INFECTION AND IMMUNITY, Sept. 1986, p. 621–627
0019-9567/86/090621-07$02.00/0

Vol. 53, No. 3

Monoclonal Antibodies to *Pseudomonas aeruginosa* Ferripyochelin-Binding Protein

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Received 18 February 1986/Accepted 12 May 1986

Hybridomas secreting specific monoclonal antibodies against the *Pseudomonas aeruginosa* ferripyochelin-binding protein (FBP) were isolated. These monoclonal antibodies reacted with FBP in immunoblots of outer membrane preparations from all serotypes of *P. aeruginosa*. Two of the monoclonal antibodies also reacted with FBP in strains of *P. putida*, *P. fluorescens*, and *P. stutzeri*. These antibodies did not react with outer membranes of *P. cepacia*, "*P. multivorans*," *P. maltophilia*, or other gram-negative organisms. The monoclonal antibodies were opsonophagocytic and blocked the binding of [^{59}Fe]ferripyochelin to isolated outer membranes of strain PAO. By indirect immunofluorescence techniques, the monoclonal antibodies were used to demonstrate that FBP is present on the cell surface of *P. aeruginosa* cells grown in low-iron but not high-iron medium. These observations were confirmed by using ^{125}I in surface-labeling techniques.

In most gram-negative bacteria, outer membrane components have been shown to play a role in the physiology of the organism as well as in the pathogenesis of diseases due to the organism. Outer membrane proteins exposed on the cell surface also have the potential to act as protective antigens against bacterial infections.

Pseudomonas aeruginosa has several major outer membrane proteins (4, 20), including some for which a physiological function has been identified (1, 5, 20). One protein has been shown to play a role in iron acquisition. The ferripyochelin-binding protein (FBP) binds iron when it is complexed with the *Pseudomonas* siderophore pyochelin (20). FBP is regulated by the iron concentration of the culture medium and is a major protein in iron-starved glucose-grown *P. aeruginosa* cells (20). An iron-regulated FBP has also been demonstrated in several other *Pseudomonas* species (19).

Polyclonal rabbit antiserum raised against purified FBP was previously shown to block ferripyochelin binding to isolated cell envelopes of *P. aeruginosa* (21) and to inhibit uptake of ferripyochelin by whole cells (22). Antibodies to FBP significantly enhanced phagocytosis of *P. aeruginosa* by human polymorphonuclear leukocytes. Passive transfer of anti-FBP antibodies was shown to protect against infection by some *P. aeruginosa* strains in a mouse burn infection model (22).

The existing evidence indicates that FBP is an important protein in the physiology of *P. aeruginosa* and may have potential as a protective antigen in the development of vaccines to combat *P. aeruginosa* infections. Therefore, in the present study we have isolated and characterized monoclonal antibodies which react specifically with FBP. We have used these monoclonal antibodies as probes to investigate the antigenic conservation of this protein among strains of *P. aeruginosa* and to reveal the antigenic heterogenicity of FBPs among *Pseudomonas* species. The monoclonal antibodies were also used in immunofluorescence experiments to demonstrate the presence of FBP on the surface of low-iron-grown *P. aeruginosa* cells. These observations were confirmed by using ^{125}I in surface-labeling techniques.

MATERIALS AND METHODS

Bacterial strains. *P. aeruginosa* PAO (7), PA103 (14), DG1 (2), M2 (24), Ps388 (9), 44821, and 50359 (22) have been previously described. The 13 *P. aeruginosa* strains representing the serotypes (A through M) of the Homma typing scheme (8) were provided by Allan Godfrey, University of Calgary. *P. cepacia* strains were previously described (15). Clinical isolates of *P. putida*, *P. fluorescens*, *Escherichia coli*, and *Serratia marcescens* were provided by M. Schaffer, Foothills Hospital, Calgary. *Salmonella* sp. strain LT2 was provided by K. E. Sanderson, University of Calgary. *E. coli* H10407 was provided by P. Shipley, Virginia Commonwealth University, Richmond.

Culture conditions. Cultures were grown in M9 minimal salts medium supplemented with 0.5% glucose at 32°C with maximum aeration to enrich for production of FBP (20). For high-iron medium, $FeCl_3$ was added to this medium to a final concentration of 100 μM. All glassware was acid washed and rinsed with deionized H_2O. All reagents were made with H_2O purified by the milli-Q system (Millipore Corp., Bedford, Mass.).

Purification of FBP. FBP was purified from cell envelopes of *P. aeruginosa* PAO as previously described (21).

Monoclonal antibody production. Dulbecco modified Eagle medium (DMEM) with sodium pyruvate (Flow Laboratories, Mississauga, Ontario, Canada) was supplemented with glutamine, penicillin, streptomycin, and 10% fetal calf serum (Myoclone; GIBCO, Burlington, Ontario). Hypoxanthine-aminopterin-thymidine and hypoxanthine-thymidine media were obtained from Flow Laboratories. The myeloma cell line NS-1 (a nonsecreting clone of P3 × 63 Ag8) was kindly provided by J. W. Costerton, University of Calgary. These cells were maintained in DMEM–10% fetal cell serum. When the cell density reached approximately 10^7 cells per ml, cells were diluted to 1 to 10^6 to 2 × 10^6 cells per ml in fresh DMEM–10% fetal calf serum and transferred. Cell cultures were maintained at 37°C under 7% CO_2.

Hybrid cell lines were prepared by published methods (10). BALB/c mice were injected four times intraperitoneally

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with 50 μg of FBP on days 0, 7, 14, and 21. Three days after the final injection, spleens were removed, and the spleen cells were collected. Spleen and NS-1 cells (10:1) were fused by 50% (wt/wt) polyethylene glycol. Fused cells were transferred into microtiter dishes at 6×10^4 input spleen cells per well and maintained for 14 days in hypoxanthine-aminopterin-thymidine medium. Hybridoma cell lines producing monoclonal antibody to FBP detectable by enzyme-linked immunosorbent assay (ELISA) (22) were transferred to hypoxanthine-thymidine medium and cloned by limiting dilution. Antibody-producing cells (2×10^6) were injected into pristane-primed mice for ascites tumor induction.

Bactericidal assay. Bactericidal activity of monoclonal antibodies to FBP was measured by a previously described modification of the procedure of Feeley and DeWitt (3, 22).

Opsonophagocytic assay. Opsonophagocytic activity was determined by the method described by Speert et al. (23). Polymorphonuclear leukocytes (PMNs) were isolated from heparinized venous human blood as previously described (22, 26). To polypropylene tubes (12 by 75 mm; Becton Dickinson Labware, Oxnard, Calif.) were added 10^7 bacteria and 10^5 PMNs, plus 100 μl of rabbit immunoglobulin G (IgG) against FBP, 50 μl of monoclonal antibodies, or 50 μl of control ascitic fluid. The reaction mixtures were incubated and processed as previously described (11, 22). The number of bacteria within the cytoplasms of each of 50 cells was quantitated. The mean number of bacteria per PMN obtained from those assays performed at 4°C was subtracted from the number obtained from the 37°C assays to obtain the final result.

Protein assay. Protein concentration was measured by using a commerical protein assay reagent (Bio-Rad Laboratories, Richmond, Calif.).

Preparation of outer membranes. Outer membranes were isolated as previously described (2, 20).

SDS-polyacrylamide gel electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed on 12.5% acrylamide–0.17% bis(acrylamide)–SDS slab gels (12).

Electrophoretic blotting procedure. Outer membrane preparations were electrophoresed as described above and transferred to nitrocellulose with a Hoeffer transblot apparatus for 30 min at 1 A. The nitrocellulose was incubated at 37°C for 1 h in 3% bovine serum albumin in 10 mM Tris (pH 7.4)–0.9% NaCl (Tris-saline) to block nonspecific binding of antiserum. The nitrocellulose paper was incubated for 2 h at 37°C with ascitic fluid at a dilution of 1:5,000. The blots were then incubated with horseradish peroxidase-conjugated protein A from *Staphylococcus aureus* (Sigma Chemical Co., St. Louis, Mo.) at a 1:2,000 dilution in Tris-saline containing 3% bovine serum albumin for 2 h at 37°C. Color was developed with Bio-Rad HRP color reagent as recommended by the manufacturer.

Whole-cell immunoblotting assay. Cultures were grown in M9 glucose medium in 96-well microtiter plates (Costar, Cambridge, Mass.) at 37°C. A 100-μl sample of each culture was transferred to nitrocellulose by using a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories). The cultures were allowed to filter under a slow vacuum. The nitrocellulose filter was removed from the apparatus, blocked with Tris-saline–3% bovine serum albumim, and reacted with antibodies as described above. Cultures which reacted with the monoclonal antibodies were visible as purple dots.

Ferripyochelin binding to cell envelopes. Ferripyochelin binding to cell envelopes was measured as previously described (20, 21). To test blocking activity, the ascitic fluid was preincubated with envelopes for 30 min at 37°C before the addition of [^{59}Fe]ferripyochelin.

Subisotyping of monoclonal antibodies. The subisotype of antibodies in the ascitic fluid was determined by using a mouse typer subisotyping kit (Bio-Rad Laboratories) as recommended by the manufacturer.

Immunofluorescence technique. *P. aeruginosa* PAO was grown overnight in M9 glucose medium. The cells were washed three times with phosphate-buffered saline (PBS) and adjusted to an optical density at 600 nm of 0.3. Samples (20 μl) were smeared on glass slides and allowed to air dry. Indirect immunofluorescence was performed by the method of Mutharia and Hancock (17), modified as follows. Bacterial smears were incubated with a 1:1,000 dilution of monoclonal antibody, normal mouse serum, or control ascites fluid. Smears of *E. coli* HB101 were also reacted with monoclonal antibody and normal mouse serum as negative controls. The slides were washed three times with PBS and then incubated with goat anti-mouse IgG serum diluted 1:100 in PBS. Controls included smears which were incubated with PBS instead of goat anti-mouse serum. After three washes with PBS, the smears were coated with fluorescein isothiocyanate-conjugated rabbit anti-goat immunoglobulin, diluted 1:40 in PBS. Smears with PBS substituted for fluorescein isothiocyanate-conjugated rabbit anti-goat serum were included to determine background levels of fluorescence. All reactions with antibody were incubated for 1 h at room temperature. After three washes, the smears were air dried and examined with a microscope (model BH-2; Olympus, Tokyo, Japan) with suitable filters for emission of fluorescein isothiocyanate.

Cell surface iodination. Iodination was performed essentially by the method of Sullivan and Williams (25). Cell suspensions were added to prepared reaction vials coated with Iodo-Gen (Pierce Chemical Co., Rockford, Ill.) one sample at a time. A 50-μl volume of ^{125}I (Amersham Corp., Arlington Heights, Ill.) diluted into PBS (10 mCi/ml) was added to the reaction vial to give 0.5 mCi of ^{125}I per reaction vial. The samples were swirled gently at room temperature for 45 s. The contents were then transferred into 8 ml of 10 mM sodium iodide–PBS (NaI-PBS) and mixed by inversion to stop the reaction. The radiolabeled cells were washed twice by centrifugation (10,000 × *g* for 20 min) and resuspension in 5 ml of NaI-PBS. The NaI-PBS-rinsed cells were washed twice with 1 ml of 0.5 M Tris hydrochloride (pH 6.8) in an Eppendorf microfuge (Brinkman Instruments, Inc., Westbury, N.Y.). After the last wash, the cell pellets were suspended in electrophoresis sample buffer (0.05 M Tris hydrochloride [pH 6.8], 2% SDS, 10% glycerol, 1% 2-mercaptoethanol). The samples were mixed and then boiled for 5 min. Samples were adjusted with sample buffer to 10,000 dpm/50 μl, and 50 μl of each sample was electrophoresed on a 12.5% SDS–polyacrylamide gel. After electrophoresis, the gel was dried onto filter paper by heating under reduced pressure. Dried gels were exposed to Kodak XAR film (Eastman Kodak Co., Rochester, N.Y.) for 2 days to obtain an autoradiogram of the labeled proteins.

RESULTS

Isolation of monoclonal antibodies against FBP. Five hybridomas producing antibodies specific for FBP were isolated from a single fusion of NS-1 cells and spleen cells from mice immunized with purified FBP. Positive clones were detected by using an ELISA with purified FBP as the

antigen. The positive hybridoma cell lines were injected into pristane-primed BALB/c mice for ascites tumor induction. The ascitic fluid obtained was shown to react with FBP in both the ELISA (Table 1) and Western blots of SDS-polyacrylamide gels. These monoclonal antibodies were designated MCA-34, MCA-36, MCA-47, MCA-75, and MCA-83.

The monoclonal antibodies were shown to have the same specificity as previously described rabbit antisera (21) in a Western blot. The reactions of MCA-47 and rabbit anti-FBP IgG with purified FBP and an outer membrane preparation of *P. aeruginosa* PAO are shown in Fig. 1. Both antibody preparations reacted with the purified FBP and the FBP band in the outer membrane preparation. Similar results were obtained with the other monoclonal antibodies (data not shown).

Classification of monoclonal antibodies. The monoclonal antibodies were classified in terms of immunoglobulin subclass, ELISA titer against purified FBP, titer against strain PAO in the whole-cell immunoblot assays, and strength of reaction in Western blots (Table 1). Three of the monoclonal antibodies were of the IgG3 subclass, and two were of the IgG1 subclass. The ascites fluid titers of the IgG3 antibodies were significantly higher than those of the IgG1 antibodies in both the ELISA and the whole-cell immunoblot assay. These antibodies also reacted much more strongly in the Western blot. This difference was not a function of antibody concentration since even low dilutions of MCA-75 and MCA-36 produced only faint reactions on the Western blots. No significant differences were seen in the results of these assays when peroxidase-conjugated anti-mouse IgG was substituted for peroxidase-conjugated protein A.

Interaction of monoclonal antibodies with FBPs of *P. aeruginosa* strains and other *Pseudomonas* species. The ability of the IgG3 monoclonal antibodies to react with FBP in *P. aeruginosa* strains of different lipopolysaccharide serotypes was examined by reacting Western blots of SDS-polyacrylamide gels of outer membrane preparations with these antibodies. MCA-83 reacted with FBP in outer membrane preparations of serotypes A through M (Fig. 2). The reaction with serotype D was much fainter, but this is most likely due to a lower amount of FBP in this preparation. MCA-47 and MCA-34 also reacted with FBP from these serotype strains (data not shown).

MCA-83 was shown to react with clinical isolates of *P. aeruginosa* in the whole-cell immunoblot assay. The following strains were tested with this assay: PAO, PA103, DG1, Ps388, M2, 44821, and 50359. The titers of MCA-83 against whole cells of these strains ranged from 1:500 to 10,000, with a mean titer of 4,188. Therefore, these monoclonal antibodies recognized antigenic sites on FBP which appear to be common among all *P. aeruginosa* strains examined.

TABLE 1. Classification of monoclonal antibodies to FBP

Monoclonal antibody	Immunoglobulin subclass[a]	Titer in ELISA[b]	Titer in whole-cell immunoblot assay	Western blot reaction[c]
MCA-47	IgG3	655,360	10,000	+
MCA-83	IgG3	655,360	10,000	+
MCA-75	IgG1	640	500	±
MCA-36	IgG1	640	ND[d]	±
MCA-34	IgG3	163,840	5,000	+

[a] These monoclonal antibodies reacted only with antiserum directed against these subclasses of immunoglobulin.
[b] Purified FBP was used at a concentration of 1 μg per well in the ELISA.
[c] Symbols: +, positive reaction; ±, only a weak reaction with FBP on Western blots.
[d] ND, No detectable reaction with ascitic fluid at a 1:100 dilution.

FIG. 1. Comparison of the reactions of MCA-47 and rabbit anti-FBP IgG with Western immunoblots of purified FBP and outer membrane preparations of *P. aeruginosa* PAO. Lane 1, Molecular weight markers ovalbumin (43,000), α-chymotrypsinogen (25,700), β-lactoglobulin (18,400), lysozyme (14,300), and bovine trypsin inhibitor (6,200); lanes 2, 4, and 6, outer membranes of strain PAO (15 μg); lanes 3, 5, and 7, purified FBP (2 μg). Lanes 1 through 3, Gel stained with Coomassie blue; lanes 4 and 5, Western blot reacted with MCA-47; lanes 6 and 7, Western blot reacted with rabbit anti-FBP IgG.

The ability of monoclonal antibodies against *P. aeruginosa* FBP to recognize FBPs from other *Pseudomonas* species was examined by reacting the antibodies with Western blots of outer membrane preparations of strains of *P. cepacia*, *P. fluorescens*, *P. putida*, "*P. multivorans*," *P. stutzeri*, and *P. maltophilia*. MCA-47 and MCA-34 reacted with FBP in strains of *P. fluorescens*, *P. putida*, and *P. stutzeri* (data not shown). They did not recognize FBPs from *P. cepacia*, "*P. multivorans*," or *P. maltophilia*. MCA-83 reacted with FBPs from *P. fluorescens* and *P. putida* but not *P. stutzeri* (data not shown). Therefore, this antibody probably reacts with a different antigenic site than that reacted with by MCA-47 and MCA-34.

The cross-reactivity of MCA-83 with several clinical isolates of *P. fluorescens*, *P. cepacia*, and *P. putida* was also determined by using the whole-cell immunoblot assay. In this assay, MCA-83 reacted with only *P. fluorescens*. No reaction was observed with the *P. cepacia* or *P. putida* clinical isolates.

The interaction of the monoclonal antibodies with FBPs from *Pseudomonas* spp. other than *P. aeruginosa* was different from that observed with rabbit anti-FBP IgG.

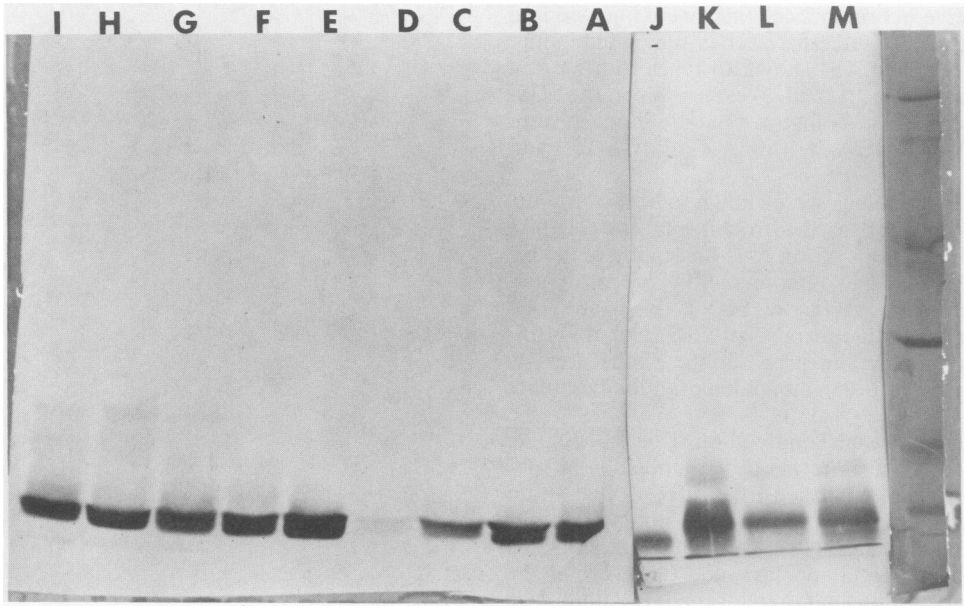

FIG. 2. Reaction of MCA-83 against outer membranes of *P. aeruginosa* lipopolysaccharide serotype strains on a Western immunoblot. Shown are outer membrane preparations of serotype strains A through M as indicated above each lane. The unmarked lane on the right side of the gel contains molecular weight markers phosphorylase *b* (molecular weight, 97,400), bovine serum albumin (68,000), ovalbumin (43,000), carbonic anhydrase (31,000), soybean tryspin inhibitor (21,500), and lysozyme (14,400).

Rabbit IgG cross-reacted with FBP in outer membrane preparations from all six of these *Pseudomonas* spp. (data not shown).

The interaction of MCA-47 and MCA-83 with outer membrane preparations from other gram-negative bacteria was also determined. Neither MCA-47 nor MCA-83 reacted with any outer membrane proteins from *E. coli* H10407, *Salmonella typhimurium* LT2, or *Serratia marcescens* on Western blots (data not shown).

Since the IgG1 monoclonal antibodies reacted with FBP poorly on electrophoretic blots of outer membrane preparations, the ability of these antibodies to cross-react with other strains was not examined.

Functional characterization of monoclonal antibodies. The ability of monoclonal antibodies to inhibit the binding of [^{59}Fe]ferripyochelin to isolated outer membranes from strain PAO was examined. Preincubation with all five monoclonal antibodies reduced the amount of ^{59}Fe bound by PAO outer membranes in this assay (Table 2). The percent inhibition was greater with the monoclonal antibodies than with the rabbit IgG, but this may reflect a difference in antibody concentration. Preincubation with MCA-75 resulted in the greatest inhibition of ferripyochelin binding. This inhibition was shown to be dose dependent (data not shown), with only 2 μl of MCA-75 resulting in a decrease of 36% compared with the control. No inhibition of [^{59}Fe]ferripyochelin binding to outer membranes was observed when membranes were preincubated with 50 μl of control ascitic fluid (GIBCO Laboratories, Grand Island, N.Y.). In fact, the amount of ^{59}Fe bound was higher than that in the PBS control. Therefore this inhibition appears to be due to antibodies against FBP and not to any nonspecific inhibition by other components of the ascites fluid.

The ability of the monoclonal antibodies to enhance the phagocytosis of *P. aeruginosa* PAO by PMNs was also examined. All of the monoclonal antibodies promoted phagocytosis of strain PAO by PMNs as compared with controls (Table 2). There were no significant differences in the degree of opsonization by any of the monoclonal antibodies.

The bactericidal activity of the monoclonal antibodies was also measured. No difference was observed in viable counts when *P. aeruginosa* was incubated with 50 μl of either monoclonal antibody or PBS in the presence of complement (data not shown). These results are not surprising since previously no bactericidal activity was demonstrated with rabbit IgG against FBP. Therefore, antibodies to FBP did not appear to be bactericidal.

Surface localization of FBP. The ability of MCA-47 to bind to the surfaces of intact *P. aeruginosa* cells was examined by using indirect immunofluorescence techniques. When

TABLE 2. Functional activity of monoclonal antibodies to *P. aeruginosa* FBP

Antibody	Effect on ferripyochelin binding to outer membranes[a]	Ability to promote phagocytosis of PAO by human PMNs[b]
MCA-34	20.2	15.2
MCA-36	11.5	14.1
MCA-47	35.3	18.4
MCA-75	8.1	12.5
MCA-83	27.4	16.0
Rabbit IgG[c]	64.7	14.2
PBS control	100.0	1.5
Ascites[d]	120.4	2.1

[a] Percent control counts per minute when outer membrane preparations were preincubated with 50 μl of monoclonal antibody before reaction with [^{59}Fe]ferripyochelin. Control mean, 8,045 cpm.
[b] Number of bacteria per PMN.
[c] A 100-μl volume of rabbit IgG was used in both assays.
[d] Ascites fluid (GIBCO Laboratories).

MCA-47 was reacted with cells of strain PAO grown in low-iron medium (M9 with no added iron), fluorescence was observed on all cells (Fig. 3A and B). It was difficult to visualize isolated bacteria, since exposure to MCA-47 resulted in agglutination of most of the bacteria (Fig. 3A). On some fields of the slide, however, individual bacteria could be visualized (Fig. 3B). No fluorescence was observed when PBS, normal mouse serum, or control ascitic fluid was substituted for MCA-47. When strain PAO was grown in high-iron medium (100 μM $FeCl_3$), MCA-47 did not react with the bacteria, as demonstrated by a lack of fluorescence (Fig. 3C). Also, no agglutination of the bacteria was observed. The reaction observed with MCA-47 and PAO grown in high-iron medium was indistinguishable from that of MCA-47 with *E. coli*. Therefore, these data suggest that FBP is present on the surface of low-iron-grown cells but not on the surface of high-iron-grown cells.

FIG. 3. Indirect immunofluorescence labeling of intact *P. aeruginosa* PAO with MCA-47. (A and B) Strain PAO grown in low-iron medium. (C) Strain PAO grown in high-iron (100 μM $FeCl_3$) medium.

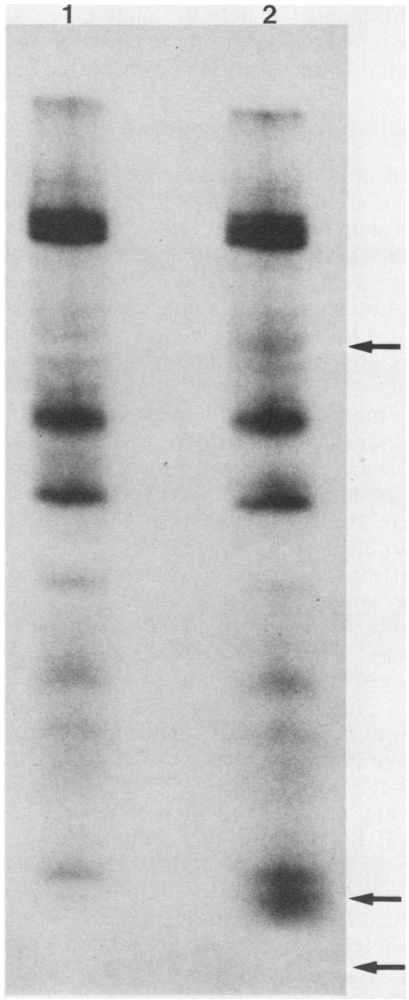

FIG. 4. Cell surface iodination of *P. aeruginosa* PAO. Whole cells of *P. aeruginosa* were radiolabeled with ^{125}I and electrophoresed on an SDS-polyacrylamide gel. The gel was dried and subjected to autoradiography. Lane 1, Strain PAO grown in high-iron medium; lane 2, PAO grown in low-iron medium. Upper two arrows indicate proteins induced in low-iron medium (66,000-molecular-weight protein and FBP). Lower arrow indicates dye front of gel.

Similar results were obtained by using cell surface iodination techniques. Whole cells of *P. aeruginosa* PAO grown in low-iron medium and high-iron medium were radiolabeled with ^{125}I by using the Iodo-Gen method of Sullivan and Williams (25). The ^{125}I-labeled cells were lysed and electrophoresed on SDS-polyacrylamide gels. The gels were dried and subjected to autoradiography. Four major and three minor bands were visible on the autoradiogram (Fig. 4). There were significant differences in two bands between the low-iron- and the high-iron-grown cells. The FBP band was dramatically increased in the low-iron-grown cells. It was by far the major protein labeled in these cells. In contrast, the band at the molecular weight of FBP was barely visible in the high-iron-grown cells. There was also a decrease in the amount of a protein with an approximate molecular weight of 66,000 in the high-iron-grown cells. The remaining proteins

were labeled to approximately the same extent in both low- and high-iron media. The results obtained in high-iron medium are similar to those previously reported (13).

DISCUSSION

Monoclonal antibodies have proven to be useful tools in the investigation of the antigenic conservation of outer membrane proteins in gram-negative bacteria. In *P. aeruginosa*, the conservation of outer membrane protein among serotype strains and clinical isolates has previously been demonstrated by using monoclonal antibodies to proteins F (17) and H_2 (6). In this study, we have shown that FBP was also antigenically conserved among all of the *P. aeruginosa* strains tested.

Two of the monoclonal antibodies also reacted with FBPs from *P. putida*, *P. fluorescens*, and *P. stutzeri* strains, whereas MCA-83 reacted with FBPs of only *P. putida* and *P. fluorescens*, in addition to *P. aeruginosa*. These data indicate that there are shared epitopes among FBPs from these *Pseudomonas* species.

It is interesting that MCA-83 reacted with FBP in Western blots of outer membrane preparations of *P. putida* yet did not react with strains of *P. putida* in the whole-cell immunoblot assay. These data suggest that the antigenic site on FBP recognized by MCA-83 is not exposed on the cell surface of *P. putida* but is exposed on the surfaces of *P. fluorescens* and *P. aeruginosa*. Outer membrane proteins of *P. putida* have previously been shown to react with monoclonal antibodies to protein F (17) and H_2 (6). Strains of *P. fluorescens* did not react with monoclonal antibodies to protein F (17, 18) but did react with antibodies to H_2 (6). Thus, there appears to be more antigenic conservation between *P. aeruginosa* and the fluorescent pseudomonads than with other *Pseudomonas* species.

Antigenic heterogeneity of iron-regulated outer membrane proteins has recently been described for *Neisseria* species (16). Mietzner et al. (16) have isolated monoclonal antibodies against a 37,000-molecular-weight protein which is iron-regulated in *Neisseria gonorrhoeae* and is speculated to be involved in iron acquisition. All of their monoclonal antibodies react with strains of *N. gonorrhoeae* and *Neisseria meningitidis*, but there is considerable variation in their reactions with other *Neisseria* species. Rabbit polyclonal antibody against the gonococcal 37,000-molecular-weight protein, however, cross-reacts with that protein in all these *Neisseria* species.

The results obtained with our monoclonals to FBP, as well as the data of Mietzner et al., indicate that although iron-regulated proteins may be antigenically similar within a genus and share common epitopes, there also exists antigenic variation, even though the proteins appear to have the same function, i.e., binding of ferripyochelin.

If monoclonal antibodies against bacterial antigens are to be successful as immunotherapeutic agents, it is essential that they have some functional activity. The monoclonal antibodies against FBP described in this study all have the ability to reduce levels of ferripyochelin binding to isolated outer membranes from *P. aeruginosa* and to promote phagocytosis of *P. aeruginosa* by human PMNs. Studies are currently in progress to explore the potential of these monoclonal antibodies in immunotherapy of *Pseudomonas* infections.

Monoclonal antibodies may also be used as probes to analyze the structure and function of bacterial antigens. By using indirect immunofluorescence techniques, we have demonstrated that FBP is present on the surface of *P. aeruginosa* cells when they are grown in low-iron medium. In high-iron medium, MCA-47 did not bind to bacteria in amounts sufficient to be detectable by immunofluorescence. Therefore, there is probably very little, if any, FBP present on the surface of high-iron-grown cells. The results obtained with the ^{125}I surface labeling of cells grown in low-iron and high-iron media also support this hypothesis.

The reactivity of the monoclonal antibodies in the whole-cell immunoblot assay also suggests that FBP is a surface protein. Mutharia and Hancock have used a similar colony immunoblot assay to examine surface-localized antigens of protein F (18). Certainly, FBP would be expected to be present on the surface of *P. aeruginosa* for it to effectively bind the ferripyochelin complex. Since there is so little of the protein expressed under high-iron conditions, it appears likely that *P. aeruginosa* may use a different mechanism to acquire iron under these conditions.

The monoclonal antibodies described in this report may have potential in immunotherapy and may also be used as probes in analyzing the structure and antigenic conservation of FBPs in different species. Perhaps a larger library of monoclonal antibodies directed against FBPs from other *Pseudomonas* species would make it possible to study further the structural and functional characteristics of FBPs.

ACKNOWLEDGMENTS

This work was supported by the Alberta Heritage Foundation for Medical Research (AHFMR), the Cancer Grant Program of the Alberta Heritage Savings Trust Fund (Alberta Cancer Board), and the Canadian Cystic Fibrosis Foundation. P.A.S. and D.E.W. are AHFMR scholars.

Excellent technical assistance was provided by M. To, C. Lewis, J. Dennis, and A. Lepage.

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