INFECTION AND IMMUNITY, Jan. 1984, p. 49–53
0019-9567/84/010049-05$02.00/0

Vol. 43, No. 1

Antibody Response of Infected Mice to Outer Membrane Proteins of *Pseudomonas aeruginosa*

RICHARD C. HEDSTROM,* OLGERTS R. PAVLOVSKIS, AND DARRELL R. GALLOWAY

Naval Medical Research Institute, Bethesda, Maryland 20814

Received 29 July 1983/Accepted 11 October 1983

The antibody response to outer membrane proteins of *Pseudomonas aeruginosa* was studied in mice experimentally infected with *P. aeruginosa* 220. The infection consisted of an abscess established by subcutaneous injection of bacteria. Sera from these mice were analyzed by indirect radioimmunoprecipitation and immunoblot methods for the presence of antibodies to proteins of the isolated outer membrane. Sera from mice 14 days postinfection were shown to contain antibodies directed against proteins that comigrated with the major outer membrane proteins F (porin), H2, and I (lipoprotein). A 16,000-dalton protein that did not appear to be a major outer membrane protein also elicited a significant antibody response in some instances. It is concluded that mice, in response to infection, elicit an immunological response to outer membrane proteins of *P. aeruginosa*.

Interest in the development of a vaccine against the opportunistic pathogen *Pseudomonas aeruginosa* has led investigators to consider outer membrane proteins from this organism as potential vaccine components (8, 12, 24). In a recent study, Mutharia *et al.* (24) found that the major outer membrane proteins F, H2, and I (nomenclature of Hancock and Carey [11]) are antigenically related in all 17 serotype-specific strains of *P. aeruginosa*. Their results imply that these major outer membrane proteins may provide "common" antigens for use in vaccines against *P. aeruginosa*. Moreover, these workers found that the original endotoxin protein vaccine against *P. aeruginosa* developed and tested by Homma (18) probably contains major outer membrane proteins that are common to all strains of *P. aeruginosa* (24).

If outer membrane proteins are to be used in the control of *P aeruginosa*, it will be essential to determine their role in pathogenesis and immunity. The outer membrane of *P. aeruginosa* is considered important in pathogenesis (4), and recent studies have attempted to elucidate the role of outer membrane proteins in this process (3, 14, 24). Precipitating antibodies to *P. aeruginosa* antigens have been demonstrated in the sera of cystic fibrosis patients (17); more recently, it was shown that, in sera from similar cystic fibrosis patients, antibodies were directed against envelope proteins (8). However, neither of these reports specified whether these antibodies were directed against the outer membrane proteins nor have any studies to date identified the outer membrane proteins of *P. aeruginosa* involved in the immunological response to infection. Since protein F is known to be exposed on the cell surface of *P. aeruginosa* (21), it was conceivable that protein F may interact with the host immune system and elicit an immunological response. The experiments reported here were undertaken to determine whether experimental infection with *P. aeruginosa* elicits an immunological response to common outer membrane proteins that may be useful in vaccine studies.

MATERIALS AND METHODS

Organism and growth conditions. The organism used for this study was *P. aeruginosa* PA220 which was described previously (26). The cells were grown in tryptic soy broth dialysate at 37°C and prepared for the challenge inoculum as detailed previously (26). For all other procedures, strain PA220 was grown in the basal salts medium previously described (6) supplemented with 14 mM glucose (final concentration) at 37°C in a rotary incubator shaker (250 rpm). The cells were harvested during late exponential growth.

Mouse infection. The infection model will be described in detail elsewhere (O. R. Pavlovskis, manuscript in preparation). Briefly, female Swiss white mice strain NMRI CV, weighing 20 ± 2 g, were each injected subcutaneously on the abdomen with 10^7 to 10^8 viable organisms suspended in 0.1 ml of saline. This challenge caused the formation of an acute abscess which was contained at the site of injection. The abscess consisted of a raised nondraining nodule of about 5 mm in diameter, and *P. aeruginosa* was always isolated from the abscess. Usually, abscesses were formed and resolved in 3 and 14 days, respectively; however, a small percentage of mice developed a systemic infection and later died. Sera from mice bled periorbitally on days 3, 7, and 14 postinfection were used in an enzyme-linked immunosorbent assay (ELISA) (5) to detect antibodies to the outer membrane of PA220. ELISA titers were defined as the absorbance value at 405 nm times the serum dilution factor, as described previously (1).

Outer membrane antigen preparations. Isolated outer membranes of PA220 were prepared by sucrose density gradient centrifugation of cell envelopes, obtained from French press lysates of whole cells, by the method of Hancock and Nikaido (13) and as described previously (16).

The protein-lipopolysaccharide (LPS) complex (PrLPS) was extracted from cells of PA220 as described previously (16) except as modified here. Briefly, the washed cell pellet was suspended to 1/10 the original culture volume in a solution of 10 mM EDTA in 333 mM Tris-hydrochloride buffer, pH 8.0, containing in final concentration 0.55 M sucrose. After extraction and centrifugation, the supernatant fraction containing the PrLPS was dialyzed for 3 to 4 h at 4°C against 10 mM Tris buffer, pH 8.0, to remove excess sucrose. This solution was then concentrated to 1/1,000 the original culture volume by ultrafiltration followed by centrifugation at 100,000 × *g* for 1 h at 4°C. Finally, the PrLPS was purified from the resultant supernatant fraction by gel filtration with Sepharose CL-4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). The supernatant fraction was applied to a column (2.6 by 93 cm) that was equilibrated with 10

* Corresponding author.

mM Tris buffer, pH 8.0, and the PrLPS was eluted in the void volume as before.

The LPS of PA220 was prepared with hot phenol-water extraction (3) and shown to be free of protein and nucleic acid by the lack of absorbance at 260 and 280 nm.

Radioiodination and indirect immunoprecipitation. Proteins of isolated outer membrane were radiolabeled with [^{125}I]iodine by the chloroglycoluril (IODO-GEN; Pierce Chemical Co., Rockford, Il.)-catalyzed method essentially as described previously (22). Briefly, a 100-μl sample of isolated outer membrane (100 μg of protein in 10 mM potassium phosphate buffer, pH 7.4) was reacted on ice with 1 mCi of [^{125}I]sodium in a chloroglycoluril-coated test tube for 20 min. The reaction was stopped by removing the sample from the reaction tube. The tube was rinsed twice each time with 100 μl of 10 mM potassium phosphate buffer, pH 7.4, and the rinses were combined with the iodinated outer membrane sample. To the sample, 1 ml of 10 mM sodium iodide in 10 mM potassium phosphate buffer, pH 7.4, was added. The radioiodinated outer membranes were separated from free iodine by Sephadex G-25M gel filtration (PD-10 column; Pharmacia). The column was preequilibrated and run with 10 mM potassium phosphate buffer, pH 7.4, containing 0.1% gelatin, and the ^{125}I-labeled membrane samples were eluted in the void volume.

The indirect immunoprecipitation method used was a modification of that described by Hansen et al. (15). To 100 μl of radioiodinated outer membrane (2×10^7 cpm) was added 100 μl of heat-inactivated serum. The suspension was mixed overnight at 4°C. A 1-ml amount of solubilization buffer was added and incubated for 1 h at 37°C, followed by an additional incubation of 1 h at ambient temperature. The solubilization buffer consisted of 10 mM Tris-hydrochloride, pH 7.8, 10 mM EDTA, 150 mM NaCl, and 1% Triton X-100. This procedure was shown to solubilize completely the isolated outer membrane of strain PA220. To the sample was then added 100 μl of a 10% suspension of Sepharose beads coupled to staphylococcal protein A (Protein A-Sepharose CL-4B; Sigma Chemical Co., St. Louis, Mo.) previously washed and equilibrated in solubilization buffer. The suspension was mixed for 1 h at ambient temperature followed by centrifugation at 12,400 × *g* for 30 s. The pelleted beads were washed five times with 50 mM Tris-hydrochloride, pH 7.5, containing 5 mM EDTA, 150 mM NaCl, 1 mg of bovine serum albumin (BSA) per ml and 0.2% sodium azide. The washed beads were resuspended in 150 μl of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) buffer (described below), heated for 5 min at 100°C, and centrifuged for 3 min at 12,400 × *g*. The resultant fraction was retained for SDS-PAGE.

SDS-PAGE and immunoblotting procedure. Components of the PrLPS complex and isolated outer membrane were extracted with SDS and analyzed by SDS-PAGE by the procedure of Hancock and Carey (11) and as described previously (16).

For immunoblotting, components of the PrLPS complex and isolated outer membrane resolved on SDS-PAGE gels were electrophoretically transferred onto a nitrocellulose sheet as described by Towbin et al. (28), except that the Tris-glycine-methanol transfer buffer contained 0.1% SDS (7) and the electrophoretic transfer time was decreased to 30 min at 0.8 to 1.4 A. After transfer, the nitrocellulose sheet was incubated in 50 mM Tris-hydrochloride, pH 7.5, containing 150 mM NaCl, 0.15% sodium azide, and 5% BSA for 1 h at 37°C. The nitrocellulose sheet was cut in strips corresponding to individual lanes of the SDS-PAGE gel. Individual strips were incubated overnight in antiserum diluted 1:100 in the above Tris-sodium chloride-azide buffer containing 1% BSA. Incubations were performed at ambient temperature on a shaking platform. The resultant immunoblots were washed extensively in phosphate-buffered saline, pH 7.5, and then incubated for 4 h with 10^5 cpm per ml of the appropriate ^{125}I-labeled second antibody which was diluted in Tris-sodium chloride-azide buffer containing 1% BSA. The second antibody was either ^{125}I-labeled goat anti-rabbit immunoglobulin G or ^{125}I-labeled goat anti-mouse immunoglobulins (New England Nuclear Corp., Boston, Mass.). Immunoblots were washed as before, dried, and analyzed by autoradiography.

Antisera. Antiserum to isolated outer membrane was prepared in white New Zealand rabbits as follows. Rabbits were immunized on days 0, 14, 28, and 42 by intramuscular and subcutaneous injections of isolated outer membrane (500 μg of protein as determined by the Lowry procedure as modified by Markwell et al. [23]) emulsified in Freund incomplete adjuvant. Serum was collected before immunization and 10 days after the last injection. Anti-LPS antiserum was prepared in Swiss white mice strain NMRI CV by subcutaneous injections of purified LPS (250 μg [dry weight]) in Freund adjuvant by using the same immunization schedule as above.

RESULTS

Analysis of major outer membrane proteins of PA220. We examined the physical and immunological characteristics of the major outer membrane proteins of PA220 as an initial step toward establishing which of these proteins are involved in the immune response to infection. Electrophoretic mobilities (Fig. 1, lane A) were identical to those previously described for *P. aeruginosa* PAO1 (11, 16). Autoradiographic analysis (Fig. 2) revealed major radioiodinated proteins that comigrated with the major outer membrane proteins F, H (we were unable to distinguish H1 from H2 here), and I (Fig. 2, lane A); furthermore, these proteins were immunoprecipitated by anti-outer membrane antiserum (Fig. 2, lane B). Thus, proteins F, H, and I were accessible to extrinsic labeling, with this system, and were immunogenic.

Antibody response to outer membrane proteins after infection. It was found, by using ELISA, that the abscess infection caused a 5 to 12-fold increase, after 14 days, in serum antibodies to the isolated outer membrane of PA220 over noninfected control levels. In two separate experiments, ELISA titers rose, over noninfected control levels, from 4 to 20 after 3 and 14 days, respectively, and from 5 to 60 after 3 and 14 days, respectively. A pooled serum from six infected mice immunoprecipitated a protein of the isolated outer membrane that comigrated with protein F (Fig. 2, lane F). Uninfected mouse control serum did not immunoprecipitate any of the labeled proteins (Fig. 2, lane E). These data suggested that experimental infection with PA220 elicited an immune response to protein F. It is unlikely that the response was due to anti-LPS antibodies because of the dissimilarity between the infected mouse serum immunoprecipitate (Fig. 2, lane F) and the anti-LPS antiserum immunoprecipitate (Fig. 2, lane D), which was predominated by a band that comigrated with protein I.

To confirm and extend the above observations, an immunoblot analysis was performed comparing the isolated outer membrane with the PrLPS complex liberated by EDTA from sucrose-stabilized cells of PA220. Since the PrLPS complex lacks the outer membrane proteins F, H2, and I (Fig. 1, lane B) but contains LPS (16), antibodies elicited during infection

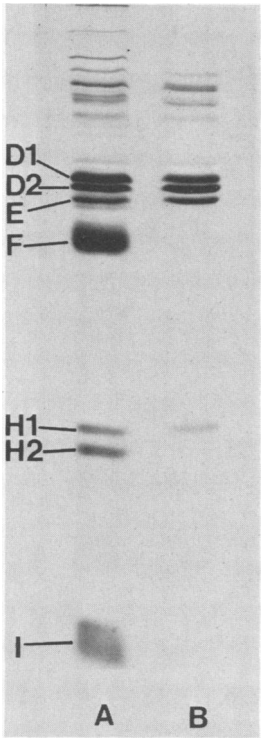

FIG. 1. SDS-PAGE profile showing the outer membrane protein pattern of the isolated outer membrane (lane A) and the PrLPS complex (lane B) of PA220. Outer membrane protein designations are shown in the margin.

and directed against these proteins can be distinguished from anti-LPS antibodies with this method. All of the major outer membrane proteins were electrophoretically transferred from SDS-PAGE gel to nitrocellulose (data not shown). Antibodies from the anti-outer membrane antiserum reacted with major bands that comigrated with proteins F, H1, H2, and I (Fig. 3, lane A). Analysis of the PrLPS complex with this antiserum (Fig. 3, lane B) revealed a banding pattern similar to that of the isolated outer membrane with the exception of a band at protein H2. Since proteins F and I are not components of the PrLPS complex, these data suggested that LPS or components of LPS migrated with the same relative mobility as proteins F and I. This was confirmed by analysis with anti-LPS antiserum (Fig. 3, lane C).

Several outer membrane proteins elicited an antibody response in mice experimentally infected with PA220 (Fig. 3 lanes D to O). Uninfected mouse control serum was negative (data not shown). In keeping with results found previously with immunoprecipitation, infected mouse sera reacted with protein F (Fig. 3, lanes D, F, H, L, and N). That this response was specifically due to anti-protein F antibodies and not anti-LPS antibodies was apparent because a band in the corresponding region of the PrLPS complex was absent (Fig. 3, lanes E, G, I, M, and O). In addition, a significant response to a band that comigrated with protein I was observed in each of the sera (Fig. 3, lanes D, F, H, J, L, and N); however, this response could be partly due to anti-LPS antibodies, since these sera also reacted, to various degrees, with the corresponding region of the PrLPS complex (Fig. 3, lanes E, G, I, K, M, and O). Mice numbered 2, 3, and especially 5, reacted specifically with a band that comigrated with the major outer membrane protein H2 (Fig. 3, lanes F, H, and L). Finally, a band of ca. 16,000 molecular weight (16K) elicited an immunological response in infected mice numbered 1, 4, 5, and 6 (Fig. 3, lanes D, E, and J to O, respectively). This band was a component of both the isolated outer membrane and the PrLPS complex (Fig. 3, lanes A and B) but did not react with anti-LPS antiserum (Fig. 3, lane C). Interestingly, the 16K-membrane component (presumably protein) does not appear to be one of the major outer membrane proteins demonstrated by Coomassie blue staining.

DISCUSSION

We have shown that experimental infection elicited an immunological response to several of the known major outer membrane proteins of *P. aeruginosa*. Immunoprecipitation analysis demonstrated precipitating antibodies to protein F. That immunoblot analysis additionally detected antibodies to proteins H2 and I may be a reflection of differences in the two methods. Antibodies in 14-day postinfection serum to proteins H2 and I may not be of the mouse immunoglobulin subclass that binds efficiently to staphylococcal protein A whereas the second antibody used for detection with immunoblot analysis was a nonspecific anti-mouse immunoglobulin capable of binding to all subclasses. Nevertheless, these results clearly indicated that a significant immunological response elicited during infection was directed to protein F, the porin protein (2, 12, 30). Whether anti-protein F antibodies are protective against *P. aeruginosa* in this infection model awaits further studies. It should be noted, however, that porin preparations from *Neisseria meningitidis* have been shown to be protective in animal experiments (9) as have porin preparations from *Salmonella typhimurium* (19, 27). Furthermore, antiporin antibodies are able to passively

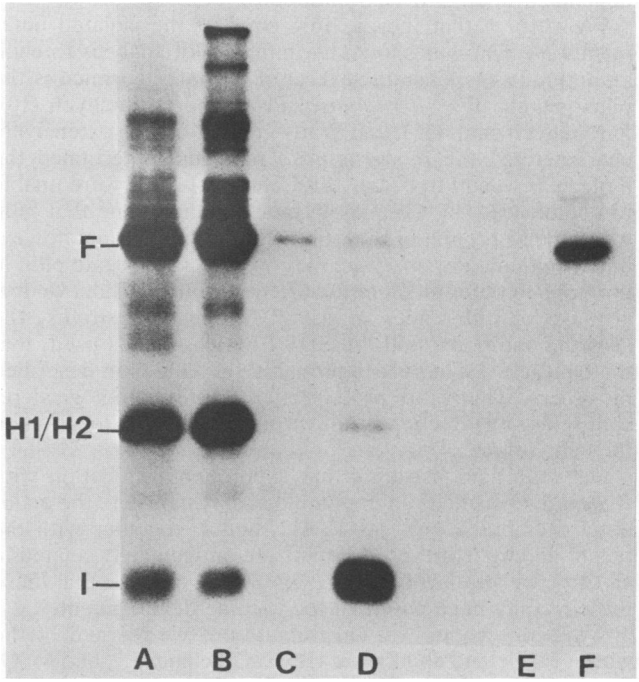

FIG. 2. Autoradiograph of SDS-PAGE profiles showing the results obtained from radioiodination of the isolated outer membrane (lane A) and from immunoprecipitation of radioiodinated, isolated outer membrane with rabbit anti-outer membrane antiserum (lane B), normal rabbit serum (lane C), mouse anti-LPS antiserum (lane D), uninfected mouse serum (lane E), and a pooled serum from infected mice (lane F).

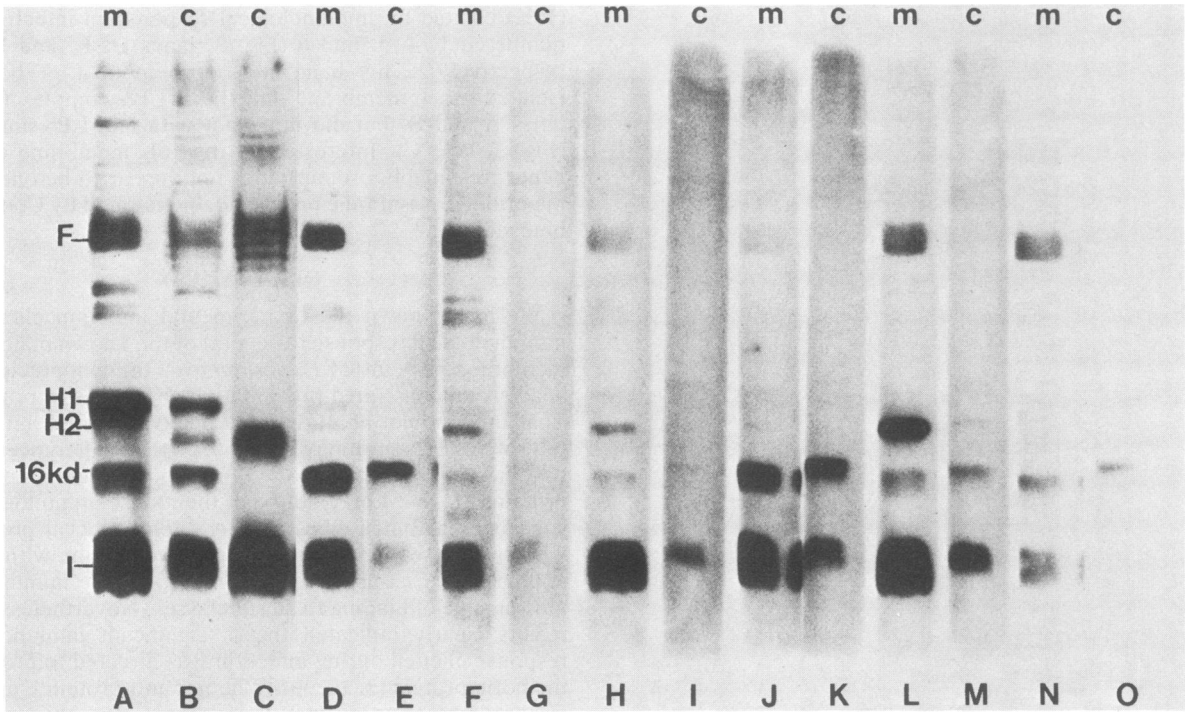

FIG. 3. Autoradiograph showing the results obtained from immunoblotting of the PrLPS complex (c) and the isolated outer membrane (m) with the following sera. Rabbit anti-outer membrane (lanes A and B), mouse anti-LPS (lane C), and infected mice (lanes D to O): mouse 1 (lanes D and E), mouse 2 (lanes F and G), mouse 3 (lanes H and I), mouse 4 (lanes J and K), mouse 5 (lanes L and M), and mouse 6 (lanes N and O).

protect mice against salmonellosis, and this protection is not dependent upon anti-LPS antibodies (20).

We suspect that the ca. 16K band of the isolated outer membrane that was shown by immunoblot analysis to elicit an antibody response in several of the infected mice is the pilus protein of *P. aeruginosa*. Frost and Paranchych (10), Paranchych et al. (25), and Watts et al. (29) have extensively characterized the *P. aeruginosa* pilin and determined the molecular weight to be ca. 18K. These workers were unable to demonstrate by Coomassie blue staining alone that pilin was a major protein of the isolated outer membrane; however, by immunoblot analysis, they were able to locate pilin in the isolated outer membrane of *P. aeruginosa*. Thus, we feel because of differences in the *P. aeruginosa* strains, the growth conditions, and the SDS-PAGE methods used, that the band we see at 16K is probably the 18K pilin described by others. That pilus protein elicits an immunological response during infection is not surprising given its location on the cell surface.

Our data demonstrating that experimental infection with *P. aeruginosa* elicits an immunological response to the major outer membrane proteins F, H2, and I, together with the recent finding that these proteins are antigenically related in all serotype strains of *P. aeruginosa* (24), indicate that these proteins may have potential for vaccine development.

While this paper was in preparation, we learned of the work of Gilleland and Parker (H. E. Gilleland, Jr., and M. G. Parker, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, E53, p. 85), who have shown significant protection against a live *P. aeruginosa* intraperitoneal challenge in mice previously immunized with a substantially purified protein F preparation. Whether a protein F vaccine will be sufficiently protective or whether complete protection will only be afforded by a multicomponent vaccine comprised of several *P. aeruginosa* antigens awaits further experimentation.

ACKNOWLEDGMENTS

This work was supported by the U.S. Naval Research and Development Command work unit no. 63750A.3M463750D808.AB.062.

We thank Gregory Dasch for the ELISA reagents and the helpful discussions. We are grateful to Emilio Weiss and Richard Walker for critical reading of the manuscript.

LITERATURE CITED

1. **Aronsson, B., M. Granström, R. Möllby, and C. E. Nord.** 1983. Enzyme-linked immunosorbent assay (ELISA) for antibodies to *Clostridium difficile* toxins in patients with pseudomembranous colitis and antibiotic-associated diarrhoea. J. Immunol. Methods **60**:341–350.
2. **Benz, R., and R. E. W. Hancock.** 1981. Properties of the large ion permeable pores formed from protein F of *Pseudomonas aeruginosa* in lipid bilayer membranes. Biochim. Biophys. Acta **646**:298–308.
3. **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.
4. **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.
5. **Dasch, G. A., S. Hale, and A. L. Bourgeois.** 1979. Sensitive microplate enzyme-linked immunosorbent assay for detection of antibodies against the scrub typhus rickettsia, *Rickettsia tsutsugamushi*. J. Clin. Microbiol. **9**:38–48.
6. **Eagon, R. G., and P. V. Phibbs, Jr.** 1971. Kinetics of transport of glucose, fructose and mannitol by *Pseudomonas aeruginosa*. Can. J. Biochem. **49**:1031–1041.
7. **Erickson, P. F., L. N. Miner, and R. S. Lasher.** 1982. Quantitative electrophoretic transfer of polypeptides from SDS polyacrylamide gels to nitrocellulose sheets: a method for their re-

use in immunoautoradiographic detection of antigens. J. Immunol. Methods **51**:241–249.
8. **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.
9. **Frasch, C. E.** 1979. Noncapsular surface antigens of *Neisseria meningitidis*, p. 304–337. *In* L. Weinstein and B. N. Fields (ed.), Seminars in infectious disease, vol. 2. Stratton Intercontinental Medical Book Corp., New York.
10. **Frost, L. S., and W. Paranchych.** 1977. Composition and molecular weight of pili purified from *Pseudomonas aeruginosa* K. J. Bacteriol. **131**:259–269.
11. **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.
12. **Hancock, R. E. W., G. M. Decad, and H. Nikaido.** 1979. Identification of the protein producing transmembrane diffusion pores in the outer membrane of *Pseudomonas aeruginosa* PAO1. Biochim. Biophys. Acta **554**:323–331.
13. **Hancock, R. E. W., and H. Nikaido.** 1978. Outer membranes of gram-negative bacteria. XIX. isolation from *Pseudomonas aeruginiosa* PAO1 and use in reconstitution and definition of the permeability barrier. J. Bacteriol. **136**:381–390.
14. **Hancock, R. E. W., A. A. Wieczorek, L. M. Mutharia, and K. Poole.** 1982. Monoclonal antibodies against *Pseudomonas aeruginosa* outer membrane antigens: isolation and characterization. Infect. Immun. **37**:166–171.
15. **Hansen, E. J., C. F. Frisch, R. L. McDade, Jr., and K. H. Johnston.** 1981. Identification of immunogenic outer membrane proteins of *Haemophilus influenzae* type b in the infant rat model system. Infect. Immun. **32**:1084–1092.
16. **Hedstrom, R. C., R. K. Schockley, and R. G. Eagon.** 1981. Ethylenediaminetetraacetate-extractable protein-lipopolysaccharide complex of *Pseudomonas aeruginosa*: characterization of protein components. J. Bacteriol. **148**:995–997.
17. **Hoiby, N., and N. H. Axelson.** 1973. Identification and quantitation of precipitins against *Pseudomonas aeruginosa* in patients with cystic fibrosis by means of crossed immunoelectrophoresis with intermediate gel. Acta Pathol. Microbiol. Scand. (B) **81**:298–308.
18. **Homma, J. Y.** 1982. Perspectives on the development of a new vaccine against *Pseudomonas aeruginosa* infection, p. 389–431. *In* C. F. S. Easmon and J. Jeljaszewicy (ed.), Medical microbiology, vol. 1. Academic Press, Inc., New York.
19. **Kuusi, N., M. Nurminen, and M. Sarvas.** 1981. Immunochemical characterization of major outer membrane proteins in experimental salmonellosis of mice. Infect. Immun. **25**:857–862.
20. **Kuusi, N., M. Nurimen, H. Saxen, and P. H. Makela.** 1981. Immunization with major outer membrane protein (porin) preparations in experimental murine salmonellosis: effect of lipopolysaccharide. Infect. Immun. **34**:328–332.
21. **Lambert, P. A., and B. R. Booth.** 1982. Exposure of outer membrane proteins on the surface of *Pseudomonas aeruginosa* PAO1 revealed by labelling with [^{125}I]lactoperoxidase. FEMS Microbiol. Lett. **14**:43–45.
22. **Markwell, M. A. K., and C. F. Fox.** 1978. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril. Biochemistry **17**:4807–4817.
23. **Markwell, M. A. K., S. M. Hass, L. L. Birber, and H. E. Tolbert.** 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. **87**:206–210.
24. **Mutharia, L. M., T. I. Nicas, and R. E. W. Hancock.** 1982. Outer membrane proteins of *Pseudomonas aeruginosa* serotype strains. J. Infect. Dis. **146**:770–779.
25. **Paranchych, W., P. A. Sastry, L. S. Frost, M. Carpenter, G. D. Armstrong, and T. H. Watts.** 1979. Biochemical studies on pili isolated from *Pseudomonas aeruginosa* strain PAO. Can. J. Microbiol. **25**:1175–1181.
26. **Pavlovskis, O. R., M. Pollack, L. T. Callahan III, and B. Iglewskis.** 1977. Passive protection by antitoxin in experimental *Pseudomonas aeruginosa* burn infections. Infect. Immun. **18**:596–602.
27. **Svenson, S. B., M. Nurinen, and A. Lindberg.** 1979. Artificial *Salmonella* vaccines: O-antigenic oligosaccharide-protein conjugates induce protection against infection with *Salmonella typhimurium*. Infect. Immun. **25**:863–872.
28. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.
29. **Watts, T. H., E. A. Worobec, and W. Paranchych.** 1982. Identification of pilin pools in the membranes of *Pseudomonas aeruginosa*. J. Bacteriol. **152**:687–691.
30. **Yoshimura, F., L. S. Zalman, and H. Nikaido.** 1983. Purification and properties of *Pseudomonas aeruginosa* porin. J. Biol. Chem. **258**:2308–2314.