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Role of Sialic Acid in Saliva-Mediated Aggregation of *Pseudomonas aeruginosa* Isolated from Cystic Fibrosis Patients

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The mechanism of saliva-mediated aggregation of *Pseudomonas aeruginosa* in subjects with and without cystic fibrosis (CF) was investigated. Virtually all saliva from CF patients that we tested strongly agglutinated the *Pseudomonas* cells and was heat stable to 56°C, whereas saliva from subjects without CF had a decreased aggregating ability and was heat sensitive. When saliva was treated with neuraminidase and proteases, and also when *P. aeruginosa* cells were treated with mixed gangliosides, there was a decrease in aggregating activities. However, neither the addition of the acid-hydrolyzed ganglioside nor the treatment of the *P. aeruginosa* cells by sugars had any effect on subsequent aggregating activities. Therefore, the release of sialic acid by enzymatic treatments of saliva, as well as the blockage of the sialic acid-binding sites on the cell wall by mixed gangliosides, resulted in the parallel loss of saliva-mediated aggregating activity of *P. aeruginosa*. The level of free sialic acid released by endogenous neuraminidase was higher in the saliva from CF patients than in that from the non-CF subjects examined. The increased aggregation of *P. aeruginosa* mediated by saliva from patients with CF seems to be directly related to the sialic acid content present, suggesting that this acid molecule acts as the salivary receptor for *P. aeruginosa*.

Cystic fibrosis (CF) is an inherited disorder of Caucasians and is characterized by progressive chronic lung infection (14, 27, 29, 44). The most common pathogen in such an infection is *Pseudomonas aeruginosa*, especially its mucoid variants (4, 13, 14, 20, 27, 29, 44). However, the mechanism of *P. aeruginosa* colonization in CF patients is still not completely understood.

There is increasing evidence which suggests that oral colonization by both gram-positive and gram-negative pulmonary pathogens is a prerequisite for their colonization in the respiratory tract and for subsequent pulmonary infection (1, 2, 15, 40, 46, 48). In a study of CF, Woods (45) suggested that aspiration of oral secretions, such as saliva, containing *P. aeruginosa* is the most likely mechanism for colonization of the lower respiratory tract. Therefore, the presence of significant numbers of *P. aeruginosa* in the saliva of CF patients may be a prerequisite for this pathogen to colonize the lower respiratory tract.

Human saliva has been known to induce aggregation of some oral bacteria (5, 7, 11, 12, 16, 26, 28). Saliva-mediated aggregation may function in vivo by causing adherence of bacteria to oral tissues or dissimilar bacteria (10, 36, 37, 43). Alternatively, this activity may function in vivo by aggregating bacteria into masses, thereby allowing easier removal from the oral cavity by swallowing or flushing actions (10, 22).

Both mucoid and nonmucoid strains of *P. aeruginosa* have been consistently isolated from various oral ecological sites (e.g., the buccal mucosa, tongue dorsum, and dental plaque) and from saliva of patients with CF, but not from subjects without CF (18, 19, 25, 48). However, the mechanism of *P. aeruginosa* colonization in the oral cavity is not known. In a study of oral streptococci, McBride and Gisslow (28) showed that there was a correlation between the amount of sialic acid released from normal saliva and its aggregating activity. They suggested that the presence of sialic acid mucins in saliva may be responsible for the aggregation of streptococci.

Further exploration of the unique characteristics of CF saliva may lead to a better understanding of the mechanism of *P. aeruginosa* colonization in CF patients. Therefore, the present investigation was carried out to survey the incidence of *P. aeruginosa* aggregation mediated by CF or non-CF saliva and to determine the unique characteristics of the salivary aggregating factor by chemical and enzymatic modifications.

MATERIALS AND METHODS

Subjects. A total of six CF patients (two female and four male) attending the CF clinic at the University Hospital, Saskatoon, Saskatchewan, Canada, were examined. The patients were in generally good health and at the time of sampling were not undergoing any drug therapy. The average age of the six CF patients was 18.8 years. Six non-CF subjects served as a control group. This investigation had been approved by the University of Saskatchewan Advisory Committee on Ethics in Human Experimentation, and informed consent was obtained from the patients or from their parents.

Assay for saliva-mediated aggregation of *P. aeruginosa*. (i) Bacterial strains and bacteriological procedures. A total of 20 strains (7 mucoid and 13 nonmucoid) of freshly isolated *P. aeruginosa* were studied. These strains were derived from various oral ecological sites, such as buccal mucosa, tongue dorsum, and dental plaque. The *P. aeruginosa* strains isolated from the oral cavity were often found to be of a different pyocine type or serotype than those found in saliva or sputum, thereby demonstrating that they were not simply present as transients. Thus, these strains were determined to be a significant component of the flora colonizing the oral cavity of CF patients (18). The methods used to isolate and identify *P. aeruginosa* were described previously (17, 18).

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Strains identified as *P. aeruginosa* were transferred into several vials containing serum-coated glass beads and were immediately frozen at −70°C (6). These beads were routinely used for subculturing to avoid further laboratory passage, which may result in alteration of characteristics of the original isolates through in vitro transfer. The *P. aeruginosa* strains were grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 24 h. The cells required for the aggregation assay were harvested by centrifugation at 10,000 × *g* for 10 min and were washed three times with TC buffer (0.05 M Tris hydrochloride–0.005 M $CaCl_2$ buffer [pH 7.2]), which was described by McBride and Gisslow (28). The cells were suspended in the same buffer and dispersed with a 1-cm^3 (25-gauge, five-eighths-in. needle) syringe.

(ii) Saliva collection and treatment. Paraffin-stimulated whole saliva was collected into ice-chilled vials and clarified by centrifugation at 10,000 × *g* for 10 min at 4°C. For experiments lasting longer than 20 min, the clarified saliva was heated at 56°C for 30 min to destroy enzymes which may inactivate the aggregating factor. For short-term experiments, saliva was not treated. If the saliva was not used immediately, it was stored at −20°C until needed. Some of the enzymatic studies were performed on a salivary pellet obtained by centrifuging the heat-clarified saliva at 10,000 × *g* at 4°C for 1 h (28). This pellet was examined by gel electrophoresis and found to be free of protein.

(iii) Aggregating assay. The reaction mixture contained 50 μl each of *P. aeruginosa* cells (10^{10} cells per ml; A_{550} = 1.5), TC buffer, and serially diluted saliva in microtiter plates and was incubated at room temperature with gentle rotation for 30 min. Controls of saliva or *P. aeruginosa* cells in TC buffer alone were included with each experiment. The degree of aggregation was examined with a dissecting microscope, and the last well showing visually detectable aggregation was taken as the endpoint. If undiluted saliva was used, aggregation was scored from 0 for no visible aggregation to +4 for maximal aggregation.

Studies of the aggregating factor. The modification studies were conducted by essentially the same methods described by McBride and Gisslow (28).

(i) Effect of heat on aggregating activity. To examine the heat sensitivity of *P. aeruginosa* salivary aggregating factor, clarified saliva was heated at 56°C or boiled for 15 min in a water bath (5). *P. aeruginosa* cells and unheated, heated, or boiled saliva were then assayed to determine their aggregation ability.

(ii) Gangliosides and sugar treatments. Various concentrations (0 to 500 μg/ml) of mixed gangliosides and acid-hydrolyzed gangliosides were preincubated with *P. aeruginosa* cells for 60 min at room temperature. Acid-hydrolyzed ganglioside was prepared by incubating type III ganglioside (Sigma Chemical Co., St. Louis, Mo.) in HCl (0.05 N) at 80°C for 90 min (28). Sugars and sugar amines (Sigma Chemical Co.) were also preincubated with the cells and assayed for aggregation activity (5). In each assay, 50 μl of saliva was added to the mixture in microtiter plates and incubated at room temperature for 30 min.

(iii) *N*-Acetylneuraminic acid. *P. aeruginosa* strains were treated with various concentrations (0.001, 0.01, 0.10, or 1.0 mg/ml) of *N*-acetylneuraminic acid (sialic acid) type IV (Sigma Chemical Co.). The cells were then incubated for 30 min at 37°C, washed three times, and suspended in TC buffer for use in the aggregation studies. As a control, bacteria were treated with TC buffer instead of with *N*-acetylneuraminic acid.

(iv) Protease. After centrifugation of clarified saliva (10,000 × *g*, 1 h at 4°C), the pellet was readily dispersed in TC buffer by vortexing for 15 s, adding 1.2 mg of proteolytic enzyme (Sigma Chemical Co.), and then incubating the mixture at room temperature for 1 h. To inactivate the proteases, the mixture was heated in boiling water for 15 min. This mixture was then used for an aggregation assay. Enzymatic disruption was studied by adding the enzymes to preformed *P. aeruginosa*-saliva aggregates and examining for dispersion by visual assessment of the microtiter plates over a period of time.

Both the effectiveness of inactivation of proteases and all the other enzymes used in these experiments, and all enzyme activities, was measured by the methods described by McBride and Gisslow (28).

(v) Glycosidase. The salivary pellet was easily suspended in 1 ml of citrate buffer (0.05 M; pH 4.6) by vortexing for 15 s. Portions (1 U each) of the glycosidases (β-galactosidase, α-mannosidase, and β-*N*-acetylhexosaminidase) (Sigma Chemical Co.) were added, and the mixture was incubated at room temperature for 48 h. A drop of $CHCl_3$ was added to prevent growth of bacteria. The mixture was then used for an aggregation assay.

(vi) Alkaline phosphatase. Clarified saliva (3 ml) was adjusted to pH 8.0 with 1 N NaOH, and then 40 U of alkaline phosphatase was added. After incubation at room temperature for 2 h, the alkaline phosphatase was inactivated by heating in a boiling water bath for 5 min. The mixture was used in an assay to determine its ability to aggregate *P. aeruginosa* cells.

(vii) Neuraminidase. The salivary pellet was readily suspended by vortexing for 15 s in 5 ml of 0.05 M sodium acetate–0.005 M $CaCl_2$ (pH 5.5), and neuraminidase type III (10 μl/ml [0.09 U/ml]; Sigma Chemical Co.) was added. The mixture was incubated at 37°C, and 1-ml samples were removed periodically. These samples were heated in boiling water for 5 min and then assayed for free sialic acid by the thiobarbituric acid assay (42) and for aggregating ability.

(viii) Acid hydrolysis. A mixture of 5 ml of 0.1 N H_2SO_4 and 5 ml of saliva was placed in a 80°C water bath. At designated intervals, samples were removed and assayed for free sialic acid and for aggregating activity.

(ix) Endogenous neuraminidase. Clarified saliva that had not been heat treated was incubated at 30°C. At designated intervals, 1-ml samples were removed and assayed for free sialic acid and also for aggregation of *P. aeruginosa* cells.

RESULTS

Saliva-mediated aggregation of *P. aeruginosa*. When CF and non-CF saliva were not heat treated, almost all of the mucoid and nonmucoid *P. aeruginosa* strains were aggregated. However, when heated at 56°C, the CF saliva still strongly aggregated most *P. aeruginosa* strains, but non-CF saliva binding ability was almost completely destroyed (Table 1). There were no significant differences between the aggregating activity by CF or non-CF saliva of the mucoid and nonmucoid *P. aeruginosa* strains. Both CF and non-CF saliva were sensitive to boiling and lost almost all aggregation properties. The heat treatment of *P. aeruginosa* cells did not have an inhibitory effect on aggregation by saliva. The controls showed that there were no self-aggregating strains of *P. aeruginosa*.

Since there was strong *P. aeruginosa* aggregation by CF saliva, the nature of the aggregating factor was further investigated. This factor was characterized in the following

TABLE 1. Aggregation ability of *P. aeruginosa* by CF and non-CF saliva after heat treatment at 56°C

P. aeruginosa strain	Aggregation by:											
	CF saliva samples						Non-CF saliva samples					
	A	B	C	D	E	F	A_1	B_1	C_1	D_1	E_1	F_1
Nonmucoid												
HSS1[a]	+4	+4	+3	+4	+4	+3	0	0	0	0	+2	0
TYT3[a]	+4	+3	+4	+4	+4	+3	+2	0	+3	0	0	0
TYT2[a]	+4	+2	+4	+3	+4	+3	0	0	0	+2	0	+1
HST2[a]	+4	+4	+4	+3	+3	+3	+1	0	0	0	+2	0
TYS2	+2	+2	+4	+3	+4	+3	0	+2	0	0	0	0
HST1	+2	+2	+3	+2	+3	+3	0	0	0	0	+1	+1
HSB1	+3	+1	+3	+3	0	+1	+1	0	0	0	+2	+2
TYB2	+2	+1	0	+3	+1	+2	0	0	+2	0	0	0
TYS1	+4	+3	+4	+3	+4	+2	0	0	0	0	0	0
CWP1	+2	0	+4	+4	+2	+2	0	0	0	0	0	0
HSP2	+4	+3	+3	+4	+4	+3	0	0	0	0	+1	0
TYB1	+4	+4	+4	+3	+2	+4	0	0	0	+3	0	0
HSS3	+3	0	+3	+2	+1	+2	0	+2	0	0	0	0
Mucoid												
CPT1[a]	+3	+3	+3	+3	+4	+4	+2	0	0	+2	+2	0
TYT1[a]	+4	+3	+4	+4	+2	+3	0	0	+1	0	0	0
KBB2[a]	+3	+3	+4	+4	+4	+4	+1	0	0	0	0	+2
CZT1[a]	+4	+2	+4	+3	+4	+2	0	0	0	+2	0	0
KBP1	+2	+2	+3	+2	+3	+4	0	0	0	0	0	0
KBP2	+2	+4	+2	+1	+3	+3	0	0	0	0	0	0
KKB1	+3	+3	+2	+3	+3	+4	0	+1	0	0	0	0

[a] Strains used in modification studies.

modification studies with eight representative strains of *P. aeruginosa* which showed strong aggregating activity by CF saliva (Table 1).

Effect of treatment of *P. aeruginosa* cells on saliva-mediated aggregation. None of the sugars, sugar amines, mucin (bovine submaxillary type I), or dextran tested had an appreciable inhibitory effect on the aggregating ability of CF or non-CF saliva (Table 2). Although a slight inhibitory effect was seen with the addition of lecithin, this effect was probably nonspecific at the concentration tested (41). Therefore, the role of lecithin may be insignificant in defining the fraction involved in saliva-mediated *P. aeruginosa* aggregation. The addition of as little as 0.01 mg of *N*-acetylneuraminic acid per ml to the *P. aeruginosa* cells inhibited aggregation by both CF and non-CF saliva. This finding indicates that the sialic acid-binding sites on the bacterial cell wall may have been occupied by the addition of *N*-acetylneuraminic acid and thus may have prevented aggregation by the salivary aggregating factor.

Saliva-induced aggregation was greatly reduced when *P. aeruginosa* cells were pretreated with a mixed ganglioside preparation, but aggregation was less affected by the acid-hydrolyzed ganglioside (Table 3). As suggested by McBride and Gisslow (28), the removal of neuraminidase-sensitive sialic acid residues by the mixed gangliosides created a molecule which was less effectively able to bind with the receptor sites within saliva.

Effect of saliva treatment on *P. aeruginosa* aggregation. The salivary factors responsible for aggregation of *P. aeruginosa* were explored by chemical and biochemical modifications. Incubation with the proteolytic enzymes (chymotrypsin, trypsin, subtilisin, and pronase) decreased the binding ability of *P. aeruginosa* by CF and non-CF saliva. Neuraminidase also had a similar effect. Collagenase, alkaline phosphatase, α-mannosidase, β-galactosidase, β-*N*-acetylglucosaminidase, and lipase had no effect on aggregating ability (Table

TABLE 2. Aggregation ability of *P. aeruginosa* by CF saliva after treatment with various compounds

Compound added	Concn (mg/ml)	Aggregation titer
Glucose	9.00	16
L-Fucose	9.00	16
Galactose	9.00	16
Galactosamine	9.00	16
D-(+)-Glucosamine	9.00	16
α-Methyl-galactoside	9.00	16
β-Methyl-galactoside	9.00	16
Lactose	9.00	16
N-Acetylglucosamine	22.00	8
Mucin (bovine submaxillary type I)	4.25	16
Dextran T2000	0.20	16
Lecithin	0.25	4
N-Acetylneuraminic acid	0.01	0
Ganglioside	0.20	0
Buffer control		16

TABLE 3. Aggregation ability of *P. aeruginosa* by CF saliva after treatment with ganglioside and acid-hydrolyzed ganglioside

Compound concn (μg/ml)	Aggregation titer	
	Ganglioside	Acid-hydrolyzed ganglioside
500	0	4
250	0	8
125	4	8
50	8	8
25	8	16
20	8	16
15	16	16
0	16	16

TABLE 4. Effect of enzymes on aggregating ability of CF saliva

Enzyme	Enzyme activity (U/ml)	Pretreated saliva aggregation titer[a]
α-Chymotrypsin	19	4
Trypsin	4.46	2
Subtilisin	3.40	8
Collagenase	180	16
β-Galactosidase	345	16
α-Mannosidase	17	16
β-*N*-Acetylhexosaminidase	57	16
Pronase	70	8
Alkaline phosphatase	50	16
Neuraminidase	1.0	0
Control		16

[a] Aggregation titer of all untreated saliva was 16.

4). The decrease in *P. aeruginosa* aggregating ability by saliva treated with proteolytic enzymes was further explored by examining the effects of these enzymes on preformed *P. aeruginosa*-saliva aggregates. The proteolytic enzymes each dispersed the *P. aeruginosa*-saliva aggregates, but the most pronounced reduction of aggregation in the shortest amount of time was seen when neuraminidase was added (Table 5).

Role of neuraminidase. Saliva-mediated *P. aeruginosa* aggregation significantly decreased with the addition of *N*-acetylneuraminic acid and mixed gangliosides to cells (Table 2) and neuraminidase to saliva (Tables 4 and 5). These treatments affected the sialic acid molecule in saliva, which resulted in a decreased binding ability of *P. aeruginosa*. To further explore the role of sialic acid, saliva was treated with neuraminidase and by acid hydrolysis. The enzymatic or chemical release of sialic acid from heat-treated CF saliva was correlated to the subsequent loss of aggregating ability by saliva (Table 6). The control did not lose its aggregating activity nor did it release sialic acid over a 60-min period.

Endogenous neuraminidase. There was a significant difference between CF and non-CF saliva *P. aeruginosa* aggregation (Table 1). To investigate these differences further, endogenous neuraminidase activity of saliva from both CF and non-CF subjects was examined. Over time, endogenous neuraminidase released a significantly higher amount of sialic acid from CF saliva than from non-CF saliva (Table 7). Furthermore, as the sialic acid was released from saliva, this release coincided with a loss of saliva-mediated *P. aeruginosa* aggregating ability.

DISCUSSION

In this study, saliva from CF patients showed a significantly higher aggregating ability of *P. aeruginosa* than did saliva from non-CF subjects. However, there were no marked differences between the activity of mucoid and nonmucoid *P. aeruginosa* strains in saliva-induced aggregation or in any of the modification studies conducted. This finding suggests that there may be something unique about CF saliva which allows both variants of *P. aeruginosa* to aggregate. The results of this investigation have provided strong evidence that the difference in salivary aggregating activity between CF and non-CF subjects is due to the different amounts of sialic acid present.

It has been suggested that there may be more than one salivary factor responsible for bacterial aggregation (5, 37). We have shown that the factor(s) in CF saliva which is responsible for *P. aeruginosa* aggregation includes a component that was resistant to heating to 56°C (Table 1) but was sensitive to boiling. In contrast, the aggregating activity of non-CF saliva was almost completely destroyed at 56°C (Table 1) and at 100°C. Modification studies were conducted to explore further the factors responsible for these aggregation differences between CF and non-CF saliva.

When *P. aeruginosa* cells were preincubated with sugars, there was no appreciable inhibitory effect on aggregation ability by saliva (Table 2). It had been suggested by Ellen et al. (5) that saliva-induced bacterial aggregation may be mediated by the interaction of sugar end groups with lectin-like ligands on the bacterial cells. This did not occur in the present study, since saliva-specific receptor sites on the cell wall of *P. aeruginosa* were not blocked by any sugar acting as an analog. This result was further substantiated in a study by Komiyama et al. (17), which also showed that interbacterial adhesion between strains of *P. aeruginosa* and indigenous oral bacteria isolated from CF patients was not inhibited by any sugars tested.

TABLE 5. Disruption by enzymes of *P. aeruginosa* saliva-mediated aggregates

Enzyme	Enzyme activity (U/ml)	Aggregation at 45 min[a]
Chymotrypsin	80	+2
Trypsin	18,955	+1
Subtilisin	145	+2
Subtilopeptidase	17	+1
Pronase	18.4	+1
Neuraminidase	0.050	0
Control	0	+4

[a] All aggregates were +4 at 0 min.

TABLE 6. Effect of neuraminidase and acid hydrolysis treatment on the aggregation ability of *P. aeruginosa* by CF saliva

Treatment	Time (min)	Free sialic acid (μmol, 10^2/ml)	Aggregation titer
Neuraminidase	0	0	16
	10	0.8	16
	20	3.2	4
	30	9.1	2
	60	24.6	0
Control	60	0	16
Acid hydrolysis	0	0	16
	15	0.88	16
	20	5.66	4
	30	20.40	0

TABLE 7. Endogenous neuraminidase activity in clarified CF and non-CF saliva

Time at 30°C (min)	Free sialic acid from CF patients (μmol, 10^2/ml)	Aggregation titer by CF saliva[a]	Free sialic acid from non-CF subjects (μmol, 10^2/ml)
0	0	16	0
10	1.97 ± 0.552	16	1.42 ± 0.339
30	2.69 ± 0.592	8	1.95 ± 0.283
60	3.47 ± 0.414	8	2.24 ± 0.096
90	3.95 ± 0.341	8	2.84 ± 0.325
120	4.52 ± 0.520	8	3.06 ± 0.438
180	4.80 ± 0.443	4	3.30 ± 0.495
240	5.48 ± 0.489	4	3.79 ± 0.823

[a] Aggregation titer of all non-CF saliva was ambiguous.

P. aeruginosa aggregating ability was reduced when the binding sites on the cell wall were blocked by the addition of mixed gangliosides and *N*-acetylneuraminic acid. Furthermore, the acid-hydrolyzed ganglioside, which had its sialidase-sensitive sialic acid molecules removed, had a decreased inhibitory effect (Table 3). Therefore, the capacity of CF saliva, and to a lesser extent non-CF saliva, to bind with the cell surface of *P. aeruginosa* may be mediated by a sialic acid-containing molecule.

This conclusion was substantiated when the removal of salivary sialic acid by proteolytic enzymes, acid hydrolysis, and neuraminidase resulted in a reduced aggregating ability by CF saliva (Tables 4 to 6). Treatment of non-CF saliva with these enzymes also reduced aggregating ability, but it was difficult to assess the degree of decreased activity because of lower overall aggregation mediated by non-CF saliva. Insight into these different aggregating abilities of CF and non-CF saliva was gained by examining the content of salivary sialic acid of both groups. Endogenous neuraminidase activity of CF saliva released a higher amount of sialic acid than did non-CF saliva, resulting in a parallel loss of aggregating ability (Table 7).

These results indicate that the activity of endogenous neuraminidase or the number of sialic acid molecules may be the factor determining the degree of *P. aeruginosa* aggregation by saliva. It is also possible that aggregating ability depends on the interaction of sialic acid with its surrounding molecules, so that with its release an environmental change not conducive to binding may occur. The addition of mucins (bovine submaxillary type I), which contain sialic acid as a terminal sugar, did not affect aggregation (Table 2). Therefore, the proximity of sialic acid in its surroundings may be as important as the size of the aggregating complex.

Reddy et al. (35) suggested that there are no apparent structural differences between neutral and sialic acid-containing units of normal and CF samples. However, our results indicate that the sialic acid-containing units in CF saliva are more available for aggregation than are those in non-CF saliva. These observations may be due to a quantitative difference in the sialic acid-containing units in saliva instead of a qualitative difference. Furthermore, Reddy et al. examined oligosaccharide structures of submandibular saliva from only one subject in each group and did not discuss the contributions of parotid or minor mucous glands. Just as there are structural differences between the sialylated mucins synthesized by goblet cells and those synthesized by mucous cells in CF patients (21), there may also be differences in the sialic acid unit contributions by the submandibular-sublingual, parotid, and minor mucous glands of CF and non-CF saliva.

Other salivary constituents implicated as possibly being involved in aggregation include such molecules as lysozyme (26, 30), secretory immunoglobulin A (3, 8), and high-molecular-weight mucinous glycoproteins (22). It is the last sialoglycoproteins which seem to be the most predominant components identified in salivary aggregating activities (7, 16, 23). Salivary sialic acid has been implicated in the aggregation of *Streptococcus sanguis* (28), *Actinomyces viscosus*, and *Actinomyces naeslundii* (5). The sialic acid constituent is also a contributing aggregating factor for several other organisms, such as *Mycoplasma pneumoniae* (39) and *Escherichia coli* (24), and serves as a receptor for virus particles in virus-induced hemagglutination (38). The other important function of sialic acid has been its ability to further the attachment and subsequent infectivity of viruses (38), which may parallel a situation in CF in which the salivary activity in *P. aeruginosa* aggregation may have a role in the colonization by this pathogen of the respiratory tract.

Bacterial adherence to host tissues is a necessary requirement for colonization. However, large numbers of cells would need to be present in saliva before it becomes statistically probable that one cell would become attached to an oral surface (9) and not be washed away by the clearing mechanisms (10, 22). Our study showed that the sialic acid moiety could provide large numbers of aggregated *P. aeruginosa* in saliva. Therefore, it is interesting to speculate that sialic acid-induced aggregation may play a role in the adherence of *P. aeruginosa* in the oral cavities of CF subjects. Moreover, since sialic acid is present in mucinous coatings of epithelial cells, adherence may also occur by this molecule acting as a receptor for the aggregated bacteria. It was demonstrated by Woods et al. (47) that removal of fibronectin facilitates adherence of *P. aeruginosa* to buccal cells. Ramphal et al. (31, 34) also showed that injured or altered tracheal cells increased adherence of *P. aeruginosa*. Thus, the difference in *P. aeruginosa* adherence and colonization in CF and non-CF subjects may be due to both the sialic acid content and some other chemical or physical change on the surfaces.

Ramphal and Pyle (32) hypothesized that the situation in cystic fibrosis may involve stagnant mucins encouraging proliferation of *P. aeruginosa*. However, it is just as likely that colonization by *P. aeruginosa* in CF patients may be facilitated by increased adherence to oral surfaces (9), by sialic acid acting as a receptor in the lower respiratory tract (32, 33), or by the inhalation of large clumps of *P. aeruginosa* formed by the sialic acid salivary aggregating factor (45).

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