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Adherence of *Pseudomonas aeruginosa* to respiratory epithelium and the effect of leucocyte elastase

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Summary. The tracheobronchial secretions from patients with cystic fibrosis often contain high amounts of free proteases. To evaluate whether human leucocyte elastase (HLE) can favour the persistence of bacterial airways infection, we exposed the frog palate mucosa to HLE and then to radiolabelled *Pseudomonas aeruginosa* and followed the sequence of events by scanning electronmicroscopy. In response to HLE there was a marked outpouring of mucus and a desquamation of the epithelium. *P. aeruginosa* was shown to adhere to recently secreted granules of mucus and to the exposed submucosal underlying connective tissues. For the eight different bacterial strains studied, a significative adherence to HLE-injured mucosa was observed only in strains that possessed internal haemagglutinating activity. Neither the presence of fimbriae, nor of the mucoid exopolysaccharide, nor of the bacterial surface haemagglutinating activity could be related to adherence of *P. aeruginosa* to the injured mucosa. These results support the hypothesis that HLE enhances bacterial infection of the respiratory mucosa both by inducing mucus hypersecretion and by exposing receptors to the microbial adhesins. It is also suggested that *P. aeruginosa* internal lectins may be implicated in adherence to host tissues.

Introduction

Infection of the tracheobronchial mucosa by *Pseudomonas aeruginosa* represents a major clinical problem in patients with chronic obstructive lung disorders, especially in cystic fibrosis (CF). In these patients, once colonisation is established, bacteria are virtually impossible to eradicate, despite the host immune response to bacterial antigens or antibiotic therapy (Geddes, 1984). *P. aeruginosa*, however, rarely infects healthy individuals; infection is usually restricted to hospitalised patients or to subjects who have some abnormality of the natural barriers that protect tissues against microbial invasion (Bodey *et al.*, 1983). Since available evidence indicates that the respiratory tract of CF patients may not be damaged initially (Sturgess, 1981), there must be some factor unique to the CF airway mucosa that allows colonisation by *P. aeruginosa*. It may be that *Staphylococcus aureus* infection, detected early in the course of the disease (Pennington *et al.*, 1979), creates local conditions favouring the adherence of *P. aeruginosa*.

Neutrophil polymorphonuclear leucocytes readily accumulate in lung tissue in response to inflammatory processes (Cohen and Rossi, 1983). During phagocytosis (Ohlsson and Olsson, 1977), leucocyte enzymes may be released extracellularly. This degranulation is known to be an important event in the amplification of the inflammatory response (Gallin, 1984). However, the presence of free leucocyte proteolytic enzymes in bronchial secretions, indicative of a local imbalance between proteases and the anti-protease system, may damage host tissues. The spectrum of the human leucocyte elastase (HLE) substrates includes structural proteins such as collagen and elastin, and macromolecules such as proteoglycans (Havemann and Gramse, 1984). Fibronectin, a glycoprotein present in extracellular matrix and at the surface of epithelial cells, is also hydrolysed by HLE (McDonald *et al.*, 1979; Suter *et al.*, 1988). The proteolytic activity of saliva from severely ill patients has been shown to be significantly correlated with the increased adherence of *P. aeruginosa*

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to buccal cells *in vitro*, due to fibronectin degradation (Woods *et al.*, 1980*a*, 1983).

The respiratory secretions of chronic bronchitic (CB) and CF patients often contain high amounts of free neutral proteases (Stockley and Burnett, 1979; Suter *et al.*, 1984; Goldstein and Döring, 1986). Besides structural protein degradation, leucocyte proteases are known to stimulate mucus secretion and to damage ciliated cells (Smallman *et al.*, 1984; Kim *et al.*, 1987), two factors known to compromise the transport of tracheobronchial secretions and to favour microbial colonisation of the airways mucosa. Despite all this evidence pointing to a role for HLE in favouring microbial persistence, there has been no systemic study on the effect of HLE on the adherence of *P. aeruginosa* to the respiratory epithelium.

The adherence of *P. aeruginosa* is a very complex phenomenon. The alginate-like exopolysaccharide produced by the majority of the isolates from CF patients has been imputed to be the bacterial adhesin allowing adherence of mucoid strains of *P. aeruginosa* to both respiratory mucins (Ramphal *et al.*, 1987) and epithelium (Marcus and Baker, 1985; Ramphal and Pier, 1985; Doig *et al.*, 1987). However, infection by non-mucoid micro-organisms often precedes the emergence of mucoid strains, suggesting the role of some other adhesin in bacteria-host mucosa interaction. Several studies have pointed to the fimbriae as the structures mediating adherence of *P. aeruginosa* to buccal cells (Woods *et al.*, 1980*b*), tracheal cells (Doig *et al.*, 1988) and human respiratory mucus (Ramphal *et al.*, 1987), whereas results of other studies conflict with this suggestion (Marcus and Baker, 1985; Hata and Fick, 1987).

P. aeruginosa has been shown to agglutinate red blood cells by two different mechanisms—*via* the production of two distinct lectins (Gilboa-Garber, 1972) and *via* hydrophobic interaction between a surface haemagglutinin and the erythrocyte membranes (Glick *et al.*, 1987). For several gram-negative bacteria, haemagglutinating activity is well correlated with adherence to epithelial cells (Dugoid and Old, 1980). Nevertheless, the role of these mechanisms on adherence of *P. aeruginosa* to the respiratory epithelium has never been investigated.

The present study was undertaken to evaluate whether HLE enhances adherence of *P. aeruginosa* to the respiratory mucosa and to determine the role of the different *P. aeruginosa* adhesins in adherence to the HLE-treated epithelium. In so far as HLE-induced mucus hypersecretion was expected to favour adherence of *P. aeruginosa*, an experimental model which allows mucus preservation—the frog palate mucosa (Plotkowski *et al.*, 1989*a*)—was used throughout this study.

Materials and methods

Bacteria

P. aeruginosa isolates 8M, 13M, 14M and 24M were mucoid (M) strains collected from expectorated sputum samples from CF patients. Isolates 25NM, 27NM, 144NM and 167NM were clinical non-mucoid (NM) strains from the same group of patients. The organisms were maintained in 1-ml volumes of glycerol 20% v/v in Trypticase Soy Broth (TSB) and stored at −20°C.

Adherence system

A tissue-culture system simulating the air-liquid interphase in which respiratory epithelial cells are found *in vivo* was used throughout this study. Briefly, a strip of frog palate mucosa (FPM) on a membrane filter (Millipore SA; pore size 0·45 μm; diameter 13 mm) was obtained as previously described (Plotkowski *et al.*, 1989). The lower parts of Swinnex filters (Millipore SA; diameter 13 mm) were sealed and filled, up to the railing, with Minimal Essential Medium (MEM) containing N-2-hydroxyethyl piperazine-N′-2-ethanesulphonic acid 1% w/v (MEM-HEPES). The membrane filters covered by the FPM were placed over the filter railing and soaked with the underlying culture medium, in order to provide the necessary nutrients for the basement side of the mucosa. Thereafter, the two parts of the filters were screwed together and the epithelial surface of the FPM was ready to be submitted to different treatments.

FPM treatment by human leucocyte elastase

HLE was isolated and purified from purulent expectorated sputum (Martodam *et al.*, 1979) and stored at −20°C until used. For analysing the response of the FPM to the leucocyte protease, 100 μl of HLE 250 μg/ml in MEM-HEPES was introduced through the upper hole of the Swinnex filters and deposited over the epithelial surface of nine different FPM preparations. These were incubated in triplicate at 37°C for 1, 2 and 4 h. Afterwards, the filters were opened, the FPM preparations were rinsed with 0·1 M phosphate-buffered saline, pH 7·2 (PBS) and examined by scanning electronmicroscopy (SEM). Control FPM preparations were treated with 100 μl of MEM-HEPES, under the same conditions.

P. aeruginosa adherence to HLE-treated FPM

A 1-ml sample of each bacterial strain was thawed and inoculated into 10 ml of TSB, and incubated for 18 h at 28°C. The temperature of 28°C rather than 37°C was chosen because it favours internal lectin and fimbriae

production (Fuerst and Hayward, 1969; Gilboa-Garber, 1972) and is closer to the environmental temperature than is 37°C. Labelling of *P. aeruginosa* with ^{99m}Technetium (^{99m}Tc) was performed as previously described (Plotkowski *et al.*, 1987) and micro-organisms were suspended in MEM-HEPES at a concentration of 10^9 cfu/ml.

In each assay, part of the final bacterial suspension was centrifuged at 2000 *g* for 30 min and the supernate was filtered on to a 0·22-μm pore size Millipore membrane. The counts of 100 μl of this supernate and of an equal volume of the ^{99m}Tc-labelled *P. aeruginosa* suspension were determined in a scintillation counter with a sodium-iodide-thallium crystal. The ^{99m}Tc counting was also related to the number of cfu/ml determined by agar plate culture of the radiolabelled bacterial suspension.

To evaluate whether HLE enhances *P. aeruginosa* adherence to the respiratory epithelium, 100 μl of the ^{99m}Tc-labelled suspension was introduced through the upper hole of the FPM-containing Swinnex filters and deposited over five FPM preparations treated with HLE solution for 4 h at 37°C, as described above, as well as over five MEM-HEPES treated FPM preparations (controls). Even though 37°C is not the optimal temperature for the maintenance of FPM, it was chosen because it is the temperature at which bacteria meet human tissues. After incubation for 1 h at 37°C, the filters were opened and the FPM preparations were rinsed with PBS, detached from the membrane filters and placed in vials to be counted in the scintillation counter. To eliminate the interference of changes in the specific activity of the *P. aeruginosa* suspension in different assays, the results were expressed according to the formula:

$$\text{Percentage of associated dpm} = \frac{\text{counted dpm}}{\text{P. aeruginosa suspension dpm} - \text{P. aeruginosa filtered supernate dpm}} \times 100$$

Scanning electronmicroscopy

SEM was used for analysing the reaction of the FPM preparations incubated with HLE for different periods, as well as for determining to which components of the HLE-treated FPM the bacteria had adhered. The FPM preparations were fixed in glutaraldehyde 2·5% w/v in PBS overnight at 4°C, dehydrated in graded concentrations of ethanol, critical point dried, coated with gold palladium and examined with a Jeol JSM 25 or a Siemens-Autoscan ETEC scanning electronmicroscope.

Detection of fimbriae

Bacteria were grown in TSB for 20 h at 28°C. A drop of the bacterial cultures was placed on collodion-coated nickel grids and dried at 56°C. The grids were rinsed with distilled water, stained with uranyl acetate 5% w/v for 3 min and examined with a Jeol 200 CX transmission electronmicroscope.

Determination of internal lectin haemagglutinating activity

To improve the detection of internal lectins, bacteria were grown in Nutrient Broth, without glucose, with shaking at 28°C for 3 days. Choline chloride solution was added each day to the growth media at a final concentration of 0·2% w/v. Cells were harvested, washed three times and resuspended in PBS to a final concentration of $(2–4) \times 10^9$ cfu/ml. They were disrupted by an ultrasonic vibrator at 20 KHz for 1 h (Gilboa-Garber, 1972). The supernatant fluids obtained by centrifugation at 30 000*g* at 4°C for 15 min were filtered through Millipore membranes (pore size 0·22 μm) and the protein concentration was determined to gauge bacterial cell disruption.

Neuraminidase-treated human blood group A erythrocytes were prepared by incubating, for 30 min at 37°C, an erythrocyte suspension 15% v/v with a *Clostridium perfringens* neuraminidase solution (Sigma) at a final enzyme concentration of 0·2 U/ml in PBS, pH 6·0. The enzyme-treated cells were washed in PBS and 100 μl of an erythrocyte suspension 0·5% v/v were added to 100 μl of serial two-fold dilutions of each *P. aeruginosa* cell-free extract. Titres were expressed as the highest dilution that showed complete agglutination of the erythrocytes. This haemagglutination test has been shown to detect both types (PAI and PAII) of *P. aeruginosa* lectins (Gilboa-Garber, 1986).

Determination of surface haemagglutinating activity (SHA)

Bacteria were grown in Trypticase Soy Agar containing choline chloride 1·4% w/v for 20 h at 28°C, resuspended in PBS containing 1 mM $MgCl_2$ (PBS-Mg) and centrifuged at 2000 *g* for 10 min. The cell pellets were washed three times and suspended to a final concentration of 10% v/v in PBS-Mg containing cysteine 0·1% w/v (Glick *et al.*, 1987). Papain-treated human blood group A erythrocytes were prepared by incubating 9 volumes of an erythrocyte 5% v/v suspension with 1 volume of papain 1% w/v (Crude preparation; Sigma) in cysteine 0·1% w/v at 37°C, for 30 min. The SHA assay was performed by adding 50 μl of a papain-treated erythrocyte 5% v/v suspension to 200 μl of serial two-fold dilutions of each *P. aeruginosa* suspension obtained as described above.

Statistical analysis

Student's *t* test was used to determine whether the differences between *P. aeruginosa* adherence to HLE-treated and control FPM were statistically significant ($p < 0·05$).

Results

Electronmicroscopy

Fig. 1a shows the control epithelium and fig. 1b the normal structure of cilia. The FPM treated with

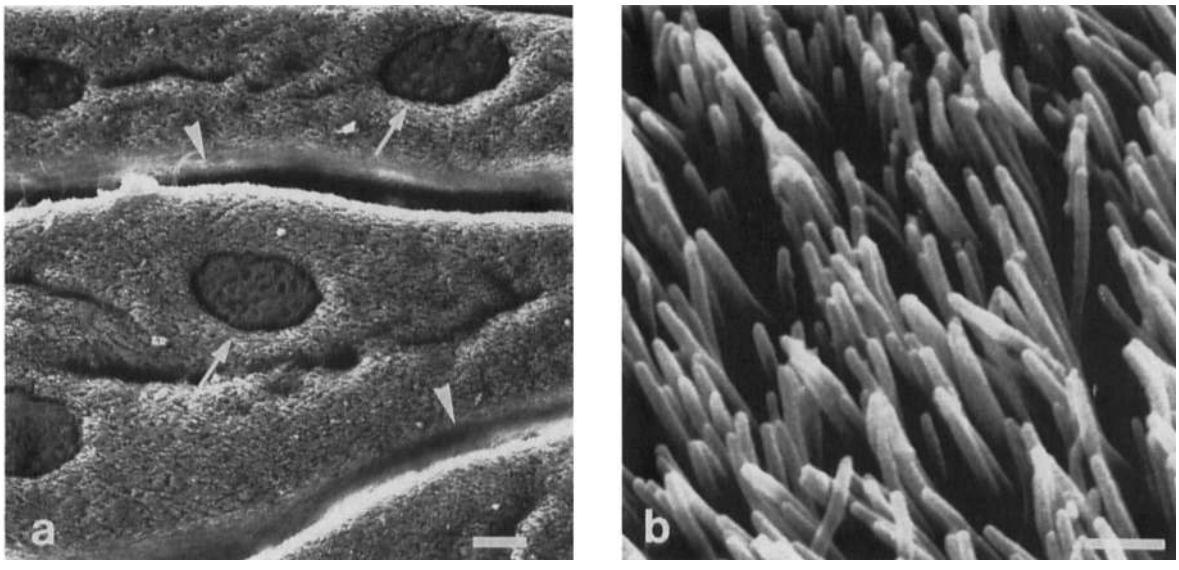

Fig. 1. SEM of control frog palate mucosa: (**a**) epithelium is covered by ciliated cells—note the presence of patches of sensory cells (arrows) and epithelial folding (arrowheads); (**b**) higher magnification showing normal structure of cilia. Bars = 50 and 1 μm respectively.

HLE for 1 h showed a mild hypersecretion of mucus but the ultrastructure of the epithelium was preserved (fig. 2a). After HLE treatment for 2 h, an abundant outpouring of mucus was noted (fig. 2b) and the tips of the cilia were severely damaged (fig. 2c). After 4 h, a marked mucus hypersecretion was accompanied by exfoliation of the epithelial cells with exposure of the submucosal underlying connective tissue (fig. 2d). In control FPM, only a few *P. aeruginosa* cells were seen attached to remaining patches of mucus (fig. 3). Bacteria rarely adhered directly to uninjured epithelium (not shown). In contrast, the HLE-treated FPM exposed to *P. aeruginosa* suspension showed an intense bacterial adherence to both recently secreted granules of mucus and to the exposed submucosal underlying connective tissue (fig. 4a, b).

P. aeruginosa adherence to HLE-treated FPM

The adherence of ^{99m}Tc-labelled bacteria to HLE-treated FPM was not uniform among the different *P. aeruginosa* strains studied. Although the percentage of radioactivity associated with the enzyme-treated mucosa was higher than that associated with control FPM in 7 of 8 cases, only strains 8M, 24M and 25NM adhered significantly more to HLE-treated than to control FPM (table I). The presence of the mucoid exopolysaccharide did not appear to be a significant factor influencing *P. aeruginosa* adherence to the HLE-treated epithelium.

Role of the different P. aeruginosa adhesins in adherence to HLE-treated FPM

By transmission electronmicroscopy, it was demonstrated that all *P. aeruginosa* strains tested exhibited fimbriae. No differences in the number of fimbriae per bacterium or in the percentage of

Table I. Adherence of *P. aeruginosa* to HLE-treated or control FPM

P. aeruginosa strain	*Adherence to FPM		p value
	HLE-treated	Control	
8 M	19·4 (3·8)	8·5 (4·0)	<0·01
13 M	6·5 (2·4)	9·7 (7·2)	NS
14 M	16·1 (8·3)	10·5 (8·3)	NS
24 M	11·5 (3·2)	5·0 (2·4)	<0·01
25 NM	9·0 (1·7)	6·9 (1·5)	<0·01
27 NM	9·5 (11·8)	6·4 (4·2)	NS
144 NM	16·3 (6·4)	9·7 (4·0)	NS
167 NM	11·0 (4·8)	7·1 (3·0)	NS

M = mucoid; NM = non mucoid.
NS = not significant.
*Adherence is expressed as the mean percentage (and SD) of added radioactivity (radiolabelled bacteria) remaining on the mucosa.

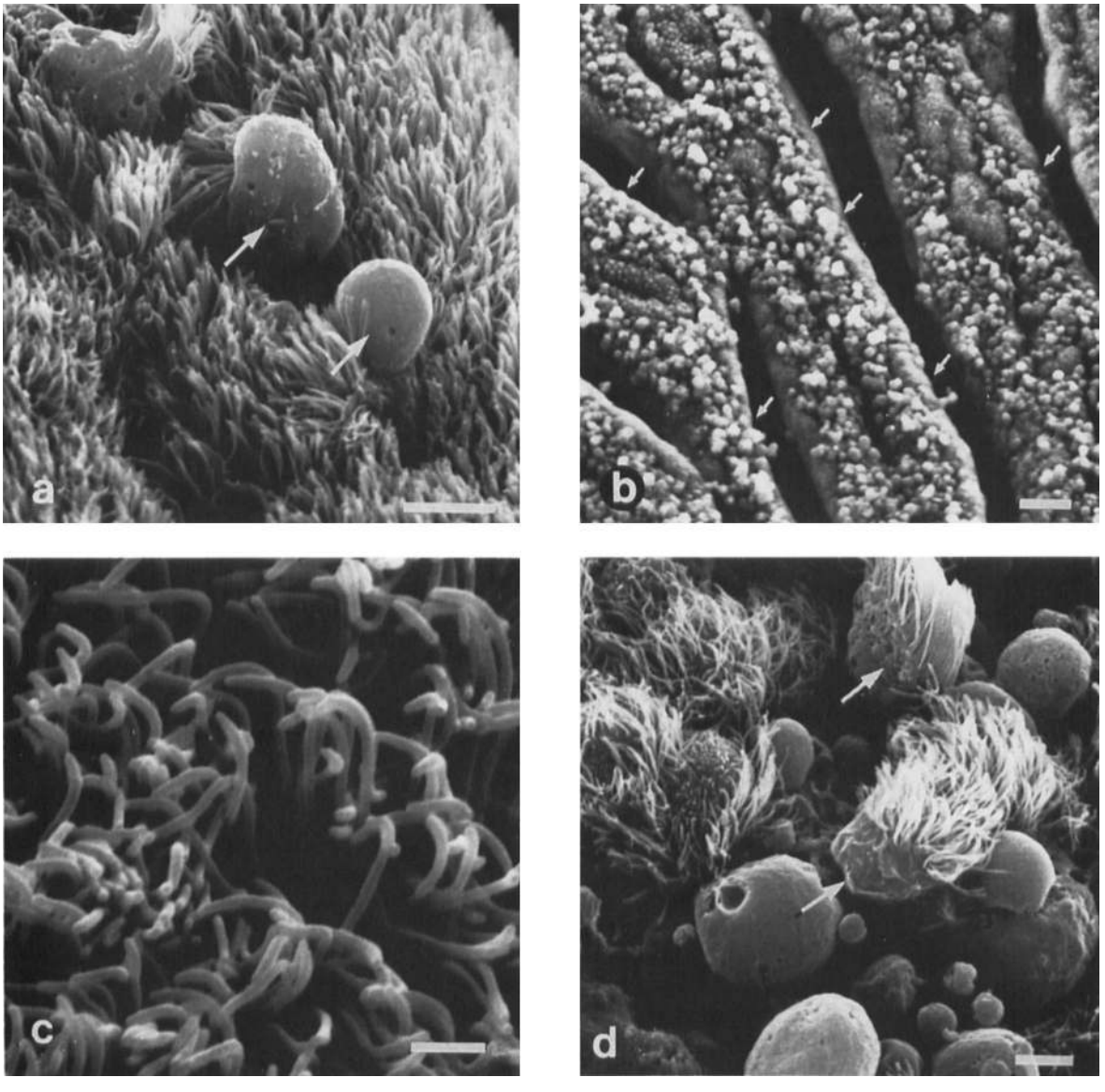

Fig. 2. SEM of frog palate mucosa treated with human leucocyte elastase: (**a**) for 1 h—note the mild secretion of mucus granules (arrows); (**b**) and (**c**) for 2 h—note the abundant outpouring of mucus granules (arrows) and the tips of the cilia severely damaged; (**d**) for 4 h—note the desquamation of the epithelial cells (arrows). Bars = 10, 50, 1 and 5 μm, respectively.

fimbriate cells could be determined between strains adherent or non-adherent to HLE-treated FPM. Table II presents the haemagglutination titres obtained with the different *P. aeruginosa* suspensions or cell-free extracts. No relationship was found between the level of the bacterial SHA and the internal lectin, between the internal lectin titre and the total protein concentration of the cell-free extract nor between the SHA and the adherence to HLE-treated FPM. In contrast, *P. aeruginosa* exhibiting higher internal lectin titres adhered significantly better to HLE-treated mucosa than to control untreated FPM.

Discussion

The ciliary clearance of mucus-entrapped micro-organisms represents a major anti-infectious defence mechanism of the airways mucosa. Therefore, any factor compromising the transport of tracheo-

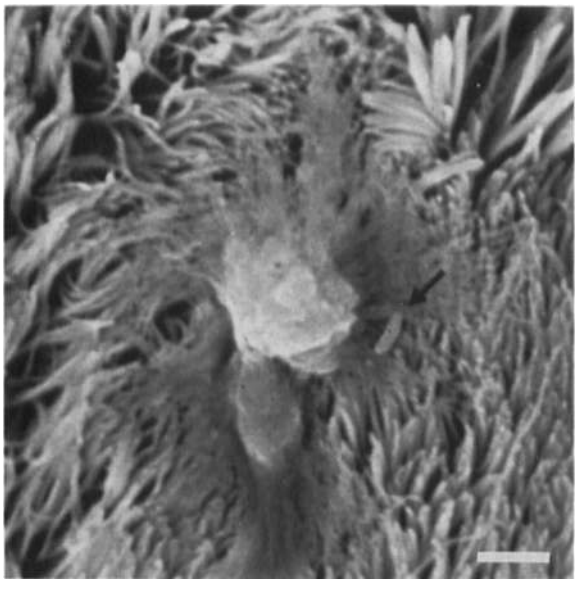

Fig. 3. SEM of control frog palate mucosa showing rare bacteria adhered to mucus (arrow). Bar = 2·5 μm.

Table II. Relationship between *P. aeruginosa* internal lectin, surface haemagglutinating activity (SHA) and cell-free extract total protein concentration

Bacterial strain	Haemagglutination titre		Cell-free extract protein concentration (μg/ml)
	SHA	Internal lectin	
8 M*	0	≥16	140
13 M	32	0	440
14 M	4	0	280
24 M*	32	≥16	670
25 NM*	64	≥16	900
27 NM	64	2	180
144 NM	ND	0	260
167 NM	0	0	220

M = mucoid; NM = non mucoid.
ND = not determined.
**P. aeruginosa* strains for which adherence to HLE-treated mucosa was increased significantly (table I).

bronchial secretions, such as mucus hypersecretion, abnormalities of mucus rheological properties or ciliary damage (Greenstone and Cole, 1985) favours the multiplication of the micro-organisms attached to the mucus gel and infection of the underlying mucosa.

Most of the models proposed to study bacterial adherence to the respiratory mucosa differ from in-vivo conditions in that there is an absence of mucus. We have recently proposed that the frog palate mucosa (FPM) may be used as a model that allows the mucus coat to be preserved (Plotkowski *et al.*, 1989*a*), in a system simulating the air-liquid interphase in which epithelial cells are found *in vivo*. The close similarity between the ultrastructure of FPM and the human tracheal mucosa (Puchelle *et al.*, 1984) and the histochemistry of their mucus-

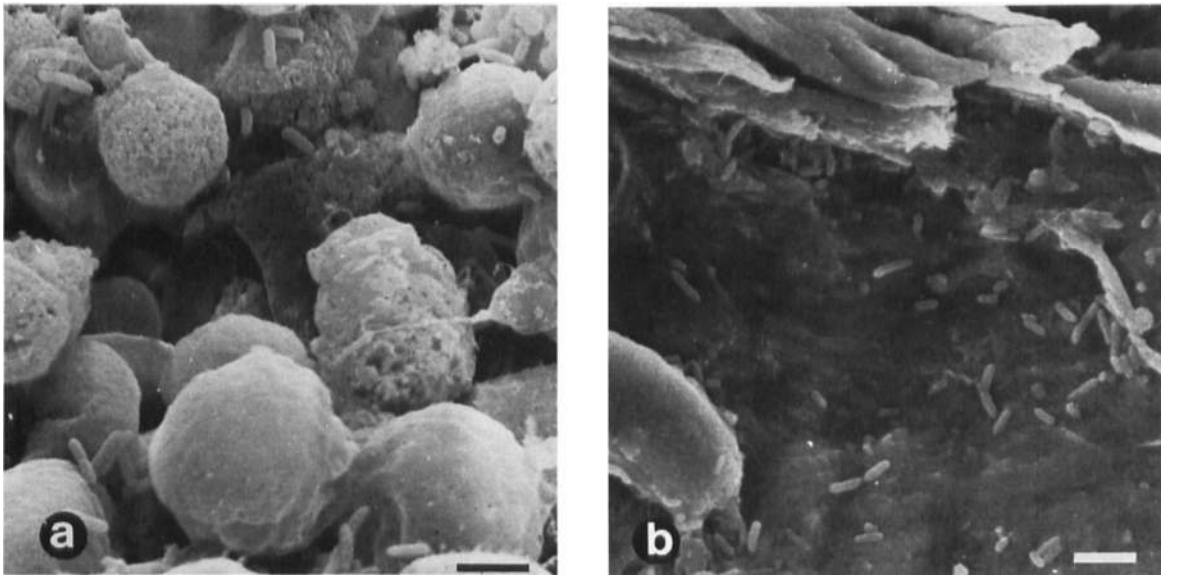

Fig. 4. SEM of frog palate mucosa treated with human leucocyte elastase exposed to *P. aeruginosa* cells showing bacteria attached to (**a**) secreted granules of mucus and (**b**) to desquamated mucosa. Bars = 2·5 μm.

secreting cells (Plotkowski *et al.*, 1989*b*) suggests that the FPM may be considered as a valuable model for studying bacterial adherence to the airways mucosa.

In the present study, we demonstrated, by SEM, that HLE, in the concentration range found in infected sputum samples from chronic bronchitis and CF patients (Stockley and Burnett, 1979; Goldstein and Döring, 1986), induces hypersecretion of mucus and that this represents an important factor promoting bacteria-host mucosa association.

Kim *et al.* (1987) have shown that, during the physiological discharge of the airways goblet cells, part of the secreted mucin is released into the exterior medium while another part remains tightly associated with the cell plasma membrane. This cell-surface mucin layer, and not the mucin packaged into secretory vesicles in the cell cytoplasm, constitutes the pool of mucin available to be released after HLE treatment. Its loss after the protease stimulus could induce a change in the reactivity of the apical surface of the goblet cells with gold-labelled lectins (Kim *et al.*, 1987). These data, as well as those from Breuer *et al.* (1989) showing that HLE releases glycoconjugates from the surface of respiratory ciliated and secretory cells, suggest that, apart from inducing the impairment of the mucociliary transport, HLE may favour the adherence of bacteria to the airways mucosa by exposing membrane receptors usually masked by bound mucin.

In the present study, we also demonstrated that the prolonged exposure of the respiratory mucosa to HLE was followed by the desquamation of epithelial cells and exposure of the submucosal underyling connective tissues. The extracellular matrix represents an important pole of attraction for the adherence of *P. aeruginosa*. The observed bacterial adherence to HLE-injured mucosa is compatible with the opportunist character of *P. aeruginosa*, a micro-organism which infects tissues that have been injured by various means (Bodey *et al.*, 1983). Adherence of *P. aeruginosa* to control mucosa was systematically low and usually associated with residual patches of mucus over the mucosa. The inability of *P. aeruginosa* to adhere to normal uninjured ciliated cells has been reported previously (Ramphal and Pyle, 1983). Also Baker and Marcus (1982) observed that, even 4–6 h after the infection of hamster tracheal explants with non-mucoid *P. aeruginosa* strains, few or no organisms were observed adhering to the tracheal epithelium. In contrast, several studies have shown the ability of *P. aeruginosa* to adhere to cilia of human tracheal cells (Niederman *et al.*, 1983; Franklin *et al.*, 1987; Doig *et al.*, 1988). This discrepancy was attributed to differences in susceptibility to the bacterial adhesins between human and animal tracheal cells (Franklin *et al.*, 1987). However, all the studies showing adherence of *P. aeruginosa* to human airways cells have been performed with suspensions of cells collected by tracheal brushing. It has been shown that ciliated cells from quail oviduct at different stages of maturation present different membrane receptors (Chailley *et al.*, 1982). Airways ciliated cells obtained by tracheal brushing correspond mainly to "pre-desquamating" cells. It may be that these cells, at a terminal differentiation stage, present receptors for microbial adhesins not found in mature airways cells. The finding of Lundberg *et al.* (1982), that bacterial adherence to desquamated nasal ciliated cells is much greater than adherence to the ciliated epithelium, seems to corroborate this hypothesis.

It has been suggested that the adherence of *P. aeruginosa* to eukaryotic cells, such as buccal, corneal, tracheal and phagocytic cells, is mediated by fimbriae (Woods *et al.*, 1980*b*; Reichert *et al.*, 1983; Paranchych *et al.*, 1986). In our study, in as much as all the bacterial strains used were fimbriate, we could not attribute the observed difference to the capability of fimbriate bacteria to adhere to HLE-treated mucosa. Similarly, the presence of the mucoid exopolysaccharide could not explain the differences in bacterial adhesion.

Many microbial species adhere to and agglutinate red blood cells in a lectin-mediated phenomenon. The recent interest in microbial lectins is due to evidence that many of them play a crucial role in mediating adherence to surfaces colonised by micro-organisms (Uhlenbruck, 1987). Indeed, microbial lectins are now considered as determinants of virulence in both animal and plant infections (Sharon, 1987). Besides the lectin-mediated agglutination of erythrocytes, bacterial lipids or basic proteins may act as haemagglutinins (Mirelman and Ofek, 1986). For instance, *P. aeruginosa* surface haemagglutinating activity (SHA) depends on hydrophobic interaction between the bacterial and the eukaryotic cells (Glick *et al.*, 1987). In the present study, no correlation could be found between SHA of *P. aeruginosa* and adherence to HLE-treated respiratory epithelium. Similarly, SHA could not be correlated with adherence to human buccal cells (Glick *et al.*, 1987).

Besides possessing SHA, *P. aeruginosa* have been shown to produce two distinct lectins, differing in their carbohydrate-binding specificity: PA-I binds D-galactose and its derivatives, whereas PA-II binds L-fucose, D-mannose and L-galactose (Gilboa-

Garber, 1972). Most of the lectin activity is distributed inside the bacterial cell (Gilboa-Garber, 1986) but it is released promptly in the extracellular medium after bacterial disruption.

Marcus *et al.* (1989) have shown that the adherence of *P. aeruginosa* to tracheal epithelium was greatly increased in the presence of galactose- and N-acetyl galactosamine-specific lectins. Since lectins present several sugar binding sites, they may act as a bridge between bacterial and host-cell polysaccharides. Similarly, it is possible that *P. aeruginosa* lectins, released *in vivo* during bacterial disruption by local host defence mechanisms, may also be necessary for binding of *P. aeruginosa* to host mucosa.

The production of *P. aeruginosa* lectins *in vitro* is greatly stimulated by the addition of choline to the culture medium (Gilboa-Garber, 1972). Under conditions of phosphate limitation, *P. aeruginosa* elaborates phospholipase C which catalyses the hydrolysis of phosphatidylcholine, yielding phosphorylcholine (Liu, 1979). In addition, an ordinary phosphatase that splits inorganic phosphate from phosphorylcholine is produced. The possibility that free choline may be found in the respiratory secretions of CF patients, as a result of the degradation of phosphatidylcholine, the major lung surfactant, would support the attractive hypothesis that *P. aeruginosa* lectins may play a crucial role in their adherence to airways mucosa *in vivo*.

Gilboa-Garber (1983) has found a close correlation between the lectin level inside *P. aeruginosa* cells and the ability of the bacteria to secrete cytotoxic factors such as protease, haemolysin and pyocyanin. If, as he proposed, the presence of internal lectin is crucial for the secretion of these toxins, their effect is not limited to the adherence phenomenon but should be also considered in relation to tissue damage.

It is evident from the foregoing data that further work is required to gain a clear understanding of the role of lectins in the virulence of *P. aeruginosa* in the respiratory tract. Nevertheless, it seems clear from the present results that during infection, and following inflammation, the leucocyte proteinase favours the persistence of *P. aeruginosa* infection by enhancing adherence to the airways mucosa.

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REFERENCES

Baker N R, Marcus H 1982 Adherence of clinical isolates of *Pseudomonas aeruginosa* to hamster tracheal epithelium in vitro. *Current Microbiology* **7**: 35–40.

Bodey G P, Bolivar R, Fainstein V, Jadeja L 1983 Infections caused by *Pseudomonas aeruginosa*. *Reviews of Infectious Diseases* **5**: 279–313.

Breuer R, Christensen T G, Niles R M, Stone P J, Snider G L 1989 Human neutrophil elastase causes glycoconjugate release from the epithelial cell surface of hamster trachea in organ culture. *American Review of Respiratory Disease* **139**: 779–782.

Chailley B, Boisvieux-Ulrich E, Sandoz D 1982 Ciliary membrane events during ciliogenesis of the quail oviduct. *Biology of the Cell* **46**: 51–64.

Cohen A B, Rossi M 1983 Neutrophils in normal lungs. *American Review of Respiratory Disease* **127**: S3–S9.

Doig P, Smith N R, Todd T, Irvin R T 1987 Characterization of the binding of *Pseudomonas aeruginosa* alginate to human epithelial cells. *Infection and Immunity* **55**: 1517–1522.

Doig P *et al.* 1988 Role of pili in adhesion of *Pseudomonas aeruginosa* to human respiratory epithelial cells. *Infection and Immunity* **56**: 1641–1646.

Duguid J P, Old D C 1980 Adhesive properties of Enterobacteriaceae. In: Beachey E H (ed) Bacterial adherence. Chapman and Hall, London, pp 187–217.

Franklin A L, Todd T, Gurman G, Black D, Mankinen-Irvin P M, Irvin R T 1987 Adherence of *Pseudomonas aeruginosa* to cilia of human tracheal epithelial cells. *Infection and Immunity* **55**: 1523–1525.

Fuerst J A, Hayward A C 1969 Surface appendages similar to fimbriae (pili) on *Pseudomonas* species. *Journal of General Microbiology* **58**: 227–237.

Gallin I 1984 Neutrophil specific granules: a fuse that ignites the inflammatory response. *Clinical Research* **67**: 320–328.

Geddes D 1984 Progress in research on cystic fibrosis. *Thorax* **39**: 721–725.

Gilboa-Garber N 1972 Purification and properties of hemagglutinin from *Pseudomonas aeruginosa* and its reaction with human blood cells. *Biochimica et Biophysica Acta* **273**: 165–173.

Gilboa-Garber N 1983 The biological functions of *Pseudomonas aeruginosa* lectins. In: Bog-Hansen TC, Spengler GA (eds) Lectins: biology, biochemistry, clinical biochemistry, vol III, Walter de Gruyter & Co, Berlin, pp 495–502.

Gilboa-Garber N 1986 Lectins of *Pseudomonas aeruginosa*: properties, biological effects and applications. In: Mirelman D (ed) Microbial lectins and agglutinins: properties and biological activities. (*Environmental and Applied Microbiology*). John Wiley & Sons, London, pp 255–269.

Glick J, Garber N, Shohet D 1987 Surface haemagglutinating activity of *Pseudomonas aeruginosa*. *Microbios* **50**: 69–80.

Goldstein W, Döring G 1986 Lysosomal enzymes from polymorphonuclear leukocytes and proteinase inhibitors in patients with cystic fibrosis. *American Review of Respiratory Disease* **134**: 49–56.

Greenstone M, Cole P J 1985 Ciliary function in healthy and disease. *British Journal of Diseases of the Chest* **79**: 9–26.

Hata J S, Fick R B 1987 Cellular mechanisms of *Pseudomonas aeruginosa* adherence to canine tracheal epithelial cell

monolayers. *American Review of Respiratory Disease* **135**: A364.

Havemann K, Gramse M 1984 Physiology and pathophysiology of neutral proteinases of human granulocytes. In: Hörl W H, Heidland A (eds) Proteases: potential role in health and disease. Advances in experimental medicine and biology, vol 167, Plenum Press, New York, London, pp 1–20.

Kim K C, Wasano K, Niles R M, Schuster J E, Stone P J, Brody J S 1987 Human neutrophil elastase releases cell surface mucins from primary cultures of hamster tracheal epithelial cells. *Proceedings of the National Academy of Sciences of the USA* **84**: 9304–9308.

Liu P V 1979 Toxins of *Pseudomonas aeruginosa*. In: Doggett R G (ed) *Pseudomonas aeruginosa*: Clinical manifestations of infection and current therapy. Academic Press Inc, New-York, pp 63–87.

Lundberg C, Lönnroth J, Nord C E 1982 Adherence in the colonization of *Streptococcus pneumoniae* in the nasopharynx in children. *Infection* **10**: 63–66.

Marcus H, Baker N R 1985 Quantification of adherence of mucoid and non-mucoid *Pseudomonas aeruginosa* to hamster tracheal epithelium. *Infection and Immunity* **47**: 723–729.

Marcus H, Austria A, Baker N R 1989 Adherence of *Pseudomonas aeruginosa* to tracheal epithelium. *Infection and Immunity* **57**: 1050–1053.

Martodam R R, Baugh R J, Twumasi D Y, Liener I E 1979 A rapid procedure for the large scale purification of elastase and cathepsin G from human sputum. *Preparative Biochemistry* **9**: 15–31.

McDonald J A, Baum B J, Rosenberg D M, Kelman J A, Brin S C, Crystal R G 1979 Destruction of a major extracellular adhesive glycoprotein (fibronectin) of human fibroblasts by neutral proteases from polymorphonuclear leukocyte granules. *Laboratory Investigation* **40**: 350–357.

Mirelman D, Ofek I 1986 Introduction to microbial lectins and agglutinins. In: Mirelman D (ed) Microbial lectins and agglutinins: properties and biological activities (*Environmental and Applied Microbiology*) John Wiley & Sons, London, pp 1–19.

Niederman M S, Rafferty T D, Sasaki C T, Merrill W W, Matthay R A, Reynolds H Y 1983 Comparison of bacterial adherence to ciliated and squamous epithelial cells obtained from the human respiratory tract. *American Review of Respiratory Disease* **127**: 85–90.

Ohlsson K, Olsson I 1977 The extracellular release of granulocyte collagenase and elastase during phagocytosis and inflammatory processes. *Scandinavian Journal of Haematology* **19**: 145–152.

Paranchych W, Sastry P A, Volpel K, Loh B A, Speert D P 1986 Fimbriae (pili): molecular basis of *Pseudomonas aeruginosa* adherence. *Clinical and Investigative Medicine* **9**: 113–118.

Pennington J E, Wolf S M, Puziss M 1979 Summary of a workshop on infections in patients with cystic fibrosis. *Journal of Infectious Diseases* **140**: 252–256.

Plotkowski M C, Beck G, Bernardo Filho M, Oliveria E F R, Hinnrasky J, Puchelle E 1987 Evaluation of ^{99m}Technetium labelling effect on *Pseudomonas aeruginosa* surface properties. *Annales de l'Institut Pasteur Microbiologie* **138**: 415–426.

Plotkowski M C, Beck G, Jacquot J, Puchelle E 1989*a* The frog palate mucosa as a model for studying bacterial adhesion to mucus-coated respiratory epithelium. *Journal of Comparative Pathology* **100**: 37–46.

Plotkowski M C, Girod-Vaquez S, Hinnrasky J, Fuchey C, Ploton D, Puchelle E 1989*b* Ultrastructural comparative distribution of carbohydrates in human tracheal and frog palate mucosa using neuraminidase and lectin-colloidal gold complexes. *Journal of Submicroscopic Cytology and Pathology* (in press).

Puchelle E, Petit A, Adnet J J 1984 Fine structure of the frog palate mucociliary epithelium. *Journal of Submicroscopic Cytology* **16**: 273–282.

Ramphal R, Pyle M 1983 Adherence of mucoid and nonmucoid *Pseudomonas aeruginosa* to acid-injured tracheal epithelium. *Infection and Immunity* **41**: 345–351.

Ramphal R, Pier G B 1985 Role of *Pseudomonas aeruginosa* mucoid exopolysaccharide in adherence to tracheal cells. *Infection and Immunity* **47**: 1–4.

Ramphal R, Guay C, Pier G B 1987 *Pseudomonas aeruginosa* adhesins for tracheobronchial mucin. *Infection and Immunity* **55**: 600–603.

Reichert R W, Das N E, Zam Z S 1983 Adherence properties of *Pseudomonas* pili to epithelial cells of the human cornea. *Current Eye Research* **2**: 289–293.

Sharon N 1987 Bacterial lectins, cell-cell recognition and infectious disease. *FEBS Letters* **217**: 145–157.

Smallman L A, Hill S L, Stockley R A 1984 Reduction of ciliary beat frequency in vitro by sputum from patients with bronchiectasis: a serine protease effect. *Thorax* **39**: 663–667.

Stockley R A, Burnett D 1979 Alpha-1-antitrypsin and leukocyte elastase in infected and noninfected sputum. *American Review of Respiratory Disease* **120**: 1081–1086.

Sturgess J M 1981 Mucus secretion and clearance in the pathogenesis of cystic fibrosis. *Monographs in Pediatrics* **14**: 60–74.

Suter S, Schaad U B, Roux L, Nydegger U E, Waldvogel F A 1984 Granulocyte neutral proteases and *Pseudomonas* elastase as possible causes of airway damage in patients with cystic fibrosis. *Journal of Infectious Diseases* **149**: 523–531.

Suter S, Schaad U B, Morgenthaler J J, Chevallier I, Schnebli H P 1988 Fibronectin-cleaving activity in bronchial secretions of patients with cystic fibrosis. *Journal of Infectious Diseases* **158**: 89–100.

Uhlenbruck G 1987 Bacterial lectins: mediators of adhesion. *Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene. Series A* **263**: 497–508.

Woods D E, Bass J A, Johanson W G, Strauss D C 1980*a* Role of adherence in the pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. *Infection and Immunity* **30**: 694–699.

Woods D E, Strauss D C, Johanson W G, Berry V K, Bass J A 1980*b* Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. *Infection and Immunity* **29**: 1146–1151.

Woods D E, Strauss D C, Johanson W G, Bass J A 1983 Factors influencing the adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. *Reviews of Infectious Diseases* **5** suppl 5: S846–S851.