INFECTION AND IMMUNITY, June 1990, p. 1489–1495
0019-9567/90/061489-07$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 58, No. 6

Mucinophilic and Chemotactic Properties of *Pseudomonas aeruginosa* in Relation to Pulmonary Colonization in Cystic Fibrosis

JAMES W. NELSON,[1] MICHAEL W. TREDGETT,[1] JOHN K. SHEEHAN,[2] DAVID J. THORNTON,[2] DEREK NOTMAN,[1] AND JOHN R. W. GOVAN[1]*

Department of Bacteriology, University Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland,[1] and Department of Biochemistry and Molecular Biology, School of Biological Sciences, University of Manchester, Manchester M13 9PT, England[2]

Received 29 November 1989/Accepted 22 February 1990

Representative isolates of nonmucoid *Pseudomonas aeruginosa* were studied to investigate the hypothesis that mucinophilic and chemotactic properties in this species act as potential factors in the initial stages of pulmonary colonization in patients with cystic fibrosis (CF). Transmission electron microscopy with a surfactant monolayer technique was used in a novel manner to demonstrate the adhesion of all 10 *P. aeruginosa* strains examined to porcine gastric mucin and tracheobronchial mucin from a patient with CF. Control experiments showed that *Escherichia coli* K-12 and single representatives of *Proteus mirabilis* and *Klebsiella aerogenes* did not bind to these mucins. The Adler capillary technique, used to measure bacterial chemotactic response, showed that purified CF mucin acted as a chemoattractant for most *P. aeruginosa* strains, with the exception of the nonmotile mutant M2Fla$^-$ and the nonchemotactic mutant WR-5. The ability of the major sugar and amino acid components of mucin to act as chemoattractants was investigated. The degree of chemotaxis was strain specific; optimum chemotaxis was observed toward serine, alanine, glycine, proline, and threonine. No strain showed chemotaxis to *N*-acetylneuraminic acid, but all strains showed a strain-dependent chemotactic response to the sugars L-fucose, D-galactose, *N*-acetyl-D-galactosamine, and *N*-acetyl-D-glucosamine. These results provide new information on the mucinophilic and chemotactic properties of nonmucoid *P. aeruginosa* and support the hypothesis that these properties could play a role in the initial stages of pulmonary colonization in patients with CF.

Cystic fibrosis (CF) is the most commonly inherited lethal disease of Caucasians and is characterized by disturbances in electrolyte transport from exocrine glands and secretory epithelia, resulting in the accumulation of abnormally viscid tracheobronchial secretions (23, 24). The rheological properties of bronchial mucus are primarily due to its mucin component (21, 33), which comprises glycoprotein macromolecules synthesized by the submucosal glands and goblet cells of the surface epithelium. Mucins consist of a polypeptide core with oligosaccharide side chains attached by *O*-glycosidic linkages (5). Intermolecular associations between mucins result in the formation of viscoelastic gels (36). Mucin hypersecretion is a characteristic feature of the pulmonary airways of patients with CF, and the tenacious viscid secretions (33) are associated with impaired mucociliary clearance and bacterial colonization which, in turn, initiates a vicious cycle of chronic inflammatory reaction. In patients with CF, initial and sometimes intermittent asymptomatic colonization with nonmucoid *Pseudomonas aeruginosa* occurs; eventually, variants expressing a mucoid-colony form (due to copious production of alginate) emerge from the colonizing strain. The establishment of these variants, and that of other phenotypes associated with chronic pulmonary colonization, is a major cause of morbidity and mortality (12, 14, 27). Most research has been directed to the properties and pathogenesis of mucoid *P. aeruginosa*; little attention has been directed to the bacterial and host factors that contribute to the initial stages of asymptomatic colonization which, arguably, forms an important microbial reservoir for the subsequent emergence of mucoid variants (13).

The aim of our study was to seek evidence which supports the hypothesis that chemotactic factors in mucin and mucinophilic properties of *P. aeruginosa* contribute to the initial stages of pulmonary colonization in patients with CF and, thereby, to provide information on which to base rational strategems for vaccination. The study also describes the novel application of an electron microscope technique, developed for the study of mucin structure (36), which demonstrates adhesion of *P. aeruginosa* to mucin monolayers.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. Nonmucoid strains of *P. aeruginosa* J1385 and J1375 were clinical isolates obtained from P. Friend (Public Health Laboratory, Truro, England) and isolated during an investigation of primary respiratory colonization in two patients with CF who had bathed in a hydrotherapy pool contaminated with multiple strains of *P. aeruginosa* (10). J1385 is the colonizing strain which was isolated from both CF patients and from the pool water; J1375 represents one of the four other environmental isolates taken from the water. The clonal relation of these isolates had been previously determined in our laboratory by pyocin typing (11) and confirmed by DNA probe analysis by M. L. Vasil and J. W. Ogle (Health Sciences Center, Denver, Colo.). *P. aeruginosa* JN8, JN47, JN61, and JN62 also express the nonmucoid phenotype. These strains were isolated from the sputa of four CF patients with no previous history of pulmonary colonization by *P. aeruginosa*; each of these four strains belonged to a different pyocin type. Additional strains of *P. aeruginosa* investigated included the well-characterized genetic strain PAO1 (15); WR-5, a nonchemotactic mutant of PAO1; M2Fla$^-$, a nonflagellate mutant of strain M2; and M2Rev, a motile revertant of M2. Strains M2Fla$^-$, M2Rev, and WR-5 were obtained from I. A.

Holder (Shriners Burn Institute, Cincinnati, Ohio). *Escherichia coli* K-12 and individual strains of *Proteus mirabilis* and *Klebsiella aerogenes* were investigated as controls for adherence to mucin monolayers.

Media. Nutrient agar was Columbia agar base (Oxoid Ltd., Basingstoke, England). Nutrient broth was Oxoid no. 2 supplemented with 0.5% (wt/vol) yeast extract. Minimal medium employed for culture of *P. aeruginosa* prior to chemotaxis assays and the chemotaxis medium (CM) used in the assays were as described by Moulton and Montie (25).

Purification of mucins. Sputum from a patient with CF was stored at −20°C, subsequently thawed into ice-cold 0.2 M NaCl–5 mM EDTA–10 mM sodium phosphate buffer (pH 6.5) containing 1 ml of diisopropyl phosphorofluoridate, and then centrifuged at 40,000 × *g* for 30 min at 5°C in a 50 Ti rotor (Beckman Instruments, Inc., Fullerton, Calif.). The pellet (gel) was then repeatedly extracted with 6 M guanidinium chloride–5 mM EDTA–5 mM *N*-ethylmaleimide–10 mM sodium phosphate buffer (pH 6.5). The mucins were then purified by a two-step isopycnic density gradient procedure, first in CsCl–4 M guanidinium chloride and then in CsCl–0.2 M guanidinium chloride, as described by Carlstedt et al. (3). Purified mucins were dialyzed into 4 M guanidinium chloride–10 mM sodium phosphate buffer (pH 6.5).

Chemotaxis assay. One milliliter of an overnight preculture grown in minimal medium was inoculated into 9 ml of minimal medium and incubated at 37°C for 3 h in an orbital incubator at 120 rpm. The exponentially growing cells were harvested by centrifugation at 4,600 × *g* for 15 min and washed twice with 10 ml of CM before suspension to a density of 2×10^8 CFU (optical density, 0.25 at 590 nm). Chemotaxis was measured by the capillary tube assay described by Adler (1). A small chamber was formed by placing a cover slip on a U-shaped piece of melting-point capillary tubing bonded to a microscope slide. Prior to use in the assays, the chambers, supported in petri dishes, were prewarmed by incubation at 37°C for at least 30 min. The chambers were then filled with 0.4 ml of bacterial suspension in CM. Capillaries (0.25-mm diameter; Phase Separations Ltd., Clwyd, United Kingdom) were filled with potential attractants dissolved in CM. Control capillaries containing CM alone were used in all experiments as a measure of background motility. The open end of a capillary was inserted into the center of a chamber containing the bacterial suspension. After incubation for 30 min (unless otherwise indicated), the capillaries were removed, their sealed ends were carefully broken, and the contents were emptied into saline with the aid of a micropipette bulb (Analtech Inc.) The contents were diluted, and plate counts were made. The accumulation of bacteria in each of two capillaries containing chemoattractant was measured as CFU per capillary, and a mean value was determined. A similar measurement was made from capillaries in which no chemoattractant was present. The ratio of these CFU values (the relative response) was then determined; the ratio normalizes for experimental or day-to-day variations. A meaningful chemotactic response as described by Moulton and Montie (25) was a relative response value of >2.

Potential chemoattractants investigated in this study included mucin purified from tracheobronchial secretions from a CF patient. Before it was used, the mucin preparation, stored as a solution in 4 M guanidinium chloride at 4°C, was diluted in CM (100 μg/ml) and dialyzed against 1 liter of CM at 4°C for 24 h. Other potential chemoattractants investigated were the major amino acids present in mucin, including alanine, aspartic acid, cysteine, glutamic acid, serine, glycine, threonine, and proline, and the major mucin sugars, including L-fucose, D-galactose, *N*-acetyl-D-galactosamine (GalNAc), and *N*-acetyl-D-glucosamine (GlcNAc). All chemicals were supplied by BDH Chemicals, Poole, United Kingdom, and were of the highest purity available. *N*-Acetylneuraminic acid (NANA; Koch-Light, Haverhill, United Kingdom) was also examined. Solutions were made in CM and, with the exception of mucin, were filtered through a 0.2-μm-pore-size membrane filter (Schleicher & Schuell, Dassell, Federal Republic of Germany).

Electron microscopy. Visualization of the mucin-bacterium association was attempted by a modification of the surfactant monolayer technique described by Sheehan et al. (36) for the study of mucin architecture. Purified porcine gastric mucin (BDH) was used in the preliminary experiments because it is reported to resemble human tracheobronchial mucin in its carbohydrate, amino acid, and sulfate ester composition (2, 34). Subsequent experiments were focused on CF respiratory mucin purified as described above. A log-phase culture of *P. aeruginosa* grown in nutrient broth was centrifuged at 4,600 × *g* for 15 min and washed twice in 1% (wt/vol) ammonium acetate in distilled water. The pellet was gently suspended in 15 ml of the porcine gastric or CF mucin solution (0.05 μg/ml in 50 mM magnesium acetate), and the mixture was transferred to a plastic petri dish and incubated for 30 min at 37°C. To prepare mucin monolayers, a small amount of fine graphite powder (BDH) was sprinkled on the surface. A mucin monolayer was obtained by touching the surface of the mucin solution with the tip of a pipette containing 1 μl of 1% (wt/vol) benzyldimethylalkyl-ammonium chloride (BAC) in distilled water. The surfactant nature of the BAC propels the carbon particles to the side of the dish, leaving a fine surface monolayer of mucin in BAC. After 15 min, carbon-coated 400-mesh electron microscope grids were touched to the surface of the monolayer, stained for 1 to 2 s in a solution of uranyl acetate (1 mM in 95% [vol/vol] ethanol), and washed for 1 to 2 s in ethanol. The grids were then allowed to dry in air before being subjected to unidirectional shadowing with platinum at a grazing angle of 7 to 12°. Transmission electron microscopy was performed with a 12A microscope (Hitachi) at 75 K. *E. coli* K-12, *Proteus mirabilis*, and *K. aerogenes* were prepared and examined in a similar manner.

RESULTS

Adherence of *P. aeruginosa* to mucin monolayers. Adherence of *P. aeruginosa* to both porcine and CF mucin monolayers was observed with each of the 10 strains of *P. aeruginosa* examined, including the nonmotile and nonchemotactic mutants. Initial experiments, performed with porcine gastric mucin, showed *P. aeruginosa* cells to be associated and often entangled in a highly aggregated mucin matrix (Fig. 1). When these experiments were repeated with mucin that had been purified from the tracheobronchial secretions of a CF patient, *P. aeruginosa* adhesion was again observed. CF mucin appeared in an aggregated form attached to the bacterial cell surface (Fig. 2A, B, and C). Attachment of aggregated mucin fragments to flagella was also observed (Fig. 2C). Many of the bacterial cells observed in the monolayers possessed flagella, and phase-contrast microscopy confirmed that all the bacteria, except strain M2Fla$^-$, were initially motile when incubated in the presence of mucin. As a control for random association or simple entanglement between mucin and bacterial cells, cultures of *E. coli* K-12, *Proteus mirabilis*, and *K. aerogenes* were

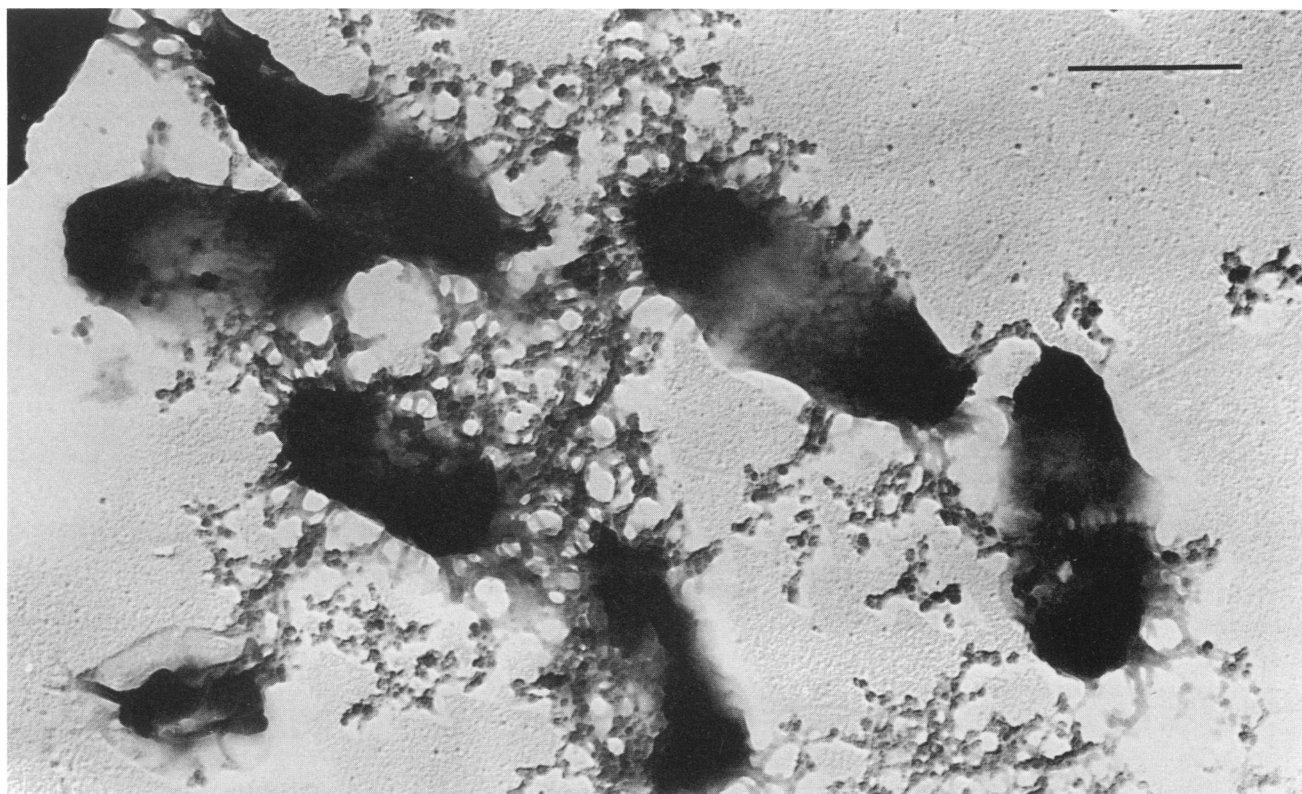

FIG. 1. Electron micrograph of *P. aeruginosa* in association with purified porcine gastric mucin (0.05 μg/ml) spread in BAC monolayer stained with uranyl acetate and subjected to unidirectional shadowing with platinum. Bar, 1 μm.

added to CF mucin and monolayers prepared as described above. In contrast to *P. aeruginosa*, only an occasional bacterial cell was observed in these monolayers, and those bacteria observed were not attached to mucin (Fig. 2D). In these control monolayers with nonadherent bacteria, the mucin appeared as lightly stained linear filaments similar in appearance to mucin in the absence of bacteria.

Chemotactic responses to *P. aeruginosa* to CF mucin and mucin components. To confirm the efficacy of the capillary chemotaxis assay in our hands, the concentration curve for serine as an attractant for *P. aeruginosa* PAO1 was determined. The chemotactic response of PAO1 was found to be similar to that reported by Moulton and Montie (25); a peak response occurred at 10^{-3} M serine.

When the chemotactic responses of nine strains of *P. aeruginosa* towards purified CF mucin were measured (Fig. 3), a meaningful response (i.e., >2.0) was observed in all strains examined, except for the nonmotile mutant M2Fla$^-$ and the nonchemotactic mutant WR-5.

The chemotactic response of *P. aeruginosa*, towards the eight major amino acids present in mucin, namely, alanine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine, and threonine, at concentrations of 10^{-1} to 10^{-5} M, was then measured. For most of the amino acids examined, considerable differences in the relevant chemotactic response of each of the strains examined were observed (Table 1). Chemoattraction to serine, alanine, glycine, proline, and threonine was particularly evident; with most of the amino acids, the peak chemotactic response occurred at a concentration of 10^{-3} M.

Chemotaxis to mucin-associated sugars. Chemotaxis assays were performed to assess the potential of the major sugar components of mucin, namely, L-fucose, D-galactose, GalNAc, GlcNAc, and NANA, to act as chemoattractants for *P. aeruginosa*. The relative chemotactic responses for these strains (Table 2) reflected the results of the previous chemotaxis assays using amino acids and emphasized a strain-dependent response to L-fucose, D-galactose, GalNAc, and GlcNAc. No strain showed a chemotactic response to NANA.

DISCUSSION

Previous studies of *P. aeruginosa* have shown its ability to adhere to tracheal epithelial cells (7, 29, 30), and it has been suggested that adhesion is the first stage in the establishment of respiratory tract infection in susceptible patients (31). In healthy individuals this adhesion probably has a protective role, enabling adherent or entangled bacteria to be removed by mucociliary clearance. In the lungs of CF patients, however, mucociliary clearance is impaired, and it is arguable that adherence of *P. aeruginosa* to CF mucin could result in potentially detrimental colonization of the mucosal surface layers. Scanning and transmission electron microscopy of CF lung tissue, done either postmortem or pre- or post-heart-lung transplantation, show that the bacteria are associated with sheets of surface secretions and not the microvillus surface of epithelial cells or deeper tissues (18; P. K. Jeffrey, personal communication).

Vishwanath and Ramphal (37) used a microdilution plate assay to demonstrate adherence of *P. aeruginosa* to wells coated with tracheobronchial mucin. In the present study we have produced further evidence of the mucinophilic properties of *P. aeruginosa*. Novel application of transmission

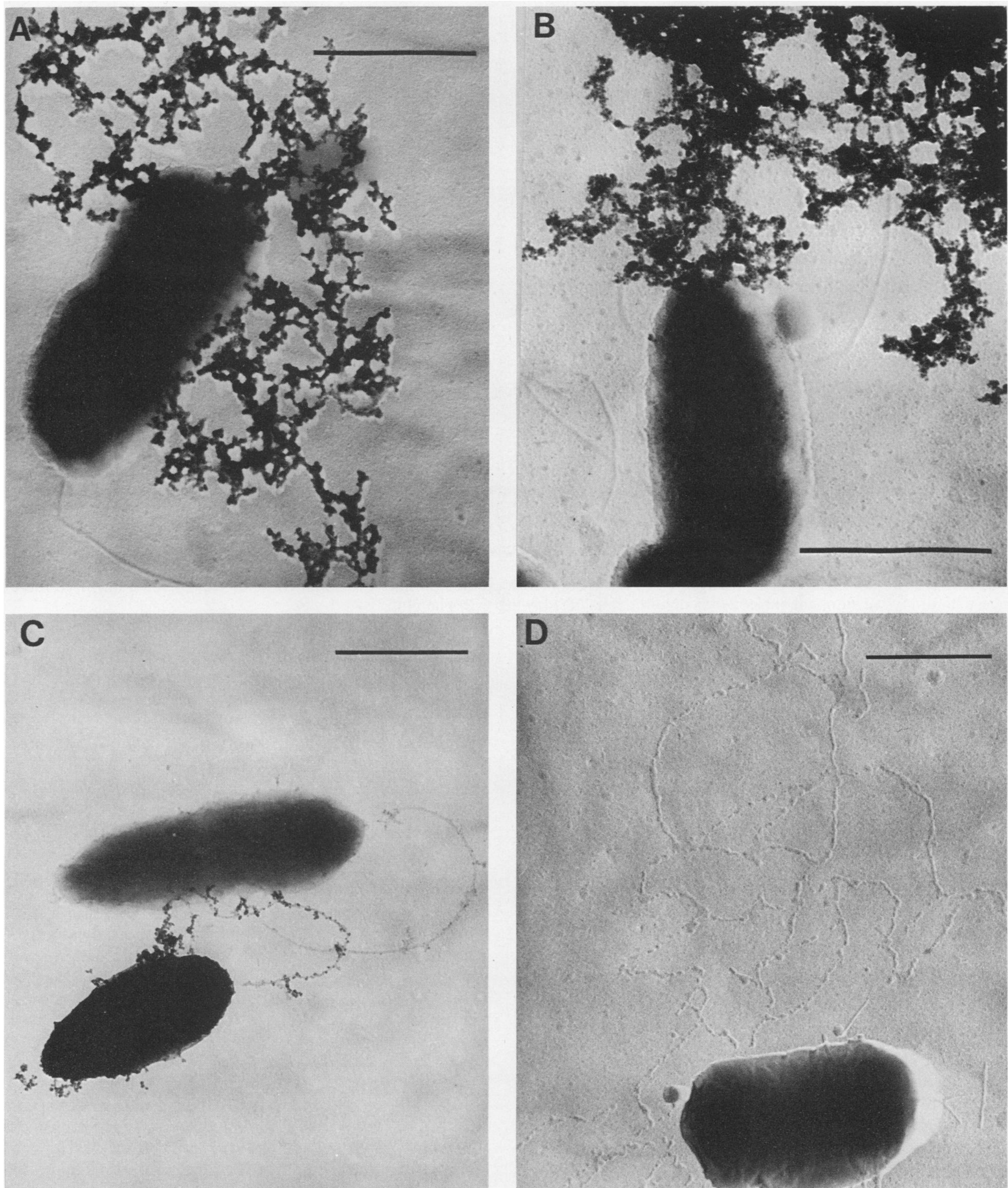

FIG. 2. (A through C) Electron micrographs of *P. aeruginosa* in association with purified tracheobronchial mucin from a CF patient showing adhesion of mucin aggregates to bacterial surfaces. (D) One of the few bacterial cells observed when *E. coli* K-12 was investigated in mucin monolayers. No direct mucin-bacterium association is evident, and the mucin appears as lightly stained linear filaments. Monolayers were stained with uranyl acetate and subjected to unidirectional shadowing with platinum. Bars, 1 μm.

TABLE 1. Chemotactic responses of *P. aeruginosa* strains toward mucin-associated amino acids

Strain	Chemotactic response[a] toward:							
	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Thr
PAO1	2.6	NC[b]	NC	2.3	2.5	3.7	6.1	NC
J1375	4.3	2.5	NC	4.9	4.2	4.8	4.0	3.0
J1385	7.2	5.0	2.2	6.5	4.7	5.2	4.7	2.9
JN8	12.4 (10^{-2})	NC	3.3	2.6 (10^{-2})	14.4 (10^{-2})	10.0	19.3	6.6
JN47	5.0	NC	NC	NC	5.0	2.6 (10^{-5})	5.5 (10^{-5})	7.2 (10^{-2})
JN61	8.2	3.1	NC	4.0	5.9	7.1	3.9 (10^{-4})	4.4
JN62	5.5 (10^{-2})	2.1 (10^{-2})	NC	2.7 (10^{-2})	8.1 (10^{-1})	3.7	2.9 (10^{-4})	3.7
WR-5[c]	NC	NC	NC	NC	NC	NC	NC	NC
M2Fla^{-}[d]	NC	NC	NC	NC	NC	NC	NC	NC
M2Rev	3.3	NC	NC	NC	3.6	3.8	3.0	2.3

[a] The chemotactic response is the ratio of accumulation of bacteria (viable count) in capillaries containing an amino acid to accumulation of bacteria in a control capillary containing no chemoattractant. A response value that is >2.0 is considered meaningful (25). The highest relative chemotactic response for each amino acid (representing the mean from duplicate assays) was at the 10^{-3} M concentration unless otherwise indicated (in parentheses).
[b] NC, No chemotaxis (i.e., <2.0).
[c] Nonchemotactic control.
[d] Nonflagellate control.

electron microscopy and BAC monolayers demonstrated bacterial entanglement and adherence to porcine gastric and purified CF mucin. In addition, quantitative measurement of the chemotactic response of strains of *P. aeruginosa* showed chemotaxis towards purified CF mucin and the major mucin-associated amino acids and sugars. The observation that individual strains of *E. coli* K-12, *Proteus mirabilis*, and *K. aerogenes* did not adhere to mucin agrees with the previous observations of Vishwanath and Ramphal (37) and suggests that the association between *P. aeruginosa* and mucin is relatively specific. In addition to mucin, other adhesion receptors for *P. aeruginosa* may include the glycosphingolipid gangliotetraosylceramide (asialo GM1), which occurs in substantial amounts in lung tissue (19).

The observation that both nonmotile and nonchemotactic mutants of *P. aeruginosa* also showed attachment to mucin indicates that adhesion is not dependent on motility or chemotaxis. The presence of mucin aggregates attached to individual cells of *P. aeruginosa* suggests that the bacterium-mucin interaction is strong enough to overcome the sheer forces as the mucin monolayer is formed. In the presence of *P. aeruginosa*, porcine and CF mucin appeared in a highly aggregated form rather than the lightly stained filamentous form observed in the case of mucin alone or in the presence of nonadherent bacteria, e.g., *E. coli*. This observation suggests that mucin molecules are degraded or undergo substantial conformational change in the presence of *P. aeruginosa*.

If initial colonization of the CF-affected respiratory tract by nonmucoid *P. aeruginosa* involves adherence to the epithelial surface or to tracheobronchial mucin or to both, chemotaxis of bacteria to mucin and mucin components would enhance the potential for bacterium-host interaction. It could be speculated that, in a given environment (e.g., the whirlpool source of J1385 and J1375), enhanced chemotaxis would confer an advantage on a potential colonizing strain, e.g., J1385. In support of this hypothesis, J1385 showed greater chemotaxis than J1375 toward CF mucin (Fig. 3) and toward the majority of mucin constituents (Tables 1 and 2). Mucin-bacterium interaction would be further enhanced if the mucin could also provide a source of bacterial nutrients. Ongoing studies indicate that mucin not only can act as a sole source of nitrogen and carbon for *P. aeruginosa* but also

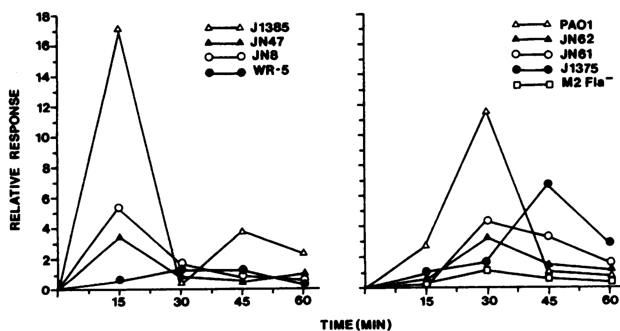

FIG. 3. Relative chemotactic responses of nine *P. aeruginosa* strains towards CF tracheobronchial mucin (100 μg/ml) from a patient with CF. The assay was performed over 60 min. Each point represents the ratio of the mean accumulation of bacterial CFU in duplicate capillaries containing mucin to the accumulation of bacterial CFU in control capillaries containing CM alone. A response greater than 2 is considered to indicate chemotaxis. *P. aeruginosa* M2Fla^{-} (nonmotile) and WR-5 (nonchemotactic) were included as controls.

TABLE 2. Chemotactic responses of *P. aeruginosa* strains toward mucin-associated sugars

Strain	Chemotactic response[a] toward:			
	L-Fucose	D-Galactose	GalNAc	GlcNAc
PAO1	2.5 (10^{-1})	15.1 (10^{-1})	NC[b]	2.3 (10^{-4})
J1375	2.8 (10^{-2})	NC	NC	3.0 (10^{-5})
J1385	5.8 (10^{-4})	3.8 (10^{-3})	3.4 (10^{-4})	4.5 (10^{-4})
JN8	5.4 (10^{-1})	3.3 (10^{-3})	4.2 (10^{-1})	4.3 (10^{-2})
JN47	2.8 (10^{-3})	3.0 (10^{-3})	2.8 (10^{-4})	2.8 (10^{-2})
JN61	NC	2.2 (10^{-1})	2.3 (10^{-1})	NC
JN62	NC	5.6 (10^{-5})	NC	NC
WR-5[c]	NC	NC	NC	NC
M2Fla^{-}[d]	NC	NC	NC	NC
M2Rev	3.1 (10^{-4})	NC	4.6 (10^{-4})	2.4 (10^{-2})

[a] The chemotactic response is the ratio of accumulation of bacteria (viable count) in capillaries containing a sugar to accumulation of bacteria in a control capillary containing no chemoattractant. A response value that was >2.0 was considered meaningful (25). Responses at five concentrations, from 10^{-1} to 10^{-5} M, were investigated. The highest relative chemotactic response for each sugar (representing the mean from duplicate assays) and the respective concentration at which it was obtained (in parentheses) are shown. There was no chemotaxis toward NANA detected.
[b] NC, No chemotaxis (i.e., <2.0).
[c] Nonchemotactic control.
[d] Nonflagellate control.

supports copious biosynthesis of alginate in mucoid *P. aeruginosa* (12; J. R. W. Govan, unpublished data).

There have been relatively few reports describing the chemotactic properties of *P. aeruginosa*. It has been shown, however, that the degree of chemotaxis towards arginine and serine is strain dependent (22). Motility has been shown to be an important virulence factor in burn infections due to *P. aeruginosa* (8), and an early antibody response to pseudomonas flagellar antigens is observed in patients with CF (35). To our knowledge there are no previous reports which demonstrate and compare the chemotactic responses of different strains of *P. aeruginosa* to mucin and mucin constituents.

The chemotactic responses to mucin and the major amino acids and sugars of mucin (32) (Fig. 3; Tables 1 and 2) clearly demonstrate a strain-specific chemotactic response of *P. aeruginosa* to different attractants. The different peak values obtained with individual strains could result from variation in the number of chemoreceptors present or in the affinities of those chemoreceptors. In the environment of the CF lung, proteolysis of mucin by proteases from *P. aeruginosa* and neutrophils (28) could explain how mucin fragments and components would be readily available in vivo to act as chemoattractants and nutritional sources for *P. aeruginosa*. The exposure and release of individual amino acids by proteolytic fragmentations of mucin may arguably influence the degree of chemotactic responses of individual strains. The presence of proteolytic-resistant glycopeptide regions within the mucin may reduce the release of serine, proline, threonine, and alanine, which are predominant in these regions. These and other amino acids, however, may be made available because of proteolysis of other proteins and glycoproteins, such as immunoglobulins and lactotransferrin, with which respiratory mucin is associated in vivo (16). Interestingly, NANA, which has been reported to be an adhesin for *P. aeruginosa* (29), did not act as a chemoattractant for any of the strains tested. NANA is a relatively strong acid in biological systems; however, the failure of NANA to act as a chemoattractant cannot be explained by a low pH value. Although the buffered preparations of NANA at 10^{-1} M did have low pHs, the other preparations examined (10^{-2} to 10^{-5} M) had neutral pHs.

Interactions between bacteria and host mucins are important in bacterial colonization of mucosal surfaces (4, 6, 20, 26). Chemotaxis of *Vibrio cholera* towards the intestinal mucosa (9) and chemotaxis of *Campylobacter jejuni* to porcine intestinal mucin (17) lend support to the hypothesis that chemotaxis towards mucins is an important bacterial virulence mechanism. From our results it seems reasonable to speculate that initial colonization of the respiratory tract in CF patients by nonmucoid *P. aeruginosa* could be assisted by chemotaxis towards, entanglement in, and adhesion to the mucin-rich mucosal surface. The chemotactic and adherence properties of *P. aeruginosa* towards purified glycopeptide components of CF and non-CF mucins is under investigation. Meanwhile, our results suggest that the mucin monolayers prepared by the BAC technique might be useful in studies of the interaction of other bacterial pathogens with respiratory and gastrointestinal mucins.

ACKNOWLEDGMENT

We thank P. K. Jeffrey for his helpful and critical review of the manuscript.

LITERATURE CITED

1. **Adler, J.** 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. **75**:77–91.
2. **Boat, T. F., P. W. Cheng, and R. E. Wood.** 1977. Tracheobronchial mucus secretion *in vivo* and *in vitro* by epithelial tissues from cystic fibrosis and control subjects. Mod. Probl. Paediatr. **19**:141–152.
3. **Carlstedt, I., H. Lindgren, J. K. Sheehan, U. Ulmsten, and L. Wingerup.** 1983. Isolation and characterization of human cervical-mucus glycoproteins. Biochem. J. **211**:13–22.
4. **Chick, S., M. J. Harber, R. Mackenzie, and A. W. Asscher.** 1981. Modified method for studying bacterial adhesion to isolated uroepithelial cells and uromucoid. Infect. Immun. **34**: 256–261.
5. **Clamp, J. R., A. Allen, R. A. Gibbons, and G. P. Roberts.** 1978. Chemical aspects of mucus. Br. Med. Bull. **34**:25–27.
6. **Cohen, P. S., R. Rossoll, V. J. Cabelli, S. L. Yang, and D. C. Laux.** 1983. Relationship between the mouse colonizing ability of a human fecal *Escherichia coli* strain and its ability to bind a specific mouse colonic mucous gel protein. Infect. Immun. **40**:62–69.
7. **Doig, P., T. Todd, P. A. Sastry, K. K. Lee, R. S. Hodges, W. Paranchych, and R. T. Irvin.** 1988. Role of pili in adhesion of *Pseudomonas aeruginosa* to human respiratory epithelial cells. Infect. Immun. **56**:1641–1646.
8. **Drake, D., and T. C. Montie.** 1988. Flagella, motility and invasive virulence of *Pseudomonas aeruginosa*. J. Gen. Microbiol. **134**:43–52.
9. **Freter, R., B. Allweiss, P. C. M. O'Brien, S. A. Halstead, and M. S. Macsai.** 1981. Role of chemotaxis in the association of motile bacteria with intestinal mucosa: in vitro studies. Infect. Immun. **34**:241–249.
10. **Friend, P. A., and S. W. B. Newson.** 1986. Hygiene for hydrotherapy pools. J. Hosp. Infect. **8**:213–216.
11. **Fyfe, J. A. M., G. Harris, and J. R. W. Govan.** 1984. Revised pyocin typing method for *Pseudomonas aeruginosa*. J. Clin. Microbiol. **20**:47–50.
12. **Govan, J. R. W.** 1988. Alginate biosynthesis and other unusual characteristics associated with the pathogenesis of *Pseudomonas aeruginosa* in cystic fibrosis, p. 67–96. *In* E. Griffiths, W. Donachie, and J. Stephen (ed.), Bacterial infections of respiratory and gastrointestinal mucosae. IRL Press, Oxford.
13. **Govan, J. R. W., J. A. M. Fyfe, C. Doherty, and W. M. McCrae.** 1984. *Pseudomonas aeruginosa* and cystic fibrosis: is the isolation of nonmucoid *Pseudomonas aeruginosa* really of no clinical significance?, p. 373. *In* D. Lawson (ed.), Cystic fibrosis: horizons. John Wiley & Sons, Inc., Chichester, United Kingdom.
14. **Hoiby, N.** 1984. The management of Pseudomonas chest infections—the way forward, p. 87–95. *In* D. Lawson (ed.), Cystic fibrosis: horizons. John Wiley & Sons, Inc., Chichester, United Kingdom.
15. **Holloway, B. W.** 1955. Genetic recombination in *Pseudomonas aeruginosa*. J. Gen. Microbiol. **13**:572–580.
16. **Houdret, N., R. Ramphal, A. Scharfman, J. M. Perini, M. Filliat, G. Lamblin, and P. Roussel.** 1989. Evidence for the *in vivo* degradation of human respiratory mucins during *Pseudomonas aeruginosa* infection. Biochim. Biophys. Acta **992**:96–105.
17. **Hugdahl, M. B., J. T. Beery, and M. P. Doyle.** 1988. Chemotactic behavior of *Campylobacter jejuni*. Infect. Immun. **56**:1560–1566.
18. **Jeffery, P. K., and A. P. R. Brain.** 1988. Surface morphology of human airway mucosa: normal, carcinoma or cystic fibrosis. Scanning Microsc. **2**:553–560.
19. **Krivan, H. C., D. D. Roberts, and V. Ginsburg.** 1988. Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAcB1-4 Gal found in some glycolipids. Proc. Natl. Acad. Sci. USA **85**:6157–6161.
20. **Liljemark, W. F., and R. J. Gibbons.** 1972. Proportional distribution and relative adherence of *Streptococcus miteor* (mitis) on various surfaces in the human oral cavity. Infect. Immun.

6:852–859.
21. **Lopez-Vidreiro, M. J., and L. Reid.** 1978. Bronchial mucus in health and disease. Br. Med. Bull. **34:**63–74.
22. **Luzar, M. A., M. J. Thomassen, and T. C. Montie.** 1985. Flagella and motility alterations in *Pseudomonas aeruginosa* strains from patients with cystic fibrosis: relationship to patient clinical condition. Infect. Immun. **50:**577–582.
23. **McPherson, M. A., and R. L. Dormer.** 1987. The molecular and biochemical basis of cystic fibrosis. Biosci. Rep. **7:**167–185.
24. **McPherson, M. A., and M. C. Goodchild.** 1988. The biochemical defect in cystic fibrosis. Clin. Sci. **74:**337–345.
25. **Moulton, R. C., and T. C. Montie.** 1979. Chemotaxis by *Pseudomonas aeruginosa*. J. Bacteriol. **137:**274–280.
26. **Nelson, E. T., J. D. Clements, and R. A. Finkelstein.** 1976. *Vibrio cholerae* adherence and colonization in experimental cholera: electron microscopic studies. Infect. Immun. **14:**527–547.
27. **Pier, G. B.** 1986. Pulmonary disease associated with *Pseudomonas aeruginosa* in cystic fibrosis: current status of the host-bacterium interaction. J. Infect. Dis. **151:**575–580.
28. **Poncz, L., N. Jentoft, M.-C. D. Ho, and D. G. Dearborn.** 1988. Kinetics of proteolysis of hog gastric mucin by human neutrophil elastase and by *Pseudomonas aeruginosa* elastase. Infect. Immun. **56:**703–704.
29. **Ramphal, R., and M. Pyle.** 1983. Evidence for mucins and sialic acid as receptors for *Pseudomonas aeruginosa* in the lower respiratory tract. Infect. Immun. **41:**339–344.
30. **Ramphal, R., and M. Pyle.** 1983. Adherence of mucoid and nonmucoid *Pseudomonas aeruginosa* to acid-injured tracheal epithelium. Infect. Immun. **41:**345–351.
31. **Rivera, M., and M. B. Nicotra.** 1982. *Pseudomonas aeruginosa* mucoid strain: its significance in adult chest diseases. Am. Rev. Respir. Dis. **126:**833–836.
32. **Rose, M. C., C. F. Brown, J. Z. Jacoby, W. S. Lynn, and B. Kaufman.** 1987. Biochemical properties of tracheobronchial mucins from cystic fibrosis and non-cystic fibrosis individuals. Pediatr. Res. **22:**545–551.
33. **Roussel, P.** 1984. Glycoproteins of cystic fibrosis mucus, p. 69–73. *In* D. Lawson (ed.), Cystic fibrosis: horizons. John Wiley & Sons, Inc., Chichester, United Kingdom.
34. **Scawen, M., and A. Allen.** 1977. The action of proteolytic enzymes on the glycoproteins from pig gastric mucus. Biochem. J. **163:**363–368.
35. **Shand, G. S., A. Fomsgard, S. S. Pedersen, and N. Hoiby.** 1988. Antibody response in cystic fibrosis serum to protein antigens of *Pseudomonas aeruginosa* outer membrane. Pediatr. Pulmonol. Suppl. **2:**125.
36. **Sheehan, J. K. I., K. Oates, and I. Carlstedt.** 1986. Electron microscopy of cervical, gastric and bronchial mucus glycoproteins. Biochem. J. **239:**147–153.
37. **Vishwanath, S., and R. Ramphal.** 1984. Adherence of *Pseudomonas aeruginosa* to human tracheobronchial mucin. Infect. Immun. **45:**197–202.