig. 6).

During the complete duration of the experiments the ciliary beat frequency lay between 12 and 14 Hz. No alteration of the frequency was observed.

Discussion

It is assumed that infection of lower parts of the respiratory tract takes place secondarily to bacterial colonisation of the oral and oropharyngeal mucosa. For bacterial colonisation of the mucosa to take place adhesion of the micro-organism to the surface epithelium is probably of critical importance. It is assumed that bacterial adhesion represents interactions between bacteria and corresponding receptors at the epithelial cell surface (Niederman et al. 1983; Franklin et al. 1987; Beachey et al. 1988).

In a series of laboratory studies adhesion between *P. aeruginosa* and human tracheobronchial mucus and receptors at the cell surface could be localised (Ramphal and Vishwanath 1987; Plotkowski et al. 1989, 1991; Ramphal et al. 1991; Yamaguchi and Yamada 1991; Reddy 1992). In our studies of the bronchial mucosa with intact mucosa we could confirm the presence of fine adhesions between the bacteria and mucosa.

Colonisation of respiratory epithelial cells from a nasal polyp was studied in primary cells cultures in vitro (Plotkowski et al. 1991). In keeping with the results of Plotkowski et al. (1991) we were able to observe in our studies, that no bacterial colonisation of the epithelium with subsequent adhesion can take place if ciliary function is intact, even if the mucous layer has been lost. The bacteria are pushed back into the medium upon contact with the cilia. Alteration of the ciliary beat frequency was not recorded.

As in experimental results obtained from rats (Yamaguchi and Yamada 1991) we were able to demonstrate in our studies of human bronchial mucosa that bacterial colonisation takes place in close proximity to mucosal defects, where both the lateral cytoplasmic zones of ciliary and basal cells and basement membrane are involved. It can thus be assumed that not only the apical cytoplasmic membrane but also the lateral cytoplasmic membrane, basal cells and basement membrane contain receptors for adhesion of *P. aeruginosa*. Evidence of bacterial colonisation of squamous metaplasia allows us to make the assumption that there are receptors present at the surface of squamous epithelial cells as in the oropharyngeal mucosa.

When ciliary loss or malfunction occurred, for example following viral infection, direct contact between bacteria and bronchial epithelium developed. This finding illustrates the important role ciliary function has in the pathogenesis of bacterial infections. Our results indicate that intact ciliary function prevents bacterial colonisation of the mucosa, even following loss of the mucous layer. Colonisation of bacteria on the bronchial epithelium is only possible when malfunctions, malformations or loss of cilia occur. Superficial and deeper mucosal defects, as well as squamous metaplasia, also promote bacterial colonisation.

From our study it is evident that adhesion to and colonisation of the mucosa was possible only after ciliary function was impaired. Ciliary function must be of crucial significance in bacterial epithelial colonisation. The

presence of specific receptors at the cell surface and development of bacterial adhesion are a second significant step in colonisation of the bronchial mucosa and will only be effective after restriction of ciliary function or in the presence of epithelial lesions.

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