INFECTION AND IMMUNITY, July 1990, p. 2361–2366
0019-9567/90/072361-06$02.00/0

Vol. 58, No. 7

Glycosphingolipid Receptors for *Pseudomonas aeruginosa*

NEIL BAKER,[1]†* GUNNAR C. HANSSON,[2] HAKON LEFFLER,[3] GERT RIISE,[4] AND CATHARINA SVANBORG-EDÉN[1]

Department of Clinical Immunology, University of Lund, Lund,[1] and Department of Medical Biochemistry, University of Göteborg,[2] and Renström's Hospital,[4] Göteborg, Sweden, and Department of Psychiatry, University of California, San Francisco, San Francisco, California[3]

Received 16 October 1989/Accepted 27 April 1990

The binding of *Pseudomonas aeruginosa* to glycosphingolipids and to buccal and bronchial epithelial cells was analyzed. Three independently expressed specificities were found by bacterial binding to glycosphingolipids separated by thin-layer chromatography. All strains bound gangliotria- and gangliotetraosylceramide. All but one of the strains bound sialic acid-containing glycosphingolipids and lactosylceramide. The latter two specificities could be separated in that the lactosylceramide binding was retained and the sialic acid binding was suppressed when bovine serum albumin was used as a blocking agent in the thin-layer chromatography assay. The attachment to buccal epithelial cells, like the binding to sialylated compounds and lactosylceramide, was abolished by Formalin treatment of the bacteria, suggesting the importance of these specificities for cell adherence. In contrast, the binding to gangliotria- and gangliotetraosylceramide was retained by nonattaching Formalin-treated bacteria.

Glycoconjugates can serve as receptors for bacterial adhesins (15, 19). Examples of this are the specific recognition of the Galα1-4Galβ-disaccharide by uropathogenic *Escherichia coli* (3, 11, 14), of GlcNAcβ1-3Gal by *Streptococcus pneumoniae* (1), of NeuAcα2-3Gal by *E. coli* type S fimbriae (21), and of mannose-containing oligosaccharides and glycoproteins by type 1 fimbriae (6, 24, 29).

Pseudomonas aeruginosa bind to buccal cells (30), to the cilia of nasal turbinate cells (20) and tracheal epithelial cells, to damaged tracheal epithelium (2, 7), and to tracheobroncheal mucins (27). A recent report concerning the binding of *P. aeruginosa* and *P. cepacia* to glycosphingolipids (GSLs) separated on thin-layer chromatography (TLC) plates concluded that *Pseudomonas* strains bind to gangliotriaosyl- and gangliotetraosylceramide and that the minimal binding unit for these organisms is GalNAcβ1-4Gal (13) (Table 1). Attachment to lactosylceramide- and sialic acid-containing GSLs was not detected. The absence of binding to sialic acid was inconsistent with sugar inhibition studies which demonstrated that *N*-acetylneuraminic acid (NeuAc) inhibited adherence of *P. aeruginosa* to buccal cells (18), to normal and damaged epithelial cells (16, 22), and to tracheobroncheal mucins (28). In this study, we demonstrate that *P. aeruginosa* also binds to sialic acid-containing GSLs and lactosylceramide and that the binding to sialic acid correlates with adherence to buccal epithelial cells.

MATERIALS AND METHODS

Bacteria. Strains 105M and 244NM were mucoid and nonmucoid clinical isolates obtained from the sputum of cystic fibrosis patients at the Children's Hospital, Columbus, Ohio. Strain 0705 was a mucoid urinary tract isolate. Strains 0979 and 0971 were nonmucoid isolates from sputum samples. Strain PAO 579, a mucoid variant of strain PAO 381, was provided by John Goran, University of Edinburgh, Edinburgh, Scotland. Strain PAO 553 was a spontaneous nonmucoid revertant of strain PAO 579. Mucoid strain 2192 was obtained from Gerald B. Pier, Harvard Medical School, Boston, Mass. Nonmucoid strain PAO 1 (ATCC 15692) was obtained from Michael Vasil, University of Colorado Health Sciences Center, Denver, Colo. All isolates were maintained on *Pseudomonas* Isolation Agar (Difco Laboratories, Detroit, Mich.) or as lyophilized stock cultures.

For adherence testing, bacterial suspensions were prepared from overnight cultures grown at 37°C without shaking in M-9 medium containing glucose (16). The optical densities of the cultures at 597 nm were determined, and the suspensions were diluted to approximately 2×10^9 CFU/ml.

Bacteria were labeled by overnight growth in 5 ml of M-9 medium in which $MgSO_4$ was replaced by $MgCl_2$ and $^{35}SO_4$ (100 μCi; specific activity, 343 Ci/mmol, Amersham). Cultures were grown with or without shaking, and the cells were collected by centrifugation at $2,500 \times g$ for 10 min. After two washes in 0.01 M phosphate-buffered saline (PBS), pH 7.4, the organisms were resuspended to approximately 10^8 CFU/ml in PBS. The specific activities of the suspensions ranged from 10 to 100 CFU/cpm.

In some studies, the bacterial suspensions were fixed before addition to cells or overlay of TLC plates. For fixation, the bacteria were suspended in PBS containing 0.5% Formalin for 2 to 3 h and washed in PBS before being used in the assays.

Glycosphingolipid extracts and TLC binding assay. Total non-acid glycosphingolipid (GSL) fractions from human meconium (12); from mouse (10), rat (4), rabbit (5), and dog (9) intestinal mucosa; and from guinea pig erythrocytes (23) were screened for binding activity. A brain ganglioside preparation, purified GM_2, and purified GM_1, were obtained from Sigma Chemical Co., St. Louis, Mo.

GSLs were separated on aluminium-backed silica gel Si60 HPTLC plates (E. Merck AG, Darmstadt, Federal Republic of Germany) by using chloroform-methanol-water (60:35:8, vol/vol/vol). One plate was sprayed with 1% anisaldehyde or copper acetate for chemical detection (12), and duplicate plates were tested for bacterial binding as previously described (3) with slight modifications. The TLC plates were immersed in 0.15% polyisobutylmetacrylate (P28; Röhm, Darmstadt, Federal Republic of Germany) in diethylether

* Corresponding author.
† Present address: Department of Microbiology, The Ohio State University, Columbus, OH 43210.

TABLE 1. Glycosphingolipids discussed in this paper and their capacities to bind *P. aeruginosa* strains on TLC plates

No.[a]	Glycospingolipid	Binding to *P. aeruginosa*:		
		Krivan[b]	244NM[c]	0705M
1	Galβ1-4Glcβ1-1Cer(h)[d]	−	+	−
2	GalNAcβ1-4Galβ1-4Glcβ1-1Cer	+	+	+
3	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer	+	+	+
4	Galα1-4Galβ1-4Glcβ1-1Cer	−	−	−
5	Galα1-3Galb1-4Glcb1-1Cer	ND[e]	−	−
6	NeuAcα2-3Galβ1-4Glcβ1-1Cer	−	+	−
7	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer 3 NeuAc2	−	+	−
8	NeuAc2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer	ND	+	−
9	Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1Cer	ND	−	−
10	Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1Cer 4 Fucα1	ND	−	−
11	Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1Cer 2 4 Fucα1 Fucα1	ND	−	−
12	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer	−	−	−
13	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer	−	−	−
14	GalNAcα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer 2 Fucα1		−	−
15	Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer 3 Fucα1	ND	−	−
16	Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer 3 Fucα1	ND	−	−

[a] 1, Lactosylceramide; 2, asialo-GM_2; 3, asialo-GM_1; 4, globotriaosylceramide; 5, isoglobotriaosylceramide; 6, GM_3; 7, GM_1; 8, sialylparaglobosid; 9, lactotetraosylceramide; 10, Le^a pentaglycosylceramide; 11, Le^b hexaglycosylceramide; 12, Forssman glycolipid; 13, globoside, 14, A-hexaglycosylceramide; 15, X-pentaglycosylceramide; 16, Y-hexaglycosylceramide.

[b] Reference 13.

[c] Strain 244NM showed the same binding pattern as strains 105M, O979, O971, 2192, PAO553, PAO579, and PAO-1.

[d] Cerh, Hydroxylated ceramide.

[e] ND, Not done.

hexane (1:1, vol/vol) for 1 min and dried overnight at room temperature. The plates were treated with 0.5 or 1% gelatin in PBS for 2 h and overlaid with 0.5×10^6 to 1.0×10^6 cpm of ^{35}S-labeled bacteria per ml (approximately 10^8 CFU/ml). Alternatively, the plates were treated with a 1% suspension of bovine serum albumin (BSA). After a 2-h incubation at room temperature, the plates were rinsed five times with PBS, air dried, and exposed to RX-100 film (Fuji) at room temperature for the indicated times.

The mobilities of the individual GSLs were compared with those of standards, the structures of which were confirmed by gas chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy (4, 5).

Adherence assays. Human buccal epithelial cells were collected from a healthy, male, nonsmoking volunteer by scraping the buccal epithelium with a premoistened cotton-tipped swab. The cells were suspended in 5 ml of PBS and washed with PBS three times by centrifugation at $500 \times g$ for 10 min. The cells were trypsinized by resuspension in PBS containing 2.5 to 5 μg of trypsin (Sigma) per ml and incubation for 30 min at 37°C. The trypsinized cells were washed three times with PBS and adjusted to a concentration of 2×10^5 cells per ml (29).

Tracheal cells were obtained by brushing the bronchial epithelium of 10 patients undergoing diagnostic bronchoscopy. The cells were suspended directly in PBS with or without 1% Histofix (Histolab AB, Göteborg, Sweden) and washed three times by centrifugation at $200 \times g$ for 10 min. The cells were suspended in PBS to approximately 10^5/ml. Cell samples that contained primarily denuded, nonciliated cells were excluded from this study, as we were primarily interested in adherence to cilia. No bacteria were observed on the cilia of the cells before the assay, and few cells displayed ciliary activity.

For the adherence assay, 200 μl of bacterial suspension was mixed with 200 μl of cells, and the mixture was centrifuged at $400 \times g$ for 10 min. After a 30-min incubation at 37°C, the mixture was resuspended and washed with 2 ml of PBS three times. The final cell pellet was suspended in 100 μl of PBS. The number of bacteria was counted on 30 to 40 cells by direct observation at a magnification of ×400 by using differential interference contrast (Nikon), and the results were expressed as the average number of bacteria per cell.

RESULTS

Binding to GSLs. The binding of *P. aeruginosa* strains to isolated GSLs on TLC plates is shown in Fig. 1 and Table 1. In agreement with previous studies (13), receptor-active components were identified as gangliotetraosyl- and gangliotriaosylceramide (Fig. 1, lanes 2 and 5).

Additional binding specificities of the organisms were, however, observed. *P. aeruginosa* 105M bound to sialylated

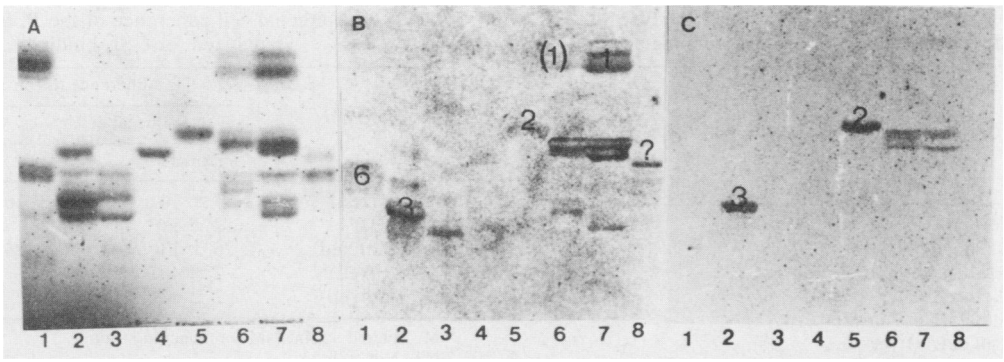

FIG. 1. Binding of *P. aeruginosa* strains to GSLs separated on thin-layer chromatograms by using chloroform-methanol-water (60:35:8). Lanes: 1, human meconium monosialylated GSLs; 2, neutral GSLs of mouse colon epithelium; 3, neutral GSLs of human meconium blood group B, Le, Se; 4, globoside; 5, neutral GSLs of guinea pig erythrocytes; 6, neutral GSLs of dog intestinal mucosa (blood group X-Y type); 7, neutral GSLs of dog intestinal mucosa (blood group A-H type); 8, neutral GSLs of mouse colon (nonepithelium). (A) Chemical detection (anisaldehyde). (B) Autoradiogram of strain 105M. (C) Autoradiogram of strain O705M. The numbers in the chromatograms refer to those in Table 1, column 1. The autoradiographs are 3-day exposures.

GSLs (Fig. 1, lane 1, and Fig. 2A, lanes 1, 2, 3, and 8) in the tri- to heptasaccharide regions. The strong binding to GSLs in the brain ganglioside fraction (lane 2), GM_1 (lane 3), and GM_3 (lane 1), and sialylparagloboside (lane 8) indicated that 105M had a broad specificity for sialic acid residues on glycoconjugates.

The same binding pattern was observed for the eight strains of *P. aeruginosa* specified in footnote *b* of Table 1. *P. aeruginosa* 0705M bound only to gangliotriaosyl- and gangliotetraosylceramide but not to the sialic acid-containing GSLs (Fig. 1C, lane 1).

Binding to a GSL migrating in the three-sugar region was observed in two different GSL preparations from dog intestinal mucosa (Fig. 1, lanes 6 and 7). In this region, only isoglobotriaosyl- and globotriaosylceramide have been detected (9). Strains 105M and 244NM bound to the trihexosylceramide region in the dog sample, as did 0705M. The strains did not bind pure globotria- or isoglobotriaosylceramide (data not shown). The binding in this region is therefore probably due to other, minor components present in these GSL preparations.

The strains 105M and 244NM, but not 0705M, also reacted with a GSL in the dihexosyl region from dog intestine, identified as lactosylceramide. This lactosylceramide contains phytosphingosine and hydroxy fatty acids (Fig. 1, lane 7) (8, 9, 24).

The use of BSA as a blocking agent in the TLC assay suppressed the binding to sialic acid but left the lactosylceramide binding intact (Fig. 3, Table 2).

Attachment to epithelial cells. The adherence characteristics of the *P. aeruginosa* strains are shown in Table 3. Strains 105M (mucoid) and 244NM (nonmucoid) adhered to the buccal cells, but strain 0705M was less adherent. Bacteria were evenly distributed over the surfaces of the buccal cells (Fig. 4A). Fixation of the bacteria with Formalin reduced this adherence. These was no difference in the binding between mucoid and nonmucoid organisms (Table 3) or between nontrypsinized and trypsinized buccal epithelial

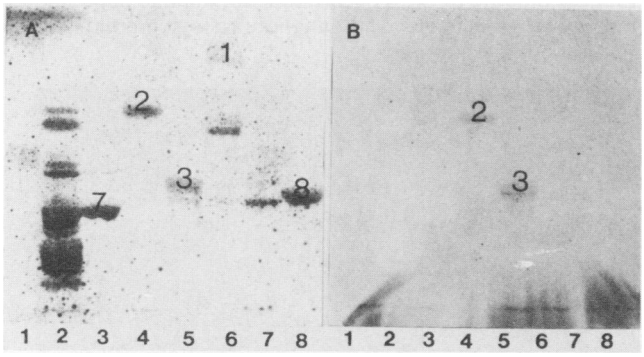

FIG. 2. Binding of unfixed and Formalin-fixed strain 105M to acidic and neutral GSLs separated on thin-layer chromatograms by using chloroform-methanol-water (60:35:8). Lanes: 1, human meconium acidic GSLs; 2, brain gangliosides; 3, GM_1; 4, neutral GSLs of guinea pig erythrocytes; 5, neutral GSLs of mouse colonic epithelium; 6, neutral GSLs of dog intestinal mucosa; 7, neutral GSLs of human meconium blood group B, Le, Se; 8, sialylparagloboside. (A) Autoradiogram of unfixed strain 105M. (B) Autoradiogram of Formalin-fixed strain 105M. The numbers in the chromatograms refer to those in Table 1, column 1. The autogradiographs are 4-day exposures.

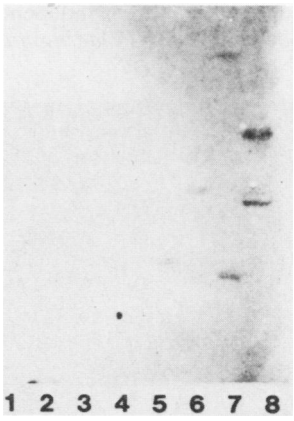

FIG. 3. Binding of *P. aeruginosa* 105M to acidic and neutral GSLs when the TLC plate was treated with 1% BSA instead of 1% gelatin before overlay with radiolabeled bacteria. Lanes: 1, brain gangliosides; 2, GM_1; 3, human meconium acidic GSLs; 4, sialylparagloboside; 5, neutral GSLs of mouse colonic epithelium; 6, neutral GSLs of guinea pig erythrocytes; 7, neutral GSLs of human meconium blood group B, Le, Se; 8, neutral GSLs of dog intestinal mucosa, blood group X-Y type. The autoradiograph is a 10-day exposure.

TABLE 2. Difference in binding of *P. aeruginosa* 105M to TLC plates with gelatin or BSA used as a blocking agent

Glycosphingolipid	*P. aeruginosa* binding with the following blocking agent	
	Gelatin	BSA
Galβ1-4Glcβ1-1Cer(h)	+	+
GalNAcβ1-4Galβ1-4Glcβ1-1Cer	+	+
Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer	+	+
Galα1-4Galβ1-4Glcβ1-1Cer	−	−
Galα1-3Galβ1-4Glcβ1-1Cer	−	−
NeuAcα2-3Galβ1-4Glcβ1-1Cer	+	−
Galβ1-3GalNAcβ1-4Galalβ1-4Glcβ1-1Cer 3 NeuAc2	+	−
NeuAc2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer	+	−
Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1Cer	−	−

TABLE 3. Epithelial cell adherence of the *P. aeruginosa* strains analyzed for GSL binding

P. aeruginosa strain[a]	Adherence (mean ± SEM) to:		
	Buccal cells		Bronchial cells
	Expt 1	Expt 2	
105M (unfixed)	17 ± 10	20 ± 16	4.4 ± 2.3
105M (fixed)	2 ± 1		NT[b]
244NM (unfixed)	26 ± 13	40 ± 23	3.5 ± 1.1
244 NM (fixed)	2 ± 4		NT
0705M (unfixed)	2 ± 4	2 ± 2	5.0 ± 2.5

[a] M, Mucoid strain; NM, nonmucoid strain.
[b] NT, Not tested.

cells. (Mean bacteria per cell for nontrypsinized and trypsinized cells, respectively, 16 and 16 for 105M, 22 and 23 for 244NM, and 2.9 and 4 for 105M).

The attachment to bronchial epithelial cells was lower than that to buccal cells (Table 3). Mucoid and nonmucoid strains bound in similar numbers. The strains bound to the cilia of the bronchial epithelial cells (Fig. 4B) but appeared also to bind to material loosely associated with the tracheal epithelial cells (Fig. 4B).

Comparison of attachment and binding to GSLs. Strains 105M and 244NM bound sialylated GSLs and lactosylceramide and attached to buccal epithelial cells. Treatment with Formalin eliminated the sialic acid and lactosylceramide binding and attachment to buccal cells. However, the binding to gangliotria- and gangliotetraosylceramide was still detectable on TLC plates. Strain 0705M bound to gangliotria- and gangliotetraosylceramide on TLC plates. It showed, however, a parallel lack of attachment to epithelial cells and binding to sialic acid-containing GSLs and lactosylceramide (Tables 1 and 3).

DISCUSSION

This report delineates three independently expressed GSL-binding specificities for *P. aeruginosa* as determined by the TLC assay. Binding to gangliotria- and gangliotetraosylceramide was found for all bacterial strains and was preserved after Formalin fixation. The binding to sialic acid and to lactosylceramide was different from the ganglioside binding in that it was absent in one strain expressing this specificity and was destroyed by Formalin. The binding to sialic acid and lactosylceramide probably reflected different specificities. First, sialic acid and lactose showed no obvious structural similarity. Second, BSA suppressed the TLC binding to sialic acid but left the lactosylceramide binding intact. An alternative interpretation is that the binding to lactose and sialic acid reflects a specificity for sialic acid linked to lactose or related structures.

The results of this study differ from those previously published. Neither the sialic acid binding nor the binding to lactosylceramide was detected by Krivan et al. (13). One explanation was that the use of BSA as a blocking agent suppressed these binding specificities, which were revealed by using gelatin as a blocker. Furthermore, Krivan et al. used lactosylceramide with a less-hydroxylated ceramide portion.

A large number of bacterial species have been proposed to bind to lactosylceramide (8, 25). Some of them also bind to gangliotria- and gangliotetraosylceramide. These specificities have been proposed to represent one adhesin with a broad epitope recognition. In contrast, here we clearly show that the binding to lactosylceramide on the one hand and the binding to gangliotria- and gangliotetraosylceramide on the

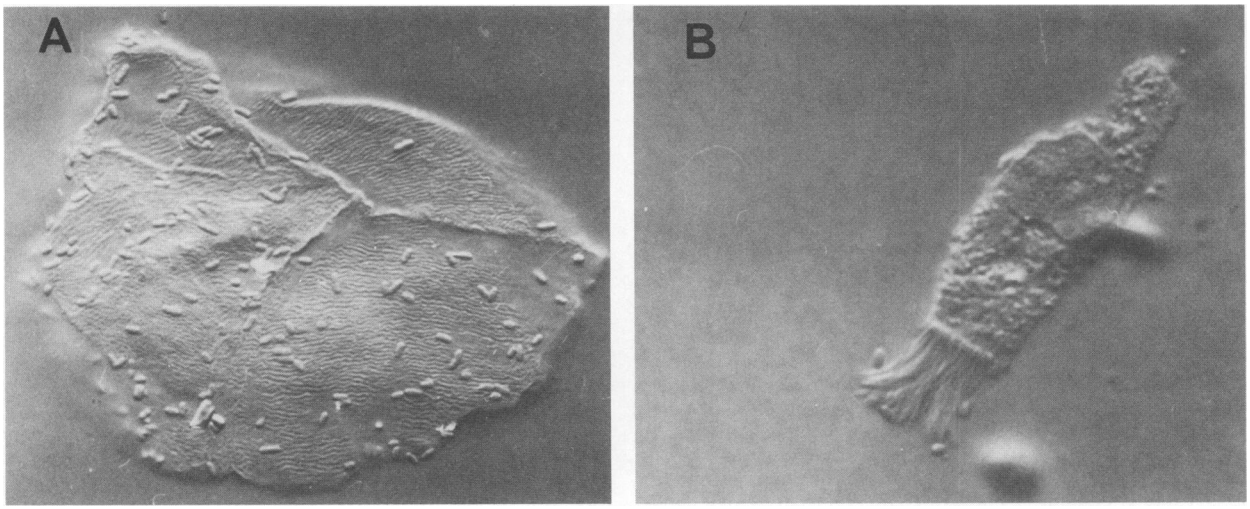

FIG. 4. Light micrographs of *P. aeruginosa* attaching to buccal cells (A) and attaching to tracheal epithelial cells (B).

other can be expressed separately by *P. aeruginosa*. They thus are likely to be due to interactions with adhesins of different specificities.

The attachment to buccal epithelial cells followed the sialic acid and lactosylceramide binding but not the binding to gangliotria- and gangliotetraosylceramide. This was consistent with previous studies showing that adherence of *P. aeruginosa* to buccal cells may be inhibited by sialic acid (18, 22). In contrast, the binding to bronchial epithelial cells, although weak, did not follow the lactosylceramide- and sialic acid-binding activity. It could be a function either of the gangliotria- and gangliotetraosylceramide-binding activity or of as yet unidentified receptor specificities.

Several components on the bacterial surface have been proposed to act as adhesins, e.g., the alginate capsule of mucoid strains and the fimbriae (2, 7, 13, 17, 20, 22, 27, 30). In this study, the binding properties did not correlate with the presence or absence of the alginate. In preliminary experiments, the purified pili were shown to bind the lactosylceramide, the gangliotria- and gangliotetraosylceramide, and the triglycosylceramide from the dog intestinal mucosa, but not the sialic acid-containing GSLs (N. R. Baker, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, B-225, p. 68).

The independently expressed binding specificities may contribute at different stages of the infectious process. *P. aeruginosa* are a significant cause of infections in the respiratory tracts of compromised patients. Clinical studies of patients with cystic fibrosis have suggested that the *P. aeruginosa* present during the initial stages of infection are nonmucoid. Such strains expressed three binding specificities. For example, the sialic acid recognition may contribute to colonization of the upper respiratory tract by attachment to buccal cells. The binding to the ganglioseries GSLs may mediate attachment to bronchial cells and there, in analogy with *E. coli* causing urinary tract infections, induce the inflammatory response which accounts for the symptoms of infection and the local damage to the mucosa (26).

ACKNOWLEDGMENTS

This study was supported by the Swedish Medical Research Council (no. 7934 and 7461) and the Swedish Board for Technical Development.

The skillful typing of A.-C. Malmefeldt is gratefully acknowledged.

LITERATURE CITED

1. **Andersson, B., J. Dahmén, T. Frejd, H. Leffler, G. Magnusson, G. Noori, and C. Svanborg-Edén.** 1983. Identification of an active disaccharide unit of a glycoconjugate receptor for pneumococci attaching to human pharyngeal epithelial cells. J. Exp. Med. **158**:559–570.
2. **Baker, N. R., and H. Marcus.** 1980. Adherence of clinical isolates of *Pseudomonas aeruginosa* to hamster tracheal epithelium *in vitro*. Curr. Microbiol. **7**:35–40.
3. **Bock, K., M. E. Breimer, A. Brignole, et al.** 1985. Specificity of binding of a strain of uropathogenic *Escherichia coli* to Galα1-4Gal-containing glycosphingolipids. J. Biol. Chem. **260**:8545–8551.
4. **Breimer, M. E., G. C. Hansson, K. A. Karlsson, and H. Leffler.** 1982. Glycosphingolipids of rat tissues. Different composition of epithelial and nonepithelial cells of small intestine. J. Biol. Chem. **257**:557–568.
5. **Breimer, M. E., G. C. Hansson, K. A. Karlsson, H. Leffler, W. Pimlott, and B. E. Samuelsson.** 1979. Selected ion monitoring of glycosphingolipid mixtures. Identification of several blood group type glycolipids in the small intestine of an individual rabbit. Biomed. Mass Spectrom. **6**:231–241.
6. **Duguid, J. P., and D. C. Old.** 1980. Adhesive properties of Enterobacteriaceae, p. 187–215. E. H. Beachey (ed.), Bacterial adherence. Chapman & Hall, Ltd., London.
7. **Franklin, A. L., T. Todd, G. Gurman, D. Black, P. M. Mankinen-Irvin, and R. T. Irvin.** 1987. Adherence of *Pseudomonas aeruginosa* to cilia of human tracheal epithelial cells. Infect. Immun. **55**:1523–1525.
8. **Hansson, G. C., K. A. Karlsson, G. Larson, A. Lindberg, N. Strömberg, and J. Thurin.** 1983. Lactosylceramide is the probable adhesin site for major indigenous bacteria of the gastrointestinal tract, p. 855–858. *In* M. A. Chester, D. Heinegård, A. Lundblad, and S. Svensson (ed.), Glycoconjugates. Rahms, Lund, Sweden.
9. **Hansson, G. C., K. A. Karlsson, G. Larson, J. M. McKibbin, N. Strömberg, and J. Thurin.** 1983. Isoglobotriaosylceramide and the Forssman glycolipid occupy different tissue compartments of dog small intestine. Biochim. Biophys. Acta **750**:214–216.
10. **Hansson, G. C., K. A. Karlsson, H. Leffler, and N. Strömberg.** 1982. Gangliotetraosylceramide is a major glycolipid of epithelial cells of mouse small intestine. FEBS Lett. **139**:291–294.
11. **Källenius, G., R. Möllby, S. B. Svensson, et al.** 1980. The p^k antigen as receptor for the haemagglutination of pyelonephritic *E. coli*. FEMS Microbiol. Lett. **7**:297–302.
12. **Karlsson, K. A., and G. Larsson.** 1981. Molecular characterization of cell surface antigens of fetal tissue. J. Biol. Chem. **256**:3512–3524.
13. **Krivan, H. C., V. Ginsburg, and D. D. Roberts.** 1988. *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from cystic fibrosis patients bind specifically to gangliotetraosylceramide (asialo GM1) and gangliotriosylceramide (asialo GM21). Arch. Biochem. Biophys. **260**:493–496.
14. **Leffler, H., and C. Svanborg-Edén.** 1981. Chemical identification of a glycosphingolipid receptor for *E. coli* attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. FEMS Microbiol. Lett. **8**:127–134.
15. **Leffler, H., and C. Svanborg-Edén.** 1986. Glycolipids as receptors for *Escherichia coli* lectins or adhesins, p. 83–110. *In* D. Mirelman (ed.), Microbial lectins and agglutinins. John Wiley & Sons, Inc., New York.
16. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 68. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Marcus, H., and N. R. Baker.** 1985. Quantitation of adherence of mucoid and nonmucoid *Pseudomonas aeruginosa* to hamster tracheal epithelium. Infect. Immun. **47**:723–729.
18. **McEachran, D. W., and R. T. Irvin.** 1985. Adhesion of *Pseudomonas aeruginosa* to human buccal epithelial cells: evidence for two classes of receptors. Can. J. Microbiol. **31**:563–569.
19. **Mirleman, D.** 1986. Microbial lectins and agglutinins. John Wiley & Sons, Inc., New York.
20. **Niederman, M. S., T. D. Rafferty, and C. T. Sasaki.** 1983. Comparison of bacterial adherence to ciliated and squamous epithelial cells obtained from the human respiratory tract. Am. Rev. Resp. Dis. **127**:85–90.
21. **Parkkinen, J., G. Rogers, T. Korhonen, W. Dahr, and J. Finne.** 1986. Identification of the O-linked sialyloligosaccharide of glycophorin A as the erythrocyte receptor for S-fimbriated *E. coli*. Infect. Immun. **54**:37–42.
22. **Ramphal, R., and M. Pyle.** 1983. Evidence for mucins and sialic acid as receptors for *Pseudomonas aeruginosa* in the lower respiratory tract. Infect. Immun. **41**:339–344.
23. **Seyama, Y., and T. Yamakawa.** 1974. Chemical structure of glycolipids of guinea pig red blood cell membranes. J. Biochem. **75**:837–842.
24. **Sharon, N., and I. Ofek.** 1986. Mannose specific bacterial surface lectins, p. 55–82. *In* D. Mirelman (ed.), Microbial lectins and agglutinins. John Wiley & Sons, Inc., New York.
25. **Strömberg, N., C. Deal, G. Nyberg, S. Normark, M. So, and K. A. Karlsson.** 1988. Identification of carbohydrate structures that are possible receptors for *Neisseria gonorrhoeae*. Proc. Natl. Acad. Sci. USA **85**:4902–4906.
26. **Svanborg-Edén, C., S. Hanson, U. Jodal, G. Lidin-Janson, K. Lincoln, H. Linder, H. Lomberg, P. deMan, S. Mårild, J.**

Martinell, K. Plos, T. Sandberg, and K. Stenqvist. 1988. Host-parasite interaction in the urinary tract. J. Infect. Dis. **157:** 421–426.

27. **Vishwanath, S., and R. Ramphal.** 1984. Adherence of *Pseudomonas aeruginosa* to human tracheobronchial mucin. Infect. Immun. **45:**197–202.
28. **Vishwanath, S., and R. Ramphal.** 1985. Tracheobronchial mucin receptor for *Pseudomonas aeruginosa*: predominance of amino sugars in binding sites. Infect. Immun. **48:**331–335.
29. **Wold, A. E., J. Mestecky, and C. Svanborg-Edén.** 1988. Agglutination of *E. coli* by secretory IgA—a result of interaction between bacterial mannose-specific adhesins and immunoglobulin carbohydrate. Monogr. Allergy **24:**307–309.
30. **Woods, D. E., J. A. Bass, and W. G. Johanson, Jr.** 1980. Role of adherence in the pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. Infect. Immun. **30:**694–699.