

Pseudomonas aeruginosa Variants Isolated from Patients with Cystic Fibrosis Are Killed by a Bactericidal Protein from Human Polymorphonuclear Leukocytes

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The susceptibility of paired mucoid and nonmucoid variants of *Pseudomonas aeruginosa* isolated from 13 patients with cystic fibrosis (CF) to killing by a 55,000-Da bactericidal protein (BP55) from human polymorphonuclear leukocytes was studied. Mucoid and nonmucoid variants were equally sensitive to killing by BP55 at both pH 5.6 and pH 7.2. Eleven of the isolates were resistant to the bactericidal activity of 10% normal human serum but were as sensitive as the serum-sensitive isolates to BP55. Similarly, the 15 isolates with lipopolysaccharides (LPS) containing O-polysaccharide side chains (smooth LPS) were as sensitive to BP55 as those isolates with rough LPS. *P. aeruginosa* isolates from patients in poor clinical condition were more likely to have LPS of the smooth type and to be resistant to killing by 10% human serum than the isolates from patients in good clinical condition. We have concluded that the susceptibility of the *P. aeruginosa* isolates from patients with CF to killing by BP55 does not correlate with mucoid or nonmucoid variations, with the presence or absence of smooth LPS, or with the sensitivity or resistance to killing by normal human serum.

Cystic fibrosis (CF) is an inherited autosomal disease characterized by frequent pulmonary infections with *Pseudomonas aeruginosa* (1). The clinical condition of the patient with CF deteriorates with the appearance of mucoid colonial forms which emerge in addition to the original nonmucoid *P. aeruginosa* colonizer (10). Despite aggressive multiple antibiotic therapy, *P. aeruginosa* often persists in the respiratory tract because of its characteristic resistance to a broad range of antibiotics. A naturally occurring antibacterial protein with remarkable potency against *P. aeruginosa* has been isolated from the cytoplasmic granules of human polymorphonuclear leukocytes (PMN) (15). This 55,000-Da bactericidal protein (BP55) binds to the target bacteria and depolarizes the cytoplasmic membrane, which results in the cessation of amino acid transport and eventual cell death (16). Like the 57,000-Da cationic antimicrobial protein (CAP57) and the 59,000-Da bactericidal and permeability-increasing protein (B/PI), which bind to the lipopolysaccharides (LPS) from the outer membranes of *Salmonella typhimurium* and *Escherichia coli* (31, 37), respectively, BP55 binds to the LPS from *P. aeruginosa* (14, 35).

The outermost portion of the LPS, the O-polysaccharide side chain, plays an important role in the pathogenicity of most gram-negative bacteria, including *P. aeruginosa* (4, 23). O-polysaccharide side chains of smooth LPS can sterically hinder the formation of the complement membrane attack complex, thus conferring serum resistance to the bacteria (8, 17). The smooth LPS can also confer resistance to β -lactam antibiotics (7). Similarly, the resistance of *S. typhimurium* and *E. coli* to the antimicrobial proteins of PMN, CAP57 and B/PI, increases with the length of the O polysaccharides of LPS (6, 38). The relationship between the susceptibility of *P. aeruginosa* to antibacterial proteins such as BP55 and the structure of its LPS has not been determined, nor has the relationship between susceptibility to

BP55 and *P. aeruginosa* colonial morphology been established.

Our laboratory has tested paired mucoid and nonmucoid *P. aeruginosa* isolated from 13 patients with CF for sensitivity to the bactericidal activity of normal human serum and for sensitivity to BP55 from normal human PMN. We have determined that less than half of these isolates were serum resistant, a characteristic that was independent of mucoid or nonmucoid colonial morphology. By contrast, all of the *P. aeruginosa* isolates were susceptible to killing by BP55, regardless of the O-polysaccharide class.

MATERIALS AND METHODS

Bacteria. Pairs of mucoid and nonmucoid *P. aeruginosa* were isolated from sputum samples or throat secretions of 13 patients with CF by the Diagnostic Microbiology Laboratory at the University of Minnesota Hospital, Minneapolis (see Table 1 for the description of patients). All 26 isolates were subcultured once on blood agar plates (BAP), and then suspensions were stored at -70°C in Mueller-Hinton broth (MHB; BBL-A Division of BioQuest, Cockeysville, Md.) plus 8% dimethyl sulfoxide as described by Speert et al. (32). *P. aeruginosa* type 1, a non-CF clinical isolate obtained in 1972 from the University of Minnesota Hospital, Minneapolis, has been cultured from lyophilized stock and has been maintained by transfer on BAP. The isolate was serotyped by the scheme of Homma (13).

Preparation of bacteria. All data reported are from experiments in which isolates were subcultured on BAP from the frozen storage described above and were grown for 18 h at 37°C . Bacteria were then suspended by brief vortexing in 1 ml of saline. The density was adjusted spectrophotometrically at 650 nm (Universal Spectrophotometer; Coleman Instruments, Maywood, Ill.) to yield approximately 10^7 CFU per ml of saline. For tests of other conditions, isolates were grown on Mueller-Hinton Agar (MHA; BioQuest, Cockeysville, Md.) and were treated as described above, except that Hanks balanced salt solution (HBSS; GIBCO Laboratories,

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TABLE 1. Sources of *P. aeruginosa* isolates: clinical characteristics of patients with CF

Isolate no.	Brasfield score ^a	Age of patient (yr)	Sex of patient ^b
1	22	18	M
2	20	11	F
3	18	28	M
4	17	11	M
5	16	15	F
6	14	19	F
7	13	11	F
8	13	18	M
9	12	18	F
10	12	33	M
11	12	29	F
12	10	35	F
13	10	33	F

^a Brasfield scores were determined at the University of Minnesota Hospital, Minneapolis, as described previously (2).

^b M, male; F, female.

Grand Island, N.Y.) was used instead of saline. Other conditions that were employed were (i) washing the overnight culture twice with saline or HBSS at room temperature, (ii) growing a transfer of the overnight culture to logarithmic phase (3 to 4 h) in MHB at 37°C, and (iii) adjusting the overnight culture to a concentration of 10⁴ CFU per ml of saline or HBSS.

Sera. Blood from five healthy AB-positive volunteers was obtained by venipuncture and allowed to clot for 60 min. After centrifugation at 1,000 × g for 15 min at 4°C, the serum was collected, pooled, and stored at -70°C.

Serum bactericidal assay. The assay utilized was a modification of the method of Young and Armstrong (40). Briefly, 0.1 ml of each prepared isolate was incubated with constant mixing in 0.8 ml of HBSS plus 0.1 ml of pooled normal human serum. Controls consisted of 0.1 ml of each prepared isolate in 0.9 ml of HBSS. After incubation at 37°C for 1 h, 0.1-ml samples were withdrawn, serially diluted in saline, and plated onto Todd-Hewitt agar (Difco Laboratories, Detroit, Mich.). Plates were incubated at 37°C overnight before being scored for serum sensitivity by the following classification: sensitive, greater than 80% killed; intermediate, less than 80% but more than 20% killed; and resistant, less than 20% killed. The following formula was used to determine the percentage of bacteria killed: [(number of colonies_{control} - number of colonies_{test})/number of colonies_{control}] × 100. Resistant isolates were tested for delayed killing by repeating the assay but by incubating for 3 h.

Bactericidal protein. A 55,000-Da bactericidal protein (BP55) was isolated from normal human PMN obtained from the Red Cross Blood Bank (St. Paul, Minn.) by acid extraction of the granules as described by Hovde and Gray (15). Their two-step column chromatography procedure was utilized and yielded pure BP55, as judged by viewing a silver-stained gel after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of a sample containing 2 to 4 μg of protein. Protein concentrations were determined by the Hartree modification of the Lowry method (12).

Bactericidal protein assay. All isolates and *P. aeruginosa* type 1 were prepared as described above. To determine the BP55 dose response, 10⁶ (± 0.5 × 10⁶) CFU per ml of saline was mixed with 80 mM citrate phosphate buffer, pH 5.6, and various amounts of BP55, for a total volume of 1 ml. Controls without BP55 were included for each isolate. All

isolates were also tested at pH 7.2. After incubation at 37°C in a reciprocal shaking water bath for 1 h, 0.1-ml samples were withdrawn, serially diluted in saline, and plated onto Todd-Hewitt agar plates. As described above, the percent killing was determined by the BP55 dose response, and the amount required for 95% killing of the particular isolate was found by extrapolation from the linear portion of the curve by using linear regression. *P. aeruginosa* type 1 was used as a standard bacterial target for each assay.

LPS isolation. The method as described by Darveau and Hancock (5) was used with few modifications. The isolates were grown overnight at 37°C on BAP, and approximately 0.1 to 0.2 g of bacteria could be removed from each plate. Therefore, a downscaled version of the method was utilized to isolate LPS. As suggested by Hancock et al. (11), the 25 mM MgCl₂ was excluded in the last centrifugation. LPS was also isolated from *P. aeruginosa* type 1, as a control for the isolation procedure.

Analysis of LPS. SDS-PAGE was performed by the method of Laemmli (21). A 14% separating gel was used with a 3% stacking gel. LPS samples in water with an equal volume of sample buffer (0.1 M Tris-HCl buffer, pH 6.8, containing 3.4% [wt/vol] SDS, 11% [vol/vol] glycerol, 0.001% [wt/vol] bromophenol blue, and 5% [vol/vol] β-mercaptoethanol) were boiled for 5 min before being loaded onto the gel. Samples were electrophoresed at 30 mA of constant current until the bromophenol blue tracking dye was at the bottom of the gel (4 to 5 h). Gels were stained by the periodate-silver nitrate method of Tsai and Frasch (34). LPS isolated from *P. aeruginosa* ATCC 27312 (List Biological Laboratories, Inc., Campbell, Calif.) was used as a smooth LPS standard in all gels.

Clinical condition of the patients. The degree of structural lung preservation of each patient was classified as good or poor by Brasfield chest roentgenographic scores (2) as follows: patients in good clinical condition had a score greater than 15, while patients in poor clinical condition had a score less than 15 (Table 1).

Statistical analysis. Data from the bactericidal protein assays were analyzed by using the Student *t* test (3), while serum assay results were analyzed by using the chi-square test and Fisher's exact test (3, 20).

RESULTS

Isolate sensitivity to serum. Of the 26 isolates from patients with CF, 38% of the mucoid and 46% of the nonmucoid *P. aeruginosa* isolates resisted the bactericidal activity of serum when exposed to 10% pooled normal human serum for 1 h at 37°C (Table 2). There was little difference between the total number of serum-resistant isolates and the total number of serum-sensitive isolates. When isolates were divided into two groups according to the clinical condition of the patient, we found that 22% of the isolates from patients in good clinical condition (Brasfield score of >15) were resistant to serum bactericidal activity, while 58% of the isolates from patients in poor clinical condition (Brasfield score of <15) were resistant; however, this difference was not statistically significant (*P* > 0.05). The age or sex of the patients did not relate to the sensitivity of the isolates to serum bactericidal activity. The overnight growth medium (BAP or MHA) did not alter the results of the assay, nor did washing the isolates in HBSS or saline. When 4 mucoid and 4 nonmucoid isolates were grown in broth (MHB) to mid-logarithmic phase, the serum bactericidal assay results were unchanged (data not

TABLE 2. Susceptibility of *P. aeruginosa* isolates to killing by normal human serum^a

Sensitivity of isolate ^b	No. (%)		Total
	Mucoid isolates	Nonmucoid isolates	
Sensitive	8 (62)	6 (46)	14 (54)
Intermediate	0 (0)	1 (8)	1 (4)
Resistant	5 (38)	6 (46)	11 (42)

^a The serum bactericidal assay was performed as follows: 10⁶ CFU of each isolate was incubated in 10% serum with HBSS for 1 h at 37°C, and the surviving bacteria were enumerated by dilution plate counts. Each isolate was assayed three to four times.

^b Sensitivity of isolates was determined as follows: sensitive, greater than 80% of the bacteria killed; intermediate, less than 80% but more than 20% of the bacteria killed; and resistant, less than 20% of the bacteria killed.

shown). None of the serum-resistant isolates demonstrated a delayed sensitivity to serum when incubated in 10% pooled normal human serum for 3 h at 37°C.

Bactericidal assay. All *P. aeruginosa* isolates were sensitive to the bactericidal activity of BP55 at both pH 5.6 and pH 7.2 (Tables 3 and 4). There was no significant difference ($P > 0.05$) in the mean amounts of BP55 required to kill the mucoid and nonmucoid isolates at pH 5.6 (0.52 ± 0.04 versus 0.53 ± 0.02 μg , respectively) or at pH 7.2 (1.80 ± 0.22 versus 1.48 ± 0.21 μg , respectively). Similarly, there was no significant difference ($P > 0.05$) in the sensitivities to BP55 between serum-resistant isolates and serum-sensitive isolates at pH 5.6 (0.55 ± 0.03 versus 0.50 ± 0.03 μg) or at pH 7.2 (1.62 ± 0.18 versus 1.71 ± 0.24 μg). At pH 5.6, there was a significant difference ($P < 0.001$) in sensitivities to BP55 between each individual isolate and *P. aeruginosa* type 1, with the mean amount of BP55 required for the 25 isolates from patients with CF being 0.53 ± 0.19 μg and that for *P. aeruginosa* type 1 being 0.20 ± 0.01 μg ($n = 6$). At pH 7.2, however, the mean amount of BP55 required to kill the isolates from patients with CF differed little from the amount for *P. aeruginosa* type 1. Indeed, only seven isolates from

TABLE 3. Susceptibility of mucoid *P. aeruginosa* to killing by the bactericidal protein BP55

Isolate	Serum assay ^a	LPS type	Mean amt ($\mu\text{g/ml}$) of BP55 \pm SEM (n) ^b at pH:	
			5.6	7.2
2M	S	Rough	0.49 ± 0.03 (3)†	1.23 ± 0.17 (6)
3M	S	Rough	0.56 ± 0.02 (3)†	0.83 ± 0.15 (6)
4M	S	Rough	0.49 ± 0.04 (3)†	2.36 ± 0.20 (5)†
5M	S	Rough	0.61 ± 0.02 (3)†	3.45 ± 0.80 (6)†
8M	S	Rough	0.55 ± 0.02 (3)†	2.90 ± 0.26 (5)†
12M	S	Rough		2.64 ± 0.12 (5)†
9M	S	Smooth	0.53 ± 0.04 (3)†	1.18 ± 0.03 (6)
13M	S	Smooth	0.54 ± 0.03 (3)†	0.91 ± 0.17 (6)
1M	R	Smooth	0.50 ± 0.02 (3)†	1.24 ± 0.17 (6)
6M	R	Smooth	0.51 ± 0.03 (3)†	2.01 ± 0.22 (5)
7M	R	Smooth	0.49 ± 0.02 (3)†	1.78 ± 0.15 (5)
10M	R	Smooth	0.48 ± 0.03 (3)†	1.19 ± 0.13 (6)
11M	R	Smooth	0.78 ± 0.08 (3)†	1.69 ± 0.17 (6)
Type 1	R	Smooth	0.20 ± 0.01 (6)	1.79 ± 0.11 (18)

^a S, sensitive to serum; R, resistant to serum (see Table 2, footnote b).

^b Mean amount of BP55 (in micrograms per milliliter) \pm one standard error of the mean required to kill 95% of 5×10^6 *P. aeruginosa*. n, number of times the assay was performed. Dagger indicates value is significantly different ($P < 0.05$) from that of *P. aeruginosa* type 1. The *P. aeruginosa* isolate 12M was not viable at pH 5.6.

TABLE 4. Susceptibility of nonmucoid *P. aeruginosa* to killing by the bactericidal protein BP55

Isolate	Serum assay ^a	LPS type	Mean amt ($\mu\text{g/ml}$) of BP55 \pm SEM (n) ^b at pH:	
			5.6	7.2
2N	S	Rough	0.46 ± 0.02 (3)†	0.83 ± 0.14 (6)
4N	S	Rough	0.48 ± 0.01 (3)†	1.49 ± 0.09 (5)
5N	S	Rough	0.53 ± 0.01 (3)†	1.12 ± 0.08 (6)
10N	S	Rough	0.50 ± 0.02 (3)†	0.93 ± 0.17 (6)
9N	I	Rough	0.47 ± 0.01 (3)†	0.86 ± 0.15 (6)
6N	S	Smooth	0.58 ± 0.02 (3)†	2.97 ± 0.24 (5)†
13N	S	Smooth	0.52 ± 0.06 (2)†	1.08 ± 0.30 (6)
1N	R	Smooth	0.47 ± 0.02 (3)†	0.74 ± 0.15 (6)
3N	R	Smooth	0.57 ± 0.06 (3)†	0.76 ± 0.16 (6)
7N	R	Smooth	0.50 ± 0.04 (3)†	2.73 ± 0.66 (5)†
8N	R	Smooth	0.68 ± 0.04 (3)†	2.38 ± 0.35 (5)†
11N	R	Smooth	0.52 ± 0.03 (3)†	1.31 ± 0.37 (6)
12N	R	Smooth	0.56 ± 0.05 (3)†	2.00 ± 0.24 (5)
Type 1	R	Smooth	0.20 ± 0.01 (6)	1.79 ± 0.11 (18)

^a S, sensitive to serum; R, resistant to serum; I, intermediate sensitivity to serum (see Table 2, footnote b).

^b Mean amount of BP55 (in micrograms per milliliter) \pm one standard error of the mean required to kill 95% of 5×10^6 *P. aeruginosa*. n, number of times the assay was performed. Dagger indicates value is significantly different ($P < 0.05$) from that of *P. aeruginosa* type 1.

patients with CF required significantly more ($P < 0.05$) BP55 than the amount required for *P. aeruginosa* type 1. Although isolates from patients with CF in poor clinical condition required more BP55 at pH 5.6 and pH 7.2 (0.55 ± 0.02 and 1.79 ± 0.18 μg , respectively) than the amount required for isolates from patients with CF in good clinical condition at pH 5.6 and pH 7.2 (0.52 ± 0.01 and 1.41 ± 0.26 μg , respectively), the difference was not significant ($P > 0.05$). The age or sex of the patient did not relate to the amount of BP55 required to kill 95% of the test inoculum of the isolate. Growing the isolates on BAP or on MHA or washing the isolates with saline or HBSS did not alter the susceptibility of *P. aeruginosa* isolates to killing by BP55 (data not shown).

Isolate LPS profile. Of the 26 *P. aeruginosa* isolates, 15 were found to have O-polysaccharide side chains on their LPS (smooth LPS), as determined by LPS appearance in silver-stained gels after SDS-PAGE. Smooth LPS profiles were detected with 54% of the mucoid isolates and 62% of the nonmucoid isolates. Like *P. aeruginosa* type 1, 100% of the 11 serum-resistant isolates were found to have smooth LPS profiles (Fig. 1, lane C), whereas 71% of the serum-sensitive isolates lacked detectable O-polysaccharide side chains on their LPS (rough LPS; Fig. 1, lane D). However, of the serum-sensitive isolates, two mucoid (Fig. 1, lanes E and F) and two nonmucoid (Fig. 1, lanes G and H) isolates were found to have smooth LPS profiles. One nonmucoid isolate was intermediate in serum sensitivity but of rough LPS type (Table 2 and Fig. 1, lane I). Sensitivity to BP55 did not relate to LPS type, with the mean amount of BP55 required to kill 95% of the smooth isolates versus that required to kill 95% of the rough isolates being 0.52 ± 0.03 versus 0.52 ± 0.02 μg at pH 5.6 and 1.69 ± 0.16 versus 1.52 ± 0.34 μg at pH 7.2. Smooth LPS profiles were detected for 75% of both mucoid and nonmucoid isolates from patients in poor clinical condition, while only 30% of the isolates from patients with CF in good clinical condition were determined to have smooth LPS ($P < 0.05$). The LPS profiles of the isolates did not relate to the sex or age of the patients.

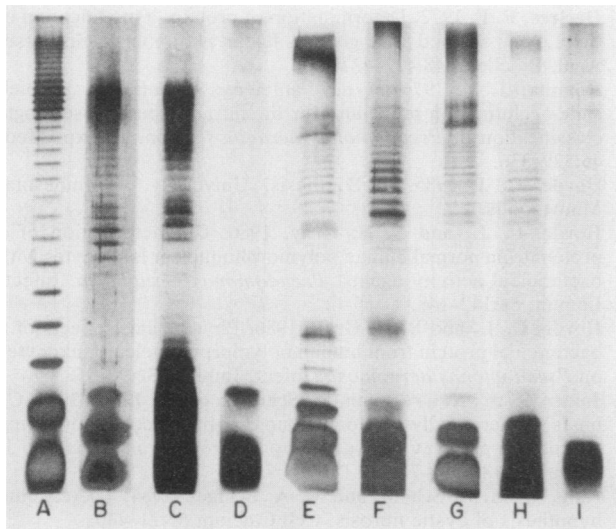


FIG. 1. SDS-PAGE of LPS from *P. aeruginosa* isolated from patients with CF. Lane A, a commercial LPS standard from *P. aeruginosa* 27312; lane B, LPS isolated from *P. aeruginosa* type 1; lane C, typical smooth LPS from a serum-resistant isolate (1N); lane D, typical rough LPS from a serum-sensitive isolate (5M); lanes E and F, mucoid, smooth LPS from serum-sensitive isolates (9M and 13M, respectively); lanes G and H, nonmucoid, smooth LPS from serum-sensitive isolates (6N and 13N, respectively); lane I, rough LPS from an isolate with intermediate sensitivity to serum (9N).

DISCUSSION

Thomassen and Demko found that of the *P. aeruginosa* isolates they obtained from the respiratory tracts of patients with CF, 46% were serum resistant (33). Likewise, Penketh et al. found 53% of their *P. aeruginosa* respiratory isolates were serum resistant (26). These results agree with our own observation that 42% of sputum isolates from patients with CF are serum resistant. Serum resistance in *S. typhimurium* and *E. coli* has been found to correlate with the presence of O-polysaccharide side chains on the LPS (smooth LPS). Indeed, Goldman et al. found that the long O-polysaccharide side chains on the LPS of *E. coli* O111 prevented the insertion of the serum complement membrane attack complex into the cell membrane (8). Similar results with *P. aeruginosa* were obtained by Schiller et al. with a serum-resistant *P. aeruginosa* isolate derived by repeated passage of a serum-sensitive strain in increasing serum concentrations (29, 30). However, we observed 4 of 14 isolates that were serum sensitive but that bore smooth LPS. Hancock et al. (11) also found 4 of 12 *P. aeruginosa* isolates from patients with CF with smooth LPS that were serum sensitive. We would conclude, as Hancock et al. did, that the presence of O-polysaccharide side chains on the LPS of *P. aeruginosa* is not sufficient to confer serum resistance, although there is a strong correlation.

The O-polysaccharide side chains of gram-negative bacteria have been found by some researchers to confer resistance to host defense factors other than serum. Rest et al. found *S. typhimurium* LT2 smooth LPS strains were less susceptible to the bactericidal activity of PMN granule extracts than were LT2 mutants that were deficient in O-polysaccharide side chains (28). More recently, *S. typhimurium* MS395 and *E. coli* strains that lack O-polysaccharide side chains on their LPS (rough LPS) were found to be more sensitive to a

bactericidal protein (B/PI) isolated from PMNs by Weiss et al. (36). Another PMN antimicrobial protein, CAP57, was found to kill a rough LPS mutant of *S. typhimurium* at a concentration lower than the concentration required to kill the smooth LPS parent strain (6). We found that both the smooth and the rough LPS isolates of *P. aeruginosa* were susceptible to killing by BP55. Moreover, we found that the killing of 95% of the smooth LPS isolates required only 6% more BP55 at pH 5.6 and 5% less BP55 at pH 7.2 than the amount required to kill 95% of the rough LPS isolates. Under similar conditions, Weiss et al. determined that at least 20-fold more bactericidal protein was required to kill the smooth LPS strains of *S. typhimurium* MS395 than was necessary to kill the rough LPS mutants (36). Farley et al. found that 11 times the amount of CAP57 required to kill the rough LPS mutant was required to kill the smooth LPS strain of *S. typhimurium* (6). This difference in sensitivity to PMN bactericidal proteins was not due to a difference in the proteins, for BP55, B/PI, and CAP57 have the same N-terminal amino acid sequence and are very likely the same protein (24, 27, 35). Furthermore, although we have not carried out a comprehensive comparison, we have previously established that killing of a smooth strain of *S. typhimurium* required 300-fold more BP55 than did killing of a rough strain of *E. coli* or of the smooth strain *P. aeruginosa* type 1 (15). The unique nature of the O polysaccharide found on the *P. aeruginosa* LPS might account for the similar susceptibilities of smooth and rough LPS isolates to BP55 activity.

The percentage of LPS in *P. aeruginosa* that possess O-polysaccharide side chains differs from that for other gram-negative bacteria. Wilkinson estimated the amount of smooth LPS in *P. aeruginosa* to be between 5 and 11 mol% of the total LPS (39), whereas Hancock et al. estimated that it was between 0.2 and 13.7 mol% (11). Conversely, the amount of smooth LPS in *S. typhimurium* LT2 was determined to be 35 mol% by Palva and Makela (25), while Goldman and Leive determined the amount of smooth LPS in *E. coli* O111 to be between 50 and 60 mol% (9). In addition, the frequency with which the O-polysaccharide side chains cap LPS molecules of *P. aeruginosa* was found to be 20% (19), whereas 33% of *S. typhimurium* LPS molecules (25) and 65% of *E. coli* LPS molecules (9) were capped with O-polysaccharide side chains. It is possible that the relative scarcity of O-polysaccharide side chains in smooth LPS of *P. aeruginosa* permits the passage of small proteins such as BP55 (molecular weight, 55,000) but not larger proteins, such as complement components (for example, C3b [molecular weight, 185,000]). In addition, the lower density of O-polysaccharide side chains in *P. aeruginosa* LPS may allow cationic bactericidal proteins access to the anionic groups in the lipid A portion of LPS. The presence of hydrophilic O-polysaccharide side chains on the LPS may also mask the hydrophobic moieties of the molecule.

Ionic and hydrophobic interactions may be essential for the interaction of bactericidal proteins with the bacterial surface. Farley et al. documented the importance of the hydrophobic interaction of the PMN bactericidal protein CAP57 with bacterial surfaces through competitive binding studies with a derivative of polymyxin B that lacks the hydrophobic grouping (6). Farley et al. also demonstrated that the O polysaccharide on *S. typhimurium* LT2 resisted the binding of CAP57 (6). The hydrophilic repulsion by the smooth LPS could be partially surmounted by increasing the electropositive charge of the bactericidal protein. Thus, for *S. typhimurium* strains with both smooth and rough LPS, the

pH optimum for binding CAP57 was determined to be 5.5 rather than 7.5 (6). We found that the pH optimum for the bactericidal activity of BP55 was 5.6 for all *P. aeruginosa* isolates, regardless of LPS type. The amount of BP55 required to kill the isolates at pH 7.2 was threefold greater than that required at pH 5.6.

It is clear that the presence of smooth LPS on the *P. aeruginosa* isolates studied here, unlike the presence of *Escherichia* or *Salmonella* smooth LPS, is not always sufficient to confer resistance to serum bactericidal activity and is never sufficient to confer resistance to the bactericidal activity of BP55. Factors other than LPS type may contribute to the survival of *P. aeruginosa* in the airways of patients with CF. For example, adherence of *P. aeruginosa* to the respiratory tract may limit the access of BP55 to the target bacteria (22). Moreover, the higher than normal physiological levels of Ca²⁺ found in respiratory secretions of patients with CF enhance the adherence of *P. aeruginosa* to the tracheal epithelium (18) and may further inhibit killing by BP55. These possibilities are being investigated.

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