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Differences in Adhesion of *Pseudomonas aeruginosa* to Mucin Glycopeptides from Sputa of Patients with Cystic Fibrosis and Chronic Bronchitis

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Pseudomonas aeruginosa **is the most prominent colonizer of the respiratory tract of patients with cystic fibrosis, but it is not known why this occurs. *P. aeruginosa* adheres to mucins from normal individuals, but mucins from cystic fibrosis patients have not been studied. To compare adhesion to mucins from cystic fibrosis with other mucins, we prepared highly glycosylated mucin glycopeptides from cystic fibrosis and chronic bronchitis patients by ion-exchange and gel-filtration chromatography and measured the adhesion of *P. aeruginosa* 1244 to these glycopeptides. We found (i) that the most mucinlike glycopeptides from *P. aeruginosa*-infected cystic fibrosis sputa showed less bacterial adhesion than did the corresponding bronchitis samples, (ii) that the most adhesive activity in cystic fibrosis samples came from a fraction that contains O and N glycopeptides and may be in part a degradation product of *P. aeruginosa* infection, and (iii) that highly glycosylated glycopeptides of the most acidic species (sialylated and sulfated) showed no adhesion at all. A single cystic fibrosis sample not infected by *P. aeruginosa* showed better binding in the adhesion-positive fractions than did the infected sputa. These studies suggest that cystic fibrosis mucins may be altered after infection is established, resulting in less binding to some fragments. However, since the clinical picture shows heavy mucus colonization, other receptors, such as cellular glycolipids which have been shed into mucus, may be contributing to this colonization.**

The reason for the predominance of *Pseudomonas aeruginosa* as the colonizing bacterium in cystic fibrosis (CF) is not known. However, this organism adheres to respiratory mucins much more avidly than other gram-negative bacteria do (23) and possesses adhesins for mucins (15). This specificity for mucins may be a clue to explain this unique predisposition, but in comparisons of the binding of *P. aeruginosa* to whole normal chronic bronchitic (CB) mucin and CF mucin, the CF mucins prepared so far do not show greater adhesion (Ramphal, unpublished observations). Nevertheless, the remarkable tropism of *P. aeruginosa* that is observed clinically and the binding specificity of this organism for mucins suggest that there may be an explanation that involves CF mucins. We hope to find an explanation for this specificity by comparing the adhesion of *P. aeruginosa* to subfractions of mucins isolated from both CF and non-CF mucins. By studying adhesion in this fashion we may be able to (i) ascertain whether there is, indeed, a difference in the binding of *P. aeruginosa* to different mucins and (ii) eventually compare the structures of binding and nonbinding fractions to study any specificity found. This latter step would be extremely difficult with whole mucins, which have molecular weights greater than 10^6 (20) and possibly contain hundreds of different oligosaccharides (4, 8) as well as associated glycolipids (5).

Mucins appear to have carbohydrate chains of differing acidity clustered on highly glycosylated regions of the polypeptide chain(s) (20). If crude mucus is reduced and then digested, the resulting mucin glycopeptides and peptides can be separated according to their net charge. Ion-exchange chromatography on ECTEOLA-cellulose resin, with increasing concentrations of NaCl-HCl as the eluting agent, yields fractions called F_1, F_2, and F_3. F_1, the fraction that is not retained on the column, is called the neutral fraction by definition. F_2, which is eluted by 0.1 M NaCl–0.01 N HCl, is slightly acidic because of the sialic acid residues present. F_3, the most acidic fraction, by virtue of the presence of sialylated and/or sulfated oligosaccharide chains is eluted by 1 M NaCl–0.1 N HCl.

These fractions can be further purified by gel filtration on Sepharose 4B to give the most highly glycosylated glycopeptides corresponding to the above fractions as follows: F_1b and F_1c from F_1, F_2b from F_2, and F_3a from F_3. Since the evidence suggests that the *P. aeruginosa* receptor in mucins is carbohydrate in nature (24), we have directed our attention to these highly glycosylated fractions.

In the studies described below we present evidence for differences in the binding of *P. aeruginosa* to the highly glycosylated subfractions of mucins. However, no specificity for CF mucin was found; instead, there appeared to be less binding to CF mucin glycopeptides after infection had occurred, and some of the most highly glycosylated mucin fractions failed to bind this organism.

MATERIALS AND METHODS

Collection of bronchial secretions. Coughed sputum was collected from 14 patients during hospitalization for respiratory failure. Volumes of between 200 ml and 1 liter were collected and immediately frozen at −20°C until they were used to prepare the mucin glycopeptides. Sputa were collected from three CB patients and pooled, from four individual CB patients and kept separately, from three CF patients with *P. aeruginosa* infection and pooled, from three individual CF patients infected with *P. aeruginosa* and kept sepa-

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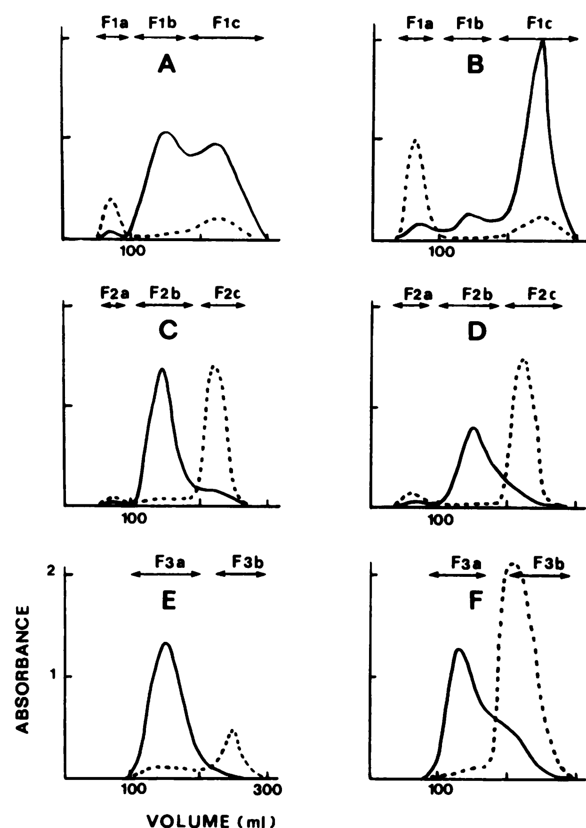

FIG. 1. Chromatographic pattern of bronchial glycopeptides (F_1, F_2, and F_3 from ECTEOLA cellulose) on a Sepharose 4B column. Glycopeptides (100 mg) were deposited on a Sepharose 4B column (2.5 by 48 cm) equilibrated and eluted with 0.1 M Tris hydrochloride buffer (pH 8.0) containing 0.2 M NaCl and 0.02% NaN_3. Fractions (5 ml) were analyzed for hexose (—) and for A_{278} (---). Fractions indicated by bars were dialyzed and lyophilized. Profiles A, C, and E correspond to bronchial glycopeptides from a CB sputum not infected by *P. aeruginosa*, and profiles B, D, and F correspond to bronchial glycopeptides from a CF sputum infected by *P. aeruginosa*.

rately, and from one CF patient without *P. aeruginosa* infection.

Preparation of mucin glycopeptides. The sputa were thawed at room temperature and reduced in phosphate buffer by 2-mercaptoethanol (Eastman Kodak Co., Rochester, N.Y.) as described previously (10). The reduced secretions were then dialyzed, lyophilized, and digested by pronase (Pronase R protease; Calbiochem, San Diego, Calif.) in 0.01 M calcium acetate buffer (pH 7.0), by using an enzyme/substrate ratio of 1:40, at 37°C. After 24 h the secretions were centrifuged, and the supernatants were dialyzed and lyophilized.

Separation of mucin glycopeptides by ion-exchange chromatography. The crude preparations of glycopeptides from the preceding steps were separated according to acidity on an ECTEOLA-cellulose column (Bio-Rad Laboratories, Richmond, Calif.) as described previously (10). Three fractions were eluted by increasing concentrations of NaCl and HCl. These were dialyzed and then lyophilized.

Separation of mucin glycopeptides by gel filtration. The glycopeptides, which were separated according to charge, i.e., neutral (F_1), slightly acidic (F_2), and acidic (F_3), were then further separated by gel filtration on Sepharose 4B (Pharmacia, Uppsala, Sweden) to separate the most highly glycosylated fractions, called F_1b, F_1c, F_2b, and F_3a, from contaminants such as peptides and nucleic acids (10).

Characterization of the highly glycosylated fractions. (i) Chemical composition. Each highly glycosylated fraction was analyzed for amino acid (10) and carbohydrates by a quantitative gas-liquid chromatographic method (8), and neuraminic acid was measured by the thiobarbituric acid assay (1). Sulfate was measured only in the case of the most acidic fractions (21).

(ii) Polyacrylamide gel electrophoresis. Polyacrylamide (5 to 15%) gel slabs were prepared by the method of Kerckaert (6), and electrophoresis was conducted with the buffer system of Laemmli (7). Protein standards were the molecular weight electrophoresis kit from Pharmacia, and the glycoprotein standard was serum α-glycoprotein from Sigma Chemical Co. (St. Louis, Mo.). After electrophoresis, proteins were stained with Coomassie brilliant blue 5250 (BDM, Dorset, England), and the carbohydrates were stained by a Schiff reagent procedure (25).

Adhesion studies. *P. aeruginosa* 1244, a piliated strain that has been described in previous studies (17), was used to test adhesion to the highly glycosylated glycopeptide fractions. The bacteria were grown overnight at 37°C in Trypticase soy broth (Difco Laboratories, Detroit, Mich.), pelleted by centrifugation, and washed with phosphate-buffered saline (pH 7.0). They were then suspended in phosphate-buffered saline at a predetermined optical density to give an inoculum between 5×10^6 and 8×10^6 CFU/ml. The adhesion assay has been described previously (23). It was modified by omitting siliconization of the polystyrene microdilution

TABLE 1. Final recovery (%) of highly glycosylated fractions after ECTEOLA cellulose and sepharose 4B chromatography of the crude glycopeptides

Treatment	Fraction	% Recovery of highly glycosylated fractions in sputum						
		Noninfected CB			Infected CF			Noninfected CF, individual (1)
		Pool	Individuals (4)[a]	Range	Pool	Individuals (3)	Range	
ECTEOLA cellulose	F_1	30.7	32.7	27.1–39.3	22.9	19.3	15.8–22.0	30.1
	F_2	7.0	9.25	8.3–11.5	5.5	9.1	7.7–10.7	1.0
	F_3	33.0	29.1	19.3–40.4	21.6	31.0	22.0–42.1	37.3
Sepharose 4B	F_1b	9.8	12.7	4.9–19.6	2.8	0.76	0.44–1.05	12.0
	F_1c	8.6	4.2	2.7–5.5	15.7	8.8	6.9–10.8	6.9
	F_2b	4.9	4.9	3.3–6.3	2.1	3.3	2.5–3.3	1.0
	F_3a	17.9	17.4	9.9–18.6	2.7	9.4	4.4–15.4	20.1

[a] Numbers within parentheses indicate numbers of individuals. Values given for individuals are means.

TABLE 2. Chemical composition of the highly glycosylated fractions of mucus glycopeptides

Sputum	Fraction	Composition (% dry wt) in residues per 100 residues[a]											
		Fucose	Mannose	Galactose	*N*-Acetylglucosamine	*N*-Acetylgalactosamine	Sialic acid (Neu Ac[b])	Glucose	Total carbohydrate	Sulfate	Total amino acid	Asp plus Glx	Thr plus Ser
CB, noninfected	F_1b	8.6	0.2	21.5	30.7	18.5	3.1	1.1	83.7	—[c]	13.8	9.9	38.1
	F_1c	7.9	8.3	15.9	18.3	4.6	3.1	0.5	58.6	—	19.5	16.4	27.5
	F_2b	10.8	Traces	26.0	25.6	13.1	8.7	0.6	86.8	—	9.1	4.6	47.3
	F_3a	11.5	0.3	26.4	26.0	14.2	2.2	0.7	81.3	0.9	7.1	5.9	47.8
CF, infected by *P. aeruginosa*	F_1b	12.7	0.2	20.2	23.7	12.2	4.6	0.8	73.8	—	8.8	7.7	41.9
	F_1c	6.9	4.3	18.0	18.8	9.2	8.1	0.8	66.1	—	24.0	22.6	19.7
	F_2b	14.5	0	20.7	17.5	11.4	10	0.8	75	—	12.7	7.2	45.1
	F_3a	14.8	0.4	30.4	24.3	14.7	3.2	2.4	88.2	1.2	7.6	5.4	51.1

[a] See reference 7.
[b] Neu Ac, *N*-acetylneuraminic acid.
[c] —, Not done.

plates (Flow Laboratories, Inc., McLean, Va.; catalog no. 76-242-05). In brief, 100 μl of solution containing 100 μg of mucin glycopeptides per ml of deionized water was used to coat the bottom of the wells overnight. The excess of glycopeptides was washed away with a hand-held microdilution plate washer (Flow). The bacteria were added (100 μl) to the wells and incubated at 37°C for 30 min. The wells were then washed 15 times with PBS, and 0.5% Triton X-100 in PBS was added to the wells for 15 min to desorb the bacteria. The Triton X-100 solution was mixed by repeated pipetting and then diluted and plated on MacConkey agar (Difco). Control wells for background binding were uncoated. Only experiments with little or no background were considered valid. Each experiment was done at least three times with two wells per determination.

Determination of glycopeptide binding to the microdilution plate. The F_3a fractions that gave little or no bacterial binding were labeled by the avidin-biotin system (2) and tested to ensure that they were bound to the microdilution plates. In brief, 1 mg of each sample was dissolved in 1 ml of 0.1 M acetate buffer (pH 5.5) with 0.02% sodium azide. Then 10 μl of 1 M sodium periodate was added, and the mixture was kept in the dark for 20 min on ice. Glycerol was then added at a final concentration of 15 mM for 5 min at 0°C, and then the oxidized samples were dialyzed at 4°C in the dark overnight. Biotin hydrazide (Pierce Chemical Co., Rockford, Ill.) in powder form was added at 10 mM concentration, and the mixture was stirred for 72 h at 4°C. The samples were then dialyzed in the dark overnight. The wells of a microdilution plate were then coated overnight with 0.1 ml of biotinylated glycopeptides at 100 μg/ml, and binding to the wells was detected by streptavidin peroxidase by using 2,2-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) (Sigma) and hydrogen peroxide as the revealing agents. Plates were read in a Titertek Multiscan plate reader (Flow) at 414 nM. Controls included whole mucins and F_1b fractions, which were known to give bacterial binding.

RESULTS

Characteristics of the highly glycosylated mucus glycopeptides. The three fractions of increasing acidic character that were obtained after ion-exchange chromatography of the crude mucin glycopeptides (F_1, F_2, and F_3) were further purified on Sepharose 4B. The elution profiles of these fractions from a CB sputum not infected by *P. aeruginosa* and a CF sputum infected by *P. aeruginosa* are shown in Fig. 1A. The differences visualized in the neutral glycopeptide fractions (Fig. 1A and B) were analyzed elsewhere (5a). To recapitulate, concurrent with *P. aeruginosa* infection, the F_1b fraction was always greatly diminished in quantity (Table 1) when compared with noninfected sputa. In contrast, the F_1c fraction was always greatly increased (Table 1). The composition of F_1b was that of O-glycosylated glycopeptides, and F_1c appeared to be a mixture of O- and N-glycosylated glycopeptides (Table 2). The slightly acidic fraction F_2b, which is highly glycosylated (19), had a similar profile in both types of mucus (Fig. 1C and D) and was similar in yield (Table 1) and chemical composition (Table 2). The chemical compositions are those of true O-glycosylated mucin glycopeptides.

Gel filtration of the most acidic fractions (F_3) allowed the separation of nucleic acids from the mucin glycopeptides (Fig. 1E and F). The chemical compositions of the remaining F_3a glycosylated fractions were also very similar in sugar and amino acid content and were those of acidic mucins because of the concentrations of sialic acid and sulfate. Thus, in a general sense all of these glycopeptides (F_1b, F_1c, F_2b, and F_3a) were qualitatively similar in the two patient groups (CF and CB).

Electrophoretic studies. Polyacrylamide gel electrophoresis of the four glycosylated fractions described above is shown in Fig. 2. One patient from each population is depicted. Fractions F_1b, F_2b, and F_3a behaved like true mucin glycopeptides, i.e., they partially migrated out of the stacking gel, were strongly stained by periodic acid-Schiff stain, and were not contaminated by proteins. In contrast, the F_1c fractions, the mixture of small glycoproteins (included in Sepharose 4B), showed many protein bands in both samples and two periodic acid-Schiff-stained bands that also stained by Coomassie blue, uniquely in the *P. aeruginosa*-

TABLE 3. Adhesion of *P. aeruginosa* 1244 to glycopeptide fractions from pools of CB and CF sputa

Fraction	No. of adherent bacteria per well (mean and range), 10^2	
	CB pool	CF pool[a]
F_1b	42 (29–100)	<1
F_1c	10 (1–31)	102 (51–135)
F_2b	77 (44–100)	45 (11–96)
F_3a	<1	<1

[a] Sputa infected by *P. aeruginosa*.

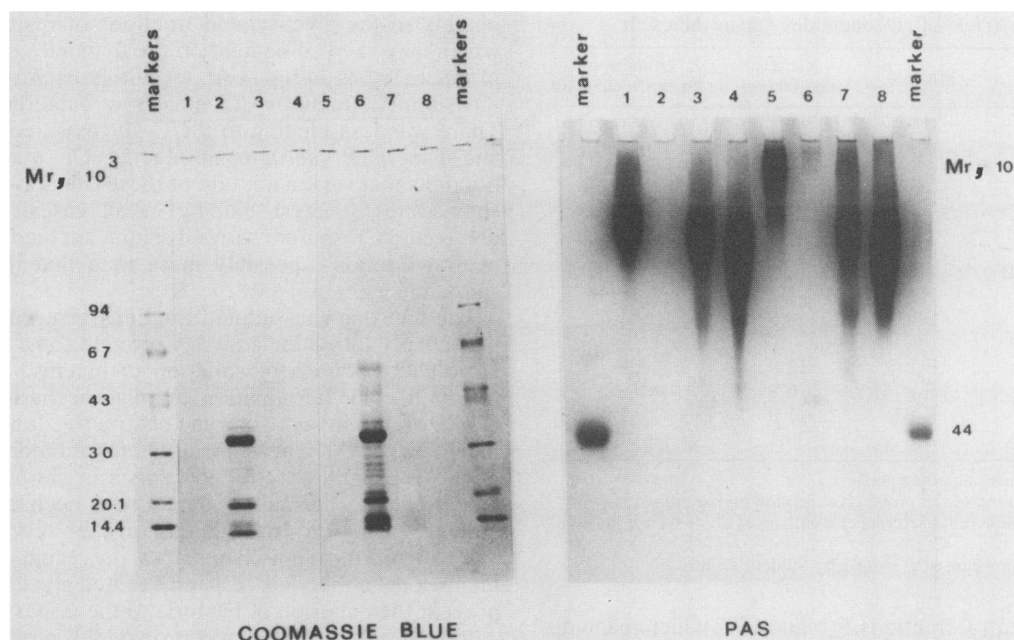

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the highly glycosylated fractions eluted from Sepharose 4B column. Lanes: 1 through 4, fractions F_1b, F_1c, F_2b, and F_3a, respectively, from a CB sputum not infected by *P. aeruginosa*; 5 through 8, fractions F_1b, F_1c, F_2b, and F_3a, respectively, from a CF sputum infected by *P. aeruginosa*. The bands specific for *P. aeruginosa* infected sputum are shown in lane 6 between 43 and 67 kilodaltons. PAS, Periodic acid-Schiff stain.

infected samples. These bands were characterized previously (5a). This is the only difference found in the sputum samples to date. The two unique bands appear to be associated with *P. aeruginosa* infection of mucus.

Adhesion. (i) Glycopeptides from pooled sputa. The adhesion studies on the pooled sputa from the two patient populations showed remarkable differences (Table 3). In the CB pool, adhesion was greatest in the neutral F_1b and slightly acidic F_2b fractions, which were more characteristic of true mucins, with a small amount in the F_1c fraction. In the CF pool, adhesion was greatest in the F_1c fraction, which may contain degradation products, probably from F_1b (5a). The F_2b fraction from the CF pool showed less adhesion than that of the CB pool, but neither F_3a fraction (the most acid fraction) showed binding.

(ii) Glycopeptides from individual patients. Adhesion studies on individual patients partially confirmed the results obtained with the pooled sputa. However, there was great variability from one patient to another (Table 4). When the noninfected CF sample was separated and the respective CB and CF values were pooled, there was less binding to the CF F_1b and F_2b fractions than to the CB fractions (Table 5), but the differences in the F_1c fractions that were seen with the pools were no longer present. Again, none of the F_3a fractions bound bacteria. Interestingly, the noninfected CF patient samples showed binding only to the neutral fractions, in numbers greater than those in both the CB and infected CF samples.

Yield of glycopeptides versus adhesion. Examination of the yields of the glycopeptides versus adhesion (Table 5) suggested that most of the adhesion to the infected CF mucus occurred with fraction F_1c, which contained a mixture of O- and N-glycosylated glycopeptides and peptides. This fraction is largest in quantity (12.3%) and shows the highest adhesion. The mucin glycopeptides, F_1b, F_2b, and F_3a, which did not contain N glycopeptides and which made up 10.8% in total of infected mucus, were much lower in binding capacity. The noninfected CF sample showed most of its

TABLE 4. Adhesion of *P. aeruginosa* 1244 to glycopeptide fractions from individual patients with CB and CF[a]

	Fraction	No. of adherent bacteria per well (mean and range), 10^2, in sputum from patient no.: 1	2	3	4
CB	F_1b	54 (20–120)	111 (32–182)	119 (68–200)	130 (49–160)
	F_1c	169 (68–250)	39 (4–85)	254 (140–412)	231 (215–247)
	F_2b	93 (48–180)	65 (11–110)	105 (54–150)	19 (3–74)
	F_3a	<1	<1	<1	<1
CF	F_1b	59 (31–68)	69 (32–100)	19 (17–21)	158 (110–235)
	F_1c	130 (49–218)	136 (91–166)	215 (200–222)	267 (150–333)
	F_2b	38 (25–71)	30 (17–50)	<1	<1
	F_3a	<1	<1	<1	<1

[a] Sputum from CF patients no. 1, 2, and 3 were infected by *P. aeruginosa*; sputum from CF patient no. 4 was not infected.

TABLE 5. Yield of glycopeptides versus adhesion

Sputum (*n*)[a]	Fraction	Yield[b] (mg/100 mg)	Adhesion[c] (mean ± SD), 10^2
CB (5)	F_1b	11.2	91 ± 32[d]
	F_1c	6.4	139 ± 101
	F_2b	4.9	75 ± 30[d]
	F_3a	17.6	<1.0
CF, infected by *P. aeruginosa* (4)	F_1b	1.8	37 ± 23[d]
	F_1c	12.3	146 ± 42
	F_2b	2.7	28 ± 16[d]
	F_3a	6.0	<1.0
CF, noninfected	F_1b	12.0	158
	F_1c	6.9	267
	F_2b	<1.0	<1.0
	F_3a	20.1	<1.0

[a] Number of samples, including pools.
[b] Recovery per 100 mg of the crude glycopeptide preparation.
[c] Mean number of adherent bacteria per well; values of <1 were counted as 1.
[d] CB versus CF significant at $P < 0.01$ by Student's *t* test.

binding in the neutral fractions F_1b and F_1c, which made up about half of the glycopeptides recovered. The largest fraction of the noninfected CF sample, the sulfated fraction F_3a (20.1%), was devoid of binding activity. Therefore, it would appear that *P. aeruginosa* infection may cause or be associated with a lowered binding capacity to all of the most highly O-glycosylated glycopeptide portions of mucus.

F_3 binding to the microdilution plates. The striking finding that the F_3a fractions from all sputa were adhesion negative, despite the high degree of glycosylation, led us to study F_3a binding to the microdilution plates (Table 6). All F_3a fractions were bound to the plates in quantities at least as great as the F_1b fractions, which showed good adhesion. Therefore, there must be structural features of F_3a that prevented adhesion, even though the sugar composition was similar to that of the other fractions (Table 2).

DISCUSSION

These studies involving the sputum samples of 14 patients with CB or CF indicate that there are differences in bacterial binding to the glycosylated fractions of respiratory mucus which may shed some light on the dynamics of colonization of mucus by *P. aeruginosa*. (i) Within each type of mucus, the sulfated fractions did not show bacterial binding. (ii) There was less binding to the O-glycosylated fractions F_1b and F_2b of *P. aeruginosa*-infected CF mucus. (iii) The fractions that were a mixture of these O and N glycopeptides showed the greatest binding in all cases. (iv) A single uninfected CF sputum showed significant binding only in the neutral fractions, possibly more than that in infected CF sputum.

TABLE 6. Optical density readings of biotin-labeled fraction F_3a bound to microdilution plates

Prepn	Optical density[a]
Whole mucin	0.391
F_1b (CB)	0.126
F_1b (CF)	0.150
CB fractions[b]	
Pool	0.413
1	0.371
2	0.348
3	0.178
4	0.223
CF fractions[b]	
Pool	0.431
1	0.229
2	0.108
3	0.142
4	0.126

[a] Means of four determinations minus background.
[b] Numbers correspond to patients in Table 4.

The fact that the sulfated fractions showed no binding is particularly intriguing and may provide some understanding as to the functions of sulfation of mucins. Sulfation may result in chain termination of oligosaccharides and commonly occurs in some chronic obstructive lung diseases (9), including CF (3). Therefore, if bacterial binding to stagnant mucus is a pathogenetic mechanism in chronic obstructive lung diseases, then sulfation may be a mechanism to reduce binding. Sulfation does appear to alter recognition, since sulfation of the i antigen masks its recognition by some monoclonal antibodies (22) and sulfated glycosaminoglycans prevent the adhesion of bacteria to the urinary bladder (13). Although sulfate groups may provide this nonadhesive property to these glycopeptides, the sequences of the oligosaccharide chains themselves could also be the reason. Some of the nonsulfated oligosaccharide sequences from CF F_3a fractions are known, but nothing unique has been found in this fraction to date (19). The sulfated oligosaccharide chains that have been elucidated also do not show any unique oligosaccharide sequences (12). Removal of the sulfate groups without altering the oligosaccharide chains could provide an answer as to whether the sulfate groups are blocking adhesion, but attempts to desulfate these fractions have resulted in the loss of fucose and sialic acid (Ramphal and Lamblin, unpublished observations). The fact that CB mucus and noninfected CF mucus have high contents of sulfated glycopeptides (Table 5) may be significant, in that *P. aeruginosa* is absent from these patients, and leads us to ask whether the high sulfated glycopeptide content plays a protective role. Alternately, *Pseudomonas* infection could be reducing the sulfated fractions.

The significance of the other findings is not clear at this time. There appear to be mechanisms that both increase and decrease binding to CF mucus; e.g., there is less of F_1b in *P. aeruginosa*-infected CF mucus and less binding to this fraction when compared with those in noninfected CF mucus. In contrast, there is less binding to F_1c of infected CF when compared with noninfected CF but more of this fraction in infected CF. There are significant amounts of F_2b and more binding in infected CF and a virtual absence in the noninfected CF samples. If one concludes that the native state of binding in CF is that seen in the noninfected CF sample, then all of these changes probably result from infection. Similarly, the F_1b and F_2b fractions of infected CF mucus also show reduced binding when compared to CB fractions. Thus the glycopeptides from infected CF mucus shows some puzzling factures, i.e., reduced binding, reduced F_1b fractions, and the appearance of an F_1c fraction, all of which may be the result of infection.

The genesis of these differences in adhesion is speculative. It is not known whether there are differences in the glycosylation of the mucin of CF patients before versus after infection, nor whether CF mucin is different from other mucins; increased sulfation of CF mucin has, however, been reported (3, 9). There is good evidence that mucin break-

down is taking place in infected CF mucus (5a, 18), and one recent study indicates that both *P. aeruginosa* elastase and neutrophil elastase are capable of degrading mucins (14). In addition, neuraminidase from *P. aeruginosa* capable of releasing sialic acid from mucin has been described (11). Thus, the differences seen in the binding of *P. aeruginosa* to the mucin glycopeptides may be the result of enzymatic activity. How these differences in adhesion and this possible degradation fit into the scheme of the pathogenesis of *P. aeruginosa* infection in CF is not clear at this time. It could be speculated that when there is some residual mucociliary clearance in CF, mucin degradation leading to decreased adhesion could protect the organism from removal.

It is clear from the above studies that it will require the study of many noninfected CF patients to answer the question of a specific tropism of *P. aeruginosa* for CF mucins. In addition, because of amount of adhesion-positive material is so low among the mucin glycopeptides of infected CF mucus, other receptor materials in infected CF mucus, e.g., membrane glycolipids (16), may be playing a role in mucus colonization.

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