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

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We tested nonmucoid Pseudomonas aeruginosa isolates obtained from cystic fibrosis (CF) patients for the expression of lipopolysaccharide (LPS) serotype antigens, serum sensitivity, and production of mucoid exopolysaccharide (MEP). When all nonmucoid isolates were compared with a set of random mucoid isolates, 20 of 52 (38%) nonmucoid isolates were typable and serum resistant, compared with 13 of 51 (24%) mucoid isolates (P = 0.16 by chi-square analysis). However, nonmucoid strains from CF patients colonized only with nonmucoid strains were more frequently typable and serum resistant (67%) than were nonmucoid isolates from patients cocolonized with mucoid 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 nonmucoid P. aeruginosa isolates tested, including nonmucoid revertants of mucoid 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 colonization nonmucoid strains produce a smooth LPS, and that once LPS-rough, mucoid 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 mucoid forms of P. aeruginosa after initial colonization with nonmucoid organisms (11). Mucoid strains produce an extracellular antigen, mucoid 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 nonmucoid strains.

Information about the antigens expressed by nonmucoid 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 nonmucoid 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 nonmucoid isolates obtained from CF sputum cultures, including strains isolated from patients colonized only with nonmucoid 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 nonmucoid isolates as a correlate of the production of smooth LPS (9). Susceptibility to killing by ≤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 nonmucoid isolates were different from mucoid isolates in the expression of serotype antigens, or whether nonmucoid isolates from patients colonized only with nonmucoid strains had a different expression of serotype antigens compared with nonmucoid isolates obtained from patients cocolonized with mucoid P. aeruginosa. For comparison, we used random mucoid 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 nonmucoid 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 mucoid strains.

MATERIALS AND METHODS

Bacterial strains. Mucoid and nonmucoid P. aeruginosa strains from sputum cultures of CF patients were isolated in the microbiology laboratories at Children’s Hospital, Bos-
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 Fish serotyping type strains of P. aeruginosa or with a commercially available antisera typing set (Bacto-Pseudomonas aeruginosa Antisa 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 μl of MEP (1 μg/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-μm-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 μl) was mixed with 100 μl 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 μg/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 μl 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 μl 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 (r²) 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 μg/ml. The derived formula (r² = −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-μl 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 Jennes (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 antisera 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 mm³) 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

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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 colonized 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 spumum 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 colonized 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 colonized 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 colonized 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*.

### 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

<table>
<thead>
<tr>
<th>IATS* type</th>
<th>No. of strains (nonmucoid only)</th>
<th>No. of strains (mucoid and nonmucoid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum resistant</td>
<td>Serum sensitive</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nontypable</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

*a* IATS, International Antigenic Typing System.

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

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 µg/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 3. Production of MEP and binding of affinity antibody in nonmucoid P. aeruginosa and other bacteria

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No. of strains</th>
<th>Mean µg of MEP per ml ± SD (range) in bacterial extracts</th>
<th>Mean OD₄₀₅nm ± SD (range) of affinity antibody binding to bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa from patients colonized with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonmucoid strains only</td>
<td>13</td>
<td>66 ± 42 (18-151)</td>
<td>0.87 ± 0.51 (0.2-1.4)</td>
</tr>
<tr>
<td>Mucoid and nonmucoid strains</td>
<td>40</td>
<td>46 ± 42 (2-182)</td>
<td>0.73 ± 0.52 (0.1-1.3)</td>
</tr>
<tr>
<td>Nonmucoid revertants</td>
<td>10</td>
<td>54 ± 22 (7-88)</td>
<td>0.88 ± 0.45 (0.3-1.5)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>8</td>
<td>&lt;0.3c</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>3</td>
<td>&lt;0.3</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3</td>
<td>&lt;0.3</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>2</td>
<td>&lt;0.3</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>1</td>
<td>&lt;0.3</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas cepacia</td>
<td>4</td>
<td>&lt;0.3</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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.
* OD, Optical density.
* Lower limit of detection of MEP in extracts.

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

<table>
<thead>
<tr>
<th>Material tested (n)</th>
<th>Amt (µg/ml ± SD) (range) of uronic acid or MEP detected by:</th>
<th>Carbazole assay</th>
<th>ELISA inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial extracts (12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEP (500 µg) in bacterial extracts (5)</td>
<td>5 ± 13 (0-43)</td>
<td>50 ± 45 (9-132)</td>
<td></td>
</tr>
<tr>
<td>Alginic acid [500 µg in bacterial extracts (5)]</td>
<td>151 ± 83 (141-208)</td>
<td>ND*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>148 ± 17 (123-172)</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* ND, Not done.

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 colonized 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 alginic 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
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, ×40,000. (B) Mucoid strain 258 treated with normal serum and ferritin conjugate. Magnification, ×105,000. (C) Mucoid strain 2192 treated with immune serum and ferritin conjugate. Magnification, ×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, ×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, ×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, ×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, ×144,000. (B) Nonmucoid strain 7066 treated with normal serum and ferritin conjugate. Magnification, ×180,000. (C) Nonmucoid strain 7066 treated with immune serum and ferritin conjugate. Magnification, ×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, ×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, ×54,000. Note intercellular deposits of ferritin (arrows) and ribbon of material binding ferritin. (F) Nonmucoid strain 7606 treated with immune serum and ferritin conjugate. Magnification, ×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 nonmucoid, 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 antibodies 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 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.

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LITERATURE CITED


