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Monoclonal Antibodies Against *Pseudomonas aeruginosa* Outer Membrane Antigens: Isolation and Characterization

ROBERT E. W. HANCOCK,* ANDREW A. WIECZOREK, LUCY M. MUTHARIA, AND KEITH POOLE
Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

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Hybridomas secreting monoclonal antibodies specific for *Pseudomonas aeruginosa* outer membrane antigens were isolated. One of the antibodies was highly specific for the O antigen of the lipopolysaccharide of International Antigen Typing Scheme serotype 5 strains, reacting only weakly with a serotype 17 strain and failing to react with the outer membranes of strains representing 15 other serotypes. This monoclonal antibody was able to agglutinate heat-killed bacterial cells as well as lipopolysaccharide-coated sheep erythrocytes. Two other monoclonal antibodies were able to interact with the outer membranes of strains representing all 17 serotypes, although they were unable to agglutinate heat-killed bacterial cells. One of these was shown to be specific for the major outer membrane lipoprotein H2. The antigenic site against which this monoclonal antibody reacted was present in the outer membranes of two *Pseudomonas fluorescens* strains, two *Pseudomonas putida* strains, a *Pseudomonas anguilliseptica* strain, and an *Azotobacter vinelandii* strain, but not in the outer membranes of five other bacterial species.

The cell surface of *Pseudomonas aeruginosa* contains a number of macromolecules which can interact with host cells during infection (see references 5, 22, and 24 for reviews). Two classes of such molecules, lipopolysaccharide (LPS) and proteins, have been shown to be components of the *P. aeruginosa* outer membrane (9, 12). The LPS of *P. aeruginosa* has been partially characterized and has been shown both chemically (15, 16) and serologically (16) to differ widely among isolates. This explains the observation that LPS-based vaccines protect only against subsequent infections by strains of the same O serotype grouping (8, 22). A number of systems of serotyping *P. aeruginosa* have been developed (see references 2, 3, and 16 for reviews); for example, the commonly used International Antigen Typing Scheme (IATS; commercially marketed by Difco Laboratories, Detroit, Mich.) contains 17 serotypes (2, 3, 16), suggesting a minimum of 17 distinct molecular forms of LPS in *P. aeruginosa*. This observation, combined with the known toxicity of LPS and LPS-based vaccines (22), has argued against the validity of using LPS as a major component of antipseudomonal vaccines.

Our recent studies have demonstrated the potential of outer membrane proteins as vaccine components (L. M. Mutharia, T. I. Nicas, and R. E. W. Hancock, submitted for publication). These studies suggest a great similarity in the major outer membrane proteins of *P. aeruginosa*, since we have demonstrated among the 17 serotype strains of the IATS (i) conservation of receptor sites for protein-specific phages, (ii) highly similar major outer membrane protein patterns on sodium dodecyl sulfate-polyacrylamide electrophoretic gels, and (iii) cross-reactions of the outer membranes of the serotype strains with antisera raised against partially purified major outer membrane proteins. Other studies have suggested that major outer membrane proteins interact with and mitogenically stimulate B lymphocytes (4).

Until now, our own and other studies on the *P. aeruginosa* cell surface antigens and their interactions with the host immune system have involved polyclonal antisera raised in animals with the use of complex antigens. Even when an attempt is made to purify the specific antigen of interest, low levels of impurities can have a very large influence on the results. The techniques for isolation of hybrid cells secreting monoclonal antisera directed against a single antigenic site (14, 20) should overcome many of the problems associated with the use of crude antisera.

In this paper, we describe the isolation and characterization of monoclonal antibodies against three separate *P. aeruginosa* outer membrane molecules and demonstrate that the antigenic site for the two anti-outer membrane protein monoclonals is conserved, whereas the anti-LPS monoclonal antibody demonstrates a serotype-specific reaction.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *P. aeruginosa* PAO1 strain H1O3 (19) was used as a standard strain for isolation of outer membrane proteins and immunization. Strains AK1160 and AK1188, obtained from A. Kropinski (Queen's University, Kingston, Ontario), were rough *P. aeruginosa* PAO derivatives with defined LPS compositions (similar to AK1012 [13] but containing one to two additional glucose residues attached to the galactosamine [Ann Ryan, M.Sc. thesis, Queen's University, Ontario, Canada, 1982]). Strain H223 was a rough, LPS-altered mutant of strain H103 selected for resistance to phage 44. Strain Z61 was a smooth, antibiotic-supersusceptible mutant of *P. aeruginosa* K799 with a lipid A alteration (1). A set of 17 serotype-specific strains was a kind gift from P. Liu. These strains were representatives of the IATS, which contains as subsets the type strains from all other commonly used *P. aeruginosa* serotyping systems with one exception: type M of the Homma typing set has no correlation in the IATS (see references 2 and 3 for relationships among the various systems). They were named as follows: type 1 (ATCC 33348), type 2 (ATCC 33349), type 3 (ATCC 33350), type 4 (ATCC 33351), type 5 (ATCC 33352), type 6 (ATCC 33354), type 7 (ATCC 33353), type 8 (ATCC 33355), type 9 (ATCC 33356), type 10 (ATCC 33357), type 11 (ATCC 33358), type 12 (ATCC 33359), type 13 (ATCC 33360), type 14 (ATCC 33361), type 15 (ATCC 33362), type 16 (ATCC 33363), and type 17 (ATCC 33364). In addition, a mucoid isolate from a cystic fibrosis patient, P1, and its spontaneous revertant was obtained from D. Speert, Children's Hospital, Vancouver. The other bacterial strains and species used were *P. fluorescens* ATCC 13525 (the type strain of this species), *P. fluorescens* ATCC 949, *P. putida* ATCC 12633 (the type strain), *P. putida* K4359, *P. anguilliseptica* ET7601, *P. acidovorans* ATCC 9355, *Azotobacter vinelandii* OP, *Aeromonas salmonicida* NCMB 1102, *Aeromonas hydrophila* ET-2, *Vibrio anguillarum* HT7602 and *Edwardsiella tarda* 79054. Further details of the growth and isolation of outer membranes from these organisms will be published (K. Nakajima, K. Muroga, and R. E. W. Hancock, Int. J. Syst. Bacteriol., in press).

P. aeruginosa cultures were maintained on 1% (wt/vol) proteose peptone no. 2 agar and used after growth in proteose peptone no. 2 broth to an optical density at 600 nm of approximately 0.5 to 0.8.

Outer membrane techniques. Outer membranes were isolated by the one- or two-step procedures described previously (9, 12). The method of isolation of proteins F, H2 (previously called 17K), and I (4) were exactly as previously detailed. Protein H1 was isolated from an H1-overproducing strain, H181 (19), by solubilization of H181 outer membranes in 2% (wt/vol) Triton X-100–20 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA. The solubilized proteins were then passed down a DEAE-Sephacel column as described for protein F (4), the resultant peak fractions containing protein H1 were pooled, and Triton X-100 was exchanged for 1% deoxycholate as follows. The pooled column fractions containing 0.1% (wt/vol) Triton X-100 were concentrated fivefold by dialysis against 30% (wt/vol) polyethylene glycol 20,000, and a fourfold excess of sodium deoxycholate over Triton X-100 was added. This sample was then applied to a Sephadex G-200 column (60 by 1.8 cm) equilibrated with 1% sodium deoxycholate–20 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA–1 M NaCl (column buffer) and eluted with column buffer. Protein H1 was found to be relatively pure (>90%) in the void volume, but it had suffered some degradation which increased with storage time.

Protein D2 was purified as described previously for protein D1 purification (10), except that the cells were grown in BM2-succinate medium to suppress protein D1 production. The resultant protein D2-containing fractions were pooled and exchanged into 1% sodium cholate as described above for the Triton-to-deoxycholate exchange, with three exceptions: (i) 1% cholate replaced 1% deoxycholate in the column buffer, (ii) an LKB AcA34 column (70 by 1.8 cm; LKB Instruments Inc., Rockville, Md.) was used in place of the Sephadex G-200 column, and (iii) after protein D2-containing fractions were pooled from the first column run, this pool was rechromatographed on the AcA34 column, and the D2-containing fractions were pooled. The purity of the proteins was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by cross titration against polyclonal antisera raised in mice, using these proteins as antigens (data not shown). Thus, we were able to estimate that protein D2 was approximately 80% pure (with protein I and LPS as significant contaminants), whereas proteins H2 and I were 70 and 80% pure, respectively, and contained 30% protein I and 10% protein H2 as contaminants, respectively. Protein F was at least 90% pure, as previously described. It was not possible to accurately estimate the purity of protein H1 because of extensive proteolysis during storage. However, only antisera to protein H1 reacted strongly against purified protein H1.

The method of LPS isolation will be described elsewhere (R. P. Darveau and R. E. W. Hancock, manuscript in preparation). In principle, cells were dissolved in 2% sodium dodecyl sulfate–10 mM EDTA–20 mM Tris-hydrochloride (pH 7) at room temperature before digestion of protein and subsequent ethanol precipitation of LPS. The resultant LPS was obtained in high yields (80% or greater) and was protein- and nucleic acid-free as judged by absorbance at 260 and 280 nm. Smooth LPS was obtained from strain H103, and rough LPS was obtained from strain AK1160 by the same method. Quantitation of the LPS was performed by using the LPS-specific sugar 2-keto-3-deoxyoctulosonate, and demonstration of the rough or smooth nature of the LPS was done by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (1).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of outer membrane proteins was described previously (9). *Azotobacter vinelandii* OP outer membranes were kindly provided by R. Moore (University of British Columbia, Vancouver).

Isolation of monoclonal antibodies. Our monoclonal antibody isolation procedure was a slight modification of the techniques of Köhler and Milstein (14, 20). BALB/c BYJ mice (Jackson Laboratories, Bar Harbor, Maine) were immunized by three intraperitoneal injections of 30 μg of *P. aeruginosa* PAO1 outer membranes at 7-day intervals, resulting in reciprocal serum antibody titers of 10^5 as determined by the

enzyme-linked immunosorbent assay (ELISA) method (see below). The medium used for suspension of mouse spleen cells and growth of NS1 myeloma cells was Dulbecco modified Eagle medium (GIBCO Laboratories, Buffalo, N.Y.) supplemented with 20% fetal calf serum (GIBCO), 2 mM glutamine, 1 mM pyruvate, 63 μg of penicillin per ml, 100 μg of streptomycin sulfate per ml, 3.5 mg of sodium bicarbonate per ml, and 5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) base. Fusion was performed with 10^8 spleen cells from immunized mice and 10^7 NS1 myeloma cells, using 50% polyethylene glycol 1,500 (BDH Chemicals, Toronto, Canada) as a fusogen. After fusion, cells were suspended at approximately 5×10^5 myeloma cells per ml in the Dulbecco medium described above, containing (per milliliter) 13.6 μg of hypoxanthine, 7.3 μg of thymidine, 0.22 μg of aminopterin, and 10^3 to 10^5 BALB/c BYJ normal thymocytes. Cloning, storage, and antibody production in ascites cells were essentially as described previously (20). Titration of monoclonal antibodies was performed by the ELISA method (23), using microtiter plates (Nunc substrate plates; Dynatech Laboratories, Inc., Alexandria, Va.) coated with outer membrane proteins (50 μg per well) that had been solubilized in 2% Triton X-100–20 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA before being precipitated with 2 volumes of ethanol and 0.1 M NaCl at −20°C overnight and suspended in coating buffer (23). LPS coating was performed by using an LPS concentration of 50 μg in 100 μl of bicarbonate coating buffer (23) to coat each well. To guard against the possibility of contaminating polyclonal antibodies in the mouse ascites, the following controls were established: all experiments were duplicated with antibodies raised in ascites cells and with culture supernatants of hybridomas grown in vitro, and normal mouse serum controls were included. In addition, the specificity of the reactions of the monoclonals with a variety of antigens (see below) argued against these results being due to contaminating polyclonal antibodies.

Miscellaneous immunological techniques. Double immunodiffusion (21) was performed in 2% (wt/vol) agarose in 0.1 M NaOH–0.12 M Barbital buffer (pH 8.6) containing 1% (vol/vol) Triton X-100. The antigen wells were filled with 20 μg of the solubilized antigen, and the antibody wells were filled with undiluted or twofold dilutions of ascites or hybridoma culture supernatants. Rocket immunoelectrophoresis was performed as described by Laurell (17), except that 1% (vol/vol) Triton X-100 was added to the antibody-containing agarose gel. Bacterial agglutination was performed by using heated cells prepared as described by Brokopp et al. (3). Agglutination was scored on a scale of +1 (weak agglutination) to +4 (strong agglutination) in undiluted ascites or hybridoma culture supernatants. Test controls were done in saline, and control ascites fluids were used to demonstrate that other components in ascites fluid were not responsible for agglutination. Passive hemagglutination was done as described by Lanyi and Bergen (16).

RESULTS

Isolation of monoclonal antibodies. After NS1 myeloma cells fused with the splenic cells of mice primed with whole outer membranes, hybridomas were cloned by limiting dilution until only a single clone in each well of the tissue culture plate was evident. A total of 15 clones (of about 400 hybrids) were isolated which produced antibody specific for outer membrane antigens; of these, 3 were chosen for detailed study. They were judged to produce monospecific antibody on the basis of their specificity towards a variety of crude antigenic fractions from outer membranes (see below) and their production of single precipitin lines in the Ouchterlony double-diffusion and rocket immunoelectrophoresis procedures, using as antigens whole PAO1 outer membranes solubilized in 1% (wt/vol) Triton X-100–20 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA. The selected hybridomas and their corresponding antibodies were named MA1-3 (monoclonal antibody fusion 1 clone 3), MA1-6, and MA1-8.

Interaction of monoclonal antibodies with LPS and LPS-altered mutants. The three monoclonal antibodies tested reacted strongly with purified outer membranes from strain PAO1 and from an antibiotic-supersusceptible, LPS lipid A-altered mutant, Z61 (1), derived from a clinical isolate of *P. aeruginosa* (Table 1). However, MA1-8 failed to interact with the outer membranes of three independently isolated rough, LPS-altered mutants derived from strain PAO1 (Table 1). In contrast, MA1-3, MA1-6, and rabbit serum raised against whole PAO1 outer membranes reacted equally well against the outer membranes of PAO1 and its rough, LPS-altered mutants. This finding was consistent with MA1-8 being specific for the O-antigen portion of PAO1 LPS. In agreement with this, MA1-8 interacted strongly with purified PAO1 LPS, but MA1-3 and MA1-6 failed to interact with LPS (Table 2). We were also able to demonstrate passive hemagglutination by MA1-8 of sheep erythrocytes coated with smooth PAO1 LPS (titer, 1,400), but we could not demonstrate hemagglutination of sheep erythrocytes coated with rough AK1160 LPS lacking O antigen (titer, <2).

Interaction of monoclonal antibodies with partially purified antigens. A variety of partially purified outer membrane proteins were tested for their ability to interact with the three monoclonal antibodies (Table 2). In agreement with the above data, MA1-8 reacted only with those antigens which could be shown by chemical means to contain LPS as a contaminant. In contrast, MA1-3 failed to interact with LPS or partially purified outer membrane proteins D2, F, and H1, but interacted well with protein I. In an Ouchterlony double-diffusion assay, MA1-3 gave a strong precipitin line when protein I was used as an antigen. The third monoclonal, MA1-

TABLE 1. Interaction of monoclonal antibodies with the outer membranes of LPS-altered strains of *P. aeruginosa*

Outer membrane antigen from strain:	LPS defect	Log_{10} ELISA titer with:[a]			
		MA1-3	MA1-6	MA1-8	Rabbit anti-whole outer membranes
PAO1	Wild type	3	3	5	7
Z61	Lipid A (semirough)	3	3	6	6
AK1160	Rough	3	3	<1	7
AK1188	Rough	3	3	<1	7
H223	Rough	3	3	<1	ND[b]

[a] Log_{10} ELISA titers were obtained by using serial 10-fold dilutions of ascites fluid or antiserum as the primary antibody in ELISA assays. The antigen-antibody complexes were then reacted with alkaline phosphatase-coupled rabbit anti-whole mouse immunoglobulin antibodies. The log_{10} of the highest dilution of ascites or antiserum giving 30% of the rate of substrate (*p*-nitrophenylphosphate) conversion to products, when compared with the 10-fold-diluted control, was taken as the log_{10} ELISA titer.

[b] Not done.

6, interacted well in ELISA with partially purified outer membrane proteins H2 and I (which was contaminated with H2), thus distinguishing it from MA1-3, which only interacted with protein I. In an Ouchterlony double diffusion test, MA1-6 gave a single precipitin line with both H2 and I as antigens. In addition, we observed a single precipitin line, using as an antigen protein H2 cut out of sodium dodecyl sulfate-polyacrylamide gels and eluted into 2% Triton X-100–20 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA solution; we also observed a positive ELISA reading when this antigen was used to coat the plates. Neither MA1-3 nor MA1-6 reacted with protein I eluted from polyacrylamide gels.

Interactions of monoclonal antibodies with the outer membranes of serotyping strains. Monoclonal antibody MA1-8, specific for the O antigen of strain PAO1, interacted strongly with the outer membrane of the serotype 5 strain (Table 3), in agreement with a previous observation that strain PAO1 is type 5 in the IATS (6). Of the other 16 serotype strains examined, only the outer membranes of type 17 interacted weakly with MA1-8. This was consistent with a series of experiments testing the ability of our monoclo-

TABLE 2. Interaction of monoclonal antibodies with partially purified outer membrane antigens of *P. aeruginosa*

Antigen	Log_{10} ELISA titer with monoclonal antiserum:		
	MA1-3	MA1-6	MA1-8
H103 outer membranes	3	4	5
Protein D2[a]	<1	<1	3
Protein F[a]	<1	<1	2
Protein H1[a]	<1	<1	2
Protein H2[b]	<1	4	<1
Protein I[b]	3	4	<1
LPS	0	0	4

[a] Partially purified proteins D2, F, and H1 were shown to contain significant amounts of LPS (about 10% [wt/wt]).

[b] Proteins H2 and I were cross-contaminated (see the text).

TABLE 3. Interaction of monoclonal antibodies MA1-3, MA1-6, and MA1-8 with the outer membrane antigens of *P. aeruginosa* strains representing the 17 serotypes of the IATS

Outer membrane antigen[a] from	Log_{10} ELISA titer[b] with antibody:		
	MA1-3	MA1-6	MA1-8
Serotype 1	3	2 (0.73)[c]	<1
2	3	2 (0.85)	<1
3	3	2 (0.54)	<1
4	3	2 (0.73)	<1
5	3	2 (0.41)	3
6	3	3 (1.08)	<1
7	3	3 (1.20)	<1
8	3	2 (0.70)	<1
9	3	3 (1.09)	<1
10	3	3 (1.21)	<1
11	3	2 (0.92)	<1
12	3	2 (1.18)	<1
13	3	2 (0.41)	<1
14	3	3 (1.25)	<1
15	3	2 (0.40)	<1
16	3	3 (0.56)	<1
17	3	3 (>2)	1
P1 mucoid	3	2 (0.38)	<1
P1 revertant	3	2 (0.20)	<1
PAO1	3	3 (1.15)	5

[a] For testing MA1-3, outer membranes were solubilized in 2% Triton X-100–20 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA and then precipitated at −20°C after the addition of 2 volumes of ethanol and 0.1 M NaCl before ELISA plates were coated. Using unsolubilized outer membranes as antigens gave inconsistent results for MA1-3. For the other monoclonal antibodies, unsolubilized outer membranes were used as the coating antigen.

[b] ELISA readings are the averages of three or more experiments.

[c] Numbers in parentheses represent the optical density at 490 nm for a single experiment after 2 h of incubation at 4°C with *p*-nitrophenylphosphate.

TABLE 4. Interaction of monoclonal antibodies MA1-3, MA1-6, and MA1-8 and anti-outer membrane sera against various bacterial strains

Antigen (outer membrane from strain:)	Log_{10} ELISA titer with:			
	MA1-3	MA1-6	MA1-8	Rabbit anti-outer membrane serum
Pseudomonas aeruginosa PAO1	3	3	5	7
P. fluorescens ATCC 13525	1	>3	<1	6
P. fluorescens ATCC 949	<1	3	<1	6
P. putida ATCC 12633	<1	3	<1	6
P. putida K4359	<1	3	<1	6
P. anguilliseptica ET 7601	<1	3	<1	6
P. acidovorans ATCC 9355	<1	<1	<1	4
Azotobacter vinelandii OP	1	3	<1	6
Aeromonas salmonicida NCMB 1102	<1	<1	<1	5
Aeromonas hydrophila ET-2	<1	<1	<1	5
Vibrio anguillarum HT 7602	<1	<1	<1	5
Edwardsiella tarda 79054	<1	<1	<1	3

nal antibodies to agglutinate whole bacteria. In these experiments, we could show strong agglutination (+4) of cells of strain PAO1, strain Z61, and our serotype 5 strain, but no bacterial agglutination reactions were observed when the other serotyping strains were used. MA1-3 and MA1-6 were unable to agglutinate PAO1 or any of the 17 serotyping strains. A rabbit anti-whole outer membrane serum agglutinated PAO1, Z61, and the serotype 16 strain strongly (+4), the serotype 5 strain moderately (+2), and the serotype 4, 6, 7, and 17 strains weakly (+1). The other 11 serotyping strains were not agglutinated.

Monoclonal antibodies MA1-3 and MA1-6 interacted with the outer membranes of all *P. aeruginosa* strains tested, although the reactions varied from strain to strain (e.g., see values in brackets for MA1-6, Table 3). Thus, the antigenic sites recognized by these antibodies are apparently common to all *P. aeruginosa* serotypes.

Interactions with the outer membranes of other species. To determine whether our monoclonal antibodies were specific for *P. aeruginosa* antigens, we screened them against the outer membranes of a variety of strains of *Pseudomonas* and other organisms (Table 4). MA1-8 was shown to be specific for *P. aeruginosa* PAO1, and MA1-3 showed weak reactions with one of two *P. fluorescens* strains tested and the *Azotobacter vinelandii* strain. However, MA1-6 reacted strongly with the outer membranes of two *P. fluorescens* strains (including the type strain ATCC 13525), two *P. putida* strains (including the type strain ATCC 12633), a *P. anguilliseptica* strain, and an *Azotobacter vinelandii* strain, but not with the outer membranes of five other organisms.

DISCUSSION

In this paper, we have described the isolation and characterization of three monoclonal antibodies directed against outer membrane antigens. One of these antibodies, MA1-8, is quite specific for the O-antigenic region of the LPS of serotype 5 strains. Based on its weak cross-reaction with the outer membrane of a serotype 17 strain, MA1-8 may well be directed against antigen 2d of the LPS which is apparently shared by serotypes 5 and 17 (16). This result agrees with the conclusion of numerous other reports that variation in the LPS O-antigen composition is responsible for serotyping differences (see, e.g., references 15 and 16).

A major finding of this paper is that all *P. aeruginosa* strains studied shared at least two separate outer membrane protein antigenic sites, as revealed by their interaction with the monoclonal antibodies MA1-3 and MA1-6. The former antibody could be distinguished from MA1-6 on the basis of interactions both with partially purified *P. aeruginosa* major outer membrane proteins (Table 2) and with the outer membranes of other bacteria (Table 4). However, the actual nature of the antigenic site against which MA1-3 is directed has remained obscure since it does not seem to interact with denatured antigens from sodium dodecyl sulfate-polyacrylamide gels, and immunoprecipitation has not given a clear result (unpublished data). It is possible that MA1-3 is directed against a minor protein or a complex of proteins H2 and I, which may be present in our partially purified protein I preparation. The existence of such a complex in vivo has been postulated from selective solubilization experiments (11) and has also been demonstrated in our laboratory by protein-protein cross-linking experiments (B. L. Angus, and R. E. W. Hancock, unpublished data).

The results reported here demonstrate that MA1-6 is specific for the major outer membrane lipoprotein H2. Mizuno (18) has demonstrated that an outer membrane protein can be immunoprecipitated from three fluorescent pseudomo-

nads, *P. aeruginosa*, *P. fluorescens*, and *P. putida*, by crude antisera against *P. aeruginosa* protein H2. Our results confirm and extend these data in demonstrating that a single antigenic site on protein H2 is shared by proteins from the outer membranes of 25 *P. aeruginosa* strains, 2 *P. fluorescens* strains, 2 *P. putida* strains, a *P. anguilliseptica* strain, and an *Azotobacter vinelandii* strain. This pattern of conservation of an antigenic site is consistent with reports suggesting that *Azotobacter vinelandii* demonstrates rRNA homology with a variety of fluorescent pseudomonads (7), whereas *P. anguilliseptica*, a pathogen of Japanese eels, cross-reacts antigenically with *P. aeruginosa*, *P. fluorescens*, and *P. putida* (Nakajima et al., in press). MA1-6 failed to interact with *P. acidovorans* outer membranes, in agreement with rRNA homology experiments suggesting that this organism is taxonomically distinct from the fluorescent pseudomonads (7).

There are at least three major practical applications of monoclonal antibodies such as those described here. A bank of serotype-specific monoclonal antibodies like MA1-8 would provide highly accurate typing sera for classification of clinical *P. aeruginosa* strains. In addition, monoclonal antibodies like MA1-6 and MA1-3 may help to identify common antigens of *P. aeruginosa* for potential use in vaccines. Finally, they may have considerable value in the taxonomy of pseudomonads. While this paper was in preparation, we learned of the work of Rabin et al. (personal communication), who have identified similar serotype-specific and -nonspecific monoclonal antibodies by using a Homma serotype M strain of *P. aeruginosa* as the injecting antigen. Unfortunately, this Homma serotype does not correspond to any of the IATS serotypes described here.

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LITERATURE CITED

1. **Angus, B. L., A. M. Carey, D. A. Caron, A. M. B. Kropinski, and R. E. W. Hancock.** 1982. Outer membrane permeability in *Pseudomonas aeruginosa*: comparison of a wild-type with an antibiotic-supersusceptible mutant. Antimicrob. Agents Chemother. **21**:299–309.
2. **Brokopp, C. D., and J. J. Farmer.** 1979. Typing methods for *Pseudomonas aeruginosa*, p. 89–133. *In* R. G. Doggett (ed.), *Pseudomonas aeruginosa*: clinical manifestations of infection and current therapy. Academic Press, Inc., New York.
3. **Brokopp, C. D., R. Gomez-Lus, and J. J. Farmer III.** 1977. Serological typing of *Pseudomonas aeruginosa*: use of commercial antisera and live antigens. J. Clin. Microbiol. **5**:640–649.
4. **Chen, Y.-H. U., R. E. W. Hancock, and R. I. Mishell.** 1980. Mitogenic effects of purified outer membrane proteins from *Pseudomonas aeruginosa*. Infect. Immun. **28**:178–184.
5. **Costerton, J. W., M. R. W. Brown, and J. M. Sturgess.** 1979. The cell envelope: its role in infection, p. 41–62. *In* R. G. Doggett (ed.), *Pseudomonas aeruginosa*: clinical manifestations of infection and current therapy. Academic Press, Inc., New York.
6. **Cox, C. D.** 1979. Passage of *Pseudomonas aeruginosa* in compromised mice. Infect. Immun. **26**:118–124.
7. **De Vos, P.** 1980. Intrageneric and intergeneric similarities of ribosomal RNA cistrons of the genus *Pseudomonas* and the implications for taxonomy. Antonie van Leeuwenhoek J. Microbiol. Serol. **46**:96.
8. **Gonggrijp, R., W. J. H. A. Mullers, and C. P. A. van Boven.** 1981. Serotype-nonspecific protection induced by ribonucleic acid isolated from the ribosomal vaccine of *Pseudomonas aeruginosa*. Infect. Immun. **33**:178–185.
9. **Hancock, R. E. W., and A. M. Carey.** 1979. Outer membrane of *Pseudomonas aeruginosa*: heat- and 2-mercaptoethanol-modifiable proteins. J. Bacteriol. **140**:902–910.
10. **Hancock, R. E. W., and A. M. Carey.** 1980. Protein D1—a glucose inducible pore-forming protein from the outer membrane of *Pseudomonas aeruginosa*. FEMS Microbiol. Lett. **8**:105–109.
11. **Hancock, R. E. W., R. T. Irvin, J. W. Costerton, and A. M. Carey.** 1981. *Pseudomonas aeruginosa* outer membrane: peptidoglycan-associated proteins. J. Bacteriol. **145**:628–631.
12. **Hancock, R. E. W., and H. Nikaido.** 1978. Outer membranes of gram-negative bacteria. XIX. Isolation from *Pseudomonas aeruginosa* PAO1 and use in reconstitution and definition of the permeability barrier. J. Bacteriol. **136**:381–390.
13. **Jarrell, K. F., and A. M. Kropinski.** 1981. Coliphage T7 receptors are present in *Pseudomonas aeruginosa* rough lipopolysaccharides. Biochem. Biophys. Res. Commun. **99**:1185–1190.
14. **Köhler, G., and C. Milstein.** 1975. Continuous culture of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
15. **Koval, S. F., and P. M. Meadow.** 1975. The relationship between aminosugars in the lipopolysaccharide, serotype, and aeruginocin sensitivity in strains of *Pseudomonas aeruginosa*. J. Gen. Microbiol. **91**:437–440.
16. **Lanyi, B., and T. Bergen.** 1978. Serological characterization of *Pseudomonas aeruginosa*, p. 93–168. *In* J. R. Norris and T. Bergen (ed.), Methods in Microbiology, vol. 10. Academic Press, London.
17. **Laurell, C. B.** 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. **15**:43–52.
18. **Mizuno, T.** 1981. A novel peptidoglycan-associated lipoprotein (PAL) found in the outer membrane of *Proteus mirabilis* and other gram negative bacteria. J. Biochem. **89**:1039–1049.
19. **Nicas, T. I., and R. E. W. Hancock.** 1980. Outer membrane protein H1 of *Pseudomonas aeruginosa*: involvement in adaptive and mutational resistance to ethylenediaminetetraacetate, polymyxin B, and gentamicin. J. Bacteriol. **143**:872–878.
20. **Oi, V. T., and L. A. Herzenberg.** 1979. Immunoglobulin producing hybrid cell lines, p. 351–371. *In* B. B. Mishell and S. M. Shiigi (ed.), Selected methods in cellular immunology. W. H. Freeman & Co., San Francisco.
21. **Ouchterlony, O.** 1958. Diffusion-in-gel methods for immunological analysis. Prog. Allergy **5**:1–78.
22. **Pennington, J. E.** 1979. Immunotherapy of *Pseudomonas aeruginosa* infection, p. 191–217. *In* R. G. Doggett (ed.), *Pseudomonas aeruginosa*: clinical manifestations of infection and current therapy. Academic Press, Inc., New York.
23. **Ruitenberg, E. J., P. A. Steerenberg, B. J. M. Brosi, and J. Buys.** 1974. Serodiagnosis of *Trichinella spiralis* infection in pigs by enzyme linked immunosorbent assays. Bull. W.H.O. **51**:108–109.
24. **Young, L. S.** 1980. The role of exotoxins in the pathogenesis of *Pseudomonas aeruginosa*. J. Infect. Dis. **142**:626–630.