INFECTION AND IMMUNITY, Sept. 1993, p. 3673–3677
0019-9567/93/093673-05$02.00/0

Vol. 61, No. 9

The K88 Fimbrial Adhesin of Enterotoxigenic *Escherichia coli* Binds to β1-Linked Galactosyl Residues in Glycosphingolipids

DEAN PAYNE,[1]* MARK O'REILLY,[2] AND DIANE WILLIAMSON[1]

Chemical and Biological Defence Establishment, Porton Down, Salisbury, Wiltshire SP4 OJQ,[1] and Department of Microbiology, St. Andrews University, St. Andrews, Fife,[2] United Kingdom

Received 16 February 1993/Returned for modification 6 May 1993/Accepted 10 June 1993

The K88 fimbrial adhesin enables certain strains of enterotoxigenic *Escherichia coli* to adhere to the porcine small intestine. In this study, the ability of the K88 adhesin to bind to glycosphingolipids was monitored by modified enzyme-linked immunosorbent assay and thin-layer chromatography overlay binding analysis. The binding of the K88 adhesin to glycosphingolipid-coated microtiter plates was saturable, with 50% maximal binding occurring with gangliotriaosylceramide, gangliotetraosylceramide, and lactosylceramide at 67 ± 21, 117 ± 21, and 73 ± 22 pM, respectively. Thin-layer chromatography overlay binding analysis demonstrated that serotype O8:K87:K88ab:H19 *E. coli* bound to hydroxylated galactosylceramide, gangliotriaosylceramide, gangliotetraosylceramide, and lactosylceramide but not to globotriaosylceramide, nonhydroxylated galactosylceramide, glucosides, glucosylceramide, or a mixture of ceramides. The K88 adhesin did not bind by either assay to globoside, the Forssman glycolipid, GM1, GM2, GM3, GD1a, GD1b, GD2, GD3, GQ1b, or GT1b. The binding pattern observed with the K88 adhesin suggests that β1-linked galactosyl residues are the minimum determinant required for binding, provided they are presented correctly. It is suggested that β1-linked galactose residues may form the molecular basis of both glycoprotein and glycolipid receptors for the K88 fimbrial adhesin in the porcine small intestine.

Enterotoxigenic *Escherichia coli* (ETEC) bearing K88 fimbriae is associated with diarrhea in both neonatal and postweaning pigs (24). Disease is thought to be facilitated by the ability of K88 fimbriae to adhere to the porcine small intestine. Subsequent colonization and release of enterotoxins result in the clinical symptoms associated with ETEC infection (18).

Several studies have investigated the molecular interaction of the K88 fimbrial adhesin with erythrocytes from various species and also porcine intestinal mucus and/or brush borders (5, 13, 17, 19, 23). Early work was based on the ability of the K88 adhesin to agglutinate guinea pig erythrocytes, which was used as a model of intestinal binding by K88$^+$ ETEC. By selectively cleaving sugar residues from terminal positions of glycoproteins which inhibited K88-mediated hemagglutination, it was found that β-D-galactosyl residues were important for binding (5). Nilsson and Svensson (15) found that oligosaccharides containing galactosyl residues that were obtained from glycolipid fractions of the pig small intestine also inhibited the agglutination of guinea pig erythrocytes by K88. More-recent work has demonstrated that ETEC expressing the K88 adhesin binds specifically to glycoproteins present in both murine and porcine mucus (11, 13, 23). Expression of the K88 receptor(s) in mucus is age related, with greater amounts detected in the intestines of older piglets. However, expression of the K88 receptor(s) on porcine brush borders seems age independent (3, 23).

The present study investigates the ability of the K88 adhesin to bind to a range of glycolipid standards. Glycolipids have been demonstrated to act as receptor molecules for a number of adhesins and toxins (4, 8). In most, if not all, cases identified, the oligosaccharide portion of glycolipids has been found to predominantly control the specificity of the ligand-receptor interaction (4, 8). Unlike glycoproteins, glycolipids contain only one oligosaccharide chain, allowing specific sequences recognized by the ligand to be deduced (2).

MATERIALS AND METHODS

Bacteria. *E. coli* serotype O8:K87:K88ab:H19 was kindly supplied by R. Sellwood (Agricultural and Food Research Council, Institute of Animal Health, Compton, Newbury, Berkshire, United Kingdom). Bacteria were grown unshaken in nutrient broth (Oxoid Ltd., Basingstoke, United Kingdom) for 16 h at 37°C to maximize expression of K88 (16).

Chemicals and reagents. All chemicals used were of Analar grade or equivalent. Abbreviations for gangliosides follow the nomenclature system of Svennerholm (21). Purified glycosphingolipids and enzymes were obtained from Sigma Chemical Company (Poole, United Kingdom), except for GQ1b, which was purchased from Calbiochem Novabiochem (Nottingham, United Kingdom).

Preparation of purified K88 antigen. The K88ab antigen was heat extracted from *E. coli* serotype O8:K87:K88ab:H19 and then subjected to ammonium sulfate and isoelectric precipitation (14, 20).

ELISA-based assay for monitoring glycolipid binding. The enzyme-linked immunosorbent assay (ELISA) was based on the method of Karlsson and Stromberg (9). Briefly, glycolipids (0.1 mg/ml) were suspended in methanol, 50 μl of the suspension was added to each well of a 96-well microtiter plate (Dynatech Immulon 2), and the methanol was allowed to evaporate overnight at room temperature. The wells were blocked with 2% bovine albumin in phosphate-buffered

* Corresponding author.

TABLE 1. Abilities of various glycolipids to recognize the K88ab adhesin

Structure[a]	Designation	Binding	
		TLC overlay	ELISA[b]
Ceramide	Cer	–	NA[c]
Galβ1-1Cer (hydroxylated fatty acids)	GalCer1	+	NA
Galβ1-1Cer (nonhydroxylated fatty acids)	GalCer2	–	NA
Glcβ1-1Cer	Glucosylceramide	–	NA
Galβ1-4Glcβ1-1Cer	Lactosylceramide (LC)	+	+
GalNAcβ1-4Galβ1-4Glcβ1-1Cer	Gangliotriaosylceramide (GM2A)	+	+
Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer	Gangliotetraosylceramide (GM1A)	+	+
Galα1-4Galβ1-4Glcβ1-1Cer	Globotriaosylceramide	–	–
GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer	Globotetraosylceramide (globoside)	–	–
GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer	Globopentaosylceramide (Forssman glycolipid)	–	–
Galβ1-3GalNAcβ1-4Gal(3←2NeuAc)β1-4Glcβ1-1Cer	GM1	–	–
GalNAcβ1-4Gal(3←2NeuAc)β1-4Glcβ1-1Cer	GM2	–	–
NeuAcα2-3Galβ1-4Glcβ1-1Cer	GM3	–	–
NeuAcα2-3Galβ1-3GalNAcβ1-4Gal(3←2αNeuAc)β1-4Glcβ1-1Cer	GD1a	–	–
Galβ1-3GalNAcβ1-4Gal(3←2αNeuAc8←2αNeuAc)β1-4Glcβ1-1Cer	GD1b	–	–
GalNAcβ1-4Gal(3←2NeuAc8←2αNeuAc)β1-4Glcβ1-1Cer	GD2	–	–
NeuAcα2-8NeuAcα2-3Galβ1-4Glcβ1-1Cer	GD3	–	–
NeuAcα2-8NeuAcα2-3Galβ1-3GalNAcβ1-4Gal(3←2NeuAc8←2αNeuAc)β1-4Glcβ1-1Cer	GQ1b	–	–
NeuAcα2-3Galβ1-3GalNAcβ1-4Gal(3←2αNeuAc8←2αNeuAc)β1-4Glcβ1-1Cer	GT1b	–	–

[a] Galβ1-linked residues are proposed as the minimum receptor structure for the K88 adhesin.
[b] Typical positive results (+) indicated an absorbance of 1.00 after 30 min at room temperature in the presence of the chromogenic substrate. Negative results (–) indicated absorbances of <0.1 and controls (no lipid) had values of <0.02 under the same conditions.
[c] NA, not applicable.

saline (PBS) for 2 h at room temperature and then washed twice with the same buffer. Purified stock K88 antigen was diluted in PBS–1% skim milk powder (BLOTTO) to a concentration of 10 μg/ml, from which doubling dilutions were prepared in the same buffer. A 100-μl volume of the dilution was then added to each microtiter plate well, and the plate was incubated at 37°C for 1 h. After a wash in 0.02% Tween 20–PBS, bound K88 was detected with rabbit anti-K88 serum in combination with a goat anti-rabbit horseradish peroxidase conjugate and with 2,2-azinobis-3-ethylbenthiazoline sulfonic acid as a chromogenic substrate (16).

Treatment of purified glycolipids with neuraminidase. Nine glycolipids were treated with *Clostridium perfringens* type 5 neuraminidase. Glycolipids were coated onto the wells of a 96-well microtiter plate as described above. Neuraminidase (0.2 U) in acetate buffer (50 mM sodium acetate, 150 mM NaCl, 9 mM $CaCl_2$ [pH 5.5]) was added, and the plate was incubated at 37°C for 2 h. The plates were washed three times in PBS and then blocked and processed as described above.

Enzymatic treatment of the gangliotetraosylceramide (GM1A). Purified GM1A (Sigma) was sequentially treated with bovine β-galactosidase and then *N*-acetylgalactosaminidase. GM1A was suspended in methanol to a final concentration of 0.1 mg/ml and added in 50-μl volumes to the wells of a 96-well microtiter plate. After evaporation of the methanol, 0.2 U of β-galactosidase in Tris buffer [10 mM Tris, 1.7 M $(NH_4)_2SO_4$, 10 mM $MgCl_2$; pH 7.3] was added to each well, and the plate was incubated for 1 h at 37°C. The plate was washed three times in PBS, and the attached glycolipids were treated with 0.0073 U of *N*-acetylgalactosaminidase in citrate-phosphate buffer (5 mM sodium citrate, 100 mM KH_2PO_4 [pH 3.7]) for 1 h at 37°C. The plate was again washed three times with PBS and then blocked for 2 h at room temperature with 100 μl of 2% bovine albumin in PBS per well. The plate was washed twice with bovine albumin-PBS buffer, and 100 μl of purified K88ab was added (10 μg/ml in PBS-BLOTTO). After incubation, bound K88 was detected as described above.

Thin-layer chromatography (TLC). TLC was performed by the method of Karlsson and Stromberg (9). Glycolipids were developed on aluminum sheets coated with silica G60 (Merck Ltd., Leicestershire, United Kingdom) with chloroform-methanol-distilled water (60:35:8 [vol/vol/vol]). Separated glycolipids and/or glucosides were visualized after being sprayed with *p*-anisaldehyde–sulfuric acid–ethanol (5:5:90 [vol/vol/vol]) and heated at 110°C for 10 min.

TLC immunostaining. Separated glycolipids and glucosides able to bind the K88 adhesin were detected by immunostaining. After development, TLC plates were immersed in 1% bovine albumin–1% polyvinylpyrrolidone in acetate-buffered saline (ABS) (0.1 M sodium acetate, 0.2% $CaCl_2$ [pH 7.2]). The TLC plate was washed twice by immersion with 3% polyvinylpyrrolidone in ABS before being incubated in a suspension of *E. coli* serotype O8:K87:K88ab:H19 (2×10^9 cells per ml in ABS) for 2 h at 37°C. Unbound bacteria were removed by washing the plate three times in ABS. Bound bacteria were visualized with rabbit anti-K88 serum and peroxidase-conjugated goat anti-rabbit immunoglobulin G (Sigma) with 4-chloro-1-naphthol as a chromogenic substrate (22).

Statistics. Where indicated, the means of samples were compared by Student's *t* test.

RESULTS

ELISA for monitoring glycolipid binding. The ability of purified K88ab to bind to 14 glycolipids was determined by the solid-phase ELISA (Table 1). Of those tested, only lactosylceramide (LC), gangliotriaosylceramide (GM2A),

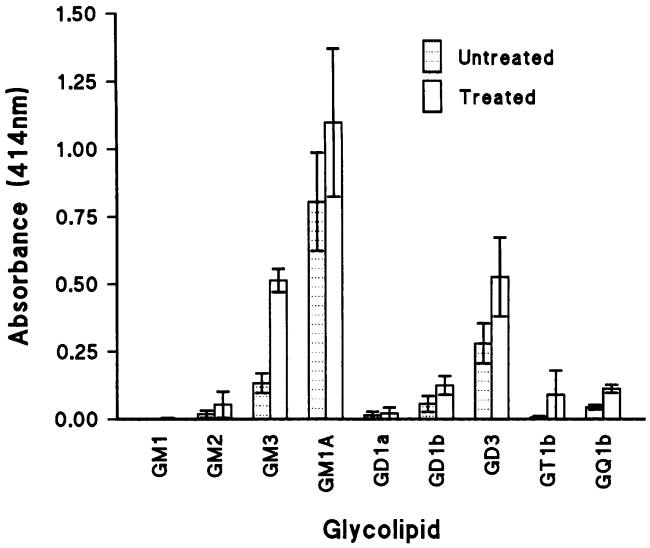

FIG. 1. Glycolipids were treated with *C. perfringens* type 5 neuraminidase to remove sialic acid residues and then assayed for their ability to bind purified K88ab adhesin. Significant ($P < 0.005$) binding of K88ab adhesin due to the generation of LC was noted after treatment of GM3 with neuraminidase. Removal of sialic acid residues from the remaining gangliosides would generate either GM1A or GM2A, both of which bind the K88 adhesin; however, it is likely that these gangliosides are resistant to the action of the neuraminidase used. The results shown are the means ± standard errors for two experiments.

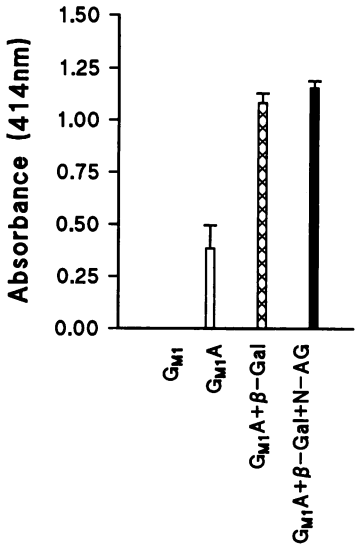

FIG. 2. Gangliotetraosylceramide (GM1A) was sequentially treated with bovine β-galactosidase (β-Gal) and then *N*-acetylgalactosaminidase (N-AG). The ability of purified K88ab to bind to the products of enzymatic treatment, i.e., gangliotriaosylceramide (GM2A) initially and LC finally, was determined by ELISA and compared with the binding of K88ab to GM1 and GM1A. The results shown are the means ± standard errors for either GM1 and GM1A (duplicates) or enzyme-treated GM1A (quadruplicates).

and gangliotetraosylceramide (GM1A) bound substantial amounts of K88ab. All glycolipids containing sialic acid residues (gangliosides) either bound purified K88ab poorly or were unable to bind it at all. In order to assess the affinity of the K88-glycolipid interaction, dilutions of K88 were prepared and the assay was performed (7). The binding of K88 to glycolipid-coated microtiter plates was found to be saturable, with 50% maximal binding occurring at 117 ± 21 (GM1A), 67 ± 21 (GM2A), and 73 ± 22 (LC) pM. Because of their insolubility in methanol, it was not possible to screen ceramide, glucosylceramide, hydroxylated galactosylceramide (GalCer1), or nonhydroxylated galactosylceramide (GalCer2) for the ability to bind purified K88ab. Attempts to use K88-expressing organisms in the ELISA were unsuccessful.

Enzymatic treatment of glycolipids. Treatment of several of the gangliosides with neuraminidase resulted in increased binding by K88ab (Fig. 1). However, only the treatment of GM3 with neuraminidase resulted in a significant ($P < 0.005$) increase. To further confirm the specificity of the binding of K88ab, GM1A was sequentially degraded from its nonreducing end. Galactose and then *N*-acetylgalactosamine residues were removed by the action of β-galactosidase and *N*-acetylgalactosaminidase, respectively. Removal of the terminal galactose residue resulted in an approximately threefold increase ($P < 0.002$) in binding compared with that of GM1A (Fig. 2). However, the subsequent removal of *N*-acetylgalactosamine to form LC did not significantly ($P = 0.17$) alter the observed binding.

Binding of intact organisms to glycolipids by TLC overlay analysis. This assay was used to demonstrate that cell-bound K88 antigen would bind to glycolipids with the same specificity as purified adhesin. *E. coli* serotype O8:K87:K88ab:H19 cells were tested for the ability to bind to 18 glycolipids and a heterogeneous ceramide preparation (Table 1). Globotriaosylceramide, globoside, the Forssman glycolipid, GM1, GM2, GM3, GD1a, GD1b, GD2, GD3, GQ1b, GT1b, GalCer2, glucosylceramide, and a heterogeneous mixture of ceramides did not bind K88$^+$ bacteria. GalCer1, LC, GM1A, and GM2A all bound intact K88$^+$ bacteria (Fig. 3 and 4).

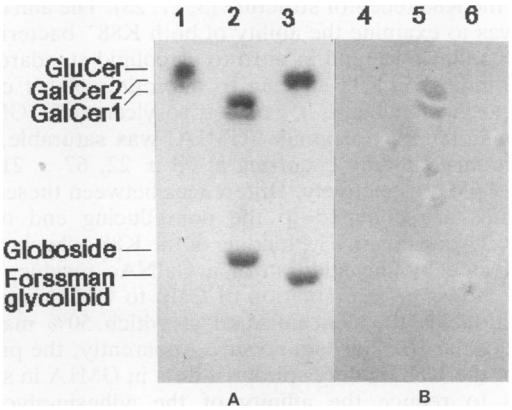

FIG. 3. TLC overlay analysis of the binding of *E. coli* serotype O8:K87:K88ab:H19 to separated glycolipids. Duplicate chromatograms were stained with *p*-anisaldehyde (A) or incubated with *E. coli* serotype O8:K87:K88ab:H19 and detected with anti-K88 serum as described in the text (B). Ten micrograms each of glucosylceramide (GluCer; lanes 1 and 4), both galactosylceramide containing 98% hydroxylated fatty acids (GalCer1) and globoside (lanes 2 and 5), and both galactosylceramide containing 98% nonhydroxylated fatty acids (GalCer2) and the Forssman glycolipid (lanes 3 and 6) was applied.

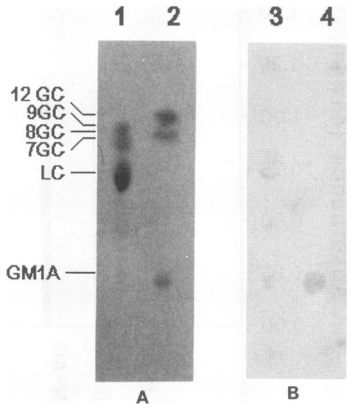

FIG. 4. TLC overlay analysis of the binding of *E. coli* serotype O8:K87:K88ab:H19 to separated glycolipids. Duplicate chromatograms were stained with *p*-anisaldehyde (A) or incubated with *E. coli* serotype O8:K87:K88ab:H19 and detected with anti-K88 serum as described in the text (B). Samples (10 μg) then applied were *N*-heptyl-β-D-glucopyranoside (7GC; lanes 1 and 3), *N*-octyl-β-D-glucopyranoside (8GC; lanes 2 and 4), *N*-nonyl-β-D-glucopyranoside (9GC; lanes 1 and 3), *N*-dodecyl-β-D-glucopyranoside (12GC; lanes 2 and 4), LC (lanes 1 and 3), and gangliotetraosylceramide (GM1A; lanes 2 and 4). Distinct binding to LC (lane 3) and GM1A (lane 4) was detected, while faint bands corresponding in R_f value to GM1A and GM2A were also present in lane 3.

DISCUSSION

Studies have indicated that the K88 receptor(s) is a glycoconjugate. Several glycoproteins present both in porcine small intestine mucus and on porcine enterocytes are able to bind the K88 adhesin (13, 23). Kearns and Gibbons (10) suggested that a polar glycolipid isolated from porcine brush borders may be the K88 receptor. Nilsson and Svensson (15) found that both the disaccharide Galα1-3Gal and the trisaccharide Galα1-3Galα1-3Gal derived from porcine small intestine glycolipids inhibited K88-mediated hemagglutination. Several other studies have implicated galactosyl residues in the K88 receptor structure (5, 17, 23). The aim of this study was to examine the ability of both $K88^+$ bacteria and the K88 adhesin to bind in vitro to glycolipid standards.

The binding of K88 adhesin to microtiter plates coated with lactosylceramide (LC), gangliotriaosylceramide (GM2A), and gangliotetraosylceramide (GM1A) was saturable, with 50% maximal binding occurring at 73 ± 22, 67 ± 21, and 117 ± 21 pM, respectively. Differences between these three glycolipids are confined to the nonreducing end of the oligosaccharide chain. The binding of the K88 adhesin to LC is unaffected by the addition of a GalNAc residue, as in GM2A. However, the addition of Galβ to GM2A approximately doubles the concentration at which 50% maximal binding of the K88 adhesin occurs. Apparently, the presentation of the K88 binding epitope differs in GM1A in such a way as to reduce the affinity of the adhesin-glycolipid interaction.

To confirm the binding specificity of the K88 adhesin, terminal monosaccharides were enzymatically cleaved from GM1A and the effect on the binding of K88 was noted. Removal of the Galβ residue from GM1A increased binding by approximately threefold. The further removal of GalNAc had no effect on the binding of the K88 adhesin. Thus, comparable results were achieved by enzymatic cleavage and direct binding. Both assays indicated that the affinity of the K88 adhesin for glycolipids is in the order GM1A < GM2A ≡ LC.

TLC overlay analysis was used to demonstrate that GM1A, GM2A, and LC all bound to $K88^+$ bacteria. In addition, GalCer1 (which contains hydroxylated fatty acids) bound $K88^+$ bacteria, while GalCer2 (which contains nonhydroxylated fatty acids), glucosylceramide, or a heterogeneous mixture of ceramides did not. The degree of hydroxylation of the ceramide portion of glycosphingolipids has been demonstrated previously to affect binding characteristics (6). For instance, both *Propionibacterium freudenreichii* and *Propionibacterium granulosum* bind to LC; however, *P. freudenreichii* binds only to LC containing nonhydroxylated fatty acids, while *P. granulosum* binds only to LC containing hydroxylated fatty acids (6). It seems that the absence of a hydroxylated fatty acid chain in GalCer2 alters the presentation of the galactose residue in such a way as to prevent the binding of the K88 adhesin.

In this study, the only common saccharide moiety of glycolipids found binding K88 was β1-linked galactose, which accordingly is hypothesized to represent the minimum structure of the K88 receptor. However, as has been noted for other adhesin systems, the presence of the minimum receptor structure in glycolipids does not necessarily correlate with binding (7). Thus, despite containing Galβ in their oligosaccharide sequence, globotriaosylceramide, globoside, the Forssman glycolipid, GM1, GM2, GM3, GD1a, GD1b, GD2, GD3, GQ1b, and GT1b do not bind or bind poorly to the K88 adhesin. The inability of K88 to bind to gangliosides may have been attributable in part to charge repulsion between the sialosyl groups on the gangliosides and the negative charge on K88 fimbriae (pI 4.2) at physiological pH. However, this is unlikely to apply to globotriaosylceramide, globoside, or the Forssman glycolipid, since none of them contain sialic acid residues. Overall, the results indicate that both the degree of hydroxylation of the ceramide portion and the saccharide sequence of the oligosaccharide moiety are critical for the binding of the K88 adhesin. Exact conformational restrictions on the presentation of Galβ may in part explain the limited binding of the K88 adhesin, despite the widespread occurrence of galactose residues in both glycoproteins and glycosphingolipids (1, 12).

The virulence of $K88^+$ ETEC is dependent on and facilitated by the ability of K88 fimbriae to recognize and bind K88 receptor molecules present in the porcine small intestine. Studies have demonstrated that K88 fimbriae bind to glycoproteins present either in porcine mucus or on porcine enterocytes (3, 13, 23). Partial biochemical characterization of the K88 receptor in inhibition studies has indicated the importance of galactosyl residues in the binding of the K88 adhesin to glycoproteins (5, 15, 17, 23). In conclusion, this study demonstrates that the K88 adhesin is able to bind to specific Galβ1-containing glycolipids with high affinity in vitro. The minimum receptor structure recognized by ETEC expressing K88 fimbriae may be β1-linked galactose; however, binding to glycolipids and/or glycoproteins may occur in vivo only when Galβ1-linked residues are favorably presented.

REFERENCES

1. **Berger, E. G., E. Buddecke, J. P. Kamerling, A. Kobata, J. C. Paulson, and J. F. G. Vliegenthart.** 1982. Structure, biosynthesis and functions of glycoprotein glycans. Experientia **38**:1129–1258.

2. **Bock, K., K. A. Karlsson, N. Stromberg, and S. Teneberg.** 1988. Interaction of viruses, bacteria and bacterial toxins with host cell surface glycolipids. Aspects on receptor identification and dissection of binding epitopes, p. 153–186. *In* A. M. Wu (ed.), The molecular immunology of complex carbohydrates. Plenum Press, London.
3. **Conway, P. L., A. Welin, and P. S. Cohen.** 1990. Presence of K88-specific receptors in porcine ileal mucus is age dependent. Infect. Immun. **58:**3178–3182.
4. **Eidels, L., R. L. Proia, and D. A. Hart.** 1983. Membrane receptors for bacterial toxins. Microbiol. Rev. **47:**596–620.
5. **Gibbons, R. A., G. W. Jones, and R. Sellwood.** 1975. An attempt to identify the intestinal receptor for the K88 adhesin by means of a haemagglutination inhibition test using glycoproteins and fractions from sow colostrum. J. Gen. Microbiol. **86:**228–240.
6. **Holgersson, J., K. A. Karlsson, P. Karlsson, E. Norrby, C. Orvell, and N. Stromberg.** 1985. Approaches to the study of receptors, p. 273–301. *In* H. Koprowski and S. A. Plotkin (ed.), World's debt to Pasteur. Alan R. Liss, Inc., New York.
7. **Karlsson, K. A.** 1989. Current experience from the interaction of bacteria with glycosphingolipids, p. 77–96. *In* L. Switalski, M. Hook, and E. Beachey (ed.), Molecular mechanisms of microbial adhesion. Springer-Verlag, New York.
8. **Karlsson, K. A., J. Angstrom, and S. Teneberg.** 1991. Characteristics of the recognition of host cell carbohydrates by viruses and bacteria, p. 9–21. *In* T. Wadstrom, P. H. Makela, A. M. Svennerholm, and H. Wolf-Watz (ed.), Molecular pathogenesis of gastrointestinal infections. Plenum Press, New York.
9. **Karlsson, K. A., and N. Stromberg.** 1987. Overlay and solid-phase analysis of glycolipid receptors for bacteria and viruses. Methods Enzymol. **138:**220–232.
10. **Kearns, M. J., and R. A. Gibbons.** 1979. The possible nature of the pig intestinal receptor for the K88 antigen of Escherichia coli. FEMS Microbiol. Lett. **6:**165–168.
11. **Laux, D. C., E. F. McSweegan, T. J. Williams, E. A. Wadolkowski, and P. S. Cohen.** 1986. Identification and characterization of mouse small intestine receptors for *Escherichia coli* K-12(K88ab). Infect. Immun. **52:**18–25.
12. **Makita, A., and N. Taniguchi.** 1985. Glycosphingolipids, p. 1–100. *In* H. Wiegandt (ed.), Glycolipids. Elsevier, Oxford.
13. **Metcalfe, J. W., K. A. Krogfelt, H. C. Krivan, S. C. Cohen, and D. C. Laux.** 1991. Characterization and identification of a small intestine mucus receptor for the K88ab fimbrial adhesin. Infect. Immun. **59:**91–96.
14. **Mooi, F. R., and F. K. de Graaf.** 1979. Isolation and characterisation of K88 antigens. FEMS Microbiol. Lett. **5:**17–20.
15. **Nilsson, G., and S. Svensson.** 1983. The role of the carbohydrate portion of glycolipids for the adherence of Escherichia coli K88+ to pig intestine, p. 637–638. *In* M. A. Chester, D. Heinegard, A. Lundblad, and S. Svensson (ed.), Proceedings of the 7th International Symposium on Glycoconjugates, Lund-Ronneby, Sweden.
16. **Payne, D. W., N. Moore, and P. Lambert.** 1993. Use of an enzyme-linked immunosorbent assay (ELISA) to measure the expression of the K88 fimbrial antigen by enterotoxigenic *Escherichia coli* (ETEC). J. Immunol. Methods **159:**283–289.
17. **Sellwood, R.** 1980. The interaction of the K88 antigen with porcine intestinal brush borders. Biochim. Biophys. Acta **632:**326–335.
18. **Smith, H. W., and M. A. Linggood.** 1971. Observations on the pathogenic properties of the K88, Hly and Ent plasmids of Escherichia coli with particular reference to porcine diarrhoea. J. Med. Microbiol. **4:**467–485.
19. **Staley, T. E., and I. B. Wilson.** 1983. Soluble pig intestinal cell membrane components with affinities for E. coli K88+ antigen. Mol. Cell. Biochem. **52:**177–189.
20. **Stirm, S., F. Ørskov, I. Ørskov, and B. Mansa.** 1967. Episome-carried surface antigen K88 of *Escherichia coli*. J. Bacteriol. **93:**731–739.
21. **Svennerholm, L.** 1963. Chromatographic separation of human brain gangliosides. J. Neurochem. **10:**613–623.
22. **Takamizawa, K., M. Iwamori, S. Kozaki, G. Sakaguchi, R. Tanaka, H. Takayama, and Y. Nagai.** 1986. TLC immunostaining characterization of Clostridium botulinum type A neurotoxin binding to gangliosides and free fatty acids. FEBS Lett. **201:**229–232.
23. **Willemsen, P. T. J., and F. K. de Graaf.** 1992. Age and serotype dependent binding of K88 fimbriae to porcine intestinal receptors. Microb. Pathog. **12:**367–375.
24. **Wilson, R. A., and D. H. Francis.** 1986. Fimbriae and enterotoxins associated with Escherichia coli serogroups isolated from pigs with colibacillosis. Am. J. Vet. Res. **47:**213–217.