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NOTES

Adherence of *Pseudomonas aeruginosa* to Cilia of Human Tracheal Epithelial Cells

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***Pseudomonas aeruginosa* was found to adhere selectively to cilia of human ciliated tracheal epithelial cells (TECs). *P. aeruginosa* bound in equal numbers to TECs of smokers and nonsmokers, with the mean adhesion index for binding of *P. aeruginosa* 492c to TECs of healthy individuals (± standard deviation) being 6.83 ± 6.00 bacteria per TEC.**

Pseudomonas aeruginosa respiratory infections are believed to be initiated by the adherence of the pathogen to the oral-pharyngeal mucosa, followed by colonization of that surface and establishment of a descending infection (2, 5, 6). *P. aeruginosa* has been reported to adhere to human tracheal epithelial cells (TECs) in higher numbers compared with adhesion to human buccal epithelial cells (9), but the importance of pili and alginate, which have been implicated in adhesion to human buccal epithelial cells (3, 8, 11–16), has not been fully elucidated. It is also unclear whether *P. aeruginosa* is capable of attaching to undamaged ciliated human TECs.

TECs were obtained by bronchoscopy from surgical patients (under general anesthetic), intubated intensive care unit (ICU) patients, and healthy volunteers. For the surgical and ICU patients, bronchoscopy was performed with a flexible Olympus Type 2 BF bronchoscope inserted through an endotracheal tube. A cytology brush was used to abrade the tracheal-bronchial mucosa, and TECs were collected in high-glucose Dulbecco modified Eagle medium containing 1% sodium citrate.

The cell suspension obtained by bronchoscopy contained both ciliated and nonciliated cuboidal and columnar epithelial cells in addition to various amounts of mucus, erythrocytes, granulocytes, and cell debris and was not suitable for direct use in an adhesion assay. The cell suspension was vortexed briefly, sequentially passed through 70- and 30-μm-pore-size-mesh nylon screens, washed twice (500 × *g* for 15 min at 4°C) with 10 ml of 0.01 M phosphate-buffered saline (pH 7.2) (PBS), and then resuspended in 1 ml of PBS. The cell suspension was then fractionated by density gradient centrifugation (500 × *g* for 15 min at 4°C in a swinging bucket rotor) on a PBS-preformed (48,000 × *g* for 40 min at 4°C) 65% (vol/vol) percoll gradient. The TEC band was collected and applied to a second percoll gradient. The ciliated TEC band was collected from the second gradient, and the cells were washed once in PBS and then resuspended in 1.5 ml of PBS. A direct cell count was performed with a hemacytometer; cell viability was determined by trypan blue dye exclusion. The cell fractionation procedure typically yielded (2.08 ± 0.34) × 10^5 cells (mean ± standard error), of which 32.8 ± 6.5% were ciliated TECs. The vast majority of these cells were viable, and in many cases the cilia were still beating. The fractionated TECs contained only epithelial cells, were essentially free of contaminating mucus, and were used directly for adhesion assays.

P. aeruginosa 492c (4) was chosen for this study because it uses both pili and alginate as adhesins (3, 8). *P. aeruginosa* 492c was labeled with L-[^{35}S]methionine, and the adherence to TECs was determined as previously described (8), except that 12-μm-pore-size filters (pretreated with 2.5 ml of 3% (wt/vol) bovine serum albumin for 5 min at 22°C and washed with 15 ml of PBS) were used to reduce nonspecific binding of bacteria to the filters. After the assay, TECs remained viable, and in many cases cilia function was maintained.

P. aeruginosa 492c bound to the TECs of ICU patients, surgical patients, and healthy volunteers in a defined and specific manner. The number of bacteria which bound to the TECs of an individual was found to be directly dependent upon the bacterial concentration used (i.e., the bacterium/TEC ratio) until bacterial receptor sites on the TEC surface were saturated. A bacterium/TEC ratio of 1,000:1 was chosen for the standard assay.

The mean adhesion index for the binding of strain 492c to TECs of healthy, nonsmoking volunteers (±standard deviation) was 5.31 ± 5.13 bacteria per TEC, whereas the mean adhesion index for smokers was 8.36 ± 6.89 bacteria per TEC (Table 1). The difference in adhesion indices for smokers and nonsmokers was not statistically significant ($P > 0.05$ by a Kruskal-Wallis nonparametric test [17]); thus, the data for the healthy volunteers were pooled, and the mean adhesion index for the binding of *P. aeruginosa* 492c to healthy human TECs was found to be 6.83 ± 6.00 bacteria bound per TEC. The adhesion indices of surgical patients

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TABLE 1. Adhesion of *P. aeruginosa* 492c to ciliated human TECs of healthy volunteers

Sex	Age (yrs)	Smoker	Adhesion index (bacteria bound/ TEC)[a]
F	31	No	2.72 ± 0.10
F	31	No	0.34 ± 0.21
F	25	No	9.53 ± 1.17
F	31	No	12.67 ± 0.13
F	27	No	6.45 ± 0.73
F	33	No	0.12 ± 0.03
F	27	Yes	7.16 ± 2.72
M	29	Yes	17.99 ± 0.66
F	27	Yes	5.19 ± 0.51
F	36	Yes	2.50 ± 1.01
M	35	Yes	15.64 ± 1.25
F	36	Yes	1.60 ± 0.32

[a] Mean ± standard deviation.

were very similar to those of the healthy volunteers, whereas the adhesion indices of ICU patients were more variable and reflected their physiological status (manuscript in preparation). The adhesion index obtained for an individual is consistent from day to day, provided that the individual's physiological status does not change radically.

P. aeruginosa 492c was found to bind directly to human cilia, usually with the major axis of the bacterium being aligned with the axis of the TEC cilia (Fig. 1). There was minimal binding of bacteria to the body of ciliated epithelial cells (these cell surfaces are not generally exposed in intact tracheal epithelium) or to unciliated epithelial cells. Experiments with tracheal biopsies indicated that strain 492c bound directly to cilia but not to the basement membrane (in areas which had been denuded of TECs) or to connective tissue (data not shown). The binding of strain 492c to TECs of healthy volunteers did not differ morphologically from that observed for TECs of surgical or ICU patients.

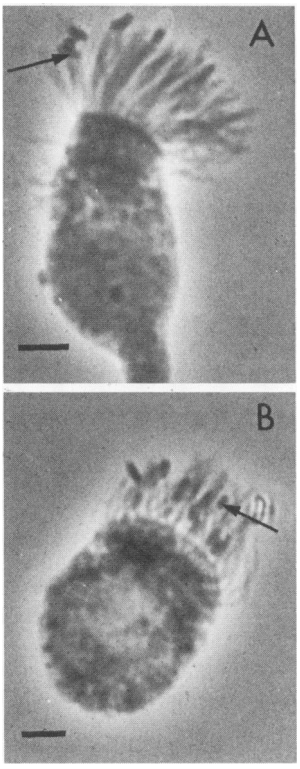

FIG. 1. Phase contrast micrograph of *P. aeruginosa* 492c bound to a ciliated columnar TEC (A) and a ciliated cuboidal TEC (B) after an adhesion assay. Arrows indicate bacteria bound to cilia. Bars, 5 μm.

Our findings and those of Neiderman et al. (9) suggest that the number, distribution, and nature of the receptor sites for binding of *P. aeruginosa* to human TECs may be different from those of human buccal epithelial cells and that these parameters thus may limit the usefulness of buccal cells in elucidating the pathogenic mechanism of *P. aeruginosa*. Animal model systems may also be of limited use because specific attachment to cilia has only been observed in the hamster tracheal organ model (1, 7, 10).

Our in vitro *P. aeruginosa* adherence model appears to be an appropriate system for examining the mechanism of *P. aeruginosa* adherence to the human tracheal epithelium.

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