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Synthetic Peptides Representing Two Protective, Linear B-Cell Epitopes of Outer Membrane Protein F of *Pseudomonas aeruginosa* Elicit Whole-Cell-Reactive Antibodies That Are Functionally Pseudomonad Specific

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Peptide 9 (TDAYNQKLSERRAN) and peptide 10 (NATAEGRAINRRVE) represent surface-exposed epitopes of outer membrane protein F of *Pseudomonas aeruginosa*. Rats immunized with four intramuscular inoculations on days 0, 14, 28, and 42 with either peptide 9 or peptide 10 conjugated to keyhole limpet hemocyanin were afforded protection against pulmonary lesions when examined 7 days subsequent to challenge (day 56) via intratracheal inoculation of *P. aeruginosa*-containing agar beads. Peptide 9 shares considerable homology with other OmpA-related outer membrane proteins in various bacteria, whereas peptide 10 displays little homology with these other proteins. Antisera directed to peptide 9 reacted weakly with cell envelope proteins from the various other OmpA-associated bacteria upon immunoblot analysis. However, antisera directed to peptide 10 reacted only with *Neisseria gonorrhoeae* cell envelope proteins upon immunoblot analysis. Antisera to both peptides 9 and 10 reacted at minimal titers with whole cells of the various other bacteria in a whole-cell enzyme-linked immunosorbent assay (ELISA) but antisera to each of the peptides reacted at high titers when various strains of *P. aeruginosa* were used as the ELISA antigen. Antibodies to peptides 9 and 10 were protective, reactive to all strains of *P. aeruginosa* tested except for a protein F-deficient mutant, and functionally specific against pseudomonads.

Outer membrane (OM) protein F of *Pseudomonas aeruginosa* has efficacy as a vaccine upon active immunization to prevent subsequent infections by *P. aeruginosa* in various animal models, including a murine acute-infection model (9), a burned-mouse model (15, 16), and a rat model of chronic pulmonary infection (3, 5–7). Protection in the chronic pulmonary infection model is of particular significance, since one potential clinical use of a protein F vaccine is for immunotherapy of children with cystic fibrosis (CF) to prevent the subsequent chronic colonization of their lungs with *P. aeruginosa*. In order to obtain a source of OM protein F that would be suitable for administration to children with CF, we recently (13) tested 10 synthetic peptides representing regions of computer-predicted antigenicity within mature protein F for their ability to elicit in immunized mice antibodies reactive with intact whole cells of heterologous immunotype strains of *P. aeruginosa*. Two of these peptides, peptide 9 (corresponding to amino acid residues 261 to 274 in mature protein F, TDAYN QKLSERRAN) and peptide 10 (corresponding to amino acid residues 305 to 318 in mature protein F, NATAEGRAINR RVE), did elicit whole-cell-reactive antibodies at sufficiently high titers to suggest they may have potential usefulness as a synthetic vaccine for *P. aeruginosa*. Furthermore, antisera to each of these two synthetic peptides were shown (13) to possess opsonic activity against wild-type cells of *P. aeruginosa*, confirming that antibodies directed against each of these two peptides have potentially protective capabilities. In this study we further examine the vaccine potential of peptides 9 and 10.

Peptides 9 and 10 were synthesized and provided as lyophilized powder by the Peptide Synthesis Unit of the Core Laboratories, Louisiana State University Medical Center Auxiliary Enterprises (New Orleans), as before (13). Each of the synthetic peptides was conjugated to keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, Calif.) as previously described (13). To obtain antisera for use in the immunological assays, 50 5-week-old female specific-pathogen-free ICR mice (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) were used per group. The mice were immunized with cross-linked KLH alone, peptide 9-KLH conjugate, peptide 10-KLH conjugate, or protein F purified from the PAO1 strain of *P. aeruginosa* by gel extraction as described previously (9). For the first two immunizations (days 0 and 14), each mouse received 0.2 ml of sterile saline containing either 150 μg of cross-linked KLH, 150 μg of peptide 9-KLH conjugate, 150 μg of peptide 10-KLH conjugate, or 10 μg of PAO1 protein F, given with aluminum hydroxide adjuvant (2.5 mg/ml) as described previously (13). The third and fourth immunizations (days 28 and 42) were given without adjuvant and consisted of 0.2 ml of sterile saline containing either 15 μg of cross-linked KLH alone, 15 μg of peptide 9-KLH conjugate, 15 μg of peptide 10-KLH conjugate, or 10 μg of the PAO1 protein F preparation. All four immunizing doses were administered intramuscularly into alternate rear hips. Two weeks after the final immunization (day 56), the mice were bled by cardiac puncture under sodium pentobarbital anesthesia, and the sera obtained were pooled for each group of mice.

Cell envelopes were prepared from each of the bacterial strains according to the procedure of Stinnett et al. (19). Proteins were extracted from the cell envelopes and separated by slab sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis as described by Gilleland and Lyle (8). Western blotting (immunoblotting) was performed as described by Towbin et al. (20), using the pooled antisera from the four groups of

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mice immunized as described above. In order to better visualize the bands cross-reactive with the antipeptide antisera, an increased concentration (15 μg) of cell envelope proteins was loaded onto the SDS-polyacrylamide gel for all strains other than the *P. aeruginosa* or *Pseudomonas fluorescens* strains, for which 2.5 μg of cell envelope proteins was used.

The enzyme-linked immunosorbent assay (ELISA) was performed as described previously (15), using whole cells of each strain of bacteria as the antigen (13). Each well of the microtiter plate was coated with 100 μl of a bacterial suspension consisting of approximately 10^8 cells per ml in phosphate-buffered saline (PBS), pH 7.2. For the *Neisseria* and *Haemophilus* strains, cells were scraped off a chocolate agar plate that had been incubated overnight at 37°C with increased CO_2 atmosphere and resuspended in sterile PBS. For all the remaining bacterial strains, cells were harvested by centrifugation during logarithmic growth phase from nutrient broth, and the pelleted cells were resuspended in PBS. Prior to coating the wells, the bacterial suspension was incubated at 56°C for 30 min. The plates with the coated wells were incubated overnight at 37°C. Antisera from the KLH control group, the peptide 9-KLH conjugate-immunized group, the peptide 10-KLH conjugate-immunized group, and the PAO1 protein F-immunized group were assayed against each of the bacterial strains in this whole-cell ELISA. For each bacterial strain, two to three assays were performed and an average titer was determined.

The strains of *P. aeruginosa* used included the following: the PAO1 Fisher-Devlin immunotype 7 strain; the KG1077 strain, which is a protein F-deficient mutant of the PAO1 strain (10) obtained from N. Gotoh (Kyoto, Japan); the previously described (7, 15) laboratory strains representing Fisher-Devlin immunotypes 1 through 6; 29 clinical strains isolated from CF patients (obtained from the Clinical Microbiology Laboratory, Schumpert Medical Center, Shreveport, La.); and 23 clinical strains isolated from non-CF patients (obtained from the Clinical Microbiology Laboratory, Louisiana State University Medical Center, Shreveport). The following clinical strains were all obtained from the Louisiana State University Medical Center Clinical Microbiology Laboratory: 1 strain of *P. fluorescens*; 2 strains of *Burkholderia* (*Pseudomonas*) *cepacia*; 10 strains of *Escherichia coli*; 3 strains each of *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae*; and 2 strains of *Salmonella typhimurium*. All *Pseudomonas* strains were grown at 30°C with shaking in BBL nutrient broth (Becton-Dickinson Microbiology Systems, Cockeysville, Md.) or on nutrient agar (Difco Laboratories, Detroit, Mich.). All enteric bacilli were grown at 37°C with shaking in BBL nutrient broth or on nutrient agar. *N. gonorrhoeae* and *H. influenzae* strains were grown on BBL chocolate agar at 37°C in an increased CO_2 atmosphere.

For performance of the rat chronic pulmonary infection model, the method of Klinger et al. (14) was used with modifications as detailed previously (7). The rats were immunized on day 0 with 0.5 ml of sterile saline containing either 1,000 μg of the cross-linked KLH preparation, the peptide 9-KLH conjugate, or the peptide 10-KLH conjugate or 25 μg of the PAO1 protein F, each adsorbed to aluminum hydroxide adjuvant. For adsorption to the aluminum hydroxide adjuvant (13), a concentration of 16 mg of aluminum hydroxide per ml was used for the KLH preparation and the two peptide-KLH preparations, whereas a concentration of 2.5 mg/ml was used for adsorption of the protein F preparation. On day 14 the rats received 0.25 ml of sterile saline containing either 500 μg of the cross-linked KLH preparation, the peptide 9-KLH conjugate, or the peptide 10-KLH conjugate or 25 μg of the PAO1 protein F, given with the adjuvant. For the third (day 28) and fourth (day 42) immunizing doses, the rats received the same concentrations of the appropriate immunogen used for the second dose but without the use of the adjuvant. All immunizations were administered intramuscularly into alternate rear hips. Two weeks after the final immunization (day 56), the rats were challenged with agar beads containing *P. aeruginosa* cells of the Fisher-Devlin immunotype 6 strain. The rats were first anesthetized with an intraperitoneal injection of sodium pentobarbital and then inoculated via a tracheal incision with 60 μl of agar bead slurry encasing approximately 5×10^4 CFU of *P. aeruginosa*. A curved, beaded-tip 20-gauge needle was gently guided to favor inoculation of the left lung. The incision was closed with sterile wound clips. Seven days after challenge (day 63), the rats were sacrificed by an overdose of halothane (Ayerst Laboratories, Inc., New York, N.Y.). The lungs were examined macroscopically for the presence of lesions. Significant lung pathology was scored as the presence of multiple (three or more) small lesions not exceeding 1 mm in diameter or the presence of any medium (2 to 5 mm in diameter) or large (exceeding 5 mm) lesion. Protection was considered to be the complete absence of lesions or no more than 1 or 2 small lesions (not exceeding 1 mm in diameter). Scoring of the pulmonary lesions was done independently by two investigators experienced in macroscopic lung lesion scoring. Statistical analyses of the scoring of the lesions between groups of immunized rats were performed with the IBM EpiStat Basic Statistics Program, with *P* values calculated by the one-tailed Fisher exact test. *P* values ≤ 0.05 were considered to be significant. All animals used in this study were handled in accordance with the guidelines of the Louisiana State University Medical Center—Shreveport Animal Care and Use Committee.

TABLE 1. Ability of peptides 9 and 10 to prevent pulmonary pathology in immunized rats in the chronic pulmonary infection model

Immunization group	No. of rats with significant lesions/ total no. of rats (%)	*P* value
Expt 1		
KLH	16/24 (66.6)	
Protein F	9/24 (37.5)	0.041
Peptide 9	7/23 (30.4)	0.014
Expt 2		
KLH	14/21 (66.6)	
Protein F	5/25 (20.0)	0.002
Peptide 10	4/23 (17.4)	0.001

We provide the first evidence that each of the peptides can protect against pulmonary lesions upon active immunization of rats in the chronic pulmonary infection model (Table 1). When compared with the control rats immunized with cross-linked KLH alone, the rats immunized with either peptide 9-KLH conjugate or peptide 10-KLH conjugate were afforded protection from significant lung lesions upon challenge with the immunotype 6 strain of *P. aeruginosa* encased within agar beads. In each case, the protection afforded by the synthetic peptide was comparable to the protection afforded by purified PAO1 OM protein F itself. In previous studies using this rat model of chronic pulmonary infection, we have shown that protection against macroscopic lung lesions is associated both with clearance of *P. aeruginosa* from the lungs (6, 7) and with the preservation of pulmonary function in the lungs (3). Thus, we believe that protection from macroscopic lung lesions is a

P. aeruginosa peptide 9	T	D	A	Y	N	Q	K	L	S	E	R	R	A	N
E. coli OmpA	S	D	A	Y	N	Q	G	L	S	E	R	R	A	Q
S. marcescens OmpA	S	D	Q	Y	N	Q	K	L	S	E	Q	R	A	Q
S. typhimurium OmpA	S	D	A	Y	N	Q	G	L	S	E	K	R	A	Q
E. aerogenes OmpA	S	E	Q	Y	N	Q	K	L	S	E	K	R	A	Q
K. pneumoniae OmpA	S	E	A	Y	N	Q	Q	L	S	E	K	R	A	Q
N. gonorrhoeae PIII	S	E	K	Y	N	Q	A	L	S	E	R	R	A	Y
H. influenzae P6	T	P	E	Y	N	I	A	L	G	Q	R	R	A	D
E. coli PAL	T	P	E	Y	N	I	S	L	G	E	R	R	A	N

FIG. 1. Homology of peptide 9 with OmpA-related proteins in various other bacteria. Amino acids identical to those of peptide 9 are shown in boldface type against a shaded background. *E. aerogenes*, *Enterobacter aerogenes*.

reliable indicator of the protective efficacy of a *Pseudomonas* vaccine in this rat model.

OM protein F of *P. aeruginosa* displays strong homology in the C-terminal region to various OM proteins from unrelated species, including OmpA from enteric bacilli in particular (2, 4, 12). These proteins are referred to as the OmpA family of peptidoglycan-associated proteins, all of which possess a conserved C-terminal domain but have unrelated N-terminal domains of various sizes (1). This raised the possibility that antibodies directed against peptides 9 and 10 cross-react to a significant degree with these other bacterial species, an undesirable attribute in any vaccine to be used against *P. aeruginosa* infections. There is considerable homology between peptide 9 and the various OmpA-related proteins, as shown in Fig. 1. The various OmpA proteins in the enteric bacilli and the PIII protein of *N. gonorrhoeae* had 9 to 11 amino acid residues identical to those in the internal 12 amino acid residues of the peptide 9 14-mer, whereas the *H. influenzae* P6 and the *E. coli* PAL proteins had less homology. The peptide 10 14-mer had less homology with the various OmpA-related proteins (Fig. 2). Multiple sequence alignment (2, 12) of the various proteins revealed at most for the *S. marcescens* OmpA protein 8 residues identical to those in peptide 10. However, a large intervening sequence separated these residues, with no more than 4 identical residues being contiguous. From the degree of homology seen between peptides 9 and 10 and the various OmpA-related proteins, one might predict cross-reaction between antibodies directed toward peptide 9 with these various proteins but not between antibodies directed toward peptide 10 with these various proteins. This was the case as shown by our immunoblot experiments.

By our routine procedure for immunoblotting, we have previously shown (16) that antisera directed against protein F give no discernible reaction upon immunoblotting with *E. coli* cell envelope proteins. In order to detect cross-reaction with the various OmpA-related proteins, we loaded the gel with a sixfold-higher protein concentration for the various other bacteria than for the pseudomonads and extended the color development. With these modifications, antibodies to both protein F and peptide 9 reacted strongly with the cell envelope proteins of *P. aeruginosa* and *P. fluorescens* and reacted more weakly with the cell envelope proteins of *E. coli*, *S. typhimurium*, *E. cloacae*, *K. pneumoniae*, *N. gonorrhoeae*, and *H. influenzae* (Fig. 3A and B). Antisera to peptide 9 reacted extremely weakly, if at all, with *S. marcescens* cell envelope proteins (Fig. 3B). The antisera to peptide 10 reacted strongly with the cell envelope proteins of *P. aeruginosa* but only reacted weakly with those of *P. fluorescens* (Fig. 3C). Of the various OmpA-related proteins tested, antisera to peptide 10 cross-reacted only with those of *N. gonorrhoeae*. None of the three antisera cross-reacted with *B. cepacia*. The pattern of cross-reactions observed by immunoblot analysis agreed well with predicted cross-reactions based on homology patterns, i.e., there was extensive cross-reaction detectable between the various OmpA-related proteins with antibodies directed toward OM protein F or peptide 9 but very little cross-reaction (only *N. gonorrhoeae* proteins) with antibodies directed toward peptide 10.

The biological significance of the antibody cross-reactions seen upon immunoblotting was investigated by ELISA with whole cells of the various bacterial species as the antigen. The ability of the antibodies to react with the surface of intact bacterial cells is a more important property, with more relevance to the role of the antibodies in protecting against *P. aeruginosa* infections. The protein F antisera reacted at high titers with the whole cells of the 7 Fisher-Devlin immunotype strains, with the 23 clinical strains of *P. aeruginosa* isolated from non-CF patients, and with the 29 clinical strains of *P. aeruginosa* isolated from CF patients (data not shown). The

P. aeruginosa peptide 10	N	A	T	A	E	—	G	R	—	—	—	—	—	—	—	—	—	—	A	I	N	R	R	V	E
E. coli OmpA	N	T	C	D	N	—	V	K	Q	R	A	—	A	L	I	D	C	L	A	P	D	R	R	V	E
S. marcescens OmpA	N	T	C	G	Y	*	G	R	A	T	K	—	A	Q	I	V	C	L	A	P	D	R	R	V	E
S. typhimurium OmpA	N	T	C	D	N	—	V	K	P	R	A	—	A	L	I	D	C	L	A	P	D	R	R	V	E
E. aerogenes OmpA	N	T	C	D	N	—	V	K	A	R	A	—	A	L	I	D	C	L	A	P	D	R	R	V	A
K. pneumoniae OmpA	N	T	C	D	N	—	V	K	A	R	A	—	A	L	I	D	C	L	A	P	D	R	R	V	E
N. gonorrhoeae PIII	Q	V	C	Q	A	—	E	V	A	K	L	*	A	L	I	A	C	I	E	P	D	R	R	V	D
H. influenzae P6	G	H	D	E	A	—	A	Y	—	—	—	—	—	—	—	—	—	—	S	K	N	R	R	A	V
E. coli PAL	G	H	D	E	A	—	A	Y	—	—	—	—	—	—	—	—	—	—	S	K	N	R	R	A	V

FIG. 2. Multiple sequence alignment (2, 12) of the C-terminal regions of various OmpA-related proteins to reveal homology with peptide 10. Amino acids identical to those of peptide 10 are shown in boldface type against a shaded background. Dashes represent gaps introduced for optimal alignment. Asterisks represent amino acid insertions. The insertion in the OmpA *S. marcescens* sequence is KS, and the insertion in the *N. gonorrhoeae* PIII sequence is GAKASKAKKRE. *E. aerogenes*, *Enterobacter aerogenes*.

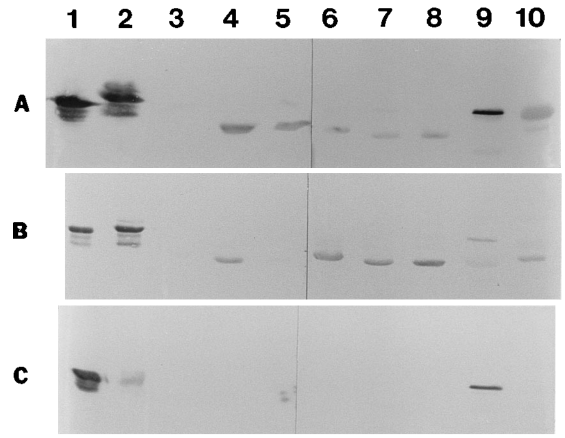

FIG. 3. Immunoblot analyses of cell envelope proteins extracted from the various bacteria reacted with antisera directed against *P. aeruginosa* purified protein F, peptide 9, or peptide 10. Lanes 1 through 10 contained proteins from the following bacteria: 1, *P. aeruginosa*; 2, *P. fluorescens*; 3, *B. cepacia*; 4, *E. coli*; 5, *S. marcescens*; 6, *S. typhimurium*; 7, *E. cloacae*; 8, *K. pneumoniae*; 9, *N. gonorrhoeae*; 10, *H. influenzae*. (A) Immunoblot with protein F-directed antisera. (B) Immunoblot with peptide 9-directed antisera. (C) Immunoblot with peptide 10-directed antisera. In each panel, the portion of the immunoblot revealing proteins in the 30- to 45-kDa range is shown. Note in panel A that anti-protein F immunoglobulin G antibodies reacted heavily with protein F bands in *P. aeruginosa* and *P. fluorescens* but reacted weakly with bands in lanes 4 to 10 even though a sixfold-higher protein concentration was loaded in these seven lanes. No reaction is seen against *B. cepacia* proteins in lane 3. In panel B, a similar pattern is seen, i.e., a strong reaction is seen in lanes 1 and 2, no reaction is seen in lane 3, and weaker reactions are seen in lane 4 and lanes 6 to 10. No reaction is seen in lane 5 in this case. In panel C, a strong reaction is seen in lane 1 only. *P. fluorescens* exhibits a very weak reaction (lane 2) against peptide 10 antisera. Of the various other bacteria in the OmpA-associated family, only *N. gonorrhoeae* in lane 9 reacted with peptide 10 antisera.

TABLE 2. Titers of immunoglobulin G antibodies in the peptide 9 antisera reactive to whole cells of the various bacterial strains as determined by ELISA

Bacterial strain (no. of strains)	No. of strains reactive at indicated titer							
	0	5–20	40–60	80–240	320–640	960–2,560	3,200–7,680	8,800–≥10,240
P. aeruginosa FD1[a]						1		
P. aeruginosa FD2					1			
P. aeruginosa FD3						1		
P. aeruginosa FD4						1		
P. aeruginosa FD5						1		
P. aeruginosa FD6						1		
P. aeruginosa PAO1							1	
P. aeruginosa KG1077[b]	1							
P. aeruginosa (23 non-CF)					2	19	2	
P. aeruginosa (29 CF)						16	13	
P. fluorescens					1			
B. cepacia (2)	2							
E. coli K-12	1							
E. coli (10 clinical)	5	4	1					
S. marcescens (3)	1	1	1					
S. typhimurium (2)	1	1						
E. cloacae (3)	1	2						
K. pneumoniae (3)	2		1					
N. gonorrhoeae (3)	2	1						
H. influenzae (3)	3							

[a] FD, Fisher-Devlin immunotype.
[b] *P. aeruginosa* KG1077 is a protein F-deficient mutant of the PAO1 strain.

only *P. aeruginosa* strain that did not react with the protein F antisera at a high titer was the protein F-deficient mutant. The protein F antisera also reacted at a high titer with *P. fluorescens* but did not react with *B. cepacia*. There was no significant titer of antibodies reactive with most of the various whole cells from the OmpA-related protein-associated bacteria. Of 11 *E. coli* strains, 10 had titers of 0 to 20, with one strain having a titer of 40. None of the strains of *S. typhimurium*, *E. cloacae*, *K. pneumoniae*, *N. gonorrhoeae*, or *H. influenzae* had a whole-cell reactive titer of greater than 20. Two of the three strains of *S. marcescens* had titers of 120 to 240, the highest titers seen for the various cross-reactions among the non-*Pseudomonas* strains. This same pattern of whole-cell reactivities was seen with the antisera directed toward peptide 9 (Table 2) and toward peptide 10 (Table 3). The peptide 9 antisera reacted with all 59 strains of *P. aeruginosa*, with titers of 320 or higher. Of 28 strains of the various non-*Pseudomonas* strains, 25 reacted with titers of 0 to 20, with only 3 strains reacting at titers of 40 to 60. The peptide 10 antisera reacted with all 59 strains of *P. aeruginosa*, with titers of 3,200 or higher. Of 28 strains of

TABLE 3. Titers of immunoglobulin G antibodies in the peptide 10 antisera reactive to whole cells of the various bacterial strains as determined by ELISA.

Bacterial strain (no. of strains)	No. of strains reactive at indicated titer							
	0	5–20	40–60	80–240	320–640	960–2,560	3,200–7,680	8,800–≥10,240
P. aeruginosa FD1[a]							1	
P. aeruginosa FD2							1	
P. aeruginosa FD3							1	
P. aeruginosa FD4							1	
P. aeruginosa FD5							1	
P. aeruginosa FD6								1
P. aeruginosa PAO1							1	
P. aeruginosa KG1077[b]	1							
P. aeruginosa (23 non-CF)							12	11
P. aeruginosa (29 CF)							6	23
P. fluorescens					1			
B. cepacia (2)	2							
E. coli K-12	1							
E. coli (10 clinical)	3	6	1					
S. marcescens (3)	1			2				
S. typhimurium (2)		2						
E. cloacae (3)		3						
K. pneumoniae (3)		3						
N. gonorrhoeae (3)	2	1						
H. influenzae (3)	3							

[a] FD, Fisher-Devlin immunotype.
[b] *P. aeruginosa* KG1077 is a protein F-deficient mutant of the PAO1 strain.

the various non-*Pseudomonas* strains, 25 reacted with titers of 0 to 20, with 1 strain of *E. coli* reacting with a titer of 40 and 2 strains of *S. marcescens* reacting at titers of 120 and 160. We conclude from these ELISA data that the level of cross-reacting antibodies would be too low (titers of 60 or less) to provide protection against infection with any of these various other bacteria, with the possible exception of some strains of *S. marcescens*. Indeed, we have previously shown that immunization with protein F cannot afford protection against infection with *E. coli* (16). Furthermore, the low level of cross-reaction with whole cells of these various other bacteria should not interfere with the high titer of *P. aeruginosa*-reactive antibodies to significantly reduce their protective efficacy against *P. aeruginosa* infection. This is as expected, especially since the C-terminal portion of OmpA proteins in enteric bacilli is contained within the periplasmic space and is not surface accessible to bind antibodies (18). Thus, we believe that peptide 9 and peptide 10 elicit antibodies that are functionally pseudomonad-specific, with the cross-reaction seen with homologous OmpA-related proteins being too weak in nature to have functional significance.

The reaction of the peptide 9- and peptide 10-directed antibodies with the various *Pseudomonas* strains requires additional comment. The antisera of neither peptide reacted with the protein F-deficient mutant, which is as expected. The antisera of both peptides reacted with *P. fluorescens*, but neither reacted with *B. cepacia* (another important pathogen in CF patients). Regarding the reaction of the antisera of each peptide with the 59 strains of *P. aeruginosa* tested, the antisera of both peptides reacted with all strains. Since all the clinical isolates, from both CF and non-CF patients, reacted at significant titers with the antisera of both peptides, there does not appear to be widespread variability among various strains of *P. aeruginosa* at the site of these two epitopes of protein F. Thus, both peptides 9 and 10 appear to have vaccine potential against all strains of *P. aeruginosa* tested.

Both the peptide 9 and the peptide 10 antisera showed the pattern of reacting at higher titers with the isolates from CF patients than with the isolates from non-CF patients. For example, only 2 of 23 (8.7%) isolates from non-CF patients reacted with the peptide 9 antisera at titers of 3,200 to 7,680, whereas 13 of 29 (44.8%) isolates from CF patients reacted at titers of 3,200 to 7,680. Similarly, only 11 of 23 (47.8%) isolates from non-CF patients reacted with the peptide 10 antisera at titers of 8,800 to $\geq$10,240, but 23 of 29 (79.3%) isolates from CF patients reacted at this titer. One possible explanation for this may be that the isolates from CF patients have lost some of the O antigens (11, 17), so that protein F becomes more accessible to antibodies. This observation supports our previous contention (7) that, theoretically, a protein F-based vaccine (such as one containing peptides 9 and 10) should elicit antibodies that react with *P. aeruginosa* upon initial colonization and which should react even better once the bacterial cells begin to lose their O antigens to become rough.

In summary, both peptides 9 and 10 appear to have potential for further development as a vaccine against infections caused by *P. aeruginosa*. Each peptide demonstrated the ability to protect against pulmonary lesions upon challenge with agar-encased *P. aeruginosa* in the rat model of chronic lung infection. Antibodies directed against peptide 9 or 10 reacted at a high titer with all strains of *P. aeruginosa* tested but reacted only quite weakly with the various other bacterial species which possess OM proteins sharing homology with protein F. Thus, peptides 9 and 10 each appear to elicit protective antibodies which are functionally pseudomonad specific.

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