

Polysaccharide Surface Antigens Expressed by Nonmucoïd Isolates of *Pseudomonas aeruginosa* from Cystic Fibrosis Patients

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Received 17 January 1986/Accepted 10 April 1986

We tested nonmucoïd *Pseudomonas aeruginosa* isolates obtained from cystic fibrosis (CF) patients for the expression of lipopolysaccharide (LPS) serotype antigens, serum sensitivity, and production of mucoïd exopolysaccharide (MEP). When all nonmucoïd isolates were compared with a set of random mucoïd isolates, 20 of 52 (38%) nonmucoïd isolates were typable and serum resistant, compared with 13 of 51 (24%) mucoïd isolates ($P = 0.16$ by chi-square analysis). However, nonmucoïd strains from CF patients colonized only with nonmucoïd strains were more frequently typable and serum resistant (67%) than were nonmucoïd isolates from patients cocolonized with mucoïd strains (31%) ($P = 0.012$, Fisher exact test). An inhibition enzyme-linked immunosorbent assay done with bacterial extracts, a direct-whole-cell enzyme-linked immunosorbent assay done with affinity-purified antibody to MEP, and immune electron microscopy all demonstrated production of MEP by all nonmucoïd *P. aeruginosa* isolates tested, including nonmucoïd revertants of mucoïd strains. No other bacterial species tested positive in these assays. These findings suggest that MEP is produced by all *P. aeruginosa* isolates obtained from CF patients, that the initial colonizing nonmucoïd strains produce a smooth LPS, and that once LPS-rough, mucoïd strains appear in the sputum, the predominant LPS phenotype is rough regardless of colony morphology.

Respiratory tract colonization of cystic fibrosis (CF) patients by bacterial pathogens is often associated with deterioration of pulmonary function and death resulting from pulmonary failure (5, 6). The disease process has many curious aspects, including the propensity of a single pathogen, *Pseudomonas aeruginosa*, to be associated with pulmonary failure in most CF patients when they experience pulmonary failure (6, 7), the physiology of the host (5, 6), and the inability of host defenses to clear organisms despite potent immune responses to bacterial antigens (10, 27). Another interesting aspect of the disease is the emergence of mucoïd forms of *P. aeruginosa* after initial colonization with nonmucoïd organisms (11). Mucoïd strains produce an extracellular antigen, mucoïd exopolysaccharide (MEP), and are usually serum-sensitive, lipopolysaccharide (LPS)-rough isolates (9, 17, 19). Little is known, however, about the surface antigen expression of the initial colonizing nonmucoïd strains.

Information about the antigens expressed by nonmucoïd isolates that initially colonize CF patients is important in understanding the pathogenesis of *P. aeruginosa* in CF patients and in developing vaccine strategies to prevent chronic colonization. Several investigators (20, 29) have suggested that an appropriate *P. aeruginosa* vaccine needs to be administered to CF patients before colonization. It is therefore desirable to know which antigens are expressed by the initial colonizing strains, so that vaccines containing appropriate antigens can be designed. In addition, little is known about factors which mediate the initial colonization of CF patients by *P. aeruginosa*. Determining antigenic expression by the initial nonmucoïd colonizers could yield important information about the surface structures involved in colonization.

The major surface antigens of *P. aeruginosa* include LPS, high-molecular-weight polysaccharide (21), pili (18), flagella (12), outer membrane proteins (8), and possibly MEP (22). Because several studies (10, 27) have shown that the *P. aeruginosa* surface polysaccharides have an important role in CF pathogenesis, we analyzed morphologically nonmucoïd isolates obtained from CF sputum cultures, including strains isolated from patients colonized only with nonmucoïd strains, for the production of serotype antigens (expressed on LPS and high-molecular-weight polysaccharide) and MEP. In addition, we analyzed the sensitivity to normal human serum of these nonmucoïd isolates as a correlate of the production of smooth LPS (9). Susceptibility to killing by $\leq 10\%$ normal human serum has been shown to be a property of LPS-rough strains of *P. aeruginosa* (9, 17). We were interested in determining whether all nonmucoïd isolates were different from mucoïd isolates in the expression of serotype antigens, or whether nonmucoïd isolates from patients colonized only with nonmucoïd strains had a different expression of serotype antigens compared with nonmucoïd isolates obtained from patients cocolonized with mucoïd *P. aeruginosa*. For comparison, we used random mucoïd isolates of *P. aeruginosa* from CF patients in two cities. As expected, these strains were similar to previously described strains (9) in serotype antigen expression and serum sensitivity. We also determined whether nonmucoïd strains produced MEP and whether there was any difference between production of MEP by isolates from patients colonized only with these strains and isolates from patients cocolonized with mucoïd strains.

MATERIALS AND METHODS

Bacterial strains. Mucoïd and nonmucoïd *P. aeruginosa* strains from sputum cultures of CF patients were isolated in the microbiology laboratories at Children's Hospital, Bos-

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ton, Mass., and British Columbia Children's Hospital, Vancouver, British Columbia, Canada. Subcultures were supplied courtesy of Anne MaCone and Donald Goldmann (Children's Hospital, Boston, Mass.) or obtained by one of us (D.P.S., Vancouver, Canada). All isolates were confirmed as *P. aeruginosa* by clinical laboratory analysis. Nonmucoid revertants were derived from mucoid strains as described previously (22). Four *Pseudomonas cepacia* strains from CF patients were obtained from Donald Goldmann. Isolates of other gram-negative and gram-positive organisms were obtained from the microbiology laboratory of the Brigham and Women's Hospital, Boston, Mass., or from laboratory stocks.

Antigens and antisera. Purified MEP from *P. aeruginosa* 2192 and rabbit antisera to this antigen were prepared as described previously (22). Affinity-purified antibody to MEP was prepared as described previously (1) by using MEP bound to epoxy-activated Sepharose as an affinity column.

Serotyping and serum sensitivity. Serotyping by agglutination was performed as described previously (23) with either rabbit antisera raised to purified LPS obtained from the Fisher immunotype strains of *P. aeruginosa* or with a commercially available antisera typing set (Bacto-*Pseudomonas aeruginosa* Antisera Set; Difco Laboratories, Detroit, Mich.). For uniformity, serotype results were expressed by the International Antigenic Typing System, which can be readily correlated with the Fisher immunotypes (23). Sensitivity to 5% normal human serum was also tested as described previously (9) with quantitative plate counts.

Inhibition ELISA for MEP. An inhibition enzyme-linked immunosorbent assay (ELISA) was developed to test for the presence of MEP in bacterial extracts. ELISA plates (Immunolon 2; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 200 μ l of MEP (1 μ g/ml) in 0.04 M phosphate buffer (pH 7.0) as described by Bryan et al. (4). Bacteria were grown overnight on tryptic soy agar plates at 37°C. Extracts were prepared by suspending the growth in 0.04 M phosphate buffer (pH 8.6) to an optical density at 650 nm of 1.0 in a screw cap tube (16 by 125 mm). These were heated at 60°C for 1 h (conditions sufficient to inactivate *P. aeruginosa* proteases [16]), the bacteria were removed by centrifugation, and the supernatant was filtered through a 0.45- μ m-pore-size filter. The extracts were diluted 1:3 in 0.04 M phosphate buffer (pH 8.6) containing 0.05% Tween 20. The diluted extract (100 μ l) was mixed with 100 μ l of a 1:800 dilution of immune rabbit serum (in 0.04 M phosphate buffer [pH 8.6] with 0.05% Tween 20) and incubated at 37°C overnight. Purified MEP at concentrations of 0.1 to 250 μ g/ml in buffer was also mixed with immune serum and incubated in a similar manner to generate a standard curve. The mixtures were then added in duplicate to sensitized wells and incubated at 37°C for 4 h. The wells were washed three times with phosphate buffer-Tween 20, and 200 μ l of a 1:3,000 dilution of goat anti-rabbit immunoglobulin G (IgG) covalently linked to alkaline phosphatase (Cooper Biomedical, Inc., West Chester, Pa.) was added. After 2 h of incubation at 37°C the wells were washed with phosphate buffer-Tween 20, and 200 μ l of *para*-nitrophenyl phosphate (1.0 mg/ml in 0.1 M glycine [pH 10.4] containing 0.001 M MgCl₂ and ZnCl₂) was added. Color development at 405 nm was determined at 60 min in an ELISA reader. For the standard curve, we calculated the linear regression formula and correlation coefficient (r^2) of the log₁₀ concentration of MEP versus the average optical density at 405 nm of duplicate samples. The curve was linear over the range of 0.3

to 250 μ g/ml. The derived formula ($r^2 = -0.97$) was used to determine the concentration of MEP in the bacterial extracts.

Direct-bacterial-cell ELISA. We also developed a whole-bacterial-cell ELISA based on the method of Borowski et al. (3) to probe different strains with an affinity-purified antibody to MEP. Bacteria were suspended in 0.04 M phosphate-0.05% Tween 20 to an optical density at 650 nm of 1.0 in screw cap tubes (16 by 125 mm). A 200- μ l sample of the solution was added to the ELISA plates and incubated at 4°C overnight. The plates were washed with phosphate buffer-Tween 20, and a 1:100 dilution of affinity-purified rabbit antibody to MEP was added. The plates were incubated at 37°C for 3 h and washed with phosphate buffer-Tween 20, and a 1:3,000 dilution of goat anti-rabbit IgG was then added. This was incubated for 2 h at 37°C, the plates were washed with phosphate buffer-Tween 20, and *para*-nitrophenyl phosphate was added as described above. The A₄₀₅ was read 60 min later, and the average optical density for strains in the various groups was calculated.

Carbazole assay for uronic acids. The modified carbazole assay of Knutson and Jeanes (13) was used to detect uronic acids in bacterial extracts.

Immune electron microscopy. Immune electron microscopy was performed on agar-grown cells of mucoid and nonmucoid strains with antiserum raised to purified MEP (22). Bacteria were suspended in saline and gently pelleted (3,000 rpm) in a glass tube. The pellet was suspended in either control (normal) or immune serum for 2 h at room temperature, washed three times with phosphate-buffered saline, and suspended in a 1:40 dilution of ferritin-labeled goat anti-rabbit IgG. After 2 h at room temperature, the cells were washed three times with phosphate-buffered saline, washed once with 0.1 M cacodylate buffer (pH 7.2), and dehydrated in graded ethanol concentrations in cacodylate buffer for 10 min each until 100% ethanol was reached. The material was kept in 100% ethanol for 1 h, placed in 50% ethanol-50% Epon 812 for 2 h, and put into 100% Epon overnight. The pellets were fixed with 2% glutaraldehyde overnight and postfixed with 1% osmium tetroxide for 2 h. Pellets (1 mm²) were placed in Beem capsules, filled with Epon, and polymerized at 65°C for 48 h. The capsules were removed, the Epon block was left at room temperature for an additional 48 h, and the block was sectioned on an ultramicrotome. Sections (600 nm) were mounted on grids, stained with 0.5% uranyl acetate and 0.4% lead citrate, and examined in a JEOL JEM 100-B electron microscope.

Statistical methods. Chi-square analyses and Fisher exact probabilities were calculated on the Harvard Medical Area Vax/Unix system with the available software packages. Mann-Whitney U tests were also performed with the Minitab software system (26) on this computer.

Clinical data. Information regarding the length of time that CF patients (from Boston only) were colonized by *P. aeruginosa* was obtained by a review of clinical records. The records were reviewed in the absence of information regarding a patient's isolates. The onset of colonization was dated from the month and year of the first sputum culture that yielded *P. aeruginosa* isolates. Patients whose isolates came from the first positive sputum culture were arbitrarily said to have been colonized for 1 month.

RESULTS

Serotypes and serum sensitivity of *P. aeruginosa* isolates. The serotype distribution and serum sensitivity patterns of

TABLE 1. Serotype distribution and serum sensitivity patterns of mucoid and nonmucoid *P. aeruginosa* isolates from CF patients

IATS ^a type	No. of mucoid strains (n = 51)		No. of nonmucoid strains (n = 52)	
	Serum resistant	Serum sensitive	Serum resistant	Serum sensitive
1	3	1	7	3
4	1	1	1	
5	4	2	2	
6	4	2	8	2
7/8			1	
9		1		1
10		1		1
11	1		1	
14		1		1
Nontypable ^b	6	23	4	20

^a IATS, International Antigenic Typing System.

^b Strains that either did not agglutinate in typing serum or agglutinated in multiple typing sera.

all nonmucoid *P. aeruginosa* isolates obtained from CF patients were compared with data from a set of mucoid isolates obtained from patients seen in the CF clinic at the same time (Table 1). Thirteen of 51 (26%) mucoid strains were both serotypable and serum resistant, compared with 20 of 52 (38%) nonmucoid strains ($P = 0.16$, chi-square analysis). Twenty-three of 51 (46%) mucoid strains were both nontypable and serum sensitive, compared with 20 of 52 (38%) nonmucoid strains ($P = 0.49$, chi-square analysis). Thus, there were no readily discernible differences between mucoid and nonmucoid *P. aeruginosa* isolates in nontypability or serum sensitivity, dual properties of LPS-rough strains.

Previous investigators (2, 28) suggested that there are no major phenotypic differences between mucoid and nonmucoid *P. aeruginosa* isolates from the same patient, with the obvious exception of mucoid growth. To determine whether nonmucoid isolates obtained from CF patients colonized only with nonmucoid strains were different from nonmucoid isolates obtained from patients cocolonized with mucoid strains, we compared the serotypes and serum sensitivities of the two groups. As determined by a review of clinical records, the median length of bacterial colonization for patients harboring only nonmucoid isolates (3.2 months) was significantly shorter ($P < 0.001$, Mann-Whitney U Test) than the median length of bacterial colonization of patients harboring mucoid isolates as well (35 months). In fact, the nonmucoid strains obtained from 17 of 24 CF patients harboring only nonmucoid strains came from the first sputum culture positive for *P. aeruginosa*, suggesting that most nonmucoid strains represented the initial colonizing *P. aeruginosa* strain from these patients. The serotype distribution and serum sensitivity of nonmucoid isolates from patients harboring only nonmucoid strains were then compared with that of nonmucoid isolates from patients cocolonized with mucoid strains (Table 2). Sixteen of 24 (67%) isolates from patients colonized only with nonmucoid strains were serotypable and serum resistant, compared with 8 of 26 (31%) nonmucoid isolates from patients cocolonized with mucoid *P. aeruginosa* ($P = 0.012$, Fisher exact test). Of 24 isolates from patients with nonmucoid strains, only 6 (25%), were nontypable and serum sensitive, compared with 15 of 26 (58%) nonmucoid strains from patients cocolonized with mucoid strains ($P = 0.019$, Fisher exact test). Interestingly,

only two serotypes (1 and 6) were documented among the isolates from patients harboring only nonmucoid strains. The implication of this finding cannot be fully determined because these strains came from patients in only one area (Boston, Mass.). Thus, strains from patients harboring only nonmucoid isolates were significantly more likely to be serotypable and serum resistant than were nonmucoid isolates from patients cocolonized with mucoid *P. aeruginosa*.

Expression of MEP by nonmucoid isolates. We tested the nonmucoid isolates for expression of MEP by the two serologic methods described in Materials and Methods. Extracts of nonmucoid strains from patients colonized only with these isolates, as well as extracts of nonmucoid strains from patients also harboring mucoid isolates, contained high levels of MEP (Table 3). There was no significant difference ($P > 0.05$, Mann-Whitney U test) between the amounts of MEP produced by these two sets of nonmucoid isolates. Extracts prepared from a set of nonmucoid revertants derived from mucoid strains also contained high levels of MEP. All other bacterial species tested, including several *P. cepacia* strains from CF patients, yielded extracts that were negative for MEP.

The second serologic assay, which used an affinity-purified rabbit antibody to MEP to probe bacterial strains immobilized in ELISA plates, yielded positive antibody reactions with all nonmucoid isolates and nonmucoid revertants of *P. aeruginosa*, whereas all other bacterial species were negative (Table 3). This assay confirmed the results of the inhibition ELISA, showing that nonmucoid isolates of *P. aeruginosa* produce MEP.

Comparison of colorimetric and serologic assays for MEP. We next used the carbazole assay for uronic acids to quantitate MEP constituents in extracts from 12 nonmucoid *P. aeruginosa* strains. Of these 12 extracts, 10 were negative in the carbazole assay, and the mean micrograms of MEP per milliliter detected by carbazole was considerably lower than that detected by the inhibition ELISA (Table 4). To determine whether extract constituents interfered with the carbazole assay, we added purified MEP (500 $\mu\text{g/ml}$) to extracts from five strains negative for MEP by the carbazole assay and measured the uronic acid content. A control containing 500 μg of alginic acid per ml in bacterial extracts was also tested. We found that only about 30% of the MEP or alginic acid was detected by the carbazole assay in the presence of bacterial extract (Table 4), indicating that components of these extracts interfere in the colorimetric assay.

Visualization of MEP on mucoid and nonmucoid *P. aeruginosa* strains by immune electron microscopy. Electron micro-

TABLE 2. Serotype distribution and serum sensitivity pattern of nonmucoid *P. aeruginosa* isolates from CF patients harboring only nonmucoid strains or both mucoid and nonmucoid strains

IATS ^a type	No. of strains (nonmucoid only) (n = 24)		No. of strains (mucoid and nonmucoid) (n = 26)	
	Serum resistant	Serum sensitive	Serum resistant	Serum sensitive
1	8	2	3	1
5			2	
6	8		2	
11			1	
Nontypable ^b		6	2	15

^a IATS, International Antigenic Typing System.

^b Strains that either did not agglutinate in typing serum or agglutinated in multiple typing sera.

TABLE 3. Production of MEP and binding of affinity antibody in nonmucoid *P. aeruginosa* and other bacteria^a

Bacterial species	No. of strains	Mean μg of MEP per ml \pm SD (range) in bacterial extracts	Mean OD ₄₀₅ ^b \pm SD (range) of affinity antibody binding to bacteria
<i>Pseudomonas aeruginosa</i> from patients colonized with			
Nonmucoid strains only	13	66 \pm 42 (18–151)	0.87 \pm 0.51 (0.2–1.4)
Mucoid and nonmucoid strains	40	46 \pm 42 (2–182)	0.73 \pm 0.52 (0.1–1.3)
Nonmucoid revertants	10	54 \pm 22 (7–88)	0.88 \pm 0.45 (0.3–1.5)
<i>Escherichia coli</i>	8	<0.3 ^c	0
<i>Streptococcus pyogenes</i>	3	<0.3	0
<i>Staphylococcus aureus</i>	3	<0.3	0
<i>Klebsiella oxytoca</i>	2	<0.3	0
<i>Klebsiella pneumoniae</i>	1	<0.3	0
<i>Pseudomonas cepacia</i>	4	<0.3	0

^a Data were obtained by an inhibition ELISA to test bacterial extracts for MEP and a direct-bacterial-cell ELISA done with affinity-purified antibody to MEP.

^b OD, Optical density.

^c Lower limit of detection of MEP in extracts.

graphs of mucoid and nonmucoid strains treated with rabbit antiserum raised to purified MEP were taken to visualize the antigen on and around the bacterial cells. The micrographs of two mucoid strains and the nonmucoid revertant of one of them (Fig. 1) showed an extracellular material reacting with the antiserum; the material was located near the bacterial surface and was much like a capsule. In addition, ferritin particles were seen bound to aggregates of intercellular debris, indicating the presence of MEP in the aggregates. Micrographs of three nonmucoid strains isolated from patients colonized only with nonmucoid strains showed that most of the material binding the ferritin label was located between the individual cells and was not tightly bound to the cell surface (Fig. 2). This indicated that the MEP synthesized by the nonmucoid strains did not remain tightly bound to the bacterial surface but was released into the intercellular milieu.

DISCUSSION

The circumstances that predispose CF patients to colonization by *P. aeruginosa* are not well understood. The most critical factors likely reside within the host. Yet for CF patients *P. aeruginosa* is by far and away the most commonly encountered pathogen that leads to severe morbidity and ultimate mortality. Thus, pathogenic mechanisms unique to *P. aeruginosa* must be involved in the disease process. Because two morphologically different types of organism are routinely obtained from CF patients, we sought to determine whether these isolates were different in the expression of serotype antigens or resistance to normal

human serum and whether nonmucoid isolates produced MEP. A number of studies (9, 17, 18) have shown that most (60 to 80%) mucoid isolates from CF patients are nontypable, serum sensitive, LPS-rough organisms. Our study extended these observations to nonmucoid isolates obtained from patients cocolonized with mucoid isolates. Our study also showed that when nonmucoid isolates were obtained from patients colonized only with these strains, they were usually serotypable, serum resistant, and, therefore, LPS-smooth organisms.

Because nonmucoid isolates from CF patients harboring only nonmucoid isolates (17 of 24 patients) were usually the first *P. aeruginosa* strains clinically obtained, our results suggest that the early colonizing strains phenotypically express a smooth LPS. When nonmucoid isolates were tested for the production of MEP, we were able to confirm the presence of MEP by two serologic assays and by immune electron microscopy. No other bacterial species tested positive for MEP in the serologic assays, implying the uniqueness of this antigen to *P. aeruginosa*. The serologic and electron microscopic demonstration of MEP production by nonmucoid *P. aeruginosa* was consistent with our previous finding that a nonmucoid strain can elicit rabbit antibody to MEP (22). Interestingly, the electron micrographs showed that the differences in MEP production between mucoid and nonmucoid strains may not have been only quantitative but may also have involved the ability of mucoid strains to retain MEP on their surface. Nonmucoid strains appeared to release most of their MEP antigen into the intercellular milieu. The production of MEP by nonmucoid strains was also consistent with the finding of Woods and Bryan (29) that antibody to MEP was able to protect rats from chronic colonization with a nonmucoid *P. aeruginosa* strain.

Our data also showed that the traditional carbazole assay for uronic acids is not sensitive enough to detect MEP in extracts of nonmucoid strains. In fact, we showed that the addition of purified MEP or alginate acid to bacterial extracts resulted in the detection of only 30% of this material by the carbazole assay. Thus, caution must be exercised in describing a *P. aeruginosa* isolate as negative for MEP when the carbazole assay is used. In fact, the two extracts of nonmucoid strains that tested positive by the carbazole assay contained the highest amount of MEP that could be detected by the serologic test.

The results reported here have multiple implications. The

TABLE 4. Comparison of ELISA and carbazole assay for detection of MEP production by nonmucoid *P. aeruginosa* isolates

Material tested (n)	Amt ($\mu\text{g}/\text{ml}$ \pm SD) (range) of uronic acid or MEP detected by:	
	Carbazole assay	ELISA inhibition
Bacterial extracts (12)	5 \pm 13 (0–43)	50 \pm 45 (9–132)
MEP (500 μg) in bacterial extracts (5)	151 \pm 83 (141–208)	ND ^a
Alginate acid [500 μg in bacterial extracts (5)]	148 \pm 17 (123–172)	ND

^a ND, Not done.

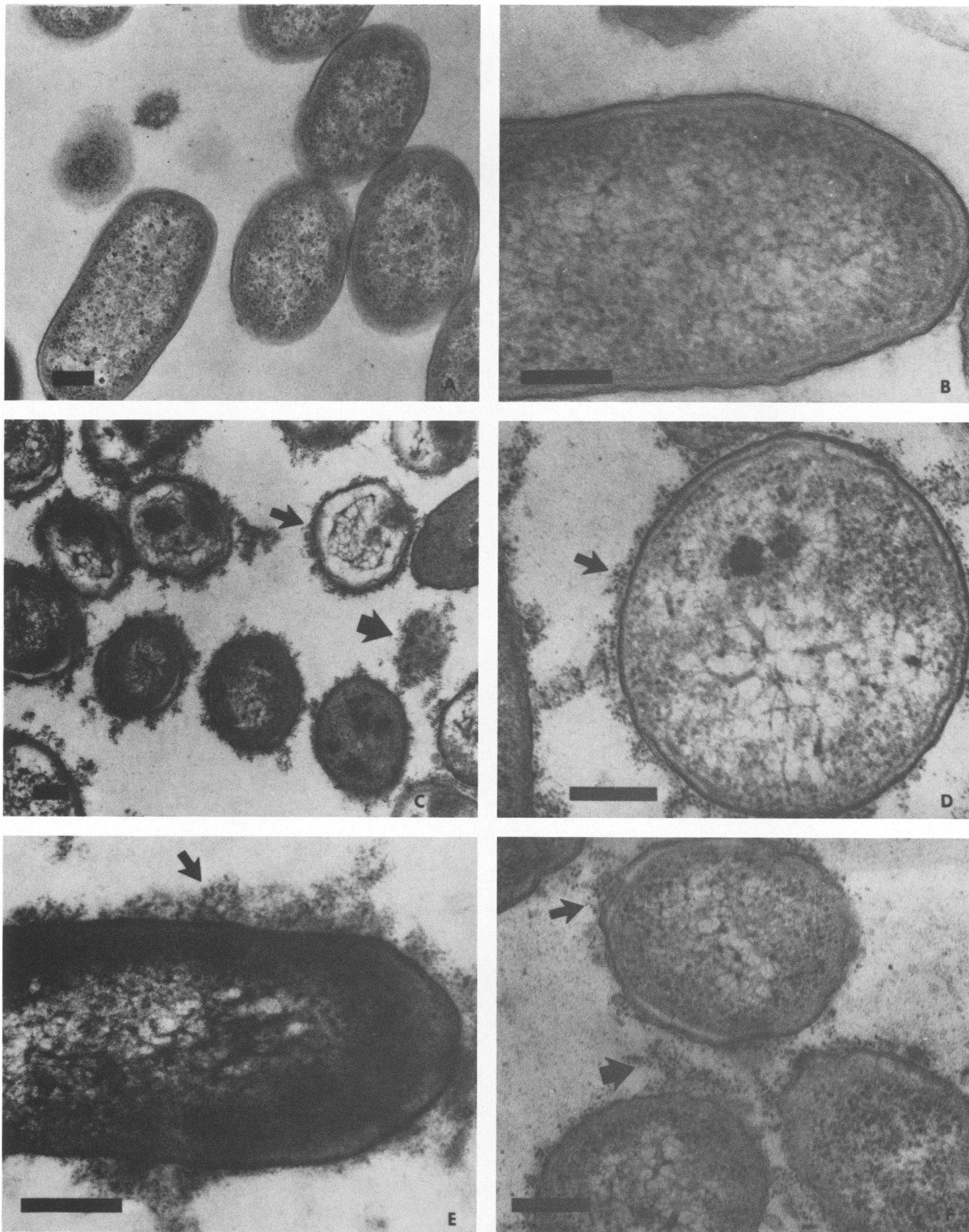


FIG. 1. Immune electron micrographs of mucoid *P. aeruginosa* 2192 and 258 and the nonmucoid revertant of strain 2192, treated with normal or immune sera raised to purified MEP and goat anti-rabbit IgG covalently coupled to ferritin. In each micrograph the bar represents 400 nm. (A) Mucoid strain 2192 treated with normal serum and ferritin conjugate. Magnification, $\times 40,000$. (B) Mucoid strain 258 treated with normal serum and ferritin conjugate. Magnification, $\times 105,000$. (C) Mucoid strain 2192 treated with immune serum and ferritin conjugate. Magnification, $\times 45,000$. Note binding of the ferritin both to the cell surface (thin arrow) and intercellular debris (thick arrow). (D) Panel C at a higher power (magnification, $\times 95,000$), showing cell-bound ferritin deposits (arrow). (E) Mucoid strain 258 treated with immune serum and ferritin conjugate, showing ferritin deposits close to the cell surface (arrow). Magnification, $\times 116,000$. (F) Nonmucoid revertant strain 2192 treated with immune serum and ferritin conjugate, showing both surface-bound (thin arrow) and intercellular (thick arrow) ferritin deposits. Magnification, $\times 90,000$.

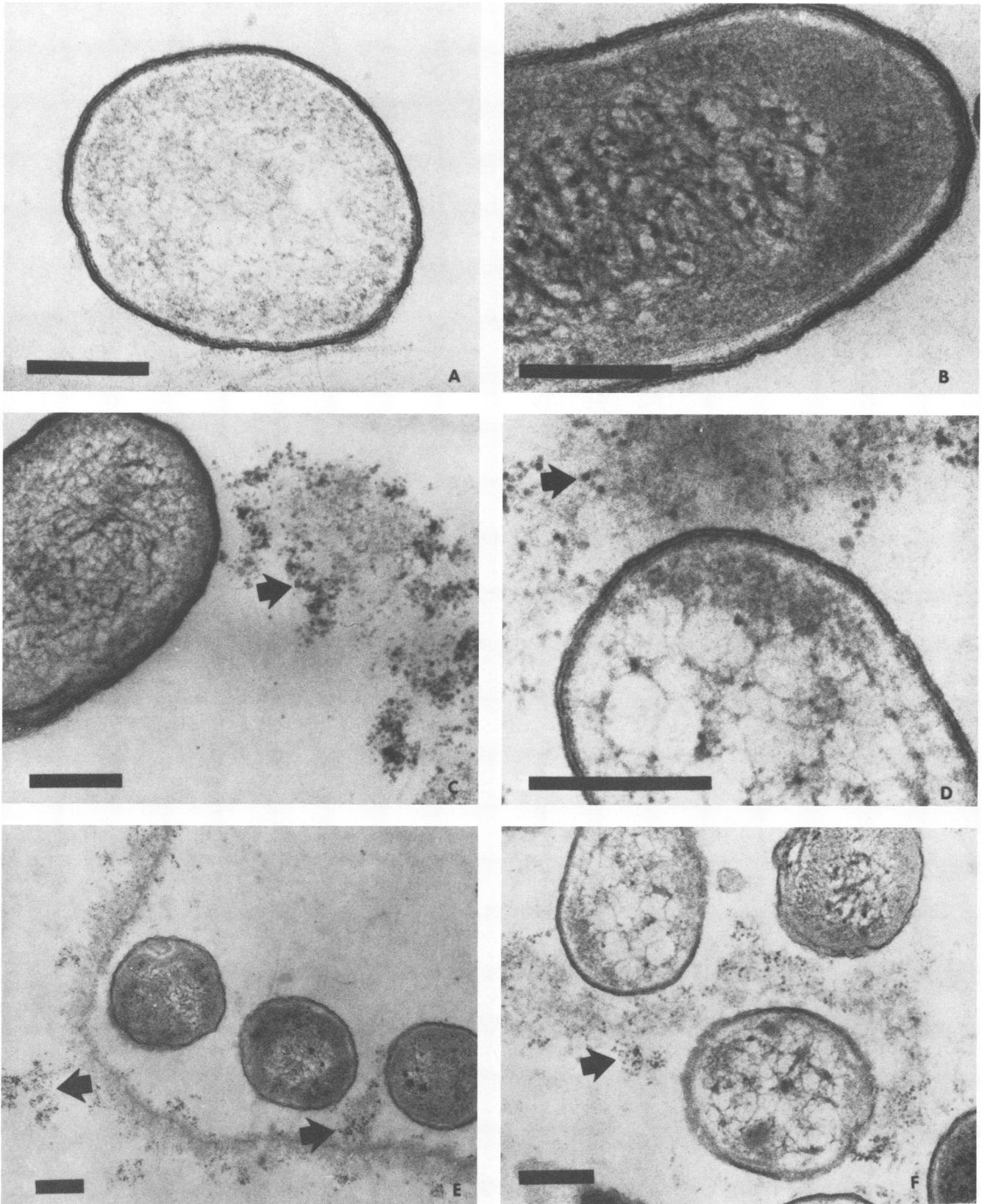


FIG. 2. Immune electron micrographs of three nonmucoid *P. aeruginosa* isolates from CF patients colonized only with nonmucoid strains. In each micrograph the bar represents 400 nm. (A) Nonmucoid strain 7606 treated with normal rabbit serum and goat anti-rabbit IgG covalently coupled to ferritin. Magnification, $\times 144,000$. (B) Nonmucoid strain 7606 treated with normal serum and ferritin conjugate. Magnification, $\times 180,000$. (C) Nonmucoid strain 7066 treated with immune serum and ferritin conjugate. Magnification, $\times 108,000$. Note lack of cell-surface-associated ferritin deposits and prominence of intercellular ferritin deposits (arrow). (D) Nonmucoid strain 7606 treated with immune serum and ferritin conjugate. Magnification, $\times 217,500$. Ferritin granules are almost exclusively seen in the intercellular material (arrow). (E) Nonmucoid strain 7441 treated with immune serum and ferritin conjugate. Magnification, $\times 54,000$. Note intercellular deposits of ferritin (arrows) and ribbon of material binding ferritin (arrow). (F) Nonmucoid strain 7606 treated with immune serum and ferritin conjugate. Magnification, $\times 90,000$. Note ribbon of material binding ferritin (arrow).

finding that the nonmucoid strains obtained from CF patients harboring only nonmucoid strains were more likely to be LPS smooth implies that CF patients become colonized by exposure to *P. aeruginosa* strains commonly found in the environment. Furthermore, it is likely that the emergence of serum-sensitive, LPS-rough mucoid isolates results from selective pressures of the host, possibly including antibody or other immune responses. However, this conclusion can be supported only by a longitudinal study that characterizes serial *P. aeruginosa* isolates and sera from individual patients. The isolation of variant strains that are serotypable yet serum sensitive, as well as strains that are nontypable yet serum resistant (Tables 1 and 2), was consistent with the hypothesis that host responses to colonization put selective pressures on *P. aeruginosa* that result in the emergence of nontypable, serum-sensitive isolates. The variant strains appeared to represent an intermediate step between the early and later colonizing strains.

The finding that the early nonmucoid colonizing strains usually express serotype antigens raises the possibility that vaccines eliciting antibody to these antigens could protect CF patients. However, the data do not support this conclusion. First, a role for type-specific antigens in the pathogenesis of *P. aeruginosa* colonization of CF patients has not been established. Ramphal et al. (24) showed that nonmucoid *P. aeruginosa* adheres to acid-injured murine tracheal tissues via pili. This observation was recently extended to the adherence of nonmucoid strains to human tracheobronchial mucins (R. Ramphal, C. Guay, and G. B. Pier, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 111, 1985). Thus, antibody to serotype determinants likely will not prevent adherence. It is possible that type-specific antibody opsonizes bacteria for phagocytic killing. However, the predominant antibody of the upper respiratory tract, secretory IgA, cannot mediate the opsonophagocytic killing of *P. aeruginosa* (25). Second, antibody to the International Antigenic Typing System serotype 1 determinant is almost universally present at high levels in the sera of normal adults and children, including CF patients not colonized by *P. aeruginosa* (unpublished observation). This serotype was the most common one among our typable strains, suggesting that the presence of antibody to this determinant is not protective. Third, Langford and Hiller (14) recently completed a trial of a *P. aeruginosa* vaccine in noncolonized CF patients that failed to demonstrate protection against colonization. This vaccine was recently characterized, and its active components appear to be type-specific antigens, such as LPS (15). Fourth, preliminary data from our laboratory (G. B. Pier and J. Saunders, Clin. Res. 33:415A, 1985), indicate that older (>15 years) CF patients who have escaped colonization by *P. aeruginosa* all have high levels of opsonophagocytic killing antibody directed at MEP and not against serotype antigens. This finding is consistent with the demonstration that all nonmucoid isolates from CF patients express MEP. Studies are under way to determine whether antibody to MEP can mediate the opsonophagocytic killing of nonmucoid strains.

These results on surface polysaccharide antigens expressed by nonmucoid *P. aeruginosa* isolates should contribute to our understanding of the interaction between this organism and CF patients. The differences between the initial colonizing nonmucoid strains and the strains isolated later in the disease process imply a dynamic interaction of host and bacterial factors that allows chronic colonization to occur. A better understanding of these interactions could

prove critical in the prevention and control of *P. aeruginosa* colonization of CF patients.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI 22806 from the National Institute of Allergy and Infectious Diseases and by a Research Scholar Award to G.B.P. from the Cystic Fibrosis Foundation.

We thank Gail Gay for secretarial assistance in the preparation of this manuscript.

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