INFECTION AND IMMUNITY, March 1986, p. 896–900
0019-9567/86/030896-05$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 51, No. 3

Characterization of Antibody to the Ferripyochelin-Binding Protein of *Pseudomonas aeruginosa*

PAMELA A. SOKOL* AND DONALD E. WOODS

Department of Microbiology and Infectious Diseases, University of Calgary Health Sciences Centre, Calgary, Alberta, Canada T2N 4N1

Received 14 August 1985/Accepted 23 November 1985

An outer membrane protein of *Pseudomonas aeruginosa* was previously shown to bind ^{59}Fe-labeled pyochelin. Antibodies to the purified ferripyochelin-binding protein (FBP) were characterized by using a variety of assays. Anti-FBP cross-reacted with several *P. aeruginosa* isolates in an enzyme-linked immunosorbent assay. Anti-FBP significantly enhanced phagocytosis of *P. aeruginosa* by human polymorphonuclear leukocytes. In a serum bactericidal assay we observed no difference in viability between cells incubated with antiserum to FBP and cells incubated with preimmune serum. Anti-FBP immunoglobulin G inhibited both binding and uptake of ^{59}Fe-labeled pyochelin by whole cells. Passive protection by anti-FBP was examined in experimental *P. aeruginosa* burn infections in mice. The protection provided by this antibody was strain dependent but lipopolysaccharide serotype independent.

Pseudomonas aeruginosa causes severe and often fatal infections in immunocompromised patients (3). Patients debilitated with neutropenia, cystic fibrosis, or burn wounds are particularly susceptible to *P. aeruginosa* infections (3, 23). Outer membrane proteins have the potential to act as protective antigens against *P. aeruginosa* infections. These proteins are highly conserved among all serotypes of *P. aeruginosa*. Antibodies to outer membrane proteins have been detected in patient sera (7, 9). Gilleland et al. (8) have shown that mice can be protected against subsequent challenges with *P. aeruginosa* by active immunization with an outer membrane protein, porin F, as well as by passive administration of antiserum against porin F. Hancock et al. (10) have also recently reported passive protection against subsequent *P. aeruginosa* infections in the mouse burn model by using monoclonal antibody to porin F.

In this study we examined the ability of antiserum against another outer membrane protein to protect against *P. aeruginosa* burn infections in mice. This outer membrane protein is the ferripyochelin-binding protein (FBP), which is involved in iron acquisition by *P. aeruginosa*. This protein binds iron when it is complexed with the *Pseudomonas* siderophore pyochelin (18). It is a major outer membrane protein in iron-starved, glucose-grown cells (18).

Recently, we described the purification of this outer membrane protein and demonstrated that the immunoglobulin G (IgG) fraction of antiserum to this protein can block iron-pyochelin binding to isolated cell envelopes of *P. aeruginosa* (19). In this study, we extended our characterization of antibodies to FBP. We demonstrated the ability of these antibodies to promote phagocytosis of *P. aeruginosa* and to passively protect burned mice against some *P. aeruginosa* infections. The effect of antibodies to FBP on iron uptake by *P. aeruginosa* was also examined.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The *P. aeruginosa* strains used in this study and their serotypes are listed in Table 1. 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 (18). All glassware was acid washed and rinsed with deionized water. All reagents were made by using water that was purified by the Milli-Q system (Millipore Corp., Bedford, Mass.).

Purification of FBP. FBP was purified from cell envelopes of *P. aeruginosa* strain PAO as previously described (19). Briefly, cells were broken by sonication, and the envelope fraction was recovered by centrifugation. Differential detergent solubilization followed by gel filtration chromatography was used to isolate pure FBP.

Preparation of specific antisera. A 1-ml mixture containing equal parts of Freund complete adjuvant and FBP (0.3 to 0.5 mg/ml) was injected into female New Zealand white rabbits (2 to 2.5 kg). The animals were injected three times at 2-week intervals. At 10 days after the last injection, the rabbits were exsanguinated by cardiac puncture. The serum was separated by centrifugation and stored in portions at −70°C. The gamma globulin fraction was obtained from the antiserum by batch binding to DEAE-Sephacel, as described previously (1). The material obtained was lyophilized and suspended in buffer to the original volume of antiserum.

LPS purification. Lipopolysaccharide (LPS) was purified from *P. aeruginosa* strains by using the method of Darveau and Hancock (5).

ELISA procedure. An enzyme-linked immunosorbent assay (ELISA) was used to determine titers of anti-FBP IgG. Purified FBP (0.5 μg/ml) or LPS (5.0 μg/ml) in sodium carbonate buffer (pH 9.6) was dispensed in 200-μl volumes into 96-well microtiter dishes and incubated for 1 h at 37°C. The plates were washed three times, and 1% bovine serum albumin in phosphate-buffered saline (PBS) was added to block nonspecific antibody binding. The plates were washed and incubated with 100-μl volumes of twofold dilutions of anti-FBP IgG for 1 h at 37°C. All washes and dilutions were done with PBS containing Tween 20 (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 0.05%. After washing, the plates were incubated with 100 μl of peroxidase-conjugated protein A (Sigma) diluted 1:1,000 for 1 h at 37°C. Color development was achieved by using the ABTS peroxidase-substrate system (Mandel Scientific, Rockwell, Ontario, Canada) as recommended by the manufacturer. Controls with uncoated wells were included to measure

* Corresponding author.

TABLE 1. *P. aeruginosa* strains and serotypes

Strain	O-antigen serotype[a]	Source or reference
PAO	2,5	11
PA103	11	14
DG1	10	4
WR35	10	2
M2	2,5	21
44821	11	FH[b]
50359	2,5	FH
Ps388	6	12
47488	2	FH
49228	9	FH
44702	3	FH

[a] Strains were serotyped by using commercial antiserum (Difco Laboratories, Detroit, Mich.).
[b] FH, Foothills Hospital, Calgary, Alberta, Canada.

nonspecific background values. The levels of absorbance were determined with a model MR580 Microelisa Auto Reader (Dynatech Laboratories, Inc., Alexandria, Va.). The titer was the last dilution which had an absorbance that was at least threefold higher than that of the controls. All assays were done in duplicate, and the values are reported below as mean antibody titers.

For the whole-cell assay, cultures were grown overnight at 32°C. The bacteria were pelleted by centrifugation at 10,000 × *g* for 10 min, washed with PBS, and suspended in sodium carbonate buffer (pH 9.6). A 50-μl sample containing approximately 10^7 bacteria was added to each well; the samples were dried onto the plates at 37°C overnight. The remainder of the ELISA was identical to the assay described above.

Bactericidal assay. The bactericidal activity of antiserum against FBP was measured by a modification of the procedure of Feeley and DeWitt (6). Cultures were grown in M9 glucose broth overnight. The bacteria were subcultured in the same medium and grown at 37°C to mid-log phase. The bacteria (10^8 CFU/ml) were diluted 100-fold in cold PBS. An equal volume of guinea pig complement (GIBCO Laboratories, Grand Island, N.Y.) diluted 1:5 in cold PBS was added to the bacterial suspension. Serum diluted 1:10 in PBS was then added. PBS alone was added for the complement controls. The bacterial suspensions were incubated at 37°C for 90 min. Viable counts of bacteria were determined by plating the organisms onto Trypticase soy agar. All assays were done in triplicate. Complement was shown to be active by assaying the hemolytic activity of a preparation against sensitized erythrocytes.

Mouse burn infection model. We used the burned mouse model of Steiritz and Holder (21), as modified by Pavlovskis et al. (15). Female Swiss Webster mice (18 ± 2 g) were immunized with either anti-FBP IgG or preimmune IgG intravenously. At 12 h after immunization the mice were anesthetized with methoxyflurane and subjected to a 10-s ethanol flame burn (2.5 by 2.5 cm). Bacteria suspended in 0.5 ml of PBS were immediately injected subcutaneously into the burn site. Five mice were used in each group. Death was used as an endpoint, and 50% lethal doses (LD_{50}) were determined by the method of Reed and Muench (16). Titers of antibody to FBP were determined by an ELISA with serum obtained by cardiac puncture.

Iron uptake assay. Bacteria were grown to a density of 10^8 CFU/ml at 37°C in a shaking water bath. The culture was divided into 2- to 5-ml portions, to which 0.5 ml (500 μg) of either anti-FBP IgG or preimmune IgG was added. The resulting cultures were incubated with IgG for 30 min at 37°C prior to the addition of ^{59}Fe-labeled pyochelin. The reactions were initiated by adding 100 μg (1 μCi) of $^{59}FeCl_3$ (Amersham Corp., Arlington Heights, Ill.) and 1.0 μg of pyochelin in a total volume of 100 μl. Samples (0.5 ml) were removed at 10-min intervals, filtered, and counted as previously described (17).

PMN isolation. To isolate polymorphonuclear leukocytes (PMNs), 60 ml of heparinized venous human blood was diluted 1:2 with PBS containing 1 mM glucose, 10-ml samples were carefully layered onto 3-ml portions of Ficoll-Paque (Pharmacia, Uppsala, Sweden) in 15-ml round-bottom centrifuge tubes, and these preparations were centrifuged at 400 × *g* for 30 min. After centrifugation, all of the layers above the granulocyte-erythrocyte layers were removed. The granulocyte-erythrocyte layers were pooled, diluted 1:2 with PBS containing 1 mM glucose, and placed in a graduated cylinder. To 5 volumes of this material we added 1 volume of a solution containing 5% dextran (Dextran T500; molecular weight, 500,000; Pharmacia), 0.7% NaCl, and 1.5% EDTA (disodium salt) (22). Following gentle mixing, the erythrocytes were allowed to sediment at room temperature for 1 h. The supernatant was then removed by using a glass pipette, and the leukocytes were sedimented by centrifugation at 600 × *g* for 10 min at 4°C. To lyse the remaining erythrocytes, 0.85% NH_4Cl was used to suspend the granulocytes (5 ml), and the preparation was incubated for 37°C for 10 min. The granulocytes were washed twice in PBS containing 1 mM glucose and adjusted by using a hemacytometer to contain 10^6 PMNs/ml. Approximately 98% of the cells obtained were PMNs. More than 99% were viable, as determined by trypan blue dye exclusion.

Opsonophagocytic assay. The assay used to measure opsonophagocytic activity was the assay of Speert et al. (20) for visual assessment of phagocytosis. To polypropylene tubes (12 by 75 mm; Becton Dickinson Labware, Oxnard, Calif.) we added 10^7 bacteria and 10^5 PMNs along with either preimmune IgG or IgG directed against FBP. The tubes were rotated end over end for 60 min at 37°C. A duplicate set of assays was incubated at 4°C to measure nonspecific attachment without ingestion (13). Following incubation, the tubes were centrifuged at 26 × *g* for 10 min. The cells were gently washed twice and suspended in 0.25 ml of PBS containing 1 mM glucose, and 1 drop of this cell preparation was deposited on a glass slide. The slides were air dried, heat fixed, and stained with crystal violet. The numbers of bacteria within the cytoplasm of each of 50 cells were determined by two separate observers who were blinded to the identity of each slide. 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.

RESULTS

Antibody specificity and cross-reactivity. Previously, FBP was purified from *P. aeruginosa* strain PAO. Rabbits were immunized with the protein to obtain specific antibodies (19). The IgG fraction of the antiserum was shown to react with FBP by reacting the IgG with an immunoblot of a sodium dodecyl sulfate-polyacrylamide gel containing an outer membrane preparation from strain PAO (19).

The cross-reactivity of anti-FBP IgG was examined by using an ELISA. Anti-FBP IgG had a titer to purified FBP of 1:40,000 whereas the titer against LPSs from three different serotypes ranged from 1:2 to 1:8 (Table 2). The antibody titer

against LPS purified from strain PAO was 1:16. Since a 1:10 dilution of anti-FBP IgG was used in most of the experiments, it seemed unlikely that any of the activities of the antiserum could be attributed to antibodies to LPS.

When whole cells of *P. aeruginosa* clinical isolates were used as antigens in the ELISA, anti-FBP IgG cross-reacted with all of the isolates tested, with mean titers ranging from 1:256 to 1:2,124 (Table 2). Therefore, this anti-FBP IgG cross-reacted with other strains of *P. aeruginosa* regardless of serotype.

Serum bactericidal activity. The bactericidal activity of antiserum raised against FBP was measured by using a modification of the vibriocidal antibody test developed by Feeley and DeWitt (6). *P. aeruginosa* strains PA103, DG1, 44821, 47488, 49229, 50359, and 44702 were incubated with a 10-fold dilution of either preimmune serum or anti-FBP serum in the presence of complement. Controls contained complement but no serum. The reaction mixtures were incubated for 90 min at 37°C, and the number of viable cells was determined at zero time and 90 min by plate counting. Although with most of the strains there was some decrease compared with the initial bacterial concentration (data not shown), there was no significant difference among the viable bacterial concentrations following incubation with preimmune serum, anti-FBP serum, or complement alone (paired *t* test). Therefore, antibodies to FBP do not appear to be bactericidal.

Effect of antibodies to FBP on phagocytosis. Anti-FBP IgG promoted phagocytosis of several strains of *P. aeruginosa* by human PMNs (Table 3). With all of the strains tested the number of bacteria per PMN was considerably higher when the bacteria were preincubated with anti-FBP IgG than when they were incubated with preimmune IgG. Anti-FBP IgG increased by two- to sixfold the phagocytosis of these strains of *P. aeruginosa* by PMNs (Table 3). These data strongly suggest that antibodies to FBP are opsonizing antibodies.

Effect of anti-FBP on iron uptake. Previously, we demonstrated that anti-FBP IgG can block the binding of ^{59}Fe-labeled pyochelin to isolated outer membranes of *P. aeruginosa* (19). In this study we examined the ability of anti-FBP to block ^{59}Fe-labeled pyochelin uptake by whole cells. When *P. aeruginosa* strain DG1 was preincubated with anti-FBP IgG for 30 min prior to the addition of ^{59}Fe-labeled pyochelin to the reaction mixture, the amount of ^{59}Fe accumulated by the bacteria was markedly reduced compared with bacteria that were preincubated with preimmune IgG (Fig. 1). Both the initial amount of ^{59}Fe bound and the rate of ^{59}Fe uptake were affected by preincubation with anti-FBP IgG. Similar results were observed when strain PAO was used in this experiment (data not shown).

Passive protection with antibodies to FBP. The ability of anti-FBP IgG to protect against *P. aeruginosa* infections was examined in a mouse burn infection model. Subcutaneous injection of viable *P. aeruginosa* cells into a burn site has previously been shown to result in lethal infections associated with bacteremia and systemic invasion (15, 21).

Mice were immunized intravenously with either 10 μg of anti-FBP IgG or 10 μg of preimmune IgG. At 12 h after immunization, the mice were burned and infected with the challenge strains. The median anti-FBP titer in the mice at the time of infection was 1:512, as determined by an ELISA

TABLE 2. Titers of anti-FBP determined by using the ELISA against purified FBP, LPS, and whole cells of *P. aeruginosa*

Antigen	Mean antibody titer
Purified FBP[a]	1:40,000
LPS from serotype 2,5[b]	1:4
LPS from serotype 11	1:8
LPS from serotype 10	1:2
LPS from strain PAO	1:16
Strain PAO whole cells[c]	1:1,024
Strain DG1 whole cells	1:1,024
Strain M2 whole cells	1:512
Strain 50359 whole cells	1:512
Strain 44821 whole cells	1:256
Strain PA103 whole cells	1:2,124
Strain Ps388 whole cells	1:256
Strain WR35 whole cells	1:256

[a] The FBP concentration used was 0.1 μg per well.
[b] The LPS concentration used was 1 μg per well.
[c] The bacterial concentration used was 10^7 CFU per well.

TABLE 3. Ability of anti-FBP IgG to promote phagocytosis of *P. aeruginosa* strains by PMNs

Bacterial strain	No. of bacteria per PMN with the following IgG preparations:		*P* value
	Anti-FBP	Preimmune	
50359	7.7 ± 0.35[a]	3.2 ± 0.20	<0.05
PA103	4.5 ± 0.25	0.8 ± 0.35	<0.02
M2	10.2 ± 0.15	2.4 ± 0.10	<0.05
PAO	5.2 ± 0.10	1.1 ± 0.05	<0.01
WR35	12.3 ± 0.7	1.8 ± 0.30	<0.05
44821	13.7 ± 0.40	3.1 ± 0.55	<0.01
Ps388	9.6 ± 0.15	2.7 ± 0.10	<0.05

[a] Mean ± standard deviation.

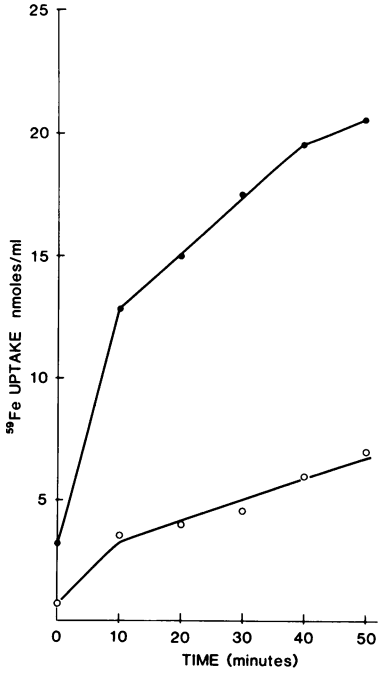

FIG. 1. Uptake of $^{59}Fe^{3+}$ by strain DG1 preincubated with 500 μg of preimmune IgG (●) or anti-FBP IgG (○). Uptake was initiated by adding ^{59}Fe-labeled pyochelin. Samples (0.5 ml) were removed and filtered. The amount of $^{59}Fe^{3+}$ accumulated was calculated from a standard curve. The data shown are the means obtained from two separate experiments.

TABLE 4. LD_{50}s for mice immunized with either preimmune IgG or anti-FBP IgG and infected with *P. aeruginosa*

Strain	LD_{50}s for mice immunized with the following IgG preparations:[a]	
	Preimmune	Anti-FBP[b]
PAO	3.6×10^2	3.7×10^5
PA103	5.3×10^2	5.5×10^4
44821	1.4×10^3	1.4×10^4
50359	<10	<10
WR35	3.3×10^3	2.5×10^4
Ps388	1.0×10^3	1.4×10^3
M2	1.1×10^2	1.3×10^1

[a] IgG was administered intravenously 12 h prior to infection in 0.1-ml volumes containing 10 μg of IgG.
[b] The median anti-FBP titer at the time of infection was 1:512.

with purified FBP as the antigen. No antibodies to FBP were detected in the mice which received preimmune IgG.

The LD_{50}s of six *P. aeruginosa* strains were compared in mice that were immunized with either anti-FBP IgG or preimmune IgG (Table 4). Anti-FBP administered prior to infection with strain PAO increased the LD_{50} by 3 logs. Passive transfer of anti-FBP increased the LD_{50} of strain PA103 by 2 logs and the LD_{50} of strain 44821 by 1 log. However, anti-FBP had no effect on the LD_{50}s of strains Ps388, 50359, and M2. Anti-FBP increased the LD_{50} of strain WR35 approximately sevenfold. Therefore, there was considerable strain variation in the ability of anti-FBP to passively protect against *P. aeruginosa* burn infections in mice. Strains 47488, 49228, 44702, and DG1 were also tested in this model, but were found to be avirulent when they were grown under the conditions which we used.

When mice were immunized with anti-FBP and challenged with 3 LD_{50}s of the strains shown in Table 4, there was considerable variation in the degree of protection obtained (Table 5). Significant protection was obtained with strains PA103 ($P < 0.05$), 44821 ($P < 0.05$), and PAO ($P < 0.01$). However, no protection was observed when mice were challenged with either strain WR35 or strain Ps388. These data strongly suggest that the protection afforded by antibodies to FBP is strain dependent.

DISCUSSION

Early studies of clinical or experimental infections with *P. aeruginosa* in which heat-stable LPS antigens were used demonstrated conclusively that the functional role of antibodies to *P. aeruginosa* is opsonization (25). More recently, the roles of cell wall components other than LPS, such as outer membrane proteins, have been studied to determine their role in the pathogenesis of *P. aeruginosa* infections (7, 9). Concomitantly, the protective role of antibodies against these antigens has also been studied (8, 10). The present study was undertaken to evaluate the immunotherapeutic potential of antibodies to an iron-binding protein (FBP) present in the outer membrane of *P. aeruginosa*.

TABLE 5. Protection of burned mice by passive immunization with anti-FBP against challenge with 3 LD_{50}s of *P. aeruginosa* strains[a]

Strain	No. of survivors/total no.		*P* value[b]
	Mice immunized with preimmune IgG	Mice immunized with anti-FBP IgG	
PA103	2/5	5/5	<0.05
44821	2/5	5/5	<0.05
WR35	3/5	3/5	NS
PAO	0/5	4/5	<0.01
Ps388	2/5	3/5	NS

[a] Mice were given 10 μg of IgG 12 h prior to infection.
[b] As determined by chi-square analysis. NS, Not significant.

Antibodies to FBP do not appear to have any bactericidal activity since there were no significant differences observed when *P. aeruginosa* strains were incubated with either anti-FBP serum or preimmune serum. Similar results have been reported by Hancock et al. (10). These authors were not able to demonstrate complement-mediated bactericidal activity with monoclonal antibodies to protein F (10). However, the role of bactericidal antibodies against *P. aeruginosa* in infections is questionable, since most strains are serum resistant, regardless of their source of isolation (25, 26).

However, opsonic antibody does seem to be important in host defense against *P. aeruginosa* infections. Patients that are bacteremic with *P. aeruginosa* have a good prognosis if they develop opsonizing antibodies, whereas the outcome is poor in the absence of these antibodies (25, 26). Anti-FBP IgG enhanced the phagocytosis of all of the *P. aeruginosa* strains tested by human PMNs, indicating that anti-FBP antibodies are opsonizing. The increase in the number of bacteria phagocytosed in the presence of anti-FBP ranged from two- to sixfold. This increase is nearly identical to that reported for monoclonal antibodies to protein F (10). These data strongly suggest that antibodies against major outer membrane proteins are opsonic and may have significant potential in the immunotherapy of *P. aeruginosa* infections.

Antibodies to FBP may have an additional advantage in immunotherapy in that they appear to reduce the amount of iron accumulated by *P. aeruginosa*, at least in vitro.

The ability of passively transferred antibodies to FBP to protect against *P. aeruginosa* infections was examined in a mouse burn infection model. Passively administered anti-FBP IgG markedly increased the LD_{50} of homologous strain PAO. Anti-FBP IgG also increased the LD_{50} of one heterologous serotype strain, strain PA103, almost to the same extent as strain PAO, and the LD_{50}s of two other strains were increased to somewhat lesser extents. However, anti-FBP did not protect against challenges with all of the *P. aeruginosa* strains tested. Anti-FBP did react with all of the strains in the whole-cell ELISA, so the lack of protection does not appear to be due to a lack of expression of this protein. However, it is possible that there may be different affinities of FBPs in different strains for anti-FBP antibodies. This could account for the differences in protection. Interestingly, anti-FBP IgG did not protect against challenges with strains 50359 and M2, even though these strains belong to the same LPS serotype as strain PAO (type 2,5). Therefore, these data provide additional evidence that the protection observed with anti-FBP IgG is not due to contaminating antibodies to LPS. The ability of antibodies to FBP to protect against *P. aeruginosa* infections appears to be LPS serotype independent, but strain dependent.

P. aeruginosa produces a variety of extracellular virulence factors which have been shown to play a role in infections with this organism (14, 24). Perhaps the strains which are not affected by anti-FBP immunization produce a combination of virulence factors which allow them to overcome any effects of the antibodies to FBP.

Hancock et al. (10) demonstrated passive protection with monoclonal antibody to protein F against subsequent chal-

lenge with *P. aeruginosa*, but in this study only one strain was examined. Gilleland et al. reported protection with polyclonal protein F antibodies with three strains (8). The protection observed with the heterologous strains was much lower than that observed with the strain from which the porin was purified (8). From these results it appears that there may be strain variation in the ability of antibodies to protein F to protect against *P. aeruginosa* infections. It would be interesting to examine the effects of these antibodies on a larger number of strains.

Passive immunotherapy may have great potential in the prevention and treatment of *P. aeruginosa* infections in immunocompromised patients. Currently we are in the process of developing monoclonal antibodies to FBP to further explore the potential of this antibody in immunotherapy. Monoclonal antibodies may also provide additional insight regarding strain differences in the affinity of FBP for antibodies to this protein. Perhaps a combination of antibodies against outer membrane proteins or extracellular toxins or both would be more effective in protection against *P. aeruginosa* infections. Antibodies used in combination might overcome the strain variation observed. Further research in this area is certainly warranted.

ACKNOWLEDGMENTS

This work was supported by the Alberta Heritage Foundation for Medical Research, the Cancer Grants 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 Alberta Heritage Foundation for Medical Research scholars.

The exceptional technical assistance of Anna LePage is gratefully acknowledged.

LITERATURE CITED

1. **Baumstark, J. S., R. J. Laffine, and W. A. Bardawil.** 1964. A preparative method for the separation of 7S gamma globulin from human serum. Arch. Biochem. Biophys. **108**:514–522.
2. **Bjorn, M. J., P. A. Sokol, and B. H. Iglewski.** 1979. Influence of iron on yields of extracellular products in *Pseudomonas aeruginosa* cultures. J. Bacteriol. **138**:193–200.
3. **Bodey, G. P., R. Bolivar, V. Fainstein, and L. Jadeja.** 1983. Infections caused by *Pseudomonas aeruginosa*. Rev. Infect. Dis. **5**:279–313.
4. **Cash, H. A., D. E. Woods, B. McCullough, W. G. Johanson, Jr., and J. A. Bass.** 1979. A rat model of chronic respiratory infections with *Pseudomonas aeruginosa*. Am. Rev. Respir. Dis. **119**:453–459.
5. **Darveau, R. P., and R. E. W. Hancock.** 1983. Procedure for isolation of bacterial lipopolysaccharides from both smooth and rough *Pseudomonas aeruginosa* and *Salmonella typhimurium* strains. J. Bacteriol. **155**:831–838.
6. **Feeley, J. C., and W. E. DeWitt.** 1976. Immune response to *Vibrio cholerae*, p. 289–294. *In* N. R. Rose and H. Friedman (ed.), Manual of clinical immunology. American Society for Microbiology, Washington, D.C.
7. **Fernandes, P. B., C. Kim, K. R. Cundy, and N. N. Huang.** 1981. Antibodies to cell envelope proteins of *Pseudomonas aeruginosa* in cystic fibrosis patients. Infect. Immun. **33**:527–532.
8. **Gilleland, H. E., Jr., M. G. Parker, J. M. Matthews, and R. D. Berg.** 1984. Use of a purified outer membrane protein F (porin) preparation of *Pseudomonas aeruginosa* as a protective vaccine in mice. Infect. Immun. **44**:49–54.
9. **Hancock, R. E. W., E. C. A. Movat, and D. P. Speert.** 1984. Quantitation and identification of antibodies to outer-membrane proteins of *Pseudomonas aeruginosa* in sera of patients with cystic fibrosis. J. Infect. Dis. **149**:220–226.
10. **Hancock, R. E. W., L. M. Mutharia, and E. C. A. Movat.** 1985. Immunotherapeutic potential of monoclonal antibodies against *Pseudomonas aeruginosa* protein F. Eur. J. Clin. Microbiol. **4**:224–227.
11. **Holloway, B. W., V. Krishnapillai, and A. F. Morgan.** 1979. Chromosomal genetics of *Pseudomonas*. Microbiol. Rev. **43**:73–102.
12. **Iglewski, B. H., J. C. Sadoff, M. J. Bjorn, and E. S. Maxwell.** 1978. *Pseudomonas aeruginosa* exoenzyme S: an adenosine diphosphate ribosyl transferase distinct from toxin A. Proc. Natl. Acad. Sci. USA **75**:3211–3215.
13. **Kozel, T. R., and R. P. Mastroianni.** 1976. Inhibition of phagocytosis by cryptococcal polysaccharide: dissociation of the attachment and ingestion phases of phagocytosis. Infect. Immun. **14**:62–67.
14. **Liu, P. V.** 1974. Extracellular toxins of *Pseudomonas aeruginosa*. J. Infect. Dis. **130**(Suppl.):S94–S99.
15. **Pavlovskis, O. R., M. Pollack, L. T. Calahan III, and B. H. Iglewski.** 1977. Passive protection by antitoxin in experimental *Pseudomonas aeruginosa* burn infections. Infect. Immun. **18**:596–602.
16. **Reed, L. J., and H. Muench.** 1938. A simple method for estimating fifty per cent endpoints. Am. J. Hyg. **27**:493–497.
17. **Sokol, P. A., C. D. Cox, and B. H. Iglewski.** 1982. *Pseudomonas aeruginosa* mutants altered in their sensitivity to the effect of iron on toxin A or elastase yields. J. Bacteriol. **151**:783–787.
18. **Sokol, P. A., and D. E. Woods.** 1983. Demonstration of an iron-siderophore-binding protein in the outer membrane of *Pseudomonas aeruginosa*. Infect. Immun. **40**:665–669.
19. **Sokol, P. A., and D. E. Woods.** 1984. Antibody inhibition of ferripyochelin binding to *Pseudomonas aeruginosa* cell envelopes. Biochemistry **23**:5076–5080.
20. **Speert, D. P., F. Eftekhar, and M. L. Puterman.** 1984. Nonopsonic phagocytosis of strains of *Pseudomonas aeruginosa* from cystic fibrosis patients. Infect. Immun. **43**:1006–1011.
21. **Steiritz, D. D., and I. A. Holder.** 1975. Experimental studies of the pathogenesis of infections due to *Pseudomonas aeruginosa*: description of a burned mouse model. J. Infect. Dis. **131**: 688–691.
22. **Williams, C. D., and J. Avigan.** 1972. *In vitro* effects of serum proteins and lipids on lipid synthesis in human skin fibroblasts and leukocytes grown in culture. Biochim. Biophys. Acta **260**:413–423.
23. **Wood, R. E.** 1976. *Pseudomonas*: the compromised host. Hosp. Pract. **11**:91–100.
24. **Woods, D. E., and B. H. Iglewski.** 1983. Toxins of *Pseudomonas aeruginosa*: new perspectives. J. Infect. Dis. **5**:S715–S722.
25. **Young, L. S.** 1974. Role of antibody in infections due to *Pseudomonas aeruginosa*. J. Infect. Dis. **130**(Suppl.):S111–S118.
26. **Young, L. S., and D. Armstrong.** 1972. Human immunity to *Pseudomonas aeruginosa*. I. *In vitro* interaction of bacteria, polymorphonuclear leukocytes and serum factors. J. Infect. Dis. **126**:257–276.