Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6157–6161, August 1988
Microbiology

Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAcβ1-4Gal found in some glycolipids

(pneumonia/glycosphingolipids/receptor)

HOWARD C. KRIVAN*, DAVID D. ROBERTS, AND VICTOR GINSBURG

Laboratory of Structural Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

Communicated by Bernhard Witkop, May 11, 1988

ABSTRACT **Pneumonia is one of the most common causes of death from infectious disease in the United States. To examine the possible role of carbohydrates as adhesion receptors for infection, several pulmonary pathogenic bacteria were studied for binding to glycosphingolipids. Radiolabeled bacteria were layered on thin-layer chromatograms of separated glycosphingolipids, and bound bacteria were detected by autoradiography. The classic triad of infectious bacteria found in cystic fibrosis, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Staphylococcus aureus*, along with other bacteria commonly implicated in typical pneumonia, such as *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and certain *Escherichia coli*, bind specifically to fucosylasialo-GM1 (Fucα1-2Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer), asialo-GM1 (Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer), and asialo-GM2 (GalNAcβ1-4Galβ1-4Glcβ1-1Cer). Bacteria maintained in nutrient medium bind better than the same cells suspended in buffer. They do not bind to galactosylceramide, glucosylceramide, lactosylceramide, trihexosylceramide, globoside, paragloboside, Forssman glycosphingolipid, or several other glycosphingolipids tested, including the gangliosides GM1, GM2, GM3, GD1a, GD1b, GT1b, and Cad. The finding that these pathogens do not bind to lactosylceramide suggests that β1-4-linked GalNAc, which is positioned internally in fucosylasialo-GM1 and asialo-GM1 and terminally in asialo-GM2, is required for binding. β-*N*-Acetylgalactosamine itself, however, is not sufficient for binding, as the bacteria did not bind to globoside, which contains the terminal sequence GalNAcβ1-3Gal. These data suggest that these bacteria require at least terminal or internal GalNAcβ1-4Gal sequences unsubstituted with sialyl residues for binding. Other bacteria, including *Mycoplasma pneumoniae*, *Streptococcus pyogenes*, *Salmonella* species, and some *E. coli*, do not bind to the GalNAcβ1-4Gal sequence. The biological relevance of these data is suggested by our finding that substantial amounts of asialo-GM1 occur in human lung tissue.**

Respiratory-tract infections are a major health problem with as many as 1.5 million cases of pneumonia occurring in the United States each year with a high mortality rate (1, 2). In addition, chronic lung infections, which are inevitable in patients suffering from cystic fibrosis, result in even higher rates of mortality, 70–90% (3, 4). The major groups of bacteria responsible for these infections are *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, and several aerobic, Gram-negative bacilli, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas* and *Haemophilus* species. To cause pneumonia, an organism must invade the normally sterile lung parenchyma and establish a large enough population at its surface to cause disease. For this to occur, the infecting microbe is likely to attach to cell surfaces. Although some type of adhesion has been described for these pathogens (5–16), the receptors that mediate their attachment have not been identified by direct binding.

Glycosphingolipids have been reported to be cell-surface receptors for some pathogenic bacteria, analogous to their proposed role as receptors in other cell–cell and cell–ligand interactions (17–20). For example, uropathogenic *E. coli* specifically bind to Galα1-4Gal sequences in globoseries glycosphingolipids that occur in the epithelial cells lining the urinary tract (21–23). Other bacteria that bind to glycosphingolipids include *Actinomyces naeslundii*, which binds to Galβ1-3GalNAc and GalNAcβ1-3Gal sequences (24), and *Propionibacterium granulosum*, which binds to Galβ1-4Glc sequences (25). We have shown (26) that *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from cystic fibrosis patients specifically bind to glycosphingolipids containing terminal or internal GalNAcβ1-4Gal sequences. In this paper, we report that other opportunistic pathogenic bacteria associated with respiratory-tract infections also specifically bind to internal or terminal GalNAcβ1-4Gal sequences occurring in glycosphingolipids such as fucosyl-asialo-GM1, asialo-GM1, and asialo-GM2 (for structures see Table 1). We also demonstrate that asialo-GM1 occurs in substantial amounts in human lung tissue.

MATERIALS AND METHODS

Materials. Mouse monoclonal anti-asialo-GM1 (IgM, κ) and mouse monoclonal anti-asialo-GM2 (2D4, IgM, ATCC TIB185) antibodies were obtained from Donald M. Marcus (Baylor College of Medicine, Houston, TX) and David Zopf (National Institutes of Health), respectively. Affinity-purified goat anti-mouse IgM (Kirkegaard and Perry, Gaithersburg, MD) and protein A (Pharmacia) were labeled with ^{125}I (ICN Biomedicals, Costa Mesa, CA) by the Iodo-Gen method (28) to a specific activity of ≈50 μCi/μg (1 μCi = 37 kBq). Bovine testes β-galactosidase and bovine serum albumin (BSA, fraction V) were purchased from Boehringer Mannheim. DL-Dihydrolactocerebroside (CDH) was obtained from Calbiochem–Behring. Ganglioside GM3 was purified from normal human kidney as described (29, 30). Paragloboside derived from sialylparagloboside and Forssman glycosphingolipids were purified from type O human and sheep erythrocytes, respectively (31, 32). Ganglioside and neutral glycosphingolipid standards were purchased from Supelco (Bellefonte, PA) and Sigma. Cad ganglioside from human erythrocytes was a gift from Donald M. Marcus (Baylor College of Medicine). Fildes enrichment and brain heart infusion (BHI), Trypticase soy, and Penassay (antibiotic medium 3) dehydrated media were purchased from

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TBS, Tris-buffered saline; BSA, bovine serum albumin.

*To whom reprint requests should be addressed.

Table 1. Structures of glycolipids tested for binding bacteria

Glycolipid	Structure*
Fucosylasialo-GM1	Fucα1-2Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer
Asialo-GM1	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer
Asialo-GM2	GalNAcβ1-4Galβ1-4Glcβ1-1Cer
Asialo-Cad	GalNAcβ1-4Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer
Sulfatide	SO_3^--Galβ1-1Cer
Galactosylceramide	Galβ1-1Cer
Glucosylceramide	Glcβ1-1Cer
Lactosylceramide	Galβ1-4Glcβ1-1Cer
Trihexosylceramide	Galα1-4Galβ1-4Glcβ1-1Cer
Paragloboside	Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer
Globoside	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer
Forssman	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer
GM3	NeuAcα2-3Galβ1-4Glcβ1-1Cer
GM2	GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1Cer
GM1	Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1Cer
GD1a	NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1Cer
GD1b	Galβ1-3GalNAcβ1-4(NeuAcα2-8NeuAcα2-3)Galβ1-4Glcβ1-1Cer
GT1b	NeuAcα2-3Galβ1-3GalNAcβ1-4(NeuAcα2-8NeuAcα2-3)Galβ1-4Glcβ1-1Cer
Cad	GalNAcβ1-4(NeuAcα2-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1Cer

*Underline indicates the probable minimum sequence required for binding. Structures are represented according to recommendations in ref. 27 and references cited therein.

Difco. GC-enriched chocolate agar plates were purchased from Remel (Lenexa, KS). Blood agar, BHI agar, Luria–Bertani broth (LB broth), and Trypticase soy agar slants were obtained from the National Institutes of Health Media Services (Bethesda, MD). Polyisobutylmethacrylate was purchased from Polyscience (Warrington, PA).

Growth and Radiolabeling of Bacteria. The bacteria used and their sources are described in Table 2. Bacteria were stored at −70°C until used and were maintained on Trypticase soy agar slants or blood and chocolate agar plates depending on the species. *M. pneumoniae* metabolically labeled with [^{3}H]palmitic acid was kindly provided by L. Olson (Mycoplasma Branch, Division of Bacterial Products, Food and Drug Administration). The other bacteria were routinely grown in 100 ml of medium (Trypticase soy broth without added glucose, LB broth, or Penassay broth) in 125-ml screw-cap bottles for 24 hr at 37°C without shaking. For growth of *H. influenzae*, Trypticase soy broth was supplemented with Fildes enrichment. Broth cultures were centrifuged at 4°C and 8000 × *g* for 20 min and pellets were washed three times in 0.01 M sodium phosphate (pH 7.2) containing 0.15 M sodium chloride. Radioiodination of bacteria was carried out as described (26). The labeled bacteria were suspended to 10^7–10^8 cells per ml either in LB broth or in 0.05 Tris·HCl (pH 7.4) containing 0.15 M sodium chloride (Tris-buffered saline, TBS) and 1% BSA (TBS/BSA).

Bacterial Overlay Assay. Bacteria bound to separated glycosphingolipids were detected as described (25, 26).

Solid-Phase Binding Assay. The binding of bacteria to purified glycosphingolipids immobilized in flat-bottomed wells of polyvinylchloride microtiter plates (Falcon 3912-III, Becton Dickinson) was done as described (26).

Human Lung Glycosphingolipids. Normal human lung tissue was obtained at autopsy through the cooperation of Barry Cook (Department of Pathology, Fairfax Hospital, Falls Church, VA) and Ralph Frates, Jr. (Department of Pediatrics, University of Texas Medical Branch, Galveston, TX). Tissue was immediately frozen at −70°C until used. Lung tissue (120 g) was homogenized in a blender with water and extracted twice with chloroform/methanol/water (4:8:3, vol/vol) (33). The total lipid extract was dried, subjected to mild alkaline degradation (34), neutralized, dialyzed extensively against distilled water (above its critical micellar concentration), and lyophilized. The neutral and acidic glycosphingolipids were separated by chromatography on DEAE-Sepharose (Pharmacia). Alkali-stable lipid contaminants were eliminated from the neutral glycosphingolipid fraction by acetylation followed by Florisil (Fisher) column chromatography and deacetylation (35).

Immunostaining of Human Lung Glycosphingolipids. Glycosphingolipid antigens were detected on thin-layer chromatograms by immunostaining followed by autoradiography for 12 hr (36).

Purification of Asialogangliosides. Fucosylasialo-GM1 and asialo-GM1 were prepared from bovine brain gangliosides by hydrolysis in 25 mM H_2SO_4 for 1.5 hr at 80°C. The hydrolysate was neutralized with NH_4OH and dried under nitrogen, the residue was dissolved in chloroform/methanol/water (60:30:4.5, vol/vol), and non-glycosphingolipid contaminants were removed by Sephadex G-25 column chromatography (37). Fucosylasialo-GM1 and asialo-GM1 were separated from residual gangliosides by column chromatography on DEAE-Sepharose and further purified by continuous thin-layer chromatography (38) on preparative silica gel G plates with chloroform/methanol/water (75:18:2.5, vol/vol) as the mobile phase. Asialo-GM2 was obtained after digestion of asialo-GM1 with bovine testes β-galactosidase (0.5 unit/ml) for 36 hr at 37°C in 0.1 M acetate buffer (pH 5.0) containing 0.2% sodium taurocholate. Polar contaminants and detergent were removed by Sephadex G-25 and DEAE-Sepharose column chromatography, respectively.

RESULTS AND DISCUSSION

Previous data have shown that *P. aeruginosa* and *P. cepacia* isolated from patients with cystic fibrosis bind specifically to asialo-GM1 and asialo-GM2 by recognizing at least the GalNAcβ1-4Gal sequence in these glycosphingolipids (26). Because these bacteria are notorious lung pathogens generally restricted to this patient population, other bacteria that cause respiratory tract infections were tested for binding to the same glycosphingolipids. Glycosphingolipid standards were subjected to thin-layer chromatography and analyzed for their ability to bind the bacteria listed in Table 2. As shown by the autoradiogram (Fig. 1*B*), compared to a similar thin-layer chromatogram of glycosphingolipids detected by orcinol reagent (Fig. 1*A*), *H. influenzae* bound specifically to fucosylasialo-GM1, asialo-GM1, and asialo-GM2 but not to other glycosphingolipids in Table 1 tested at 1–5 μg. Similar results were obtained with the other bacteria listed as positive for binding in Table 2. Interestingly, the lung pathogen *M.*

Table 2. Bacteria tested for binding to GalNAcβ1-4Gal sequences found in fucosylasialo-GM1, asialo-GM1, and asialo-GM2

Microorganism	Source*	Binding†	Comment‡
Streptococcus pneumoniae 33400	ATCC	+	Type strain
Streptococcus pneumoniae 6303	ATCC	+	Type III capsule
Streptococcus pneumoniae 27336	ATCC	+	Rough phase
Staphylococcus aureus 12600	ATCC	+	Neotype strain
Staphylococcus aureus 8095	ATCC	+	
Haemophilus influenzae 33391	ATCC	+	Type strain
Haemophilus influenzae 9795	ATCC	+	Type B capsule
Haemophilus parainfluenzae 33392	ATCC	+	Type strain
Klebsiella pneumoniae 27736	ATCC	+	
Pseudomonas aeruginosa CT3	Human	+	CF patient, mucoid
Pseudomonas aeruginosa CT4	Human	+	CF patient, mucoid
Pseudomonas aeruginosa CT5	Human	+	CF patient, mucoid
Pseudomonas aeruginosa 17648	ATCC	+	
Pseudomonas aeruginosa 19142	ATCC	+	Mucoid
Pseudomonas aeruginosa 33347	ATCC	+	Rough phase
Pseudomonas aeruginosa 21472	ATCC	+	
Pseudomonas cepacia 25416	ATCC	+	Type strain
Pseudomonas cepacia ML1	Human	+	CF patient
Pseudomonas cepacia 13945	ATCC	+	
Pseudomonas maltophilia 13637	ATCC	+	Type strain
Escherichia coli VJ1	NIH	+	
Escherichia coli 6883	ATCC	+	
Mycoplasma pneumoniae M129	FDA	−	
Streptococcus pyogenes 12344	ATCC	−	Type strain
Salmonella milwaukee U4 4407-50	CDC	−	
Salmonella enteritidis 13076	ATCC	−	Type strain
Escherichia coli K1	NIH	−	
Escherichia coli K99 1472 (B44)	URI	−	09:K30, K99, F41:NM

*ATCC, American Type Culture Collection; CDC, Centers for Disease Control; NIH, National Institutes of Health; FDA, Food and Drug Administration; URI, University of Rhode Island.
†Bacteria were tested for binding to glycosphingolipids by the bacterial overlay assay. Plus (+) indicates binding and minus (−) indicates no binding to at least 2 μg of glycosphingolipid containing the GalNAcβ1-4Gal sequence.
‡CF, cystic fibrosis.

pneumoniae did not bind, nor did the group A streptococci or several enteric bacteria.

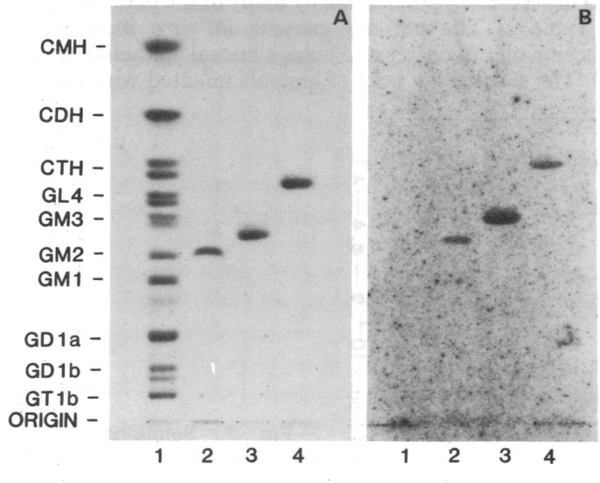

FIG. 1. Binding of ^{125}I-labeled bacteria to glycosphingolipids separated by thin-layer chromatography. (*A*) Standard glycosphingolipids detected with orcinol reagent. (*B*) Glycosphingolipids detected by autoradiography (12 hr) with radioiodinated *H. influenzae* ATCC 9795. Lanes 1, galactosylceramide (CMH), lactosylceramide (CDH), trihexosyl ceramide (CTH), globoside (GL4), and gangliosides GM3, GM2, GM1, GD1a, GD1b, and GT1b (2 μg of each compound); lanes 2, fucosylgangliotetraosylceramide (fucosylasialo-GM1, 1 μg); lanes 3, gangliotetraosylceramide (asialo-GM1, 1 μg); lanes 4, gangliotriaosylceramide (asialo-GM2, 1 μg). For structures, see Table 1.

The pulmonary pathogens bind to asialogangliosides after they are cultured in either Trypticase soy broth, LB broth, or Penassay broth under static conditions at 37°C for 24 hr. *P. aeruginosa* does not bind well when grown on agar (other bacteria were not tested). Thus, binding of this organism may depend on culture conditions as has been described for other bacterial adhesins (10). To exclude the possibility that a high molecular weight component of the growth medium was mediating binding of bacteria to these glycosphingolipids, *P. aeruginosa* CT4 was grown in Trypticase soy broth that had been filtered through a membrane with a molecular weight cut-off of 3000. This treatment did not affect the binding of *P. aeruginosa* to asialo-GM1.

After bacteria were radiolabeled and washed, the cells were suspended in either LB broth or in isotonic buffer (TBS/BSA). Cells maintained in a nutrient medium bound better than cells suspended in TBS/BSA and remained viable for a longer time (data not shown). Therefore, LB broth was used as diluent for bacteria in all experiments in this investigation. Most nonviable bacteria did not bind to glycosphingolipids in our assays. When maintained in non-nutrient media, some bacteria, particularly the pneumococci, quickly lost their ability to bind to asialogangliosides. These results are consistent with reports that bacterial attachment to carbohydrate receptors requires favorable growth conditions so that at least minimal protein synthesis can occur (39) and that bacteria that bind to glycosphingolipid receptors on thin-layer chromatograms are viable while attached (23).

The pulmonary pathogens do not bind to 5 μg of lactosylceramide. Thus, β-*N*-acetylgalactosamine, which is posi-

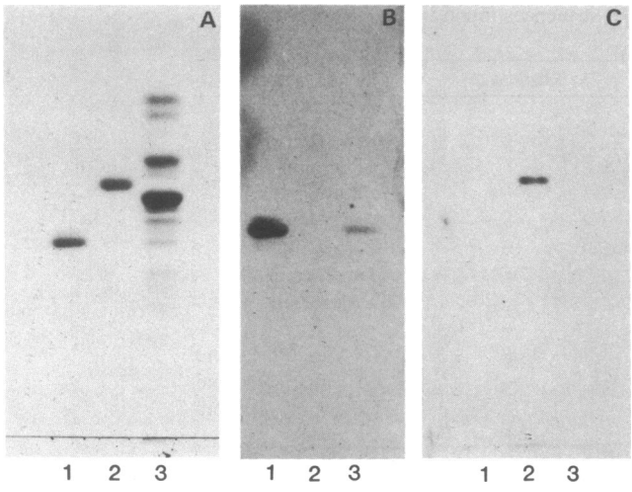

FIG. 2. Detection of asialo-GM1 in human lung extracts by immunostaining of thin-layer chromatograms. (*A*) Glycosphingolipids detected by orcinol spray. (*B*) Immunostaining with mouse monoclonal anti-asialo-GM1 antibody (1 μg/ml). (*C*) Immunostaining with mouse monoclonal anti-asialo-GM2 antibody (5 μg/ml). Lanes 1, asialo-GM1 (25 ng); lanes 2, asialo-GM2 (50 ng); lanes 3, total human lung neutral glycosphingolipid fraction from 100 mg (wet weight) of tissue.

tioned internally in fucosylasialo-GM1 and asialo-GM1 and terminally in asialo-GM2, is required for binding. *N*-Acetylgalactosamine alone, however, is not sufficient, because the bacteria do not bind to globoside, which contains a terminal GalNAcβ1-3Gal sequence, or to the Forssman glycosphingolipid, which contains a terminal GalNAcα1-3GalNAc sequence (Table 1). In addition, these pulmonary pathogens do not bind to the gangliosides GM1, GM2, GD1a, GD1b, GT1b, and Cad, which all contain the GalNAcβ1-4Gal sequence, suggesting that the sialyl residues prevent binding.

The biological relevance of these data is suggested by the presence of asialo-GM1 in human lung extracts. Neutral glycosphingolipids from human lung were separated by thin-layer chromatography and immunostained with monoclonal antibodies specific for asialo-GM1 (40) and asialo-GM2. As shown in Fig. 2*B*, anti-asialo-GM1 antibody bound only to authentic asialo-GM1 and to an antigen with the same mobility in the neutral lung fraction, indicating the presence of asialo-GM1 in this fraction. The antigen was also verified to be asialo-GM1 by comigration with the authentic glycosphingolipid in two other solvent systems as detected by orcinol and immunostaining (data not shown). Asialo-GM2 was not detected by immunostaining in the neutral lung fraction (Fig. 2*C*).

The binding avidity of a representative group of bacteria from Table 2 for asialo-GM1 was estimated by dilution curves from microtiter wells (Fig. 3). All three pathogens tested exhibit similar binding curves in the solid-phase binding assay for asialo-GM1 and compared with our previous data for *P. aeruginosa* and *P. cepacia* (26). Immobilized glycosphingolipids that did not contain the GalNAcβ1-4Gal sequence were not receptors for the bacteria. Metabolically labeled bacteria produced similar binding curves, demonstrating that the specificity for asialo-GM1 is not an artifact of radioiodination (data not shown).

The data presented in this paper show that *S. pneumoniae*, *S. aureus*, *H. influenzae*, *K. pneumoniae*, certain *E. coli*, and several species of *Pseudomonas* specifically recognize the carbohydrate sequence GalNAcβ1-4Gal found in fucosyl-asialo-GM1, asialo-GM1, and asialo-GM2. The ability of these bacteria to recognize internal or terminal sequences clearly enhances their adhesion potential as lung pathogens, since cells could contain several different glycosphingolipids with this sequence. This is true in the case of certain *E. coli* that cause urinary-tract infections, which recognize terminal and internal Galα1-4Gal sequences in glycosphingolipids (23). Asialo-GM1, but not asialo-GM2, was detected in glycosphingolipids from human lung tissue, but other glycosphingolipids or glycoproteins containing the GalNAcβ1-4Gal sequence may also bind the bacteria. For example, glycoproteins with blood group Sd^a and Cad specificity, which contain this sequence (41, 42), occur in many human secretions (43). When desialylated, glycosphingolipids with the blood group Cad determinant (44) (see Table 1) bind *P. aeruginosa* (data not shown).

Some viruses, including influenza, express a neuraminidase and change the morphology of the lung by destroying ciliated epithelium (45, 46). Neuraminidase decreases the viscosity of the lung mucus, which may predispose individuals to secondary pneumonia by many of the pathogens listed in Table 2. The purified enzyme *in vitro* increases the adhesion of oral bacteria to human buccal epithelial cells (47, 48). The respiratory tract of animals infected with influenza

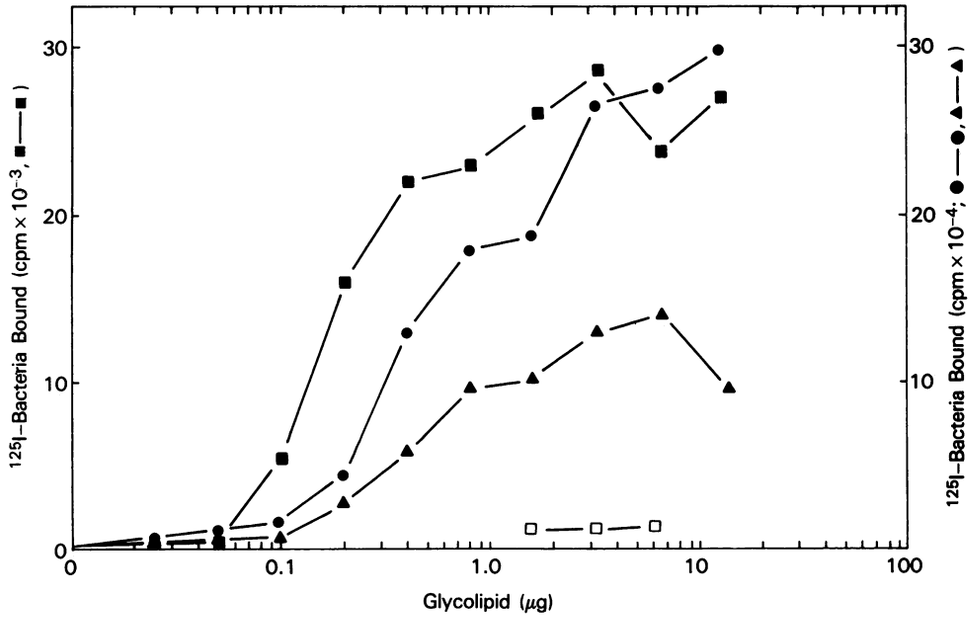

FIG. 3. Solid-phase binding assay for some pathogenic bacteria. The assay was carried out by using ≈10^7 cells per well with glycosphingolipids serially diluted in microtiter wells. Solid symbols, binding of ^{125}I-labeled *S. pneumoniae* (■), *S. aureus* (●), *H. parainfluenzae* (▲) to asialo-GM1. □, Binding of *S. pneumoniae* to galactosylceramide, glucosylceramide, lactosylceramide, trihexosylceramide, or GM1.

virus exhibits enhanced adhesion of several bacteria, including *P. aeruginosa* (49), *H. influenzae* (50), *S. aureus* (51, 52), and *S. pneumoniae* (53). In addition, mice are susceptible to bacterial pneumonia only after persistent infection with influenza A virus (50, 54). A possible explanation as to why influenza virus and pathogenic bacteria might interact in the pathogenesis of pneumonia is that the viral neuraminidase may desialylate carbohydrate sequences on the cells lining the respiratory tract and increase the number of structures containing unsubstituted GalNAcβ1-4Gal residues. Interestingly, *P. aeruginosa* adhere better to mouse tracheas treated with 0.1 M HCl (55), again possibly due to hydrolysis of acid-labile sialyl residues.

In summary, several pathogenic bacteria, both Gram-positive and Gram-negative, bind specifically to GalNAcβ1-4Gal sequences found in fucosylasialo-GM1, asialo-GM1, and asialo-GM2. Although these microorganisms belong to different genera, when infectious they all have in common a high degree of tissue tropism for the respiratory tract. The possible use by these organisms of the same cell-surface receptors for colonization might explain this common tropism. Consistent with this possibility is our finding that asialo-GM1 occurs in substantial amounts in human lung tissue.

We thank Drs. B. Cook and R. Frates, Jr., for the human lung samples; Dr. D. M. Marcus for the monoclonal anti-asialo-GM1 antibody and Cad ganglioside; Dr. D. Zopf for the monoclonal anti-asialo-GM2 antibody; Drs. V. Jimenez and John Foulds (National Institute of Diabetes and Digestive and Kidney Diseases) for the *E. coli* and *P. aeruginosa* isolates CT3, CT4, and CT5; and Dr. P. S. Cohen (Department of Microbiology, University of Rhode Island, Kingston) for *E. coli* K99. We are grateful to Dr. L. Olson (Mycoplasma Branch, Division of Bacterial Products, Food and Drug Administration) for providing ^{3}H-labeled *M. pneumoniae*.

1. Pennington, J. E. (1986) *Am. Fam. Physician* **33,** 153–160.
2. Eickhoff, T. C. & Rytel, M. W. (1985) *Am. J. Med.* **78,** 58–62.
3. Reynolds, H. Y., Levine, A. S., Wood, R. E., Zerdt, G. H. & Dale, D. C. (1975) *Ann. Intern. Med.* **82,** 819–831.
4. Wood, R. E., Boat, T. F. & Doershuk, C. F. (1976) *Am. Rev. Respir. Dis.* **113,** 833–878.
5. Selinger, D. S. & Reed, W. P. (1979) *Infect. Immun.* **23,** 545–548.
6. Andersson, B., Eriksson, B., Falsen, E., Fogh, A., Hanson, L. A., Nylen, O., Peterson, H. & Svanborg-Eden, C. (1981) *Infect. Immun.* **32,** 311–317.
7. Aly, R., Shinefield, H. I., Strauss, W. G. & Maibach, H. I. (1980) *Infect. Immun.* **17,** 546–549.
8. Baseman, J. B., Cole, R. M., Krause, D. C. & Leith, D. K. (1982) *J. Bacteriol.* **151,** 1514–1522.
9. De Graaf, F. K. & Mooi, F. R. (1986) *Adv. Microb. Physiol.* **28,** 65–135.
10. Jones, G. W. & Isaacson, R. E. (1983) *CRC Crit. Rev. Microbiol.* **10,** 229–260.
11. Clegg, S. & Gerlach, G. F. (1987) *J. Bacteriol.* **169,** 934–938.
12. Fader, R. C., Avots-Avotins, E. E. & Davis, C. P. (1979) *Infect. Immun.* **25,** 729–737.
13. Ramphal, R. & Pyle, M. (1983) *Infect. Immun.* **41,** 339–344.
14. Woods, D. E., Straus, D. C., Johanson, W. G., Jr., Berry, V. K. & Bass, J. A. (1980) *Infect. Immun.* **29,** 1146–1151.
15. Woods, D. E., Bass, J. A., Johanson, W. G., Jr., & Straus, D. C. (1980) *Infect. Immun.* **30,** 694–699.
16. Lampe, R. M., Mason, E. O., Jr., Kaplan, S. L., Umstead, C. L., Yow, M. D. & Feigin, R. D. (1982) *Infect. Immun.* **35,** 166–172.
17. Leffler, H. & Svanborg-Eden, C. (1986) in *Microbial Lectins and Agglutinins: Properties and Biological Activity*, ed. Mirelman, D. (Wiley, New York).
18. Barondes, S. H. (1981) *Annu. Rev. Biochem.* **50,** 207–231.
19. Frazier, W. & Glaser, L. (1979) *Annu. Rev. Biochem.* **48,** 491–523.
20. Sharon, N. (1987) *FEBS Lett.* **217,** 145–157.
21. Svanborg-Eden, C. & Leffler, H. (1979) *Scand. J. Infect. Dis. Suppl.* **24,** 144–147.
22. Leffler, H. & Svanborg-Eden, C. (1980) *FEMS Microbiol. Lett.* **8,** 127–134.
23. Bock, K., Breimer, M. E., Brignole, A., Hansson, G. C., Karlsson, K.-A., Larson, G., Leffler, H., Samuelsson, B. E., Stromberg, N., Svanborg-Eden, C. & Thurin, J. (1985) *J. Biol. Chem.* **260,** 8545–8551.
24. Brennan, M. J., Joralmon, R. A., Cisar, J. O. & Sandberg, A. L. (1987) *Infect. Immun.* **55,** 487–489.
25. Hansson, G. C., Karlsson, K.-A., Larson, G., Stromberg, N. & Thurin, J. (1985) *Anal. Biochem.* **146,** 158–163.
26. Krivan, H., Ginsburg, V. & Roberts, D. D. (1988) *Arch. Biochem. Biophys.* **260,** 493–496.
27. IUPAC-IUB Joint Commission on Biochemical Nomenclature (1986) *Eur. J. Biochem.* **159,** 1–6.
28. Fraker, P. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80,** 849–857.
29. Rauvala, H. (1976) *Biochim. Biophys. Acta* **424,** 284–295.
30. Rauvala, H. (1976) *J. Biol. Chem.* **251,** 7517–7520.
31. Siddiqui, B. & Hakomori, S.-I. (1973) *Biochim. Biophys. Acta* **330,** 147–155.
32. Siddiqui, B. & Hakomori, S.-I. (1971) *J. Biol. Chem.* **246,** 5766–5769.
33. Hakomori, S. & Siddiqui, B. (1974) *Methods Enzymol.* **32,** 345–367.
34. Tadano, K. & Ishizuka, I. (1982) *J. Biol. Chem.* **257,** 1482–1490.
35. Saito, T. & Hakomori, S. (1971) *J. Lipid Res.* **12,** 257–259.
36. Magnani, J. L., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, Z., Koprowski, H. & Ginsburg, V. (1982) *J. Biol. Chem.* **257,** 14365–14369.
37. Wells, M. A. & Dittmer, J. C. (1963) *Biochemistry* **2,** 1259–1263.
38. Young, W. W., Jr., & Borgman, C. A. (1987) *Methods Enzymol.* **138,** 125–132.
39. Yu, C., Lee, A. M. & Roseman, S. (1987) *Biochem. Biophys. Res. Commun.* **149,** 86–92.
40. Gillard, B. K., Jones, M. A. & Marcus, D. M. (1987) *Arch. Biochem. Biophys.* **256,** 435–445.
41. Donald, A. S. R., Soh, C. P. C., Watkins, W. M. & Morgan, W. T. J. (1982) *Biochem. Biophys. Res. Commun.* **104,** 58–65.
42. Blanchard, D., Cartron, J.-P., Fournet, B., Montreuil, J., van Halbeek, H. & Vliegenthart, J. F. G. (1983) *J. Biol. Chem.* **258,** 7691–7695.
43. Pickles, M. M. & Morton, J. A. (1977) in *Human Blood Groups*, Fifth International Convoc. Immunology, eds. Mohn, J. F., Plunkett, R. W., Cunningham, R. K. & Lambert, R. M. (Karger, Basel), pp. 277–286.
44. Blanchard, D., Piller, F., Gillard, B., Marcus, D. & Cartron, J.-P. (1985) *J. Biol. Chem.* **260,** 7813–7816.
45. Jackson, G. G. & Muldoon, R. L. (1975) *J. Infect. Dis.* **131,** 308–357.
46. Sweet, C. & Smith, H. (1980) *Microbiol. Rev.* **44,** 303–330.
47. Yamazaki, Y., Ebisu, S. & Okada, H. (1981) *Infect. Immun.* **31,** 21–27.
48. Saunders, J. M. & Miller, C. H. (1983) *J. Dent. Res.* **62,** 1038–1040.
49. Ramphal, R., Small, P. M., Shands, J. W., Jr., Fischlschweiger, W. & Small, P. A., Jr. (1980) *Infect. Immun.* **27,** 614–619.
50. Francis, T. & Vicente de Torregrosa, M. (1945) *J. Infect. Dis.* **76,** 275–284.
51. Jansen, R. Y., Chappell, W. A. & Gerone, P. J. (1963) *Am. J. Hyg.* **78,** 275–284.
52. Sanford, B. A., Davison, V. & Ramsay, M. (1986) *Proc. Soc. Exp. Biol. Med.* **181,** 104–111.
53. Plotkowski, M.-C., Puchelle, E., Beck, G., Jacquot, J. & Hannoun, C. (1986) *Am. Rev. Respir. Dis.* **134,** 1040–1044.
54. Nugent, K. M. & Pesanti, E. L. (1983) *Infect. Immun.* **42,** 1102–1108.
55. Ramphal, R. & Pyle, M. (1983) *Infect. Immun.* **41,** 345–351.