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Evidence that In Vitro Adherence of *Klebsiella pneumoniae* to Ciliated Hamster Tracheal Cells Is Mediated by Type 1 Fimbriae

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Clinical isolates of fimbriated and nonfimbriated *Klebsiella pneumoniae* were examined for the ability to adhere to hamster tracheal cells cultured in vitro. Fimbriated-phase *K. pneumoniae* adhered preferentially to ciliated cells, whereas nonfimbriated-phase organisms were not adherent. The adherence was inhibited by D-mannose but not D-glucose, suggesting that type 1 fimbriae serve as the adhesin in the attachment process.

The ability of an organism to adhere to and colonize a mucosal surface is often a crucial step in an infectious process. This is likely to be the case in the respiratory tract, where colonization of the trachea would enable an organism to avoid the action of mucociliary clearance. Colonization and overgrowth of the area may then lead to the development of pneumonia, which is a common occurrence during extended hospital stays. A common organism causing bacterial pneumonia in this patient population is *Klebsiella pneumoniae*. The ability of encapsulated and nonencapsulated *K. pneumoniae* to adhere to buccal cells (9) and mouse tracheal rings (13) has been examined under in vitro conditions; however, these studies did not address the mechanism of attachment. To better understand how *K. pneumoniae* colonizes the respiratory tract, we have developed an in vitro adherence system by using hamster tracheal cells in tissue culture to study the initial step in the colonization process—bacterial adherence.

The methods involved in establishing the hamster tracheal cells in culture have been previously described in detail (11). Briefly, the tracheae of Syrian golden hamsters were surgically removed, rinsed in medium 199 (GIBCO Laboratories, Grand Island, N.Y.), and incubated overnight at 4°C in 0.1% pronase dissolved in medium 199 to enzymatically dissociate the tracheal cells. The cells were harvested by using a tuberculin syringe fitted with a 16-gauge needle, centrifuged at 180 × *g*, and suspended in medium 199. The cells were then centrifuged for a second time and suspended in a supplemented growth medium. The resulting cell preparation was seeded onto collagen-coated 12-mm-diameter Millicell-HA filters (Millipore Corp., Bedford, Mass.) and placed into 24-well culture plates containing 0.4 ml of growth medium. Under these conditions, the cells differentiate into functional ciliated and secretory cells within 5 days.

The *K. pneumoniae* strains used in the study were isolated from burn patients at the Shriners Burns Institute, Galveston, Tex. The organisms were examined for the presence of type 1 fimbriae by serial passage through tryptic soy broth containing 5 g of D-glucose (TSB; Difco Laboratories, Detroit, Mich.) per liter. Previous studies have demonstrated that this procedure enhances the production of fimbriae (5), the presence of which can be monitored by the ability of the organism to hemagglutinate guinea pig erythrocytes in a mannose-sensitive manner (4). Two strains were initially selected for use in this study: an organism recovered from a blood culture that failed to produce fimbriae after repeated passage (Fim^-) and a fimbriated isolate (Fim^+) recovered from a sputum culture. Stock cultures of these organisms were maintained in TSB at −70°C. Fim^- and Fim^+ isolates were prepared for adherence testing by inoculating TSB with a loopful of the stock culture and incubating the medium overnight at 37°C. The organisms were washed once in phosphate-buffered saline (PBS; pH 7.2) and suspended in PBS to a final concentration of 10^8 bacteria per ml. The resulting suspension was tested for mannose-sensitive hemagglutination of guinea pig erythrocytes (2) immediately prior to the addition of bacteria to the tracheal cells.

The in vitro adherence system was a modification of the procedure described by Ramphal and Pyle (13). Fim^- and Fim^+ bacterial suspensions (0.5 ml) were added to the wells containing the tracheal cells, and the wells were incubated for 1 h at 37°C. Nonadherent organisms were removed by five rinses with PBS, and the filters were overlaid with 2.5% glutaraldehyde in 0.1 M sodium cacodylate. The filters were rinsed in 0.1 M sodium cacodylate, post-fixed in osmium tetroxide, dehydrated in ethanol, critical point dried, and coated with gold (20 nm) for viewing with a scanning electron microscope.

The filters were examined with an ISI DS 130 scanning electron microscope (International Scientific Instruments, Milpitas, Calif.). Adherent bacteria in 20 randomly selected ciliated and nonciliated fields were quantitated at ×4,000 magnification, and the mean number of adherent bacteria per field was determined. Working distance (20 mm), angle (30°), and voltage (20 kV) remained constant throughout the study. A grid was attached to the viewing screen to facilitate quantitation. All results expressed are the means of four tests plus or minus the standard error of the mean unless otherwise noted.

Initially, the Fim^+ and Fim^- organisms were evaluated for the ability to adhere to the tracheal cells. The results indicated that only the Fim^+ bacteria adhered to any appreciable degree and the binding was almost exclusively to the ciliated tracheal cells (23.7 ± 10.5 bacteria per ciliated field versus 1.5 ± 0.5 bacteria per nonciliated field). The Fim^- organism showed only occasional adherence (0.7 ± 0.3 bacterium per ciliated field and 1.3 ± 0.6 bacteria per nonciliated field). The degree of this preferential binding of the Fim^+ organism to the ciliated tracheal cells can be observed in Fig. 1.

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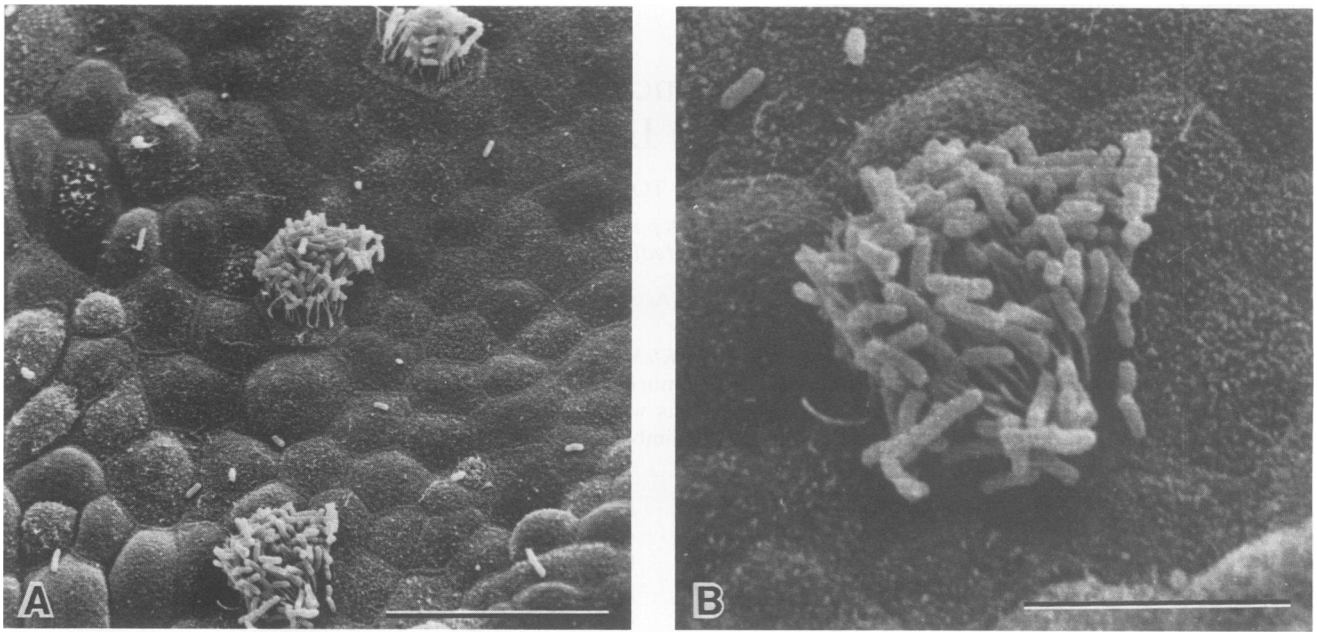

FIG. 1. Bacterial adherence of ciliated tracheal cells. (A) Note that the bacteria bind predominantly to the ciliated cells. Bar, 20 μm. (B) Higher-magnification view of the field shown in panel A with large numbers of bacteria closely associated with the ciliated surface of a tracheal epithelial cell. Bar, 10 μm.

Since the Fim$^-$ organism represented only a single clinical isolate that could not be induced to produce fimbriae, the Fim$^+$ isolate was serially passaged on agar medium to induce its nonfimbriated phase as previously described (7). After five passages on agar medium and an overnight growth in TSB, the organism was devoid of fimbriae as revealed by transmission electron microscopy (Fig. 2) and by the failure to hemagglutinate guinea pig erythrocytes. The fimbriated phase of the organism, maintained throughout in TSB, continued to express fimbriae (Fig. 2) and hemagglutinated guinea pig erythrocytes in a mannose-sensitive manner. The organism in its fimbriated and nonfimbriated phases was then tested for the ability to adhere to the tracheal cells. As before, fimbriated organisms adhered to the ciliated cells (23.5 ± 11.8 bacteria per ciliated field). However, the organism in its nonfimbriated phase lost the ability to bind to the cells (0.06 ± 0.05 bacterium per ciliated field). Neither phase showed any appreciable adherence to nonciliated cells.

D-Mannose is a known inhibitor of bacterial adherence to mammalian cells mediated by type 1 fimbriae (2, 4–6). Since the results of the initial studies suggested that type 1 fimbriae mediated the adherence of *K. pneumoniae* to the ciliated tracheal cells, experiments were initiated to determine the

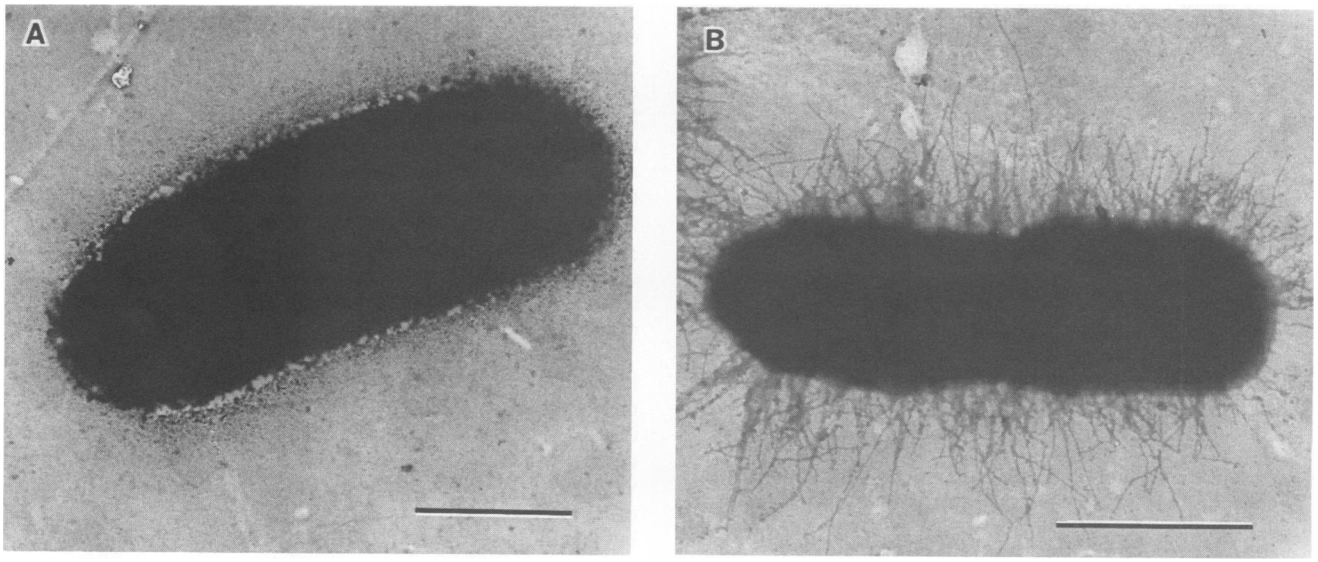

FIG. 2. Transmission electron microscopy of the Fim$^+$ *K. pneumoniae* isolate in the nonfimbriated (A) and fimbriated (B) phases. The organisms were stained for 30 s with 0.1% phosphotungstic acid. Bar, 1 μm.

TABLE 1. Effect of D-mannose and D-glucose on adherence of fimbriated *K. pneumoniae* to hamster tracheal cells

Treatment	Bacteria/field[a]	% Inhibition
Control	13.5 ± 5.1	
D-Mannose[b]	0.2 ± 0.1	98.5
D-Glucose[c]	16.0 ± 11.4	

[a] Mean number of adherent bacteria per ciliated field at ×4,000 magnification ± standard error of the mean.
[b] Results are mean of four tests.
[c] Results are mean of duplicate tests.

effect of the carbohydrate on adherence (Table 1). D-Mannose and D-glucose, at concentrations of 10 mg/ml in PBS, were used to suspend the Fim^+ strain for adherence testing. All other procedures were as previously described. The results indicated that D-mannose almost totally inhibited the adherence of the organism to ciliated tracheal cells (98.5% decrease; $P \leq 0.05$). D-Glucose had no inhibitory effect on adherence.

The results of this study suggest that type 1 fimbriae serve as the adhesin that binds *K. pneumoniae* to ciliated tracheal epithelial cells. The specificity of the organism for adherence only to ciliated tracheal cells is not surprising, since other respiratory tract pathogens such as *Bordetella pertussis* (1, 12) and *Pseudomonas aeruginosa* (8) have also demonstrated preferential binding to these cells. Whereas *P. aeruginosa* appears to have at least two adherence mechanisms for the respiratory tract epithelium (3, 10, 14), it appears that type 1 fimbriae act as the sole adhesin for *K. pneumoniae*. This is based on the observations that D-mannose almost totally inhibited the ability of the organism to adhere to the tracheal cells and that induction of the nonfimbriated phase eliminated the ability of the organism to adhere. Further adherence studies are warranted to determine whether other members of the family *Enterobacteriaceae* use type 1 fimbriae as a means of attachment in the respiratory tract. Such knowledge could facilitate preventive therapy to avoid respiratory tract colonization of hospitalized patients with these potential pathogens.

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