INFECTION AND IMMUNITY, Sept. 1991, p. 2859–2863
0019-9567/91/092859-05$02.00/0

Vol. 59, No. 9

Pseudomonas aeruginosa Exoenzyme S Is an Adhesin

N. R. BAKER,[1] V. MINOR,[1] C. DEAL,[2] M. S. SHAHRABADI,[3] D. A. SIMPSON,[3] AND D. E. WOODS[3]*

Department of Microbiology, The Ohio State University, Columbus, Ohio 43210[1]; Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20307[2]; and Department of Microbiology and Infectious Diseases, University of Calgary Health Sciences Centre, 3330 Hospital Drive, N.W., Calgary, Alberta, Canada T2N 4N1[3]

Received 10 December 1990/Accepted 13 May 1991

Exoenzyme S from *Pseudomonas aeruginosa* has been studied as an adhesin for glycosphingolipids and buccal cells. Binding of exoenzyme S to gangliotriosylceramide (GalNAcβ1-4Galβ1-4Glcβ1-1Cer), gangliotetraosylceramide (Galβ1-3GalNAcTβ1-4Galβ1-4Glcβ1-1Cer), and lactosylceramide (Galβ1-4Glcβ1-1Cer) separated on thin-layer chromatograms was observed. Binding curves for exoenzyme S with dilutions of gangliotetraosylceramide immobilized on plastic plates were similar to previously reported results for the intact bacteria. Binding of exoenzyme S to sialylated counterparts of these glycosphingolipids was not seen, indicating that the addition of a sialic acid residue interferes with binding. Exoenzyme S and monoclonal antibody to exoenzyme S inhibit the binding of *P. aeruginosa* to buccal cells. The presence of exoenzyme S on the surface of *P. aeruginosa* was detected by immunogold labeling of bacteria with antibodies to exoenzyme S. Results of these studies led us to conclude that exoenzyme S is an important adhesin of *P. aeruginosa*.

Pseudomonas aeruginosa is an important opportunistic pathogen of immunocompromised patients (7). Infections of the respiratory tract are of particular concern because they are associated with high mortality rates (2). Cystic fibrosis patients are at high risk for lung infections due to *P. aeruginosa* which lead to significant morbidity and contribute to death of the patients (15, 17). Identification of the factors responsible for colonization of the lungs of cystic fibrosis patients by *P. aeruginosa* is particularly important since the organism is virtually impossible to eradicate once a chronic infection has been established.

Gangliotriosylceramide (GalNAcβ1-4Galβ1-4Glcβ1-1Cer), gangliotetraosylceramide (GgO_4; Galβ1-3GalNAcTβ1-4Galβ1-4Glcβ1-1Cer), and lactosylceramide (Galβ1-4Glcβ1-1Cer) have been identified as possible receptors for *P. aeruginosa* (1, 13), but the adhesin responsible for this specificity has not been identified. Pili, which have been identified as an important adhesive factor for buccal cells (28), damaged tracheal epithelium (22), and mucous proteins (21), are not the glycosphingolipid (GSL) adhesin since mutant strains which do not produce pilin retain the binding specificity of the parent strain (1a).

Exoenzyme S is one of several toxic products of *P. aeruginosa* which contribute to its pathogenicity (29). This enzyme ADP-ribosylates several membrane-associated eucaryotic proteins (3). Preliminary studies on the binding of exoenzyme S to cells indicate that it may recognize the same carbohydrate sequences as the bacteria. Thus, we have examined the binding of exoenzyme S to GSLs. In this study, we show that exoenzyme S has the same binding specificity for GSLs as the whole bacteria and that it is found on the outer surface of the bacteria where it can function as an adhesin. Furthermore, exoenzyme S and antibodies to exoenzyme S inhibit the attachment of *P. aeruginosa* to buccal cells, evidence which is in support of a role for this protein in adherence.

* Corresponding author.

MATERIALS AND METHODS

Exoenzyme S and antibodies to exoenzyme S. Exoenzyme S was purified from *P. aeruginosa* DG1 as described previously (26). The composition of the preparations used in the GSL binding studies varied because some samples contained lipopolysaccharide (LPS; see Fig. 4). The preparation used in the adherence inhibition assays produced a single protein band on sodium dodecyl sulfate (SDS)-polyacrylamide gels as visualized by Coomassie blue staining. Polyclonal antiserum to exoenzyme S raised in New Zealand rabbits was prepared as described previously (26). Western blotting (immunoblotting) with this antibody preparation revealed reactivity with exoenzyme S and LPS. Therefore, antiserum used in gold labeling studies was absorbed with *P. aeruginosa* DG1-ExS5, a mutant strain which is not totally exoenzyme S deficient but which produces significantly lower amounts of exoenzyme S than the parent strain (27). The production of monoclonal antibodies to exoenzyme S has been described previously (26).

GSLs and TLC binding assay. Purified GSLs were purchased from Sigma Chemical Co. (St. Louis, Mo.). The total nonacid GSL fraction from dog intestinal mucosa, a gift from G. Hansson (University of Goteborg, Goteborg, Sweden), was described previously (9). The thin-layer chromatography (TLC) plate binding assay was done as described previously (1) with some modifications. GSLs were separated on aluminum-backed silica gel Si60 high-performance TLC plates in chloroform-methanol-water (60:35:8). One plate was sprayed with cupric acetate for chemical detection, and duplicate plates were used for bacterial or exoenzyme S binding assays. The TLC plates were immersed in 0.3% polyisobutyl methacrylate in diethylether-hexane (1:1) for 1 min and air dried. Plates were wet with phosphate-buffered saline (PBS) containing 0.2% Tween (PBS-T) and overlaid with 2 to 3 ml of exoenzyme S diluted in PBS-T or a suspension of strain DG1 diluted to an optical density at 600 nm (OD_{600}) of 0.5 in PBS-T. After a 2-h incubation period at room temperature, the plates were washed five to six times with PBS-T. Bound exoenzyme S or bacteria were detected by sequential 1-h overlays with 2 to 3 ml of 1:1,000 dilutions of anti-exoenzyme S and alkaline-phosphatase-

labeled goat anti-rabbit immunoglobulin G (IgG) in PBS-T. After the last incubation period, the plates were washed three times with PBS-T and two times with 0.05 M Tris-saline (pH 7.4). The plates were developed by immersion in Tris-saline containing 2 mg of fast red and 1 mg of naphthol phosphate per ml. Plates were observed for development of a red precipitate.

Solid-phase binding assay. GSLs were diluted in methanol, and 25-μl aliquots were added to individual wells of 96-well microtiter trays. The plates were dried slowly overnight at room temperature and rinsed once with PBS-T, and 50 μl of exoenzyme S diluted in PBS-T was added to each well. The plates were incubated for 2 h at room temperature and rinsed four or five times with PBS-T. Bound exoenzyme S was detected by sequential addition of 50 μl of anti-exoenzyme S and alkaline phosphatase-labeled goat anti-rabbit IgG with intermittent and final washes with PBS-T. The plates were developed by the addition of 100 μl of 1 mg of nitrophenol phosphate in 10% diethanolamine buffer (pH 9.6). The plates were read at 405 nm.

Electrophoretic blotting of exoenzyme S eluted from immobilized GSLs. Since the preparations of exoenzyme S contained some LPS and possibly other minor proteins, it was important to confirm that exoenzyme S was the binding ligand. Exoenzyme S was reacted with GSLs immobilized on plastic plates as described above. After a 2-h incubation period, the plates were washed six to seven times with PBS-T, and the bound material was eluted in sample buffer containing 0.5 M Tris hydrochloride (pH 6.8), 50 mM dithiothreitol, 2% SDS, and 10% glycerol. The eluates were heated at 100°C for 90 s and separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% acrylamide gels as described by Laemmli (16). The gels were blotted onto nitrocellulose sheets for 30 min at 0.8 A as described by Towbin et al. (25). The nitrocellulose sheets were immersed in PBS-T and then incubated for 1 h in PBS-T containing anti-exoenzyme S (dilution, 1:1,000). After washing, the blots were incubated for 1 h in PBS-T containing alkaline-phosphatase-conjugated goat anti-rabbit serum (dilution, 1:1,000), washed, and reacted with the fast red-naphthol phosphate substrate. Prestained low-molecular-weight standards (Bio-Rad, Richmond, Calif.) were used to estimate the molecular weights of the separated proteins.

Immunogold labeling of strain DG1. The antibody-gold conjugate labeling procedure used was a modification of that described previously (8). Formvar-coated grids were coated with 0.1% bovine serum albumin in distilled water. A 5-μl bacterial suspension was placed on the grids and allowed to settle for 5 min. The excess fluid, containing bacteria, was drained from the grids, and the grids were immediately floated over a drop of a solution containing a 1:10 dilution of anti-exoenzyme S monoclonal antibody in PBS and incubated at 37°C for 30 min. The grids were then washed successively three times by being floated over drops of PBS (5 min for each wash). The grids were then floated over a drop of anti-rabbit antibody-gold conjugate (Sigma) and incubated at 37°C for 30 min. The grids were washed again as described above, briefly rinsed in distilled water, and examined (unstained) by use of a Philips EM400 electron microscope.

In vitro adherence. The ability of purified exoenzyme S (26), purified pili (28), and monoclonal antibodies to exoenzyme S (26) to inhibit binding of intact *P. aeruginosa* to buccal epithelial cells was examined as previously described (28).

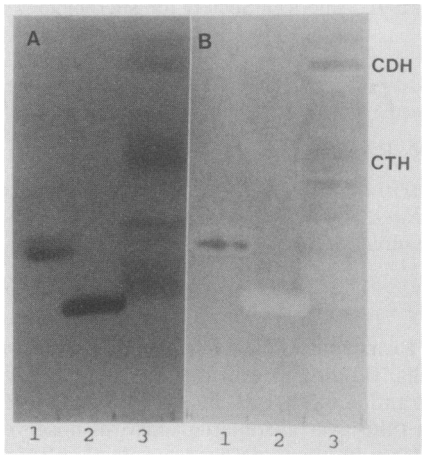

FIG. 1. Binding of exoenzyme S to GSLs separated by TLC by using chloroform-methanol-water (60:35:8). (A) Chemical detection (cupric acetate); (B) plate overlaid with exoenzyme S, followed by rabbit anti-exoenzyme S antiserum and alkaline phosphatase-conjugated goat anti-rabbit IgG. The plate was developed with fast red-naphthol phosphate substrate. Lanes: 1, GgO_4 (2 μg); 2, GM_1 (2 μg); 3, neutral GSLs of dog intestinal mucosa (10 μg). CDH, lactosylceramide; CTH, trihexosylceramide.

RESULTS

Binding of exoenzyme S to GSLs. The binding of exoenzyme S and *P. aeruginosa* DG1, from which the enzyme was purified, to GSLs on TLC plates is shown in Fig. 1 and 2, respectively. Binding to GgO_4 and lactosylceramide was clearly evident, as was binding to the trihexosylceramide region of the dog mucosa sample. Binding to gangliotriosylceramide was detected (results not shown). The same binding specificity has been detected with iodinated or biotinylated exoenzyme S, demonstrating that results are not due to binding of the antibodies to GSLs. Furthermore, no bands were detected if the overlay with exoenzyme S was omitted

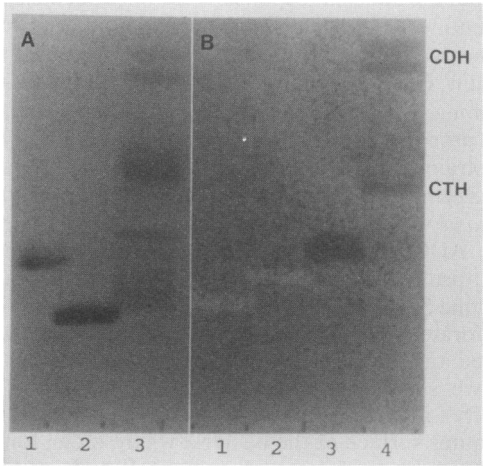

FIG. 2. Binding of *P. aeruginosa* DG1 to GSLs separated by TLC by using chloroform-methanol-water (60:35:8). (A) Chemical detection (cupric acetate); (B) plate overlaid with strain DG1, followed by rabbit anti-exoenzyme S antiserum and alkaline phosphatase-conjugated goat anti-rabbit IgG. The plate was developed with fast red-naphthol phosphate substrate. Lanes: A1 and B3, GgO_4 (2 μg); A2 and B1, GM_1 (2 μg); A3 and B4, neutral GSLs of dog intestinal mucosa (10 μg); B2, GM_2 (2 μg). CDH, lactosylceramide; CTH, trihexosylceramide.

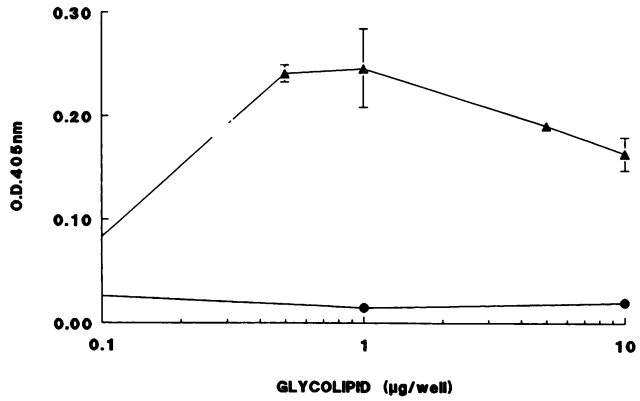

FIG. 3. Binding of exoenzyme S to GgO_4 immobilized on microtiter plates. Exoenzyme S (10 µg per well) diluted in PBS-T was added to serial dilutions of GgO_4 coating wells of microtiter plates. Bound exoenzyme S was detected by sequential incubations with rabbit anti-exoenzyme S antiserum and alkaline phosphatase-conjugated goat anti-rabbit IgG. The plate was developed with nitrophenol phosphate in diethanol-amine buffer, and the optical density at 405 nm was read. Symbols: ▲, asialo GM-1; ●, GM_1.

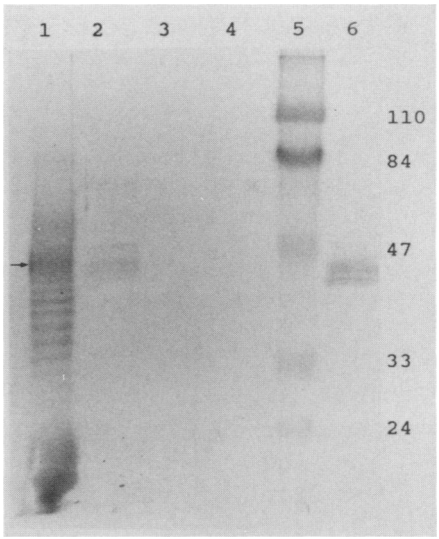

FIG. 4. Immunoblot analysis of eluates from GSL-coated wells incubated with exoenzyme S preparations. GSL-bound material was eluted with sample buffer and separated by SDS-PAGE on 12% gels. The gels were transferred to nitrocellulose and reacted with rabbit anti-exoenzyme S antiserum and alkaline phosphatase-conjugated goat anti-rabbit IgG. The sheet was developed with fast red-naphthol phosphate substrate. Lanes: 1, exoenzyme S; 2, eluate from GgO_4; 3, eluate from GM_1; 4, eluate from control well (no GSL); 5, prestained molecular weight markers; 6, eluate from GgO_4 incubated with a different exoenzyme S preparation. The arrow indicates the position of migration of purified exoenzyme S.

from the assay. This binding specificity is in agreement with previously published reports for many strains of *P. aeruginosa* (1, 13). Neither exoenzyme S nor strain DG1 bound to the sialylated GSLs GM_1 or GM_2. Curves of exoenzyme S binding to dilutions of GSLs immobilized on plastic plates reveal optimum binding at approximately 1 µg of GgO_4 (Fig. 3). The reduced binding at 10 µg is most likely an artifact of the assay related to the coating of the plate at high concentrations of GSL since it is also observed with whole bacteria (results not shown).

Elution of exoenzyme S from GSLs. The presence of LPS in some of the exoenzyme S preparations was detected in immunoblots with polyclonal antiserum. To prove that exoenzyme S was binding to GgO_4 the bound material was eluted from immobilized GSLs, separated by SDS-PAGE, and analyzed by immunoblot. The only bands detectable in the eluted samples were in the same molecular weight region as purified exoenzyme S (Fig. 4). The higher-molecular-weight protein bands which were observed were consistent with previous results obtained with purified exoenzyme S (26). Different preparations of exoenzyme S produced the same results (Fig. 4, lanes 2 and 6). Samples eluted from uncoated wells or wells coated with GM_1 did not produce any bands (Fig. 4).

Immunogold labeling of bacteria. Exoenzyme S must exist on the surface of bacteria for it to function as an adhesin. The presence of exoenzyme S on the bacterial cell surface was detected by immunogold labeling. The gold beads were observed as a uniform coating on the cell surface, indicating that the enzyme was evenly distributed (Fig. 5A). Controls incubated in the absence of antibody to exoenzyme S did not reveal any beads on the cell surface (Fig. 5B).

Adherence inhibition studies. The role of exoenzyme S in adherence of *P. aeruginosa* to buccal cells was determined by using exoenzyme S and monoclonal antibodies to exoenzyme S as inhibitors. Incubation of buccal cells with purified exoenzyme S inhibited the binding of bacteria to the cells in a dose-dependent manner (Fig. 6). Exoenzyme S eliminated adherence at 200 µg of protein per 10,000 cells, whereas the same concentration of purified pili only reduced adherence by approximately 50%. Furthermore, monoclonal antibody MCA1, which neutralizes the cytotoxicity of exoenzyme S but has no affect on enzymatic activity, reduced adherence of bacteria to background levels (Table 1). Monoclonal antibody MCA3, which neutralizes enzymatic activity but not cytotoxicity, did not inhibit the adherence of bacteria to buccal cells.

DISCUSSION

During the last decade, much of the work on adherence of *P. aeruginosa* has focused on pili as the primary adhesin. Extensive evidence has been presented which demonstrates that pili are important in the attachment of *P. aeruginosa* to various cells (6, 22, 28) and mucous proteins (21). A binding domain has been localized to the carboxy-terminal end of pilin by studying the binding of peptides to cells (11) and by adherence inhibition studies with monoclonal antibodies directed against the peptides (5). However, review of the literature reveals that the maximum inhibition of adherence of bacteria to cells achievable with isolated pili or antipilin antibodies is approximately 60% (5, 6). Other adhesins which account for the residual adherence must exist.

Previous studies have shown that *P. aeruginosa* binds to specific sequences of carbohydrates on GSLs (1, 13). Nonpiliated mutants of *P. aeruginosa* which do not produce pilin detectable by immunoblotting of whole-cell extracts retain this binding specificity, indicating that pili are not the adhesins for GSLs (1a). The results of the present study show that exoenzyme S has the same binding specificity for GSLs as do the bacteria. Exoenzyme S has previously been described as an exoproduct of *P. aeruginosa* which ADP-ribosylates membrane proteins, leading to cell death (26). This study shows that exoenzyme S can be found on the cell surface, a finding which is relevant to its potential to act as an adhesin.

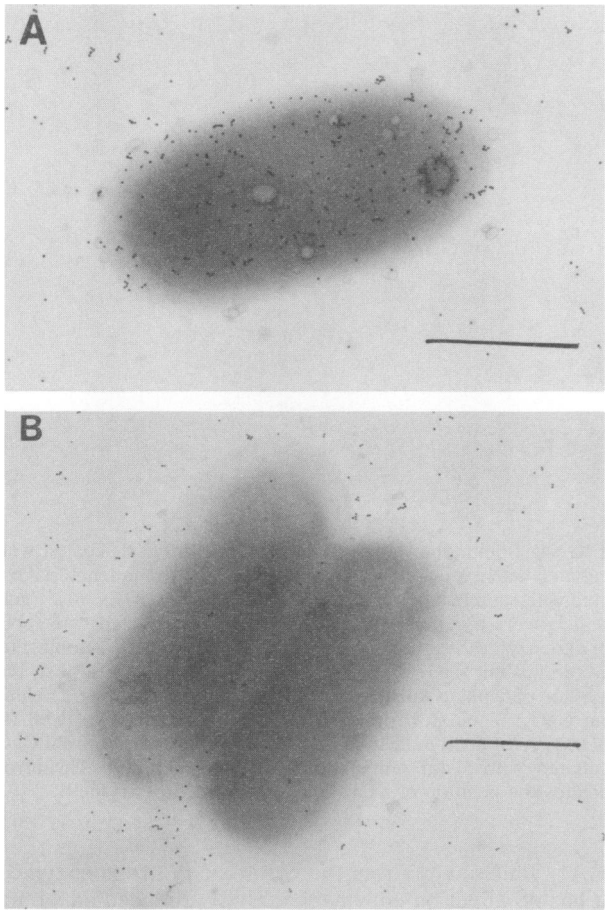

FIG. 5. Reaction of *P. aeruginosa* DG1 to monoclonal antibody raised against purified exoenzyme S (A) or PBS (B), followed by protein A-colloidal gold labeling. The protein A-gold conjugate used for antibody detection contained colloidal gold as 15-nm particles. Bars, 1 μm.

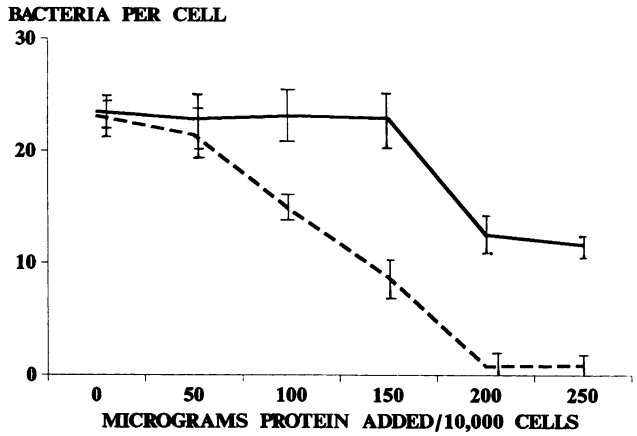

FIG. 6. Dose-response curves reflecting the ability of purified exoenzyme S (– – –) or purified pili (——) to block the adherence of intact *P. aeruginosa* DG1 to epithelial cells. Buccal epithelial cells were exposed to increasing amounts of purified protein for 1 h at 37°C before being tested for intact *P. aeruginosa* adherence.

The ability of purified exoenzyme S and monoclonal antibodies which neutralize the cytotoxicity of exoenzyme S to virtually eliminate the adherence of *P. aeruginosa* to buccal cells is definitive proof that this protein is a major adhesin for this organism. It is interesting to note that, in contrast to results obtained with antiserum to pili (25), the ability of antibody to exoenzyme S to inhibit adherence does not appear to be strain specific (Table 1).

We have not proven that *P. aeruginosa* or exoenzyme S binds to GSLs in the cell membranes. The presence in buccal cells (24) and lung extracts (14) of some of the GSLs recognized by the bacteria and exoenzyme S has been shown, indicating that they may be the receptors. The possibility exists that the binding specificity observed reflects a specificity found on glycoproteins. Additional studies will need to be done to identify the actual receptor molecule for this adhesin.

Previous studies have shown that a number of respiratory tract pathogens have the same binding specificity as *P. aeruginosa* and exoenzyme S (14). *Neisseria gonorrhoeae* also shares this binding specificity, which is associated with the production of a 36-kDa surface protein (20). It will be interesting to determine if these organisms produce an analogous protein which accounts for this specificity. *P. aeruginosa* and *N. gonorrhoeae* have been shown to possess adhesins which recognize the same carbohydrate sequences on GSLs and are not produced as integral components of pili. Thus, they are distinct from the uropathogenic *Escherichia coli* adhesin for digalactose, which is a tip protein of the pilus (19). By using the available information, a reasonable model of adherence by these organisms can be developed. The long filamentous pili are probably necessary for initial interaction of the bacteria with the cell surface. The precise specificity of this interaction is not currently clear, but evidence for binding to specific cell proteins exists (4, 11). Following this binding, multiple interactions of bacterial surface proteins, such as exoenzyme S, with cell surface receptors lead to irreversible attachment of the bacteria. Other adhesins and receptors are likely to be important in the process.

Previous results have also detected binding of many strains of *P. aeruginosa* to sialylated GSLs (1), although the strains used in the present study did not have this binding characteristic. Other investigators have shown that sialic acid residues may be important in adherence of *P. aeruginosa* to immature or scarified ocular epithelium (10, 23) and salivary proteins (12). The properties of *P. aeruginosa* which account for this specificity have not been identified, but they

TABLE 1. Effect of monoclonal antibodies to purified exoenzyme S on *P. aeruginosa* adherence to buccal epithelial cells

Monoclonal antibody[a]	Strain	Adherence[b]
None (control)	PAO	22.1 ± 2.5
	DG1	26.7 ± 3.4
MCA1[c]	PAO	1.7 ± 0.3[d]
MCA1	DG1	0.8 ± 0.1[d]
MCA3[e]	PAO	21.9 ± 3.6
MCA3	DG1	25.8 ± 4.2

[a] Monoclonal antibodies prepared as previously described (26).
[b] Adherence assay performed as previously described (28). Values represent means ± standard deviations.
[c] Neutralizes cytotoxicity but not enzymatic activity.
[d] Significantly different from control ($P < 0.01$).
[e] Neutralizes enzymatic activity but not cytotoxicity.

may be associated with the ability of the organisms to produce neuraminidases (18). The significance of sialic acids in adherence of *P. aeruginosa* is currently not clear and requires further study.

Identification of exoenzyme S as an important adhesin for *P. aeruginosa* could lead to new approaches in the prevention of lung infection. Exoenzyme S is immunogenic and could be considered as a target for vaccine development since generation of a specific mucosal immune response may prevent colonization. Since exoenzyme S has been shown to be a membrane protein, the generation of opsonizing antibodies could also play an important role in protection. Alternatively, it may be possible to prevent colonization by instilling receptor analogs which would block adherence.

ACKNOWLEDGMENTS

Funding for this project was provided by the Department of the Army, contract DAAL03-86-0001, and the Cystic Fibrosis Foundation, grant 2155 01-1, to N.R.B. and by Medical Research Council of Canada grant MA-8117 to D.E.W.

Excellent technical assistance was provided by Lynda Goebel.

REFERENCES

1. **Baker, N., G. C. Hansson, H. Leffler, G. Riise, and C. Svanborg-Eden.** 1990. Glycosphingolipid receptors for *Pseudomonas aeruginosa*. Infect. Immun. **58**:2361–2366.
1a. **Baker, N. R.** 1989. Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, B 225, p. 68.
2. **Bryan, C. S., and K. L. Reynolds.** 1984. Bacteremic nosocomial pneumonia: analysis of 172 episodes from a single metropolitan area. Am. Rev. Respir. Dis. **129**:668–671.
3. **Coburn, J., R. T. Wyatt, B. H. Iglewski, and D. M. Gill.** 1989. Several GTP-binding proteins, including $p21^{c\text{-}H\text{-}ras}$, are preferred substrates of *Pseudomonas aeruginosa* exoenzyme S. J. Biol. Chem. **264**:9004–9008.
4. **Doig, P., W. Paranchych, P. A. Sastry, and R. T. Irvin.** 1989. Human buccal epithelial cell receptors of *Pseudomonas aeruginosa*: identification of glycoproteins with pilus binding activity. Can. J. Microbiol. **35**:1141–1145.
5. **Doig, P., P. A. Sastry, R. S. Hodges, K. K. Lee, W. Paranchych, and R. T. Irvin.** 1990. Inhibition of pilus-mediated adhesion of *Pseudomonas aeruginosa* to human buccal epithelial cells by monoclonal antibodies directed against pili. Infect. Immun. **58**:124–130.
6. **Doig, P., T. Todd, P. A. Sastry, K. K. Lee, R. S. Hodges, W. Paranchych, and R. T. Irvin.** 1988. Role of pili in the adhesion of *Pseudomonas aeruginosa* to human respiratory epithelial cells. Infect. Immun. **56**:1641–1646.
7. **Farmer, J. J., III.** 1976. *Pseudomonas* in the hospital. Hosp. Pract. **10**:63–70.
8. **Godfrey, A. J., M. S. Shahrabadi, and L. E. Bryan.** 1986. Distribution of porin and lipopolysaccharide antigens on a *Pseudomonas aeruginosa* permeability mutant. Antimicrob. Agents Chemother. **30**:802–805.
9. **Hansson, G. C., K . A. Karlsson, G. Larson, J. A. McGibbon, N. Stromberg, and J. Thurin.** 1983. Isoglobotriaosylceramide and the Forssman glycoplipid occupy different tissue compartments of dog small intestine. Biochim. Biophys. Acta **750**:214–216.
10. **Hazlett, L. D., M. Moon, and R. S. Berk.** 1986. In vivo identification of sialic acid as the ocular receptor for *Pseudomonas aeruginosa*. Infect. Immun. **51**:687–689.
11. **Irvin, R. T., P. Doig, K. K. Lee, P. A. Sastry, W. Paranchych, T. Todd, and R. S. Hodges.** 1989. Characterization of *Pseudomonas aeruginosa* pilus adhesin: confirmation that the pilin structural protein subunit contains a human epithelial cell-binding domain. Infect. Immun. **57**:3720–3726.
12. **Komiyama, K., B. F. Habbick, and S. K. Tumber.** 1987. Role of sialic acid in saliva-mediated aggregation of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients. Infect. Immun. **55**:2364–2369.
13. **Krivan, H. C., V. Ginsburg, and D. D. Roberts.** 1988. *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from cystic fibrosis patients bind specifically to gangliotetra-osylceramide (asialo GM_1) and gangliotriaosylceramide (asialo GM_2). Arch. Biochem. Biophys. **260**:493–496.
14. **Krivan, H. C., D. D. Roberts, and V. Ginsburg.** 1988. Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAcβ1-4Gal found in some glycolipids. Proc. Natl. Acad. Sci. USA **85**:6157–6161.
15. **Kulczyki, L. L., T. M. Murphy, and J. A. Bellanti.** 1978. *Pseudomonas* colonization in cystic fibrosis: a study of 160 patients. J. Am. Med. Assoc. **240**:30–34.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Laraya-Causey, L. R., K. R. Kundy, and N. N. Haung.** 1976. *Pseudomonas* carrier rates of patients with cystic fibrosis and members of their families. J. Pediatr. **89**:23–26.
18. **Leprat, R., and Y. Michel-Briand.** 1980. Extracellular neuraminidase production by a strain of *Pseudomonas aeruginosa* isolated from a cystic fibrosis patient. Ann. Microbiol. (Paris) **131B**:210–222.
19. **Lund, B., F. Lindberg, B. I. Marklund, and S. Normark.** 1987. The PapG protein is the α-D-galactopyranosyl-(1→4)-β-D-galactopyransose-binding adhesin of uropathogenic *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:5898–5902.
20. **Paruchuri, D. K., H. S. Seifert, R. S. Ajioka, K. A. Karlsson, and M. So.** 1990. Identification and characterization of a *Neisseria gonorrhoeae* gene encoding a glycolipid-binding adhesin. Proc. Natl. Acad. Sci. USA **87**:333–337.
21. **Ramphal, R., C. Guay, and G. B. Pier.** 1987. *Pseudomonas aeruginosa* adhesins for tracheobronchial mucin. Infect. Immun. **55**:600–603.
22. **Ramphal, R., J. C. Sadoff, M. Pyle, and J. D. Silipingi.** 1984. Role of pili in the adherence of *Pseudomonas aeruginosa* to injured tracheal epithelium. Infect. Immun. **44**:38–40.
23. **Singh, A., L. D. Hazlett, and R. S. Berk.** 1990. Characterization of *Pseudomonas aeruginosa* adherence to mouse corneas in organ culture. Infect. Immun. **58**:1301–1307.
24. **Stromberg, N., and K. A. Karlsson.** 1990. Characterization of the binding of *Actinomyces naeslundii* (ATCC 12104) and *Actinomyces viscosus* (ATCC 19246) to glycosphingolipids, using a solid-phase overlay approach. J. Biol. Chem. **265**:11252–11258.
25. **Towbin, M., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
26. **Woods, D. E., and J. U. Que.** 1987. Purification of *Pseudomonas aeruginosa* exoenzyme S. Infect. Immun. **55**:579–586.
27. **Woods, D. E., and P. A. Sokol.** 1985. Use of transposon mutants to assess the role of exoenzyme S in chronic pulmonary disease due to *Pseudomonas aeruginosa*. Eur. J. Clin. Microbiol. **4**:163–169.
28. **Woods, D. E., D. C. Straus, W. G. Johanson, Jr., V. K. Berry, and J. A. Bass.** 1980. Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. Infect. Immun. **29**:1146–1151.
29. **Woods, D. E., M. To, and P. A. Sokol.** 1989. *Pseudomonas aeruginosa* exoenzyme S as a pathogenic determinant in respiratory tract infections. Antibiot. Chemother. (Basel) **42**:27–35.