Identification of the O-Linked Sialyloligosaccharides of Glycophorin A as the Erythrocyte Receptors for S-Fimbriated Escherichia coli

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The erythrocyte receptors for S-fimbriated Escherichia coli, which causes sepsis and meningitis in newborn infants, were investigated. Neuraminidase and trypsin treatments of erythrocytes abolished the hemagglutination ability of the bacteria. To identify the receptor glycoproteins, we separated the receptor membrane proteins by gel electrophoresis, bled them to nitrocellulose, and incubated them with 125I-labeled bacteria. The only bacterium-binding bands identified corresponded to glycophorin A dimer and monomer, and the binding was abolished by neuraminidase treatment of the blot. Radiolabeled bacteria also bound to purified glycophorin A adsorbed to polyvinyl chloride microswells, and the binding was inhibited by other sialoglycoproteins and isolated sialyloligosaccharides containing the NeuAcα2-3Gal sequence. Oligosaccharides which contain the NeuAcα2-3Galβ1-3GalNAc and NeuAcα2-3Galβ1-3GalNAc sequence and which are identical to the O-linked saccharides of glycophorin A were twofold more effective inhibitors of binding than were other oligosaccharides containing the NeuAcα2-3Gal sequence. The replacement of sialic acid in asialoerythrocytes with a purified Galβ1-3GalNAc α2-3 sialyltransferase, which forms the O-linked NeuAcα2-3Galβ1-3GalNAc sequence in asialoerythrocytes, restored bacterial hemagglutination. These results indicated that the major erythrocyte receptor for S-fimbriated E. coli is the NeuAcα2-3Galβ1-3GalNAc sequence of the O-linked oligosaccharide chains of glycophorin A.

The ability of bacteria to adhere to host epithelial cells is regarded as a prerequisite for the establishment of infectious diseases (2) such as pyelonephritis (34) and diarrhea (11) caused by Escherichia coli. The adhesion of E. coli and other gram-negative bacteria takes place through the binding of bacterial fimbriae to specific receptors on the host cell surface (2, 15). Most of the few bacterial receptors identified at the molecular level are carbohydrates. Carbohydrate structures considered to be cell surface binding sites for E. coli include the Galα1-4Gal sequence of P blood group antigens (14, 20), α-mannosides (25), and terminal N-acetylgalacosamine residues (38). Although there is evidence for the glycoprotein nature of the receptors for mannose-binding E. coli (7) and for Mycoplasma pneumoniae (22), all the carbohydrate receptors for bacteria ascribed to single molecular species have been glycolipids (13, 14, 20).

We have recently shown that several E. coli strains bind to sialic acid-containing structures on human erythrocytes (28). Hemagglutination inhibition studies with isolated sialyloligosaccharides indicated that this binding activity has specificity for the NeuAcα2-3Gal sequence. The bacterial components mediating the binding to sialylgalactosides were identified as a novel type of fimbriae, designated as S fimbriae (16). S fimbriae were recently shown to be associated with E. coli serotype O18:K1:H7, which causes meningitis and septicaemia in newborn infants, suggesting that S fimbriae may have a pathogenic role in these infections (17). However, the molecular nature of the host cell sialoglycoconjugates recognized by S fimbriae is not known.

To identify the pathophysiological receptors for S fimbriae, knowledge of the molecular nature of these receptors and methods of investigation are needed. In the present study, the erythrocyte receptor for S-fimbriated E. coli was identified, by a novel application of the protein blot overlay method, as glycophorin A, the MN sialoglycoprotein. The bacterium-binding site in glycophorin A was found to be the O-linked NeuAcα2-3Galβ1-3GalNAc sequence.

MATERIALS AND METHODS

Abbreviations. The abbreviations used in this paper are as follows: Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine; GlcNAc, N-acetyl-D-glucosamine; NeuAc, N-acetylneuraminic acid; Pi/NaCl, 10 mM sodium phosphate–0.15 M NaCl (pH 7.4).

Materials. Trypsin (type XIII, tolylsulfonyl phenylalanine chloromethyl ketone treated), fetuin (type IV), and methyl α-d-mannose were from Sigma Chemical Co., St. Louis, Mo. Endo-β-galactosidase was from Seikagaku Kogyo, Tokyo, Japan, and wheat germ agglutinin was from Pharmacia, Uppsala, Sweden. Iodo-Beads were from Pierce Chemical Co., Rockford, Ill., and polyvinylpyrrolidone K 30 was from Fluka, Buchs, Switzerland. Titerette flat-bottomed polyvinyl chloride immunosassay microplates (highly activated) were from Flow Laboratories, Ayrshire, Scotland. Nitrocellulose membrane (type HAWP; pore size, 0.45 μm) was from Millipore Corp., Molsheim, France. XAR-5 X-ray film was from Eastman Kodak Co., Rochester, N.Y. α1-Acid glycoprotein was kindly provided by G. Myllylä (Finnish Red Cross Blood Transfusion Service). Sialyloligosaccharides were purified from human urine (26, 27) and bovine colostrum (28) as described before. NeuAcα2-3Galβ1-3GalNAc was liberated from the corresponding 1-phosphate (27) by phosphatase treatment, followed by purification on a
column of AG 1-X2 (26). All sialyloligosaccharides and other compounds used for bacterium-binding inhibition studies were dissolved in Pi/NaCl adjusted to pH 7.4. Glycoporphins A^M^ and A^N^ were isolated from human MM and NN blood group erythrocytes by previously published procedures (3, 10).

**Bacteria.** From previously characterized sialylgalactoside-binding, S-fimbriated E. coli strains (16, 17, 28), IH3037 (serotype O18:K1:H7) and IH11054 (serotype O2:K1) were selected for the present study. Both also express mannoselbinding, type 1 fimbriae. The strains were stored in nutrient agar at 4°C and subcultured on colonization factor antigen agar (5). Since the expression of S fimbiae shows phase variation, an S-fimbriated bacterial population was selected by adsorption to erythrocytes (24). Briefly, 1 ml of Pi/NaCl containing about 10^{10} bacteria and 50 μl of packed human erythrocytes was incubated on an ice bath for 15 min. The erythrocytes were pelleted by centrifugation, washed twice with 2 ml of Pi/NaCl, lysed with 0.2 ml of distilled water, and plated on colonization factor antigen agar.

**Labeling of bacteria.** Erythrocytes were labeled with 125I by using N-chloro-benzenesulfonamide-derivatized poly styrene beads (Iodo-Beads) (23). The reaction was carried out in 0.3 ml of 0.1 M sodium phosphate buffer (pH 7.0) containing 6 × 10^9 bacteria and 0.5 mM of carrier-free Na^125I with one washed Iodo-Bead for 15 min at 4°C. After removal of the Iodo-Bead and the addition of 20 μl of 0.5 M NaI, the reaction mixture was layered on top of 2 ml of Pi/NaCl containing 6% (wt/vol) bovine serum albumin, and the bacteria were pelleted by centrifugation (15 min, 1,200 × g) in the cold. The radioactivity incorporated was 0.05 to 0.1 cpmp per bacterium. Labeled bacteria were used immediately or stored frozen at −70°C in Pi/NaCl containing 10% (vol/vol) glycerol.

**Hemagglutination tests.** Hemagglutination tests were carried out in 50 μl of Pi/NaCl containing 0.1 M methyl α-D-mannose with 2% human erythrocytes on glass slides over crushed ice (28). Hemagglutination titers were expressed as the reciprocal of the lowest dilution of bacteria causing hemagglutination after 5 min.

**Enzyme treatments of erythrocytes.** Trypsin treatment of erythrocytes was carried out as described before (4) with trypsin concentrations of 0.2 and 1.0 mg/ml. Endo-β-galactosidase treatment (39) and NaIO_4 treatment (12) were performed as previously described. Neuraminidase treatment of erythrocytes and subsequent resialylation with purified sialyltransferases were carried out as described before (31).

**Protein blot overlay studies.** Erythrocyte membranes were prepared as described before (6). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in slab gels (9% acrylamide, 1.5 mm thick) as described by Laemmli (18). After electrophoresis the gels were equilibrated for 30 min in 25 mM Tris–192 mM glycine buffer containing 20% (vol/vol) methanol, and the proteins were transferred from the gels to a nitrocellulose sheet (37) in the same buffer by using an electric current of 140 mA for 16 h in the cold. The nitrocellulose sheet was washed with Pi/NaCl and stored frozen at −20°C. Proteins were stained with amido black (37). Since glycopharin A did not stain, it was visualized by being overlaid with 125I-labeled wheat germ agglutinin after incubation of the blot in 2% (wt/vol) polyvinylpyrrolidone (6).

For bacterium-binding studies, the nitrocellulose sheet was cut into strips and incubated in Pi/NaCl containing 3% (wt/vol) bovine serum albumin at 37°C for 1 h. The strips were overlaid with radiolabeled bacteria (10^6 cpm/ml) in Pi/NaCl containing 3% (wt/vol) bovine serum albumin and 0.1 M methyl α-D-mannose (1 ml for a 1- by 12-cm strip) and incubated without shaking in a humid atmosphere at 4°C for 3 h. The strips were washed six times with cold Pi/NaCl for 5 min each time on a tilting table, dried, and exposed to XAR-5 X-ray film by using an intensifying screen at −70°C for 1 to 16 h.

For desialylation experiments, the strips were washed once after albumin treatment with 50 mM sodium acetate buffer (pH 5.5) containing 5 mM CaCl_2 and 0.1% (wt/vol) bovine serum albumin and incubated in the same buffer with or without Vibrio cholerae neuraminidase (50 μU/ml) at 37°C for 16 h. The strips were washed three times with Pi/NaCl for 5 min each time and overlaid with 125I-labeled bacteria as described above.

**Bacterium-binding assay in microwells.** Purified glycoporphin A^M^ or A^N^ was adsorbed to polyvinyl chloride microtiter wells by incubation at various concentrations in 50 μl of Pi/NaCl at 23°C for 2 h. The wells were washed four times with 200 μl of Pi/NaCl and incubated with 200 μl of Pi/NaCl containing 0.1% (wt/vol) bovine serum albumin at 23°C for 30 min. After the wells were emptied, 125I-labeled bacteria (about 10^6 cpm per well) were added in 50 μl of Pi/NaCl containing 0.1% (wt/vol) bovine serum albumin with or without inhibitors and incubated at 4°C for 16 h. The wells were washed six times with 200 μl of cold Pi/NaCl and cut from the plate for radioactivity determination in a gamma scintillation spectrometer.

**RESULTS**

Trypsin and endo-β-galactosidase treatments of erythrocytes. In agreement with earlier results (28), neuraminidase treatment of erythrocytes destroyed the bacterial receptors (Table 1). Mild periodate treatment of erythrocytes, which oxidizes mainly the glyceral side chain of sialic acid (12), also abolished bacterial hemagglutination (Table 1). Trypsin treatment of erythrocytes either markedly decreased bacterial hemagglutination or abolished it, depending on the trypsin concentration (Table 1). Trypsin is known to specifically cleave the N-terminal part of glycopharin A (4). Thus, the trypsin sensitivity of the erythrocyte receptors for S-fimbriated E. coli indicated the proteinaceous nature of these molecules and also suggested that the major receptor was glycopharin A. Bacterial hemagglutination was not affected by endo-β-galactosidase treatment of erythrocytes (Table 1), which is known to cleave the polysialotide chains of band 3, band 4.5, and poly(glycosylv)ceramides (8). This finding indicated that the sialyloligosaccharide termini of the polysialotide chains do not significantly contribute to the binding of S-fimbriated E. coli to the erythrocyte surface.

**Binding of bacteria to glycoprotein receptors on protein blots.** To accomplish more direct identification of the glyco-
FIG. 1. Binding of radiolabeled bacteria to erythrocyte membrane proteins and purified glycophorin A transferred to nitrocellulose. Solubilized erythrocyte membrane proteins (lanes A and B, 200 μg of protein per lane) and glycophorin AM (lanes C and D, 5 μg of protein per lane) were run in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Lane A was visualized by amido black staining, and lane C was visualized by incubation with 125I-labeled wheat germ agglutinin followed by autoradiography. Lanes B and D were incubated with 125I-labeled bacteria and bound bacteria were identified by autoradiography as described in Materials and Methods. The numbering of the bands is according to Steck and Yu (32).

protein receptors of S fimbriae, we used conditions most suitable for the binding of radiolabeled bacteria to glycoprotein receptors on a nitrocellulose membrane. The use of freshly labeled bacteria was crucial for the suppression of nonspecific binding. Polyvinylpyrrolidone and Tween 20 were no more effective at blocking nonspecific binding than was bovine serum albumin. In fact, specific binding was also reduced when Tween 20 was used as the blocking agent. Total erythrocyte membrane proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and overlaid with 125I-labeled bacteria. After the blot was washed, bound bacteria were detected by autoradiography. Using this technique, we identified two bacterium-binding bands with mobilities similar to those of glycophorin A dimer and monomer (Fig. 1). Purified glycophorin A bound the bacteria in a similar fashion (Fig. 1), confirming the identity of the bacterium-binding components. Neuraminidase treatment of the blot before it was overlaid with bacteria abolished bacterial binding both to the whole membranes and to purified glycophorin A, indicating that the binding was mediated by sialic acid. Also, binding was specific for the S-fimbriated bacteria, since preparations enriched in these bacteria but not unfraccionated preparations containing unfimbriated, type 1-fimbriated, and only a minor portion of S-fimbriated bacteria (24) bound to glycophorin A.

Specificity of bacterial binding to glycophorin A. The sialyloligosaccharide sequence recognized by S-fimbriated \textit{E. coli} was studied by comparing the inhibitory effects of isolated sialyloligosaccharides on bacterial binding. To develop a more quantitative bacterial binding assay than hemagglutination, we studied the binding of 125I-labeled bacteria to glycophorin A adsorbed onto microtiter wells. Bacterial binding was found to depend on the concentration of glycophorin A in the coating buffer, with the highest binding occurring at 100 μg of glycophorin A per ml (Fig. 2). The binding was inhibited by the sialoglycoproteins fetuin and α1-acid glycoprotein, as well as by the isolated sialyloligosaccharides (Fig. 3), indicating that the binding was specific. No difference was observed in bacterial binding between glycophorins AM and AN.

In agreement with the results obtained by hemagglutination inhibition (28), oligosaccharides containing the NeuAcα2-3Gal sequence were potent inhibitors of bacterial binding, whereas corresponding oligosaccharides containing the NeuAcα2-6Gal or NeuAcα2-8NeuAcα2-3Gal sequences did not induce inhibition at the concentrations used; free sialic acid or lactose also did not induce inhibition (Table 2). The three different sialyloligosaccharides with the NeuAcα2-3Gal β1-4 sequence were equally good inhibitors, irrespective of

FIG. 2. Binding of radiolabeled bacteria to glycophorin A adsorbed to microwells. Glycophorin AM (●) and bovine serum albumin (○) were adsorbed at the concentrations indicated onto microtiter wells, and the wells were incubated with 125I-labeled bacteria as described in Materials and Methods.

FIG. 3. Inhibition of bacterial binding to glycophorin A. Glycophorin AM was adsorbed at a concentration of 100 μg/ml onto microtiter wells, and the wells were incubated with 125I-labeled bacteria and with inhibitors at different concentrations expressed as sialic acid concentration for the glycoproteins and as oligosaccharide concentration for the oligosaccharides. The inhibitors were as follows: fetuin (●), α1-acid glycoprotein (○), NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc (▲), and NeuAcα2-3Galβ1-4Glc (▲).
whether the subsequent residue was glucose, glucitol, or GlcNAc (Table 2). However, the oligosaccharide with the NeuAcα2-3Galβ1-3GalNAc sequence and its derivative sialylated at C-6 of GalNAc, which are identical to the O-linked sugar chains of glycoporphin A (21, 35), were about twofold better at inhibition than were the other oligosaccharides with the NeuAcα2-3Gal sequence (Table 2). The majority (85 to 90%) of sialic acid incorporated by this enzyme into asialoerythrocytes is found in glycoporphins A and B (29). Thus, these results confirmed that the primary erythrocyte receptor for S-fimbriated E. coli is the O-linked NeuAcα2-3Galβ1-3GalNAc sequence of glycoporphin A.

The ability of bacteria to agglutinate the derivatized erythrocytes was dependent on the amount of sialic acid incorporated, with a threshold limit between 46 and 89 nmol of sialic acid per ml of packed cells (Table 3). It should be noted that the O-linked oligosaccharides occur on the erythrocyte surface at a higher concentration than do the N-linked chains containing the Galβ1-4GlcNAc termini. The incorporation of sialic acid into the latter structures remained lower than the threshold amount for bacterial hemagglutination (Table 3). Thus, these results cannot be used for the comparison of the relative binding activities of the various sialyloligosaccharide sequences.

**DISCUSSION**

Although the role of glycolipids as the host cell receptors for bacterial adhesion has been much emphasized recently (13), the present study shows that protein-bound saccharides also serve as receptors for bacteria. The results presented in this report indicate that the erythrocyte receptor for S-fimbriated E. coli is the MN sialoglycoprotein, glycoporphin A. This conclusion is supported by several lines of evidence. First, bacterial receptors were destroyed by trypsin treatment of erythrocytes, which is known to primarily cleave glycoporphin A (4). Second, following electrophoresis and transfer of erythrocyte membrane proteins to nitrocellulose, only the monomeric and dimeric forms of glycoporphin A were detected by binding of S-fimbriated bacteria. Third, bacteria bound to purified glycoporphin A that was transferred to nitrocellulose or attached to polyvinylchloride microtiter wells. Finally, selective replacement of sialic acid in asialoerythrocytes with Galβ1-3GalNAc α-2-3 sialyltransferase, which incorporates sialic acid mainly into glycoporphins A and B (29), restored bacterial hemagglutination.

**TABLE 2. Inhibitory effect of isolated oligosaccharides on bacterial binding to glycoporphin A**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Conc (mM) causing 50% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>NeuAcα2-3Galβ1-4Glc</td>
<td>4.9</td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-4GlcOβ1</td>
<td>5.0</td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-4GlcNAc</td>
<td>4.8</td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc</td>
<td>2.3</td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-3GalNAc</td>
<td>2.3</td>
</tr>
<tr>
<td>NeuAcα2-6Galβ1-4Glc</td>
<td>&gt;10'</td>
</tr>
<tr>
<td>NeuAcα2-6Galβ1-4GlcNAc</td>
<td>&gt;10'</td>
</tr>
<tr>
<td>NeuAcα2-8NeuAcα2-3Galβ1-4Glc</td>
<td>&gt;10'</td>
</tr>
<tr>
<td>Galβ1-4Glc</td>
<td>&gt;50'</td>
</tr>
</tbody>
</table>

* Microtiter wells were coated with glycoporphin A and incubated with radiolabeled bacteria and different concentrations of inhibitors as described in the legend to Fig. 3. Inhibitor concentrations causing 50% inhibition were obtained from the corresponding inhibition curves.

**TABLE 3. Restoration of bacterial hemagglutination of neuraminidase-treated erythrocytes by resialylation**

<table>
<thead>
<tr>
<th>Erythrocytes</th>
<th>NeuAc incorporated* (mmol/ml of cells)</th>
<th>Agglutination titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Neuaminidase treated</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Resialylated* to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-4(GlcNAc</td>
<td>36 0</td>
<td></td>
</tr>
<tr>
<td>NeuAcα2-6Galβ1-4GlcNAc</td>
<td>64 0</td>
<td></td>
</tr>
<tr>
<td>Galβ1-3(NeuAcα2-6)GalNAc</td>
<td>77 0</td>
<td></td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-3GalNAc</td>
<td>240 16</td>
<td></td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-3GalNAc</td>
<td>110 8</td>
<td></td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-3GalNAc</td>
<td>89 8</td>
<td></td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-3GalNAc</td>
<td>46 0</td>
<td></td>
</tr>
</tbody>
</table>

* Native erythrocytes contained about 750 nmol of NeuAc per ml of packed cells. Treatment with V. cholerae neuraminidase removed at least 90% of the total sialic acid.

* Neuaminidase-treated cells were resialylated with CMP-NeuAc and purified sialyltransferases (31) to produce the structures shown.

Glycoporphin A is one of the best-characterized membrane proteins. Its amino acid sequence has been determined (36), and it is known to contain an average of 15 O-linked sugar chains (36) and 1 N-linked chain of the complex biantennary type (40). The structure of most of the O-linked chains has been identified as NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc (21, 35). The binding site for S-fimbriated E. coli in glycoporphin A is the NeuAc α2-3Galβ1-3GalNAc sequence of the O-linked chains, as revealed by the following two findings. First, Galβ1-3GalNAcα2-3 sialyltransferase, which forms the O-linked NeuAcα2-3Galβ1-3GalNAc sequence in asialoglycoporphins (29) restored the hemagglutination ability of the bacteria. Second, isolated sialyloligosaccharides containing this sugar chain were potent inhibitors of bacterial binding to glycoporphin A, whereas oligosaccharides which only shared the NeuAcα2-3Gal sequence with this structure were somewhat less effective. The α2-6-bound sialic acid residue in the O-linked chains does not seem to contribute to bacterial binding, since the free oligosaccharides containing the NeuAcα2-3Galβ1-3GalNAc and NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc sequences were equally effective inhibitors. This result also suggests that the GalNAc residue is recognized only partially by the bacteria, since its substitution at the C-6 position did not affect the inhibitory effect of the oligosaccharides. On the other hand, the importance of the terminal α2-3-linked sialic acid moiety in bacterial binding is indicated by the findings that the removal of terminal sialic acid by neuraminidase treatment and even the removal of its glycerol side chain by mild periodate treatment totally abolished bacterial hemagglutination.
S-fimbriated *E. coli* apparently requires a relatively high density of the appropriate sialyloligosaccharides for optimal binding. This is demonstrated by the finding that agglutination of erythrocytes resialylated to contain the NeuAcα2-3Galβ1-3GalNAc sequence exhibited a threshold limit between 46 and 89 nmol of sialic acid per ml of packed cells. In contrast, influenza viruses with similar receptor specificity (31) agglutinate erythrocytes with as little as 20 nmol of sialic acid per ml of packed cells (G. N. Rogers and J. C. Paulson, unpublished results). The polyvalent nature of the S-fimbriated *E. coli* receptor is further illustrated by the finding that the highly sialylated glycoproteins fetuin and α1-acid glycoprotein were about 30-fold more effective at inhibiting bacterial binding to glycoporin A than were the free sialyoligosaccharides (Fig. 3). In this respect, S fimbriae are similar to other lectins, such as the galactose-specific lectin of mammalian liver, for which it has been shown that the ability of glycoproteins to bind to the lectin increases exponentially as the number of appropriate sugars attached to the protein increases (33).

The minor sialoglycoproteins of the erythrocyte membrane (glycoporphins B and C) appear to carry O-linked sialyoligosaccharides similar to those of glycoporphin A (9). Our experiments with erythrocyte membranes (Fig. 1) did not reveal bacterial binding to these minor sialoglycoproteins, a result which may have been due to their lower concentrations in the erythrocyte membrane compared with that of glycoporphin A (9). The fact that α1-acid glycoprotein, fetuin, and isolated sialyoligosaccharides were potent inhibitors of bacterial binding to glycoporphin A (Fig. 3) suggests that no protein-dependent determinants are recognized by S-fimbriated *E. coli*. Thus, protein-bound sialyoligosaccharides could also serve as binding sites for these bacteria on other cells. Our recent finding that S fimbriae bind to human vascular endothelium (T. K. Korhonen, J. Parkkinen, Jörg Hacker, J. Finne, A. Pere, M. Rhen, and H. Holthöfer, manuscript in preparation) suggests that the biological function of S fimbriae might be to mediate bacterial adhesion to vascular endothelium and possibly to facilitate their spread to the cerebrospinal fluid.

The NeuAcα2-3Galβ1-3GalNAc sequence also occurs in gangliosides of the ganglio series, which are especially abundant in the brain (19). However, no binding of radiolabeled S-fimbriated *E. coli* to thin-layer chromatograms of these gangliosides (13) could be detected (J. Parkkinen and J. Finne, unpublished results). This result suggests that the O-linked sialyoligosaccharides of glycoporphin A are more accessible to bacteria than are the sialyloligosaccharide chains of gangliosides, at least in the in vitro binding assays used.

Taken together, our results indicate that on the erythrocyte surface, S-fimbriated *E. coli* binds to the NeuAcα2-3Galβ1-3GalNAc sequence of the O-linked saccharides of the MN sialoglycoprotein glycoporphin A. The novel method of overlaying protein blots with radiolabeled bacteria should be a useful general method for the identification of glycoprotein receptors for bacteria in different cells and tissues. The pathogenic role of S fimbriae in neonatal meningitis caused by *E. coli* and the molecular nature of the receptors on possible target tissues are currently under study.

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**LITERATURE CITED**


