Sialic Acid-dependent Adhesion of Mycoplasma pneumoniae to Purified Glycoproteins*

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Several purified glycoproteins including laminin, fetuin, and human chorionic gonadotropin promote dose-dependent and saturable adhesion of Mycoplasma pneumoniae when adsorbed on plastic. Adhesion to the proteins is energy dependent as no attachment occurs in media without glucose. Adhesion to all of the proteins requires sialic acid, and only those proteins with α2-3-linked sialic acid are active. The α-subunit of human chorionic gonadotropin also promotes attachment, suggesting that a simple biantennary asparagine-linked oligosaccharide is sufficient for binding. Soluble laminin, asparagine-linked sialyloligosaccharides from fetuin, and 3'-sialyllactose inhibit attachment of M. pneumoniae to laminin. M. pneumoniae also bind to sulfatide adsorbed on plastic. Dextran sulfate, which inhibits M. pneumoniae binding to sulfatide, does not inhibit attachment on laminin, and 3'-sialyllactose does not inhibit binding to sulfatide, suggesting that two different receptor specificities mediate binding to these two carbohydrate receptors. Both 3'-sialyllactose and dextran sulfate partially inhibit M. pneumoniae adhesion to a human colon adenocarcinoma cell line (WiDr) at concentrations that completely inhibit binding to laminin or sulfatide, respectively, and in combination they inhibit binding of M. pneumoniae to these cells by 90%. Thus, both receptor specificities contribute to M. pneumoniae adhesion to cultured human cells.

Adhesion of Mycoplasma pneumoniae to many cell types in vitro may be mediated by recognition of sialyloligosaccharides on the host cell surface (1, 2, reviewed in Ref. 3). Based on selective restoration of binding of neuraminidase-treated erythrocytes using CMP-sialic acid and purified sialyltransferases, adhesion of the erythrocytes on surface grown sheet cultures of M. pneumoniae specifically requires sialic acid-linked α2-3- to N-acetyllactosamine sequences (4). Inhibition studies using glycolipids, glycoproteins, and oligosaccharides suggested that sialylated linear or branched polylactosamine sequences on both glycoproteins and glycolipids are receptors on erythrocytes for M. pneumoniae (4, 5). Although glycolipids including gangliosides inhibited attachment in this and some other adherence assays (6), other workers have concluded that binding is mediated by glycoproteins but not glycolipids (7, 8). In the latter study (8), no sialic acid was found in a purified receptor protein from lung fibroblast (MRC5) cells.

In the accompanying paper (9), we demonstrated that M. pneumoniae binds avidly to some sulfated glycolipids but does not bind to glycolipids containing α2-3-sialyllactosamine sequences. Based on these results, we have reexamined the role of sialyloligosaccharides on glycoproteins in adhesion by measuring the activity of several glycoproteins with known carbohydrate structures in promoting M. pneumoniae attachment using a direct adhesion assay. We report here that several glycoproteins containing α2-3- but not α2-6-linked sialic acid can support attachment of M. pneumoniae and that simple biantennary asparagine-linked oligosaccharides are sufficient to efficiently mediate adhesion. Based on inhibition studies, this binding specificity is distinct from sulfatide binding and both mechanisms are involved in adhesion to cultured human cells.

EXPERIMENTAL PROCEDURES

Materials—Laminin purified from the mouse Engelbreth Holm Swarm tumor was provided by Dr. Lance Liotta, National Cancer Institute, National Institutes of Health (NIH). Thrombospondin was purified from thrombin-stimulated human platelets (10). Human plasma fibronectin was from Collaborative Research, Inc. Human chorionic gonadotropin (hCG) and the purified α-subunit were provided by Drs. Bruce Weintraub and Peter Gyves, National Institute of Diabetes and Digestive and Kidney Diseases, NIH. Most other proteins, dextran sulfate M, 5,000,000, and neuraminidase (Clostridium perfringens, Type VI) were obtained from Sigma.

6'-Sialyllactose from human milk was provided by Dr. David Smith, Department of Biochemistry and Nutrition, Virginia Polytechnic Institute and State University. 3'-Sialyllactose was isolated from human milk or from a mixture of sialyllactose isomers from bovine colostrum (Boehringer Mannheim).

Contamination of the 6'-sialyllactose with 3'-sialyllactose was less than 2% as determined by ion exchange chromatography on an AS-4 column (Dionex Corp., Sunnyvale, CA).

Oligosaccharides from 500 mg of bovine fetuin (Sigma) were released by digestion in 0.2 mM sodium phosphate, pH 8.6, containing 10 mM β-mercaptoethanol, 1 mM EDTA, and 0.1 mM phenylmethanesulfonfyl fluoride with 20 units of peptide-N(N-acetylglucosaminyl) asparagine amidase F from Flavobacterium meningosepticum (Boehringer Mannheim) (11). For quantitative removal of asparagine-linked oligosaccharides, 10 mg of fetuin was digested with 10 units of enzyme for 48 h at 37 °C. Complete release of N-linked sugars was

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1 The abbreviations used are: hCG, human chorionic gonadotropin; RPMI-BSA, RPMI 1640 medium containing 25 mM Hepes, pH 7.3, and 1% bovine serum albumin; Tris-BSA, 50 mM Tris-HCl, pH 7.6, 110 mM NaCl, 5 mM CaCl₂, 0.2 mM phenylmethanesulfonfyl fluoride, 1% bovine serum albumin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
confirmed by change in migration of the protein on sodium dodecyl sulfate-gel electrophoresis (11). Following enzyme treatment, protein was precipitated with ethanol, and the oligosaccharides released from 500 mg of fetuin were desalted on Sephadex G-25 in 50 mM pyridinium acetate, pH 5, yielding 30 mg of oligosaccharides. The oligosaccharides (15) were fractionated on a 25-ml column of concanavalin A-Sepharose. Triantennary oligosaccharides were eluted in the void volume, and the biantennary fraction (0.5 mg) was eluted with 20 mM methyl-ß-D-glucoside. The oligosaccharides were desalted by gel filtration and lyophilized. Sialic acid was determined by the periodic acid-schiff assay (12) and carbohydrate composition was determined by anion exchange chromatography on a Dionex AS-6 column (13). The triantennary and biantennary fractions contained 3.2 and 1.9 mol of sialic acid/mol of oligosaccharide, respectively. Analysis of the sialyloligosaccharides by anion exchange chromatography in 50 mM NaOH with 100 mM sodium acetate on a Dionex AS-6A column confirmed that the biantennary oligosaccharides were quantitatively bound on the concanavalin A column and that the biantennary fraction was free of triantennary oligosaccharides. The biantennary fraction eluted as a triplet of peaks on the AS-6A column with similar retention times as authentic biantennary disialyoglycosides released from human transferrin and the ß-subunit of hCG using peptide-N-acetylglucosaminyl asparagine amidase P (11).

O-Linked oligosaccharides from fetuin were released by alkaline borohydride degradation of 20 mg of fetuin pronase-resistant glycopeptides for 16 h at 45 °C in 1 M NaBH4, 0.05 M NaOH (14). The released sialyloligosaccharides were purified by gel filtration on Biogel P-4 (400 mesh) eluted in 50 mM pyridinium acetate, pH 5. Hexose and sialic acid were determined using the phenol-sulfuric acid (15) and resorcinol assays, respectively.

*M. pneumoniae Adhesion to Immobilized Glycoproteins—Glycoproteins dissolved in 0.01 M sodium phosphate buffer, pH 7.4, containing 150 mM NaCl, 1% bovine serum albumin, and 0.01% NaN3 were adsorbed onto plastic (Falcon 3912 polyvinylchloride 96-well microtiter plates) by incubation for 16 h at 4 °C. Immulon 2 Removeawell plates, or Falcon 1007 bacteriological polystyrene were also used in some experiments. The unbound proteins were removed, and the wells were filled with Tris-BSA and incubated for 30 min at room temperature. The wells were rinsed with RPMI 1640 containing 25 mM HEPES, pH 7.3, and 1% bovine serum albumin (Sigma fatty acid free). *M. pneumoniae strain M129 labeled with [3H]palmitate (16) were dispersed in RPMI-BSA by passing 4 times through a 26-gauge needle, and 50 µl of the suspension was applied to the wells. After incubation for 60 min at 37 °C, the wells were washed 5 times with saline, and the labeled *M. pneumoniae bound to the proteins were quantified by scintillation counting in Aquasol.

For inhibition studies, sugars in 25 µl of RPMI-BSA were added to wells coated with laminin (10 µg/ml) followed by 25 µl of *H-labeled *M. pneumoniae. Binding was determined to both laminin-coated and uncoated wells in triplicate at each inhibitor concentration and in the absence of inhibitor. In some experiments the adsorbed proteins were pretreated with neuraminidase. After adsorption of the proteins and incubation in Tris-BSA, the wells were rinsed 3 times with 50 mM sodium acetate, pH 5.5, containing 150 mM NaCl, 5 mM CaCl2, 1 mg/ml bovine serum albumin, and 1 mM phenylmethylsulfonfyl fluoride. The wells were incubated with 0.05 units/ml neuraminidase in the same buffer or with buffer without enzyme overnight at 20 °C. The wells were rinsed 3 times with Tris-BSA, and *M. pneumoniae binding was determined as described above.

Binding of monoclonal antibody My-28 (provided by Dr. Curt Civan, Johns Hopkins Oncology Center, Baltimore) to the immobilized proteins before or after digestion with neuraminidase was determined using a 1:1000 dilution of ascites fluid in Tris-BSA. After incubation for 2 h at room temperature, the wells were washed 3 times with Tris-BSA. Bound antibody was detected using goat anti-mouse IgM (Kirkegaard and Perry) labeled with 125I by the iodogen method (17).

*M. pneumoniae Adhesion to WtDr Cells—Adhesion of labeled *M. pneumoniae to WtDr cells on glass coverslips was determined as described in the accompanying paper (9). For inhibition studies, dextran sulfate and 3'-sialyllactose were dissolved in RPMI-BSA, and the pH was adjusted to 7.4 with NaOH. The inhibitors were added to wells containing washed coverslips with attached WtDr cells or 25 µl of WtDr fractions incubated in 50 µl of Tris-BSA. Labeled *M. pneumoniae were added immediately and incubated with slow rocking for 60 min at 37 °C. After washing the coverslips by dipping 6 times in saline, bound *M. pneumoniae were determined by scintillation counting in Aquasol.

RESULTS

Several glycoproteins including laminin, fetuin, and hCG support dose-dependent and saturable adhesion of *M. pneumoniae when adsorbed on plastic (Fig. 1). Typically, 20-60% of the added *M. pneumoniae bound to the wells at saturating protein concentrations. Nonspecific binding to uncoated wells was 0.3-3% of the total radioactivity applied. As was reported for *M. pneumoniae attachment to glass substrates (18) and binding to sulfated glycolipids (9), binding is energy dependent and no binding was detected in a Tris-albumin buffer without glucose. Most proteins, however, are inactive in this assay (Fig. 1 and Table I). The relative activities of several proteins for promoting *M. pneumoniae adhesion were estimated by comparing the dose response curves and are summarized in Table I. The proteins laminin, fetuin, thrombospondin, hCG, and the ß-subunit of hCG have similar activity and promote adhesion to wells coated with less than 10 ng of glycoprotein. Glycophorin and α1-acid glycoprotein are weakly

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**Fig. 1. *M. pneumoniae* binding to immobilized glycoproteins.** [H]-Labeled *M. pneumoniae, 630,000 cpm/5 × 10^5 color changing units, were incubated for 60 min at 37 °C in microtiter wells coated in duplicate with laminin (♦), fetuin (○), hCG (■), or transferrin (□) at the indicated concentrations. After washing to remove the unbound organisms, the bound *M. pneumoniae* were determined by scintillation counting. Binding to uncoated wells was 3% of the applied radioactivity.

**TABLE I**

*M. pneumoniae* binding to glycoproteins adsorbed on plastic

| Protein | Relative binding activity |
|---------|--------------------------|
| Murine laminin | 1.5 |
| Bovine fetuin | 1.0 |
| PNGase F-treated fetuin | 0.09 |
| hCG | 0.7 |
| hCG ß-subunit | 0.8 |
| Human platelet thrombospondin | 0.7 |
| Human type MM glycophorin | 0.06 |
| Human α1-acid glycoprotein | 0.03 |
| Hen ovomucoid | <0.01 |
| Human transferrin | <0.01 |
| Human plasma fibrinogen | <0.01 |
| Human plasma fibronectin | <0.01 |
| Bovine serum albumin | <0.01 |

* Binding of [3H]-labeled *M. pneumoniae* was determined to polyvinyl chloride microtiter wells coated with 0.006–2 µg of the respective proteins. Relative binding activities of the proteins were determined by the amount accumulated in minimum required to give a labeled *M. pneumoniae* (typically 10–30% of the total added) and are expressed relative to fetuin which was included as a positive control in each experiment and was assigned a value of 1.0. Results are the mean values of two or three experiments for each protein.
active, whereas the other proteins are essentially inactive, promoting binding of less than 10% of the added *M. pneumoniae* at the highest levels tested (1–5 μg/well).

Immuno-5 microtiter plates and bacteriological polystyrene were also examined as substrates for *M. pneumoniae* adhesion to adsorbed proteins. Although binding varied with the plastic used, the distinction between the active and inactive glycoproteins was consistently observed with all three types of plastic. Thus, the differences in activity are probably not an artifact of selective adsorption of the active glycoproteins.

*N*-Deglycosylated fetuin was tested in the assay to examine the role of the *O*-linked sialyloligosaccharides of fetuin in adhesion of *M. pneumoniae* (Table I). The protein promoted adhesion of *M. pneumoniae* at higher concentrations but was approximately 10-fold less active than intact fetuin. The low activity of glycoporphin (Table I) also suggests that *α*2-3-linked sialic acid on *O*-linked oligosaccharides is not as active as on *N*-linked oligosaccharides.

Binding to all of the active glycoproteins requires sialic acid, as neuraminidase treatment of the adsorbed proteins (Fig. 2) or pretreatment with neuraminidase in solution before adsorption (results not shown) abolishes all binding activity. Several of the inactive glycoproteins also contain sialic acid but the linkage reported in human transferrin (19), fibrinogen (29), and plasma fibronectin (21) is exclusively *α*2-6 to galactose. The linkage in hCG (22) and a majority of *N*-linked fetuin oligosaccharides (23–25) is *α*2-3. Thus, in agreement with previous studies of erythrocyte adhesion to surface grown sheet cultures of *M. pneumoniae* (4), binding of the labeled *M. pneumoniae* to immobilized glycoproteins appears to be specific for *α*2-3-linked sialic acid.

With the exception of hCG, all of the active glycoproteins have extensive heterogeneity in their carbohydrate structures or have only partially characterized structures. hCG contains only mono- and biantennary asparagine-linked oligosaccharides on both subunits (22, 26) and 4 *O*-linked oligosaccharides on the β-subunit (27). Since the α-subunit of hCG binds *M. pneumoniae* as well as the intact protein (Table I and Fig. 2), the *O*-linked carbohydrates on the β-subunit are not required for binding. Thus, a biantennary asparagine-linked carbohydrate with *α*2-3-linked sialic acid is sufficient for binding of *M. pneumoniae*.

Inhibition of binding after neuraminidase treatment is not due to a contaminating protease as all of the adsorbed proteins bind monoclonal antibody My-28 after neuraminidase treatment (results not shown). This antibody recognizes *N*-acetyllactosamine sequences found in glycolipids and glycoproteins (28). In most cases, antibody binding is detected only after neuraminidase digestion. Excepting glycoporphin, which binds about 10-fold less antibody, all of the asialoglycoproteins have similar binding curves with antibody My-28. Uniform binding of the antibody to all of the neuraminidase-treated glycoproteins confirms that all of the proteins are adsorbed on plastic to a similar extent under the conditions used and that sialylated *N*-acetyllactosamine sequences on the immobilized proteins are accessible for binding antibodies and *M. pneumoniae*.

Binding of *M. pneumoniae* to adsorbed laminin is inhibited by soluble laminin with 50% inhibition at 80 μg/ml (Table II). Binding is also inhibited by 3′-sialyllactose (Table II) at comparable concentrations as were reported to inhibit erythrocyte adhesion to *M. pneumoniae* surface grown sheet cultures (4), 6′-Sialyllactose is 20-fold less active. Neither laminin nor 3′-sialyllactose inhibit *M. pneumoniae* attachment to sulfatide. Conversely, dextran sulfate is a potent inhibitor of binding to sulfatide (9) but has no effect on attachment on laminin (Table II). Thus, the two binding specificities probably require two independent carbohydrate-binding sites on the *M. pneumoniae* pathogen.

Asparagine-linked oligosaccharides released from fetuin also inhibit *M. pneumoniae* binding to laminin (Table II). Unfractionated oligosaccharides (data not shown) and triantennary oligosaccharides (23, 24) eluted in the unbound fraction from chromatography on concanavalin A-Sepharose (Table II) have similar inhibitory activity to 3′-sialyllactose. The bound fraction from the concanavalin A column, which contains the biantennary oligosaccharides of fetuin (24, 29), is about 20-fold more active, inhibiting *M. pneumoniae* binding by 60% at 12 μM.

The reduced *O*-linked tri- and tetrasaccharides from fetuin also inhibit binding of *M. pneumoniae* (Table II). The tri- and tetrasaccharide NeuAcα2-3Galβ1-3GalNAcα is about 1.5-fold more active than the tetrasaccharide NeuAcα2-3Galβ1-3NeuAcα2-6GalNAcα but both are at least 10-fold less active than the biantennary fetuin oligosaccharide.

3′-Sialyllactose inhibits *M. pneumoniae* adhesion to monolayers of WI-38 cells (Fig. 3). Inhibition was dose dependent,

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**TABLE II**

| Inhibitor                      | Substrate | Laminin | Sulfatide |
|-------------------------------|-----------|---------|-----------|
| 3′-Sialyllactose              | 0.3 mM    | >5 mM   | ND        |
| 6′-Sialyllactose              | 6 mM      | ND      | ND        |
| NeuAcα2-3Galβ1-3GalNAcα      | 0.13 mM   | ND      | ND        |
| NeuAcα2-3Galβ1-3NeuAcα2-6GalNAcα | 0.4 mM  | ND      | ND        |
| Triantennary fetuin oligosaccharides | 0.3 mM | ND      | ND        |
| Biantennary fetuin oligosaccharides | 0.012 mM | ND      | ND        |

* Concentration of inhibitor giving 50% inhibition of control binding.

+ ND, not determined.

* Binding was 112% of control at 200 μg/ml dextran sulfate M, 500,000.

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**Fig. 2. Effects of neuraminidase treatment on *M. pneumoniae* binding to immobilized glycoproteins.** Microtiter wells were coated with fetuin (circles), hCG (squares), or α-subunit of hCG (triangles) and treated for 16 h with 0.05 units/ml neuraminidase (open symbols) in sodium acetate buffer, pH 5.5, or buffer alone (closed symbols). "H-Labeled *M. pneumoniae* binding was determined as described under "Experimental Procedures."
but, at concentrations more than 10-fold higher than the ID₉₀ for inhibiting binding to laminin, 40% of the control adhesion remained. In the same experiment, dextran sulfate also gave partial inhibition of M. pneumoniae adhesion (Fig. 3). However, when the two inhibitors were combined, adhesion was inhibited by 90%. In two additional experiments, the relative inhibition by the two separate inhibitors varied, but more than 90% inhibition was reproducible obtained with both inhibitors. Thus, both binding specificities probably participate in adhesion to WiDr cells, and almost complete inhibition of M. pneumoniae adhesion to these cells can be achieved using a combination of inhibitors for both binding mechanisms.

**DISCUSSION**

The adhesive glycoproteins laminin and thrombospondin and several other glycoproteins when adsorbed on plastic strongly promote adhesion of M. pneumoniae (Table I and Fig. 1). The adhesive activities of all these proteins require terminal sialic acid on their oligosaccharides and is lost after neuraminidase treatment (Fig. 2). All or most of the sialic acid on these proteins is linked α-2-3 to galactose (22–25, 41). All sialic acid in human plasma fibronectin (22) and fibrinogen (20) and except for a minor triantennary oligosaccharide in transferrin (30) is linked α-2-6 and no binding of M. pneumoniae was detected. Thus, the specific requirement for α-2-3-linked sialic acid for binding to purified glycoproteins is in agreement with previous results for adhesion of erythrocytes to M. pneumoniae (4, 5).

Whereas the oligosaccharide structures on some of the active glycoproteins are heterogeneous or only partially characterized, the α-subunit of hCG contains only one monoa-ntenary and one biantennary asparagine-linked oligosaccharide (22, 26) and is as active as the other glycoproteins with more complicated oligosaccharide structures (Table I). Therefore, the minimum structure for adhesion of M. pneumoniae (Fig. 4) is probably a simple biantennary oligosaccharide with sialic acid-linked α-2-3 to the terminal galactoses. It cannot be determined from the present data whether the monoa-ntenary sialyloligosaccharide on the α-subunit of hCG binds to the M. pneumoniae adhesin with high affinity. Strong inhibition by sialyl biantennary oligosaccharides from fetuin but not the triantennary or O-linked oligosaccharides (Table II) suggests that this is the preferred structure of M. pneumoniae binding. However, the disialyl-biantennary oligosaccharides of fetuin contain a mixture of isomers, some of which may contain both α-2-3- and α-2-6-linked sialic acid (24, 31). Further studies will be required to determine the relative affinity of each isomer. Biantennary oligosaccharides are common structures that are probably present on the surface glycoproteins of many cells and could account for the broad range of cell types that show sialic acid-dependent M. pneumoniae binding (1–6).

The binding activity of N-deglycosylated fetuin (Table II) suggests that the O-linked sialyloligosaccharides may also contribute to binding. Although the O-linked tri- and tetra- saccharides and N-linked triantennary oligosaccharides of fetuin are relatively weaker inhibitors of M. pneumoniae binding than is the biantennary oligosaccharide (Table II), when present at high density they may both mediate avid attachment by low affinity binding to multiple adhesin molecules on these microorganisms.

The same terminal sequence that is found on the aspara-gine-linked oligosaccharides of the glycoproteins that bind M. pneumoniae, Siaα2-3Galβ1-4GlcNAcβ1-2Man1-6, occurs on glycolipids yet does not support binding of M. pneumoniae when the glycolipids are immobilized on this layer plates or in a phosphatidylcholine/cholesterol monolayer on plastic (9). The orientation of the sequence may be different in glycolipids so that it is not recognized or is sterically inhibited from binding by M. pneumoniae, or additional sugar residues such as the manose found only on the glycoproteins may be required for high avidity binding.

Loomes and co-workers (4, 5) proposed that polylactosa-mine sequences are required for M. pneumoniae binding. Of the most active proteins, laminin has polylactosamine sequences (32, 33), but fetuin (23, 25), thrombospondin, and hCG do not. Furthermore, human α1-acid glycoprotein has polylactosamine sequences (34), but they are uncommon in glycoporin (35) and neither protein binds M. pneumoniae well. This contrasts with the finding that the latter two proteins inhibit erythrocyte adhesion to M. pneumoniae better than fetuin (4). Glycoporin contains hydrophobic regions, however, and inhibition of sialic acid-dependent erythrocyte binding and invasion by merozoites of Plasmodium falciparum malaria results from a toxicity of the hydrophobic peptide of glycoporin (36). A similar toxicity may account for inhibition.

**FIG. 3. Inhibition of M. pneumoniae adhesion to the human adenocarcinoma WiDr cell line.** Adhesion of 3H-labeled M. pneumoniae to WiDr cells growing on 13-mm glass coverslips was determined as described under "Experimental Procedures." Inhibition by dextran sulfate or 3'-sialyllactose at the indicated concentrations or by a combination of 100 μg/ml dextran sulfate and 5 mM 3'-sialyllactose (D.S.) was calculated relative to control binding determined in RPMI-BSA without inhibitors. Results are presented as percent inhibition (mean ± S.D., n = 4 with n = 8 for determination of control binding without inhibitors).

**FIG. 4. Structures of sialylated oligosaccharides on the α-subunit of human chorionic gonadotropin (23) proposed to mediate M. pneumoniae adhesion.** The biantennary oligosaccharide is the minimal structure required for binding based on the present results. It is not known whether the monoa-ntenary oligosaccharide can bind M. pneumoniae with high affinity.

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2 Sialic acid on asparagine-linked oligosaccharides of mouse laminin is linked α-2-3 to galactose (R. Knibbs, F. Ferimi, and I. Goldstein, personal communication).
of M. pneumoniae adhesion, as a "receptor" for glycoprophin was isolated from M. pneumoniae membranes, but its binding was inhibited as well by a hydrophobic peptide of glycoprophin which lacks carbohydrate as by the intact glycoprotein (37).

The ability of fetuin to bind M. pneumoniae was isolated from calf serum (38) which is a component of the growth medium preincubated in medium containing fetal calf serum is specific to coverslips preincubated in Tris-BSA. Whether the increased "specific" sialic acid-dependent binding of M. pneumoniae to WiDr cells shown in Fig. 3 is due to sialylated glycoproteins produced by these cells or more efficient adsorption of fetuin from the growth medium onto the cell surface than on the glass coverslip remains to be determined. Specific adsorption of fetuin was observed onto the surface of a differentiated leukemia cell line (24).

Inhibition studies (Table II and Ref. 9) indicate that M. pneumoniae has two distinct adhesins that recognize sulfated glycolipids and o2-3-linked sialyl oligosaccharides on glycoproteins, respectively. Based on the complete dependence on erythrocyte sialyloligosaccharides for binding (5), only the latter receptor is required for binding erythrocytes. Inhibition of M. pneumoniae adhesion to cultured cell lines by an inhibitor of sulfatide binding (9) or following neuraminidase treatment (39, 40), however, is usually incomplete. As shown in Fig. 3, the effects of 3'-sialyllactose and dextran sulfate are additive, and nearly complete inhibition is obtained with both inhibitors, suggesting that both types of carbohydrates are utilized by M. pneumoniae to adhere to these cells in vitro. Based on these results, it is unlikely that agents inhibiting binding to either carbohydrate receptor could prevent infection by blocking adhesion to host tracheal epithelium which contains both sulfated glycoproteins and sulfatides (9), but a combination of the two types of inhibitors may prevent infection by M. pneumoniae.

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