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Surface Localization of *Pseudomonas aeruginosa* Outer Membrane Porin Protein F by Using Monoclonal Antibodies

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Hybridomas secreting highly specific monoclonal antibodies against porin protein F of *Pseudomonas aeruginosa* were isolated. These antibodies interacted with protein F in outer membranes isolated from strains representing the 17 serotypes of *P. aeruginosa* and from another 15 clinical isolates from patients with cystic fibrosis. The cell surface localization of antigenic sites on protein F was shown by indirect immunofluorescent techniques with these monoclonal antibodies. No fluorescence was observed on a protein F-deficient strain H283 of *P. aeruginosa*. Another monoclonal antibody specific for outer membrane lipoprotein H2 of *P. aeruginosa* showed no fluorescence on intact, wild-type bacterial cells, but was able to interact with a rough, LPS-deficient mutant.

The outer membranes of gram-negative bacteria are composed of two major classes of macromolecules, lipopolysaccharide (LPS) and proteins (9). Most of the studies on the antigenicity of these surface components have focused on LPS (15, 19). In principle, the antigenicity of outer membrane polypeptides has been shown directly for *Escherichia coli* (5, 13) and *Neisseria gonorrhoea* (4), as well as indirectly by active protection against eye infections with *Shigella* spp. (1) and against experimental salmonellosis in mice (17). More recently, Hansen et al. (12) have demonstrated the immunogenicity of the outer membrane proteins of *Haemophilus influenzae* type b in infant rat models.

When outer membranes of *Pseudomonas aeruginosa* are resolved by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis, up to nine major polypeptides can be identified, depending on growth conditions (6, 9). One of these, protein F, is present in 1×10^5 to 3×10^5 copies per cell (21) and is the major hydrophilic channel of *P. aeruginosa* (21), with an exclusion limit for saccharides of ca. 6,000 daltons (9), equivalent to a channel diameter of 2.3 nm (3). Despite this large channel size, protein F shows low activity with only about 0.2% of the available protein F forming active functional channels (21). The consequent low total channel area available for antibiotic diffusion explains in part the well-known high intrinsic resistance of *P. aeruginosa* to antibiotics.

Lambert and Booth (18) have recently demonstrated surface labeling of protein F with ^{125}I-labeled lactoperoxidase. However, Sullivan and Williams (26) reported that radio-iodination of whole cells of *N. gonorrhoea* by this technique was not specific and labeled other, non-outer membrane, proteins, whereas surface labeling by dansylation of cell envelope proteins of *P. aeruginosa* only occurred after Tris-EDTA treatment (25).

Bacterial pathogens interact with their hosts via their surface components. Some of these bacterial "antigens" elicit, in hosts, immune responses specific for the injecting antigen. Characterization of outer membrane proteins exposed on bacterial surfaces is thus important in the search for potential vaccines and in studies on the interaction of these proteins with host immune mechanisms as well as in the study of the composition of these surfaces. Recently, surface-exposed protein antigens on the cell surfaces of *H. influenzae* type b (11) and gonococci (27) were demonstrated in studies with antiserum directed against the intact organism.

We previously demonstrated conservation of some of the major outer membrane polypeptides among the 17 *P. aeruginosa* serotype strains of the International Antigen Typing Scheme (19) by protein patterns on SDS-polyacrylamide gel electrophoretograms and by cross-reaction of the major outer membrane proteins of these serotype strains with rabbit antisera raised against partially purified proteins and whole outer membranes (20). Furthermore, the finding that there was conservation of receptor sites for protein-specific phages on these serotype strains suggested that some of these conserved proteins are exposed on the surface (18).

To study the surface antigenic structure of *P. aeruginosa* outer membrane proteins, we applied hybridoma technology developed by Kohler and Milstein (16) to the production of highly

specific monoclonal antibodies that react with major outer membrane protein F of *P. aeruginosa*. In this paper we demonstrate by using these monoclonal antibodies, the conservation of specific antigenic determinants on protein F among a wide variety of *P. aeruginosa* strains including isolates from patients with cystic fibrosis, and, by using immunofluorescence techniques, the surface localization of antigenic sites of the protein on the intact bacterial surface.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *P. aeruginosa* PAO1 strain H103 (6) was used as a standard strain in the isolation of outer membrane proteins and immunization. A set of 17 serotype-specific strains (10), representative of the International Antigen Typing Scheme for *P. aeruginosa* (17), was a kind gift from P. Liu. In addition, 14 strains isolated from cystic fibrosis patients were obtained from G. Pier, Harvard Medical School, Boston, Mass. These strains were named CF3660-1, CF9490, CF221, CF4349, CF4522, CF832, CF3790, CF1278, L, CF1452, CF6094, CF2314, CF283, and CF284. A mucoid isolate P1 and its spontaneous nonmucoid revertant were obtained from D. Speert, Children's Hospital, Vancouver, British Columbia, Canada. The protein F-deficient strain H283 was isolated from strain H103 as described previously (21). Strain H223 was a rough, LPS-altered mutant of strain H103 (10). All other bacterial strains and species (see Fig. 3 legend) as well as all growth and culture conditions were as described previously (10).

Outer membrane techniques. Outer membranes were isolated by the one-step procedure described previously (6). Protein F was purified by L. Chan in our laboratory as previously described (7).

Isolation of monoclonal antibodies and ELISA methods. Monoclonal antibodies were isolated by minor modifications of the methods of Kohler and Milstein (16) as described previously (10). Titration of monoclonal antibodies was performed by using a modification of the enzyme-linked immunosorbent assay (ELISA) method (10, 24). Polyvinyl chloride microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated for 1 h at 37°C with 50 or 100 μl of 20-μg/ml outer membranes or 10- to 15-μg/ml of purified outer membrane proteins in bicarbonate coating buffer (24). Plates were then washed three times with phosphate-buffered saline (PBS; 137 mM NaCl, 1.47 mM KH_2PO_4, 20.4 mM $Na_2HPO_4 \cdot 7H_2O$, 3.1 mM NaN_3, 2.68 mM KCl) (pH 7.4) containing 5 mM $MgCl_2$. Further nonspecific binding was blocked by incubation with 1% fetal calf serum (FCS; GIBCO Canada, Ontario) in PBS for 30 min at 37°C. The plates were subsequently incubated with dilutions of the test antibody, serum, or culture supernatant in 1% FCS–PBS for 1 h at 37°C, washed three times in PBS, and incubated for a further 1 h at 37°C with a 1,000-fold dilution, in 1% FCS–PBS, of goat anti-mouse F(ab)' fragments conjugated to alkaline phosphatase (Helix Biotech Ltd., Richmond, British Columbia, Canada). Plates were then washed three times in PBS, and alkaline phosphatase substrate (Sigma Chemical Co., St. Louis, Mo.) in 10% diethanolamine buffer (pH 9.8 with HCl) was added. The color developed was read after 45 min with an ELISA reader (Titertek Multiscan; Flow Laboratories, McLean, Va.) set at 405 nm. Control experiments with normal mouse serum or ascites of the NS-1 myeloma cell line as sources of first antibody or heterologous proteins as antigens produced only background responses in our ELISA procedure.

Heavy-chain isotype analysis of the monoclonal antibodies was performed by double immunodiffusion (23) in 2% (wt/vol) barbital buffer (pH 8.6), using culture supernatants of the monoclonal antibody-producing hybridomas and subclass-specific antisera against mouse immunoglobulin G1 (IgG1), IgG2a, IgG2b, IgG3, and IgM from Miles Laboratories Ltd. (Ontario, Canada).

Electrophoretic blotting procedure and immunological detection of proteins. The outer membrane proteins of *P. aeruginosa* strains were separated by SDS-polyacrylamide gel electrophoresis as described previously (10), and the separated proteins were transferred to nitrocellulose paper by the western blotting procedure described by Towbin et al. (28). Subsequent immunostaining of outer membrane components followed closely the method of O'Connor and Ashman (22), except that PBS (pH 7.4) was used as the buffer in all steps and all incubations were for 1 h at 37°C.

Immunofluorescence techniques. *P. aeruginosa* cells grown in 1% (wt/vol) Proteose Peptone no. 2 broth (Difco Laboratories, Detroit, Mich.) to an optical density at 600 nm of 0.5 to 0.8 were washed twice in PBS and resuspended at an optical density of 0.3 to 0.5 in PBS for subsequent procedures.

Indirect immunofluorescence was performed by a modification of the procedures described previously by Hofstra et al. (14). Bacterial smears on glass slides were incubated with a 50- to 100-fold dilution of monoclonal antibody or a 1,000-fold dilution of polyclonal rabbit-anti *P. aeruginosa* outer membrane serum (20) in 1% FCS–PBS for 30 min. After three washes in PBS, the smears were incubated with goat anti-mouse IgG serum (Flow Laboratories Inc., Ontario, Canada) diluted 100-fold in 1% FCS-PBS for 30 min at 23°C. After three washes in PBS, the smears were then coated with fluorescein isothiocyanate-conjugated rabbit anti-goat immunoglobulin (Sigma) diluted 20-fold in 1% FCS–PBS and incubated for 30 min. After being washed, the smears were air dried and examined with a Zeiss microscope (Standard RA, with a condenser for fluorescence microscopy) containing a halogen lamp and suitable filters for emission of fluorescein isothiocyanate at 525 nm.

RESULTS

Isolation of protein F-specific monoclonal antibodies. After fusion of NS-1 or SP2/0 myeloma cells with spleen cells of mice immunized with purified protein F, hybridomas producing antibodies specific for outer membrane protein F were cloned by limiting dilution until a single clone in each well was obtained. By using this procedure, six monoclonal antibodies producing clones specific for protein F were isolated from different fusions. The selected hybridomas and their corresponding antibodies were named

MA2-10, MA3-11, MA4-2, MA4-4, MA4-10, and MA5-8 (Table 1). Each of these monoclonal antibodies gave a single precipitin line in Ouchterlony double immunodiffusion analyses against antisera specific for only one of the five mouse immunoglobulin isotypes tested (Table 1). Furthermore, they specifically interacted with only protein F on western electrophoretic blots of either the purified immunizing antigen or outer membranes.

Specificity of monoclonal antibodies and interaction with outer membranes of serotyping strains and of cystic fibrosis *P. aeruginosa* isolates. Monoclonal antibodies specific for protein F were tested by ELISA for binding to outer membranes of the 17 serotype strains of *P. aeruginosa* and 16 cystic fibrosis patient isolates. Results for MA4-4 and MA2-10 are presented in Table 2. These monoclonal antibodies reacted with all of the outer membranes of the *P. aeruginosa* strains tested, although the reactions varied from strain to strain and with the different monoclonal antibodies. Thus, the antigenic sites recognized by these monoclonal antibodies on protein F are apparently common to all of these *P. aeruginosa* strains.

This was confirmed for all protein F-specific monoclonal antibodies by interaction with electrophoretic blots of separated outer membrane proteins from *P. aeruginosa* serotyping strains (Fig. 1). Generally, only one band identified as protein F by comparison with identical blots

TABLE 1. Classification of the monoclonal antibodies to porin protein F

Designation	Immunoglobulin subclass	ELISA titers against:[a]		
		Purified protein F[b]	Strain H103 outer membranes	Strain H103 whole cells
MA2-10	IgG1	10^5	10^4	10^4
MA3-11	IgG1	10^4	10^2	ND
MA4-2	IgG3	10^2	10^1	10^2
MA4-4	IgG2a	10^5	10^2	10^2
MA4-10	IgG1	10^5	10^3	10^3
MA5-8	IgG2b	10^5	10^3	ND

[a] ELISA titers were obtained by using ascites fluid as the primary antibody in ELISA assays. The ELISA titer was the highest 10-fold dilution giving 30% of the rate of substrate (paranitrophenyl phosphate) conversion to products when compared with the 10-fold-diluted ascites. Control ascites fluid gave only background responses. Whole cell ELISAs were performed as described in the text except that 10^2 cells in 0.1 ml PBS were used as antigens to coat polyvinyl chloride plates which had been precoated with poly-L-lysine (1 μg in 0.1 ml of PBS) for 1 h at 37°C. ND, Not determined.

[b] Purified protein F was derived from strain H103.

TABLE 2. Interaction of monoclonal antibodies MA2-10 and MA4-4 with outer membranes of *P. aeruginosa* serotype strains and cystic fibrosis isolates

Source of outer membrane antigen	ELISA titer of monoclonal antibody[a]	
	MA4-4	MA2-10
PAO1 (H103)	10^2 (0.66)[b]	10^4 (1.42)
Purified protein F (from H103)	10^5 (1.16)	10^5 (1.54)
Serotype strains		
1	10^4 (0.58)	10^4 (0.62)
2	10^3 (0.24)	10^4 (0.25)
3	10^3 (0.27)	10^4 (0.24)
4	10^4 (0.40)	10^5 (0.32)
5	10^4 (2.0)	10^4 (2.0)
6	10^5 (1.54)	10^4 (2.0)
7	10^4 (2.0)	10^4 (1.79)
8	10^4 (0.89)	10^5 (0.92)
9	10^4 (1.3)	10^5 (1.0)
10	10^4 (1.4)	10^4 (1.29)
11	10^4 (1.67)	10^4 (2.0)
12	10^4 (0.72)	10^4 (0.72)
13	10^4 (1.11)	10^4 (1.2)
14	10^4 (0.13)	10^4 (0.20)
15	10^4 (0.37)	10^4 (0.42)
16	10^4 (0.71)	10^4 (0.69)
17	10^4 (1.17)	10^4 (1.09)
Cystic fibrosis isolates		
P1 (mucoid)	10^3 (0.2)	10^3 (0.54)
P1 (nonmucoid)	10^4 (0.6)	10^3 (1.11)
CF3660-1	10^4 (0.24)	10^3 (0.29)
CF9490	10^5 (0.5)	10^3 (0.61)
CF221	10^5 (0.58)	10^3 (0.62)
CF4349	$<10^2$ (0.15)[b]	$<10^2$ (0.13)[b]
CF4522	10^5 (0.69)	10^3 (0.72)
CF832	10^3 (0.73)	10^3 (0.78)
CF3790	10^4 (0.48)	10^3 (0.55)
CF1278	10^2 (0.22)[b]	10^2 (0.34)[b]
L	10^5 (0.7)	10^3 (0.71)
CF1452	10^5 (0.43)	10^3 (0.37)
CF6094	10^2 (0.15)	10^3 (0.18)
CF2314	10^4 (0.46)	10^3 (0.41)
CF283	$<10^1$ (0.10)[b]	$<10^1$ (0.10)[b]
CF284	10^4 (0.53)	10^4 (0.48)

[a] ELISA readings are the average of three experiments. Numbers in parentheses represent the average optical density at 490 nm after 1 h of incubation at 37°C with *p*-nitrophenyl phosphate. Controls lacking antigen or using ascites fluid from NS-1 myeloma cells gave optical densities of 0 to 0.04.

[b] Although interaction of antibodies with these antigens was weak or barely above background, after overnight incubation color appeared for all except CF4349 and CF283. All of the strains except CF283 interacted with these monoclonal antibodies on blots. CF4349 showed weak interaction.

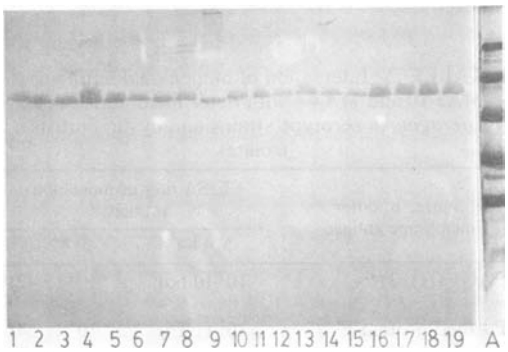

FIG. 1. Western electrophoretic blots of outer membranes of the serotyping strains of *P. aeruginosa* after treatment with monoclonal antibody MA5-8. The blot was made by electrophoretic transfer of separated outer membrane proteins from SDS-polyacrylamide gels onto nitrocellulose paper. These electrophoretic blots were treated with the monoclonal antibodies followed by a goat antimouse alkaline phosphatase-conjugated antibody and addition of the substrate (Napthol AS MX phosphoric acid and fast red TR salt). Outer membrane samples are: lane 1, serotype 17; lane 2, serotype 16; lane 3, serotype 15; lane 4, serotype 14; lane 5, serotype 13; lane 6, serotype 12; lane 7, serotype 11; lane 8, serotype 10; lane 9, purified protein F; lane 10, serotype 9; lane 11, serotype 8; lane 12, serotype 7; lane 13, serotype 6; lane 14, serotype 5; lane 15, serotype 4; lane 16, serotype 3; lane 17, serotype 2; lane 18, serotype 1; lane 19, wild-type H103. Lane A shows molecular weight standards: bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400).

stained with amido black was evident in the immunolabeled blots. Exceptions were purified protein F fractions (Fig. 1, lane 9) and outer membrane (lanes 7 and 8) or overloaded outer membrane protein gels (see Fig. 3, lanes 1 and 10) for which higher-molecular-weight forms of protein F were stained, presumably due to either native oligomers of protein F or protein F-LPS complexes which were also identified in a cross-linking study (2). With these exceptions, single bands were obtained with the other protein F-specific monoclonal antibodies, and the intensity of the label on the blots correlated with the ELISA results for the different outer membrane isolates. We did not observe any labeled band in electrophoretic blots with outer membranes of a protein F-deficient mutant of strain H283 (see below, Fig. 3, lane 2).

The SDS-polyacrylamide gel electrophoresis outer membrane protein patterns of the cystic fibrosis *P. aeruginosa* isolates were similar to those of strain H103 (data not shown). Protein F from these isolates interacted well with monoclonal antibodies MA4-4 and MA2-10 (Fig. 2) on electrophoretic blots. There was no interaction of monoclonal antibody MA4-4 with outer membrane proteins of *Edwardsiella tarda*, *Vibrio anguillarum*, *P. anguilliseptica*, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, or *Azotobacter vinelandii*, although it interacted strongly with a protein from *Pseudomonas putida* outer membranes (Fig. 3).

Surface localization of antigenic sites on protein F. Monoclonal antibodies were tested for their ability to bind to the cell surfaces of intact *P. aeruginosa* cells, using indirect immunofluorescence techniques. Whole cells of *P. aeruginosa* H103 interacted with all protein F-specific monoclonal antibodies (Fig. 4). The three monoclonal antibodies tested against the protein F-deficient mutant strain H283 did not interact, as demonstrated by lack of fluorescence on these cells (Table 3). In contrast, these three monoclonal antibodies interacted well with a rough, LPS-deficient mutant of strain H103 as well as a serotype 1 strain, ATCC 33348 (Table 3; Fig. 4). Both *P. aeruginosa* H103 and H283 interacted strongly with monoclonal antibody MA1-8, specific for serotype 5 O antigen (Fig. 4). As a control, we demonstrated that neither strain ATCC 33348 of different serotype nor strain H223 which lacked serotype-specific antigens showed fluorescence with MA1-8 as the first antibody (Table 3). Monoclonal antibody MA1-6

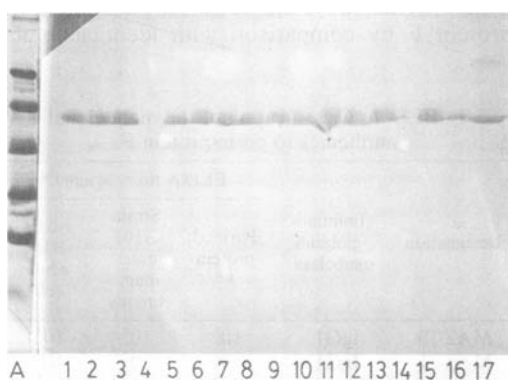

FIG. 2. Interaction of monoclonal antibody MA2-10 with outer membranes from cystic fibrosis *P. aeruginosa* isolates. The electrophoretic blots of the outer membranes were prepared as described in the legend to Fig. 1. The outer membrane samples in Fig. 2 are as follows: lane 1, CF3660-1; lane 2, CF9490; lane 3, CF221; lane 4, CF283; lane 5, CF4522; lane 6, CF832; lane 7, CF3790; lane 8, CF1278; lane 9, strain H103; lane 10, L; lane 11, CF1452; lane 12, CF6094; lane 13, CF2314; lane 14, CF4349; lane 15, CF284; lane 16, P1; lane 17, purified protein F. Lane A shows molecular weight standards: bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400). Only one band identified as protein F by comparison with stained electrophoretic blots was labeled in any of the membranes.

did not bind to intact cells of strains H103, H283, or ATCC 33348 (Table 3); however, it showed fluorescence with the rough mutant H223. All strains tested reacted strongly with a polyclonal rabbit anti-outer membrane serum as judged by indirect immunofluorescence with goat anti-rabbit IgG as second antibody.

In agreement with the above data, we observed binding of all protein F-specific monoclonal antibodies to whole cells of strain H103 when these cells were used as the antigen in ELISA assays involving poly-L-lysine-coated polyvinyl chloride plates (Table 1). In these experiments, the antibody titers obtained were similar to those obtained for H103 outer membranes (Table 1), although backgrounds tended to be higher due to nonspecific binding of the second antibody to bacterial cells.

DISCUSSION

In this paper, we report the production of six monoclonal antibodies specific for porin protein F of *P. aeruginosa*. Using these monoclonal antibodies, we demonstrated cross-reactivity and conservation of specific antigenic sites on protein F among 34 strains of *P. aeruginosa*. The monoclonal antibodies did not react with unrelated species, as shown on western immunoelectrophoretic blots, suggesting conservation of the antigenic sites on *P. aeruginosa* and taxonomically closely related strains only. We previously obtained similar results with a mono-

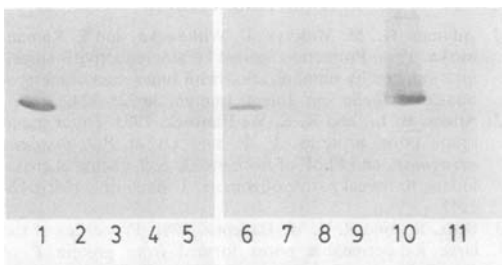

FIG. 3. Cross-reactivity of protein F-specific monoclonal antibody MA4-4 with outer membrane proteins from other bacterial species. Western electrophoretic blots of separated outer membrane proteins and immune staining were performed as described in the legend to Fig. 1. Lanes 1 and 6, *P. aeruginosa* H103; lane 2, *P. aeruginosa* H283, a protein F-deficient mutant; lane 3, *E. tarda* 79054; lane 4, *V. anguillarum* HT7602; lane 5, *A. hydrophila* ET-2; lane 7, *P. anguilliseptica* ET7601; lanes 8 and 9, *P. fluorescens* ATCC 13525 and ATCC 949, respectively; lane 10, *P. putida* ATCC 12633; lane 11, *A. vinelandii* OP. The bands of higher and lower molecular weight revealed by this monoclonal antibody were probably oligomeric associations of protein F and proteolytic degradation products, respectively (see Fig. 1 legend and text).

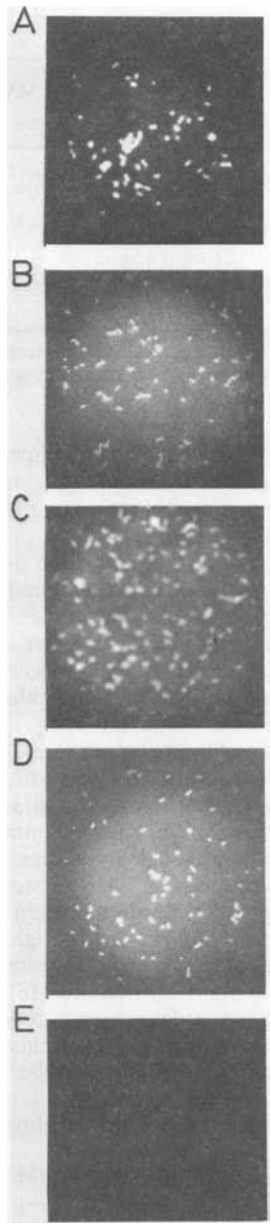

FIG. 4. Indirect immunofluorescent labeling of intact *P. aeruginosa* tagged with monoclonal antibodies to outer membrane component. (A) Strain H103 interacted with protein F-specific monoclonal antibody MA4-4. (B) Strain H223 interacted with MA2-10. (C) Strain H103 interacted with LPS O antigen-specific monoclonal antibody MA1-8. (D) Rough, LPS-deficient mutant strain H233 interacted with lipoprotein H2-specific monoclonal antibody MA1-6. (E) Strain H283 (F-minus mutant) interacted with protein F-specific monoclonal antibody MA2-10.

TABLE 3. Demonstration of cell surface localization of outer membrane components of *P. aeruginosa* by using indirect immunofluorescence

Bacterial strain[a]	Phenotype	Cell surface binding of antibodies against outer membrane components[b]					
		Protein F			Protein H2(MA1-6)	LPS O-antigen (MA1-8)	Whole outer membranes (rabbit antisera)
		MA2-10	MA4-4	MA4-10			
H103	Wild type; serotype 5	+	+	+	−	+	+
H283	Protein F deficient	−	−	−	−	+	+
H223	LPS deficient; rough	+	+	+	+	−	ND
ATCC 33348	serotype 1	+	+	+	−	−	+

[a] Strains were isogenic with the exception of ATCC 33348, the type strain for serotype 1 of *P. aeruginosa*.
[b] +, Positive fluorescence; −, no fluorescence; ND, not determined.

clonal antibody specific for an antigenic determinant on protein H2, although this antigenic site showed stronger conservation among taxonomically related pseudomonads (10).

We observed differences in the interaction of the protein F-specific monoclonal antibodies with protein F from different *P. aeruginosa* strains. This may be due either to different amounts of protein F in the various membranes or to the alteration of the antigenic site recognized by the monoclonal antibody, giving rise to differing antibody-antigen affinities.

A major observation of this study was that protein F is surface exposed and accessible to antibodies, as shown by indirect immunofluorescence with monoclonal antibodies specific for antigenic determinants on the protein. These antibodies did not bind a protein F-deficient mutant of *P. aeruginosa*. Although surface localization of protein F has been previously suggested by Lambert and Booth (18) using ^{125}I-labeled lactoperoxidase, some reports in the literature have shown that proteins other than outer membrane proteins can be labeled by using this technique, suggesting disruption of the outer membrane during the labeling procedure (25, 26).

Monoclonal antibody MA1-6 did not bind to protein H2 in intact smooth *P. aeruginosa* cells (Table 3). It did, however, bind to a rough, LPS-deficient mutant. This may be due either to the unmasking of antigenic determinants by loss of the O side chains of LPS or to rearrangements of the outer membrane components. The interaction of MA1-6 with a rough strain is significant since we have recently shown that many *P. aeruginosa* strains from patients with cystic fibrosis are deficient in LPS O side chains (8) and therefore might have protein H2 as a surface antigen.

In conclusion, we have reported the production of monoclonal antibodies specific for antigenic determinants present on protein F of *P. aeruginosa*. We have shown the use of these monoclonal antibodies as sensitive immunological probes in the study of the surface properties of the bacteria, and specifically the surface localization of protein F on intact cells. These should be useful in investigation of the interaction of pseudomonal surface antigens and the host.

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