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Pseudomonas aeruginosa Isolates from Patients with Cystic Fibrosis: A Class of Serum-Sensitive, Nontypable Strains Deficient in Lipopolysaccharide O Side Chains

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Twenty-six *Pseudomonas aeruginosa* strains from patients with cystic fibrosis were typed by the Fisher immunotyping scheme. Only 6 strains were agglutinated by a single typing serum, whereas 15 strains were agglutinated with more than one serum and 5 were not agglutinated by any serum. Neither the polyagglutinable nor the nonagglutinable strains were typable by hemagglutination inhibition or immunodiffusion, suggesting that these polyagglutinable strains did not express multiple serotype antigens, but were instead being agglutinated by antibody to nonserotype determinants. Four typable isolates were resistant to pooled normal human serum, whereas the 12 polyagglutinable and nonagglutinable isolates studied were very sensitive to normal human serum. The outer membranes of 16 strains were isolated and characterized. The data suggested, in general, strong conservation of outer membrane protein patterns. Lipopolysaccharides (LPS) were purified by a new technique which allowed isolation of both rough and smooth LPS in high yields. Three of four typable, serum-resistant strains examined had amounts of smooth, O-antigen-containing LPS equivalent to our laboratory wild type, *P. aeruginosa* PAO1 strain H103. In contrast, 10 of 12 polyagglutinable or nonagglutinable, serum-sensitive strains had very little or no smooth, O-antigen-containing LPS, and the other two contained less smooth LPS than our wild-type strain H103. In agreement with this data, five independent, rough, LPS O-antigen-deficient mutants of strain H103 were nontypable and serum sensitive. We suggest that the LPS defects described here represent a significant new property of many *P. aeruginosa* strains associated with cystic fibrosis.

The basis of serotyping *Pseudomonas aeruginosa* is variations in the O-antigenic side chain composition of the lipopolysaccharides (LPS) of different strains (2). A variety of typing systems have been described (1, 18) demonstrating as many as 17 distinct serotypes. The most common method of serotype determination is slide agglutination of heat-killed bacteria with typing sera. One of the problems (18) with such characterizations, particularly with cystic fibrosis isolates (27, 31), is the high frequency of non-, self-, or polyagglutinable strains. Since it has been demonstrated that some rough mutants of *P. aeruginosa* which are lacking the O-antigenic portion of LPS are capable of self-agglutinating in 4% NaCl solutions (16), whereas the lack of O-antigen might be expected to result in nontypability by O-antigen-specific sera, we considered here the possibility that some of the nontypable or polyagglutinable isolates of *P. aeruginosa* from patients with cystic fibrosis were deficient in O-antigen.

Interestingly, evidence has been obtained (2, 30) which suggests that some *P. aeruginosa* strains are very deficient in O-antigenic LPS. One can calculate that the mole percent of smooth-type molecules in *P. aeruginosa* LPS varies from 0.2 to 13.7 mol% of the total LPS (calculated from data [2, 30] using molecular weights of 14,000 and 3,000, respectively, for smooth and rough LPS oligosaccharides [13]). This agrees with an independent calculation of 5 to 11 mol% by Wilkinson in a recent review (S. G. Wilkinson, Rev. Infect. Dis., in press). In the above studies, the LPS of a number of strains which were typed as Habs serotypes 7, 8, and 12 in the study of Chester et al. (2) as well as others of serotypes 9 and 13 in the study of

Wilkinson and Galbraith (30) contained <1.7 mol% of smooth O side chains. Thus, it can be concluded that *P. aeruginosa* isolates contain less smooth-type LPS than, e.g., natural *Salmonella* sp. or *Escherichia coli* isolates (8).

Recently, Darveau and Hancock (3) demonstrated a new method of LPS isolation which is capable of isolating both smooth- and rough-type LPS with equal efficiency and in high yield (60 to 80% of the total cellular LPS). Such a method was shown to have advantages over the classical Westphal and Jann (29) procedure, which favors isolation of smooth LPS, as well as the Galanos et al (7) procedure, which favors rough LPS isolation. For the isolation of *P. aeruginosa* LPS, this is important due to the lack of correlation between colony morphology and LPS type (19), as well as to the heterogeneous composition of the LPS in wild-type organisms and the demonstration that the Westphal and Jann phenol-water technique is not effective in the isolation of LPS from some *P. aeruginosa* strains (3, 26). In this paper, a strong correlation between the apparent numbers of O side chains, sensitivity to serum, and agglutination patterns with Fisher typing sera was found for isolates from patients with cystic fibrosis.

MATERIALS AND METHODS

Bacterial strains. The strains used as a basis of comparison for these experiments were *P. aeruginosa* PAO1 strains H103 (9) and K799 (17), which were originally obtained as human clinical isolates. Twenty-six distinct clinical isolates from patients with cystic fibrosis were obtained from Ann Macone, Children's Hospital Medical Center, Boston, Mass., and used for serotyping. Twelve of these were selected for further study and named as follows: CF 221, CF 283, CF 284, CF 832, CF 1278, CF 1452, CF 2314, CF 3790, CF 4349, CF 4522, CF 6094, and CF 9490. In addition, two mucoid isolates, CF P1M (L. M. Mutharia and R. E. W. Hancock, submitted for publication) and CF C96M, were obtained from cystic fibrosis patients at the University of Minnesota Hospitals, Minneapolis, Minn., and the Children's Hospital, Vancouver, B.C., respectively. From these strains, spontaneous nonmucoid derivatives CF P1NM and CF C96NM arose. Serum-sensitive blood culture isolate strain L of *P. aeruginosa* was also used. For the serotyping experiment, 21 strains of *P. aeruginosa* representing three each of the seven Fisher serotypes (23) were used as controls. All clinical isolates from cystic fibrosis patients were stored as frozen broth cultures (−70°C) with 8% dimethyl sulfoxide and subcultured as infrequently as possible to prevent mutations during handling of strains. A variety of LPS-deficient rough mutants of *P. aeruginosa* PAO were obtained from A. Kropinski, Queen's University, Kingston, Ontario, including AK 1160 (10), AK 1188 (10), AK 1012 (14), and AK 1121 (16). Strain H223 was obtained as an LPS-specific bacteriophage 44-resistant mutant of strain H103 (10) and has an undefined LPS rough mutation. All strains were routinely grown in Protease Peptone no. 2 medium (Difco Laboratories, Detroit, Mich.) or Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.).

Outer membrane isolation and SDS-polyacrylamide gel electrophoresis. Outer membranes were isolated by the one-step procedure described previously (9). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of outer membrane proteins was performed as described previously, using a 14% acrylamide running gel containing 0.07 M NaCl (9). SDS-polyacrylamide gel electrophoresis of LPS and subsequent periodate treatment and silver staining were performed as described by Tsai and Frasch (28), except that 0.1 M sodium EDTA (pH 6.8) was present in the solubilization mix before gel electrophoresis. Staining of LPS in SDS-polyacrylamide gel electrophoretograms of separated outer membranes was performed by the technique of Tsai and Frasch (28), except that isopropanol was substituted for ethanol in all steps, resulting in preferential staining of LPS (P. Hitchcock, personal communication).

LPS isolation. The technique of Darveau and Hancock (3) used for LPS isolation has been shown to result in high yields from both smooth and rough mutant strains of *P. aeruginosa* (57 to 81% of the LPS present in whole cells as quantitated with hydroxy fatty acid, heptose, and 2-keto-3-deoxyoctonate yields), with a high degree of purity. When LPS was isolated from mucoid strains, $MgCl_2$ was omitted from the final ultracentrifugation step to prevent potential contamination by mucoid polysaccharide, which is water soluble but complexed by Mg^{2+} ions. The LPS extractions were repeated three times on separate batches of cells, and for some strains up to twofold variations in yields were observed from time to time. However, no differences were observed in the SDS-polyacrylamide gel profiles of the isolated LPS despite these variations in yields. This suggested that our method was not subfractionating the LPS.

Monoclonal antibody techniques. The isolation and characterization of monoclonal antibodies specific for LPS O-antigen of *P. aeruginosa* PAO1 strain H103 (MA1-8), lipoprotein H2 (MA1-6), and porin protein F (MA2-10, MA4-4, MA5-8) have been described (10; Mutharia and Hancock, submitted). LPS rough core-specific monoclonal antibodies MA3-5 and MA3-6 were isolated by using LPS from strain AK1012 as the antigen for priming mice. Isolation of hybridomas closely followed the technique of Kohler and Milstein (15). These monoclonal antibodies were shown to be rough LPS specific on the basis of their ability to interact with purified rough (i.e., O-antigen-deficient) LPS from a variety of strains in both enzyme-linked immunosorbent assay and electrophoretic blot analyses. The antigenic site against which monoclonal antibody MA3-5 was directed was shared by LPS from serotype strains representing types 5, 7, 8, 10, and 16 of the International Antigen Typing Scheme, whereas monoclonal antibody MA3-6 reacted with strains from serotypes 5, 7, 8, 10, 14, and 16.

Serum sensitivity testing. Due to previous observations that the apparent sensitivity of *P. aeruginosa* to normal human serum was dependent on the technique used (4), two different methods were used. In method 1, bacteria were plated on Trypticase soy agar plates and then scraped off and resuspended in Trypticase soy broth at an optical density at 650 nm of 0.1. They

were then grown to an optical density at 650 nm of 0.4, centrifuged down, and resuspended in an equal volume of minimal essential medium. This cell suspension was diluted 1:1,000 to 1:10,000 into minimal essential medium, and 0.1 ml of the diluted cell suspension was mixed with 0.1 ml of fresh normal human serum. After 1 h at 37°C the bacteria were plated for viable counts on Trypticase soy agar plates. Serum was routinely stored at −70°C before use.

Method 2 was based on the recently published method of Schneider and Griffiss (24) and the well-known broth dilution method for testing of antimicrobial susceptibility. Logarithmic cultures of cells were centrifuged down and resuspended in Proteose Peptone no. 2 broth (Difco) at a final concentration of 5×10^4 cells per ml. A 20-μl portion of cells was added to 80 μl of fresh normal human serum which had been serially diluted in protease peptone no. 2 broth to give final serum concentrations (after addition of cells) ranging from 80 to 1.25% in seven serial twofold dilutions. As a control, 80 μl of medium was added to 20 μl of cells. For convenience, the assay was performed in 96-well microtiter trays (Linbro tissue culture plates; Flow Laboratories, Inc., Richmond, Va.), allowing comparison of 12 different strains on a single microtiter tray. The plate was incubated for 18 h at 37°C. Endpoints were recorded as the highest concentration of serum allowing full growth compared with the control without serum. Usually a twofold-higher concentration of serum completely prevented growth.

Serotyping. Serotyping was performed with Fisher immunotyping sera raised to phenol-water-extracted (29) LPS from the seven Fisher immunotype strains of *P. aeruginosa*, as described previously (23).

Confirmation of the serotype was performed by both hemagglutination inhibition assays and immunodiffusion assays (22). For the hemagglutination inhibition assay, sheep erythrocytes were sensitized with phenol-water-extracted LPS from the seven Fisher immunotype strains as previously described (22). Antigens for inhibition were prepared by growing the *P. aeruginosa* strains in 10 ml of Trypticase soy broth for 18 h at 37°C. The bacteria were then removed by centrifugation, and the LPS antigen was recovered by the addition of 4 volumes of alcohol to the culture supernatant. The resultant precipitates were recovered by centrifugation, redissolved in 0.5 ml of 0.15 M NaCl, and heated at 90°C for 1 h. This same antigen was also used for immunodiffusion studies. Antisera for hemagglutination inhibition and immunodiffusion studies were raised in rabbits by immunization with Formalin-fixed whole cells of the seven Fisher immunotype strains of *P. aeruginosa*, followed by immunization with live organisms as described before (23).

Miscellaneous immunological techniques. Western blotting of outer membrane proteins or LPS from SDS-polyacrylamide gel electrophoretograms onto nitrocellulose and immune staining were performed as described previously (22; Mutharia and Hancock, submitted).

RESULTS

Agglutinability of cystic fibrosis isolates. The agglutination pattern of 26 cystic fibrosis clinical isolates of *P. aeruginosa* is shown in Table 1. Of the six typable isolates, three were Fisher immunotype 1, one was Fisher immunotype 4, and two were immunotype 6 as confirmed by hemagglutination inhibition assays and gel immunodiffusion (Table 1). None of the 20 poly- or nonagglutinable strains was able to inhibit the reaction of any typing antisera with sheep erythrocytes sensitized with the corresponding LPS. Similar data were obtained utilizing immunodiffusion, where a precipitin line was observed for the typable strains and controls utilizing rabbit antisera raised to whole organisms, but no precipitin lines were observed for any of the multiply agglutinable or nontypable strains. Thus, we were unable to confirm the presence of multiple Fisher immunotype antigens in the polyagglutinable strains by using hemagglutination inhibition or immunodiffusion techniques. These strains were perhaps being agglutinated by antibody in the typing sera directed at nonserotype determinants.

TABLE 1. Agglutination patterns of *P. aeruginosa* isolates from cystic fibrosis

Pattern[a]	No. (%)	% Typable by:	
		HAI[b]	Immunodiffusion
PA	15 (57.7)	0	0
NA	5 (19.2)	0	0
Typable	6 (23.1)	100	100
Typable controls	21 (100)	95	100

[a] PA, Polyagglutinable, agglutinated by two or more Fisher typing sera; NA, no agglutination in any typing sera. Typable is defined as agglutination by a single typing serum. Controls were three each of the seven Fisher immunotypes. Use of nonmucoid revertant strains did not change the agglutination patterns of mucoid isolates.

[b] HAI, Hemagglutination inhibition.

Serum sensitivity. The work of De Matteo et al. (4) has demonstrated that the measured susceptibility of *P. aeruginosa* to serum bactericidal activity can vary according to the in vitro test used for analysis. Thus, two different methods were used. In the first, survival after 1-h exposure to 50% fresh human serum was measured (Table 2). Most of the typable strains studied here were found to be serum resistant, and only a single typable blood isolate strain, L, was serum sensitive. In contrast, all of the polyagglutinable and nontypable isolates tested were found to be serum sensitive. When a related methodology, that of Hirsch and Strauss (11), was used (data not shown), the serum resistance of H103 and CF 2314 was confirmed, whereas the polyagglutinable cystic fibrosis isolates CF P1M, CF P1NM, CF C96M, and CF C96NM were found to be serum sensitive as were the

TABLE 2. Resistance of *P. aeruginosa* isolates to normal human serum

Strain	Fisher type[a]	Serum resistance	
		% Survivors after exposure to 50% serum for 1 h, 37°C	Resistance level (% serum allowing growth after 18 h)
H103		>100	40
CF 283	6	>100	40
CF 2314	6	89	40
CF 4349	4	>100	40
CF 6094	1	86	40
L	2	0	2.5
CF 832	PA	0	1.25
CF 3790	PA	6	<1.25
CF 4522	PA	0	2.5
CF 9490	PA	0	1.25
CF P1M	PA	0	1.25
CF P1NM	PA	0	1.25
CF C96M	PA	0	1.25
CF C96NM	PA	0	1.25
CF 221	NA	0	1.25
CF 284	NA	0	1.25
CF 1278	NA	0	1.25
CF 1452	NA	0	1.25
AK 1160			<1.25
AK 1188			1.25
AK 1012			1.25
AK 1121			1.25
H223			1.25

[a] PA, Polyagglutinable; NA, no agglutination.

rough, LPS-altered mutants of strain H103 (i.e., strains AK 1160, AK 1188, AK 1012, AK 1121, and H223).

In addition to these methods, a screening technique for serum sensitivity was initiated to provide comparative information about the level of serum allowing growth after 18 h of exposure (Table 2). All strains found to be serum resistant by the above methods were capable of growing in the presence of 40% serum. In contrast, strain L as well as all of the polyagglutinable and nontypable cystic fibrosis isolates and the rough LPS-altered mutants were susceptible to lower concentrations of serum (usually to 1.25% or less). Thus, these data were in basic agreement with the results of the other methods.

Outer membrane isolation and characterization. The outer membranes of the 18 cystic fibrosis isolates studied here easily obtained by our standard method (9), which had been shown to be effective in the isolation of outer membranes from all of the serotype strains of *P. aeruginosa* (21). A number of minor alterations in outer membrane protein profiles were obtained but, as observed previously (21), the general patterns were strongly conserved (Fig. 1). The most substantial alterations were observed in strains CF 283 and CF 4349. Strain CF 283 had a protein F band which ran with higher mobility than protein F from the other strains. This band was 2-mercaptoethanol modifiable like protein F and interacted with two protein F-specific monoclonal antibodies (e.g., MA2-10, MA4-4 [Mutharia and Hancock, submitted]) but not with another (MA5-8) on Western electrophoretic blots. Due to its lower molecular weight, this protein may well represent a small deletion in the protein F structural gene, although this remains to be confirmed. In addition

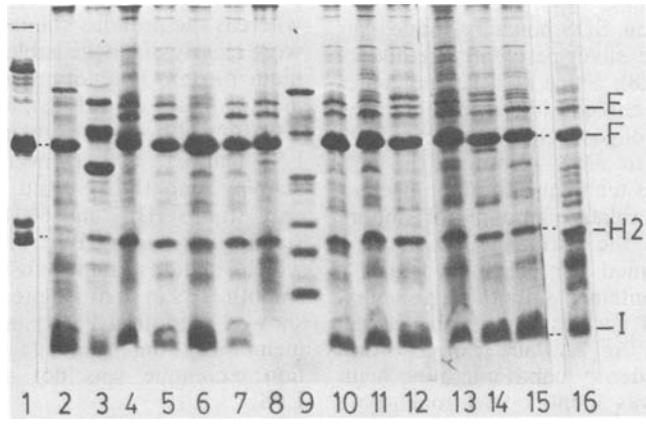

FIG. 1. SDS-polyacrylamide gel electrophoretograms of outer membrane proteins of *P. aeruginosa* isolates from patients with cystic fibrosis. Outer membranes were dissolved in solubilization reduction mix at 88°C in the absence of 2-mercaptoethanol. The overall effect of this solubilization schedule is described in Fig. 1 of reference 21. Lane 1, Strain CF 4349 (other minor polypeptides were visible upon overloading of this outer membrane); lane 2, CF P1M; lane 3, CF 283; lane 4, CF 2314; lane 5, CF 6094; lane 6, CF 1452; lane 7, strain L; lane 8, CF 1278; lane 9, molecular weight standards—bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soyabean trypsin inhibitor (21,500), myoglobin (16,900), and lysozyme (14,400); lane 10, CF 3790; lane 11, CF 832; lane 12, CF 4522; lane 13, CF 221; lane 14, CF 9490; lane 15, CF C96NM; lane 16, H103.

to this alteration, strain CF 283 contained two extra proteins of 43 and 61 kilodaltons. Strain CF 4349 contained a number of extra polypeptides, as well as an apparent deficiency in protein I.

To better define the outer membrane polypeptide composition of these strains, outer membrane proteins were transferred to nitrocellulose by the Western blot method and interacted with a variety of monoclonal antibodies. All of the strains demonstrated a protein which interacted with protein F-specific monoclonal antibodies MA2-10 and MA4-4 (Mutharia and Hancock, submitted). In addition, all strains contained a cross-reacting protein, H2, as revealed by monoclonal antibody MA1-6 (10). In contrast, a monoclonal antibody specific for the O side chain of LPS of strain H103 (serotype 5 in the International Antigen Typing Scheme), MA1-8 (10), was unable to interact with any of the outer membranes, whereas two LPS rough core-specific monoclonal antibodies, MA3-5 and MA3-6, were only able to interact with strains CF 1452 and CF 4522, respectively.

LPS of cystic fibrosis strains. It has been previously observed that the standard techniques used in the extraction of bacterial LPS are relatively specific for smooth (29) or rough (7) LPS. However, recently we were able to devise a new technique (see Materials and Methods) which enabled the isolation of both types of LPS with equal efficiency. Using our method, we found that we could isolate an average of 1.6 $\pm$ 0.56 μg of 2-keto-3-deoxyoctonate per mg of cell dry weight from the cystic fibrosis isolates. This represents around 60% of the total LPS in these cells (3).

LPS were run on SDS-polyacrylamide gels and stained by the silver-periodate method of Tsai and Frasch (28). Since all LPS molecules revealed by this technique had in common the lipid A and rough oligosaccharide core of LPS which is sufficient to allow staining of the LPS (Fig. 2, lane 2), this technique could be used as an estimate of the relative amounts of smooth and rough LPS in the various *P. aeruginosa* strains. We confirmed our previous data (17) that strain H103 contained smooth oligosaccharide side chains of various lengths (hence the multiple bands; see Fig. 2A, lane 1) on a portion of its LPS. The dense band migrating with highest mobility was identified as rough-type LPS (i.e., rough core oligosaccharide plus lipid A) by comparison with a rough LPS-altered mutant, AK 1121 (see Fig. 2, lane 2). The other bands in lane 1 were identified as smooth-type LPS (i.e., O-antigen plus rough core oligosaccharide plus lipid A) since they interacted with the O-antigen-specific (10) monoclonal antibody MA1-8 on Western electrophoretic blots (3). In contrast, the dense fast-migrating bands of strains H103 and AK1121 did not interact with MA1-8. All LPS bands derived from these strains interacted with the rough specific monoclonal antibodies MA3-5 and MA3-6. In addition to these data, we confirmed using our method, the data of Goldman and Leive (8) showing that *Salmonella typhimurium* LT2 demonstrated substantial heterogeneity in its LPS with little if any rough-type LPS (Fig. 2A, lane 3).

The results for most of the cystic fibrosis *P. aeruginosa* isolates are shown in Fig. 2. Only one of the isolates, CF283, contained more smooth-type LPS than our laboratory wild-type strains H103 (cf. Fig. 2A, lanes 1 and 5) and K799 (8). Two other strains, CF 4349 (Fig. 2B, lane 13) and CF 6094 (Fig. 2B, lane 15), contained levels of smooth LPS comparable to strain H103. Of the other strains only CF 284 and CF 9490 contained moderately high levels of O side chains. No O side chains were observed for strains CF 4522, CF 221, and CF 1278 even when 300 times as much LPS was loaded onto gels as the minimum loading required to see O side chains for strain CF 283. The results are presented in Table 3 and are expressed as the minimum amount of LPS applied to SDS-polyacrylamide gels in order to see smooth-type LPS. As a comparison, as little as 20 to 50 ng of LPS was required for rough-type LPS to be visible.

To confirm that we were not subfractionating cells, we ran SDS-polyacrylamide gels of outer membranes and stained for LPS by a modification of the periodate-silver staining technique. Although this method did result in some staining of minor protein bands, the LPS stained orange whereas the proteins stained brown or grey and were clearly distinguishable. By using this technique to stain 30 μg of outer membrane (equivalent to about 5 μg of LPS), we saw that strain CF 283 showed a strong band equivalent to smooth LPS and CF 4349 showed a slightly weaker staining, smooth LPS band. Of the other strains, only strains H103 and 6094 showed significant staining of smooth LPS. Since only those strains which demonstrated substantial amounts of smooth LPS in their isolated LPS (Table 3) also showed smooth LPS staining in intact outer membranes, this again suggested that our isolation technique was not subfractionating the LPS.

DISCUSSION

Zierdt and Williams (31) previously demonstrated that 48% of 173 isolates from patients with cystic fibrosis were agglutinated by more than one of the seven Fisher immunotyping sera. We found that similar strains did not express serotype-specific determinants. The polyagglu-

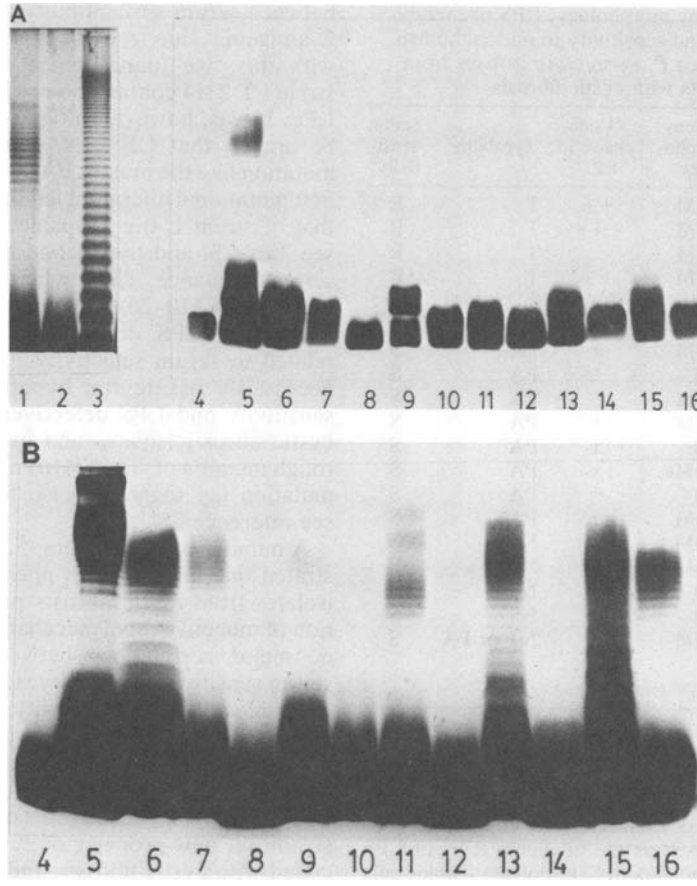

FIG. 2. LPS from control strains and *P. aeruginosa* isolates from patients with cystic fibrosis analyzed by SDS-polyacrylamide gel electrophoresis and periodate-silver staining. Lane 1, *P. aeruginosa* strain H103; lane 2, *P. aeruginosa* rough mutant AK 1121; lane 3, *S. typhimurium* LT2; lanes 4 to 16, LPS of *P. aeruginosa* isolates from cystic fibrosis patients as follows: lane 4, CF 221; lane 5, CF 283; lane 6, CF 284; lane 7, CF 832; lane 8, CF 1278; lane 9, CF 1452; lane 10, CF 2314; lane 11, strain L; lane 12, CF 3790; lane 13, CF 4349; lane 14, CF 4522; lane 15, CF 6094; lane 16, CF 9490. (A) A 5-μg portion of the LPS of the control strains (lanes 1 to 3) and 1 μg of the LPS of the cystic fibrosis isolates were added (assuming that 2-keto-3-deoxyoctonate was 4.3% of the dry weight of LPS; 16, 17). Although as summarized in Table 3 some O side chain-containing smooth LPS was seen for strains CF 4349 and CF 9490 at this loading, we were unable to photographically reproduce this. Note that strain CF 1452 apparently has two bands equivalent to rough LPS. For (B), 30 μg of LPS was added to each lane. Strains CF 1452, CF 2314, and CF 3790 demonstrated low but reproducible levels of smooth O side chain-containing LPS (not easily visible on this reproduction). Note that the rough LPS band is very overloaded at this concentration of LPS.

tinability of these strains might be due to deficiencies in LPS O-antigenic side chains (Table 3), since rough strains self-agglutinate under the appropriate salt conditions (16). Since all except three of the polyagglutinable or nontypable strains showed a small amount of smooth-type LPS on SDS-polyacrylamide gels, it might be expected that they would be typable by more extensive typing schemes. The demonstration by Zierdt and Williams that some of the strains which are polyagglutinable by the Fisher typing sera are monoagglutinable by one of the Homma typing sera may well relate to the quality of the sera used, with factors such as salt and serum protein content being potentially important. Another possibility is that some of the Homma sera are directed at antigenic determinants in the LPS core. The concept of LPS core heterogeneity in *P. aeruginosa* is strongly supported by the observation that the rough core-specific monoclonal antibodies MA3-5 and MA3-6 interacted with only 6 of the 17 serotype strains (see Materials and Methods) and 2 of the 16 cystic fibrosis isolates. It should be noted that a significant number (12.5%) of the polyagglutinable cystic fibrosis isolates studied by Zierdt and Williams

TABLE 3. Colony morphology, LPS phenotype, serotypability, and sensitivity to normal human serum of selected *P. aeruginosa* isolates from patients with cystic fibrosis

Strain	Colony morphology[a]	O side chains in LPS[b]	Typability[c]	Serum sensitivity[d]
H103	NM	++	T	R
CF 283	NM	+++	T	R
CF 2314	NM	±	T	R
CF 4349	NM	++	T	R
CF 6094	PM	++	T	R
L	NM	±	T	S
CF 832	NM	±	PA	S
CF 3790	M	±	PA	S
CF 4522	NM	−	PA	S
CF 9490	NM	+	PA	S
CF P1M	M	±	PA	S
CF P1NM	NM	±	PA	S
CF C96M	M	±	PA	S
CF C96NM	NM	±	PA	S
CF 221	NM	−	NT	S
CF 284	NM	+	NT	S
CF 1278	M	−	NT	S
CF 1452	M	±	NT	S
Rough, LPS-altered mutants	NM	−	NT or PA	S

[a] NM, Nonmucoid, smooth colony appearance; M, mucoid; PM, partially mucoid.

[b] SDS-polyacrylamide gels similar to those seen in Fig. 2 were run with different amounts of each LPS and stained by the procedure of Tsai and Frasch (28). Results are expressed as the minimum level of LPS which had to be loaded onto SDS-polyacrylamide gels to see smooth-type LPS (containing O side chains) upon subsequent staining. +++, 100 ng of LPS; ++, 100 to 500 ng of LPS; +, 1 to 10 μg of LPS; ±, 10 μg of LPS; −, no smooth-type LPS observed with 30 μg of LPS.

[c] T, Typable by agglutination; PA, agglutinable with more than one serum, nontypable by other means; NT, nontypable.

[d] R, Resistant to 10% normal human serum; S, sensitive to 10% or less normal human serum.

were nontypable in the Homma typing scheme (31).

Five of the cystic fibrosis isolates contained higher levels of smooth LPS than the strains discussed above. Of these, only three contained amounts of smooth LPS similar to our laboratory wild-type strain H103, a *P. aeruginosa* PAO1 derivative. Interestingly, like strain H103, these three strains, CF 283, CF 4349, and CF 6094, were typable and serum resistant. However, Meadow et al. (20) previously demonstrated a spontaneous serum-resistant mutant, PAC610, which was derived from the rough LPS-defective mutant PSC605. This double mutant still lacked O-antigenic side chains and was nontypable, suggesting that no absolute relationship between serum susceptibility and possession of O-antigenic side chains exists. In agreement with this, we found that the serum-resistant strain CF 2314 contained very little smooth-type LPS. It was, however, a typable strain. It could be argued that CF 2314 represents a double mutation like the mutant PAC610 (20), where the first mutational alteration led to a phenotype like that of strain L (i.e., typable, serum sensitive; see Table 3) and the second mutation restored serum resistance. Thus, whereas the results for mutant PAC610 (20) and strain CF 2314 demonstrate that LPS defectiveness is not strictly related to serum sensitivity, these data do not necessarily negate the hypothesis that serum sensitivity and LPS defectiveness in the other cystic fibrosis isolates and in our spontaneous rough mutants of strain H103 arose from a single mutation (as suggested also for *Neisseria* sp.; see reference 25).

A number of researchers (5, 12) have demonstrated that an important phenotypic feature of isolates from cystic fibrosis patients is production of mucoid exopolysaccharide. We observed no major correlations between mucoidy and serum sensitivity, typability, LPS phenotype, or outer membrane protein patterns of our cystic fibrosis isolates or for the two pairs of mucoid cystic fibrosis isolates and their spontaneous nonmucoid revertants that we studied. Thus, the LPS phenotypes described here demonstrate a significant new property of *P. aeruginosa* associated with cystic fibrosis. Indeed, the property of serum sensitivity which seems to be associated with the LPS phenotype may well explain why patients with cystic fibrosis rarely suffer from *P. aeruginosa* bacteremia. Another important implication of these data relates to the advisability of using LPS-based vaccines for strains which are deficient in O-antigen.

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