INFECTION AND IMMUNITY, Mar. 1989, p. 907–911
0019-9567/89/030907-05$02.00/0

Vol. 57, No. 3

Ganglioside Epitope Recognized by K99 Fimbriae from Enterotoxigenic *Escherichia coli*

ERIKO ONO,[1] KENJI ABE,[2] MUNEO NAKAZAWA,[3] AND MASAHARU NAIKI[1]*

Department of Biochemistry, Faculty of Veterinary,[1] and Research Center for Molecular Genetics,[2] Hokkaido University, Sapporo 060, and National Institute of Animal Health, 3-1-1 Kannondai, Tsukuba, Ibaraki 305,[3] Japan

Received 31 August 1988/Accepted 4 December 1988

The receptor structure recognized by *Escherichia coli* possessing K99 fimbriae and by isolated K99 fimbrial fractions was examined by using an equine erythrocyte hemagglutination inhibition test. Both K99-positive organisms (strain B41) and fimbrial preparations reacted with *N*-glycolylneuraminyl-lactosyl-ceramide (NeuGcLacCer) purified from equine erythrocytes with very high potency. Fimbrial preparations were 253 times more potent than intact organisms, indicating that isolated fimbriae more precisely recognize the structure of NeuGcLacCer than do fimbriae located on the bacterial cell wall. Structurally, the *N*-glycolyl group of the sialic acid was shown to be essential because substitution of the N-acetyl group for the *N*-glycolyl group caused the reactivity to completely disappear. The substitution of the *O*-acetyl group for the C4 hydroxyl group of the sialic acid (4-*O*-Ac-NeuGcLacCer) also diminished the reactivity by about 500 times, indicating that the fine structure of NeuGc is necessary for recognition. *N*-Glycolylneuraminyl-neolactotetraosyl-ceramide (NeuGcnLc$_4$Cer) and *N*-glycolylneuraminyl-neolactohexaosyl-ceramide (NeuGcnLc$_6$Cer), both of which have identical disaccharides at the nonreducing terminal and longer carbohydrate chains, showed reduced reactivity, indicating that the ceramide of NeuGcLacCer is also involved in the recognition. Indeed, NeuGcLac oligosaccharides altered by cleavage of the ceramide or the terminal sialic acid (NeuGc) showed dramatically reduced reactivities. Ten other *E. coli* strains (isolated from diseased calves) and two strains (isolated from diseased piglets) which possessed the same K99 antigen and various O antigens were used for the recognition test. The results obtained were similar to those mentioned above.

Enterotoxigenic *Escherichia coli* is a common cause of diarrhea in neonatal animals. Its pathogenesis requires both adhesion and colonization of bacteria on the mucosal surface of the small intestine (18, 28) and the production of enterotoxins (23, 29). For adhesion, *E. coli* recognizes a specific receptor on the host cell. It is known that such receptor epitopes are carbohydrates of glycolipids or glycoproteins or both (4).

Enterotoxigenic *E. coli* have fimbriae, which have been classified by their antigenicities and subunit molecular weights (7). The fimbriae are composed of hydrophobic fimbrillins, which are structural domains of fimbriae, and adhesins, which are recognition proteins.

K99 fimbrial antigen is often found in enterotoxigenic *E. coli* isolated from calves, piglets, and lambs suffering from diarrhea. A major subunit of the K99 fimbriae has a molecular weight of 18,500 (18.5K) and consists of 159 amino acids, which were deduced by their gene sequence (27). The adhesive subunits which recognize *N*-glycolylneuraminic acid-α,2-3 D-galactose-β,1-4 D-glucose-ceramide (NeuGcLac Cer) on equine erythrocytes (31) and piglet intestinal mucosa (Teneberg et al., submitted for publication) should be localized at the tips of fimbriae (16). For this report, we analyzed the fine carbohydrate structure recognized by K99 fimbriae by using various *N*-glycolylneuraminic acid (NeuGc)-containing glycoconjugates and carbohydrates and studied the differences in substrains possessing the same fimbriae (12 strains from calves and 2 strains from piglets).

MATERIALS AND METHODS

Organisms and growth conditions. *E. coli* strains were prepared as follows. Strain B41 (O101:K$^-$:K99$^+$:F41$^+$), which was isolated from a calf, was used as a standard strain of *E. coli* possessing K99 fimbrial antigen. Strains H1763 (O9:K35:K99$^+$), H1764 (O9:K35:K99$^+$), H1914 (O101:K28:K99$^+$), H1915 (O8:K25:K99$^+$), and H1918 (O8:K85:K99$^+$), which were also isolated from calves, were kindly supplied by P. A. M. Guinée, the National Institute of Public Health, Bilthoven, The Netherlands. Strain B117 (O8:K85:K99$^+$) (24) was kindly supplied by I. Ørskov, World Health Organization Collaborative Centre for Reference and Research on *Escherichia*, Statens Seruminstitut, Copenhagen, Denmark. Strains 880K$^-$ (O101:K99$^+$) (20), S-32 (O141:K$^+$:K99$^+$) (32), I-1 (O20:K$^+$:K99$^+$) (32), I-11 (O20:K$^+$:K99$^+$) (32), and NAS10K$^-$ (O9:K99$^+$) were isolated from calves, while 431K$^-$ (O101:K99$^+$:F41$^+$) (1) was a K$^-$ mutant of strain 431 kindly supplied by K. Toriyabe of the Kyoritsu Ltd. Co. EmiliK$^-$ (O101:K99$^+$) (21) was isolated from a piglet.

The bacteria were cultured on Minca agar plates for the hemagglutination (HA) test or in minimal medium for preparation of the fimbrial fraction. The Minca agar (1 liter) consisted of Casamino Acids (Difco Laboratories) (1 g), KH_2PO_4 (1.36 g), Na_2HPO_4 (10.1 g), glucose (1 g), a trace salt solution (1 ml), and Difco agar (12 g). The trace salt solution (1 liter) contained $MgSO_4 \cdot 7H_2O$ (10 g), $MnCl_2 \cdot 4H_2O$ (1 g), $FeCl_3 \cdot 6H_2O$ (0.135 g), and $CaCl_2 \cdot 2H_2O$ (0.4 g). The minimal medium (1 liter) contained KH_2PO_4 (3.0 g), Na_2HPO_4 (7.5 g), NaCl (0.5 g), NH_4Cl (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.12 g), $FeSO_4 \cdot 7H_2O$ (5 mg), CaCl · $2H_2O$ (11 mg), and glucose (5 g).

Isolation of K99 fimbriae. The K99 fimbrial fraction was isolated from *E. coli* B41 by incubation at 60 to 70°C for 20 min in 50 mM phosphate buffer (pH 7.5) containing 2 M urea, by the method of Jacobs et al. (11). After centrifugation at 20,000 × *g* for 30 min, the supernatant was precipitated by 30

* Corresponding author.

TABLE 1. Chemical structures of glycolipids, glycoprotein, and oligosaccharides used

Compound[a]	Structure
NeuGcLacCer	NeuGc(α,2-3)Gal(β,1-4)Glc-Cer
4-*O*-Ac-NeuGcLacCer	Ac-4-*O*-NeuGc(α,2-3)Gal(β,1-4)Glc-Cer
NeuAcLacCer	NeuAc(α,2-3)Gal(β,1-4)Glc-Cer
$NeuGcnLc_4Cer$	NeuGc(α,2-3)Gal(β,1-4)GlcNAc(β,1-3)Gal(β,1-4)Glc-Cer
$NeuGcnLc_6Cer$	NeuGc(α,2-3)Gal(β,1-4)GlcNAc(β,1-3)Gal(β,1-4)-GlcNAc(β,1-3)Gal(β,1-4)Glc-Cer
NeuGcLac	NeuGc(α,2-3)Gal(β,1-4)Glc
NeuAcLac	NeuAc(α,2-3)Gal(β,1-4)Glc
Bovine erythrocyte major glycoprotein	NeuGc(α,2-3)Gal(β,1-3)GalNAc-Thr (or Ser) |(α,2-6) NeuGc

[a] Abbreviations: Glc, D-glucose; Gal, D-galactose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; NeuGc, *N*-glycolylneuraminic acid; NeuAc, *N*-acetylneuraminic acid; Lac, lactose; nLc_4, neolactotetraosyl; nLc_6, neolactohexaosyl; Ac, acetyl; Thr, threonine; Ser, serine; Cer, ceramide.

to 60% saturated ammonium sulfate, and the precipitate was dissolved and dialyzed against the same phosphate buffer. The fimbrial fraction gave two subunits (18.5K and 20K) by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and both bands were stained with anti-K99 fimbriae antibody after being blotted on nitrocellulose paper. The fimbriae of the fimbrial preparation appeared to be smaller by electron microscopy than did the native fimbriae on the organisms.

Inhibitor substances used in the HA inhibition test. NeuGc LacCer (35) and 4-*O*-acetyl-NeuGcLacCer (4-*O*-Ac-NeuGc LacCer) (8) were isolated from thoroughbred horse erythrocytes. NeuGc-neolactyltetraosyl-Cer ($NeuGcnLc_4Cer$) (19), NeuGc-neolactylhexaosyl-Cer ($NeuGcnLc_6Cer$) (3), and a major glycoprotein (22) were isolated from bovine erythrocytes. NeuGcLac was prepared from NeuGcLacCer by ozonolysis followed by alkaline treatment (34). Free NeuGc was prepared from NeuGcLacCer by treatment with neuraminidase (10). NeuAcLacCer was isolated from dog erythrocytes (6). NeuAcLac (a mixture of 85% NeuAc-α,2-3Lac and 15% NeuAc-α,2-6Lac) and NeuAc were purchased from Sigma Chemical Co. The molecular weight of each glycolipid was calculated on the basis of its possessing lignoceric acid as the acyl group and 4-sphingenine as the long-chain base. For HA inhibition, each glycosphingolipid was dissolved in 0.01 M phosphate buffer (pH 7.2) containing 0.85% NaCl and 0.05% sodium taurodeoxychorate (Sigma).

Bacterial concentration was measured by A_{660}. Each hemagglutinin solution (25 μl) containing 2 HA units was incubated with the same volume of twofold serially diluted inhibitor solution at 4°C for 12 h. A 0.5% erythrocyte suspension (25 μl) was then added, and HA was read after incubation at 4°C for 2 h.

RESULTS

E. coli B41 bacterial suspension (3.9×10^7 organisms per ml), which contained approximately 142 ng of fimbrial proteins in the same number of organisms, hemagglutinated equine erythrocytes. The isolated fimbrial fraction did so at concentrations of more than 313 ng/ml, indicating that the HA potency of fimbriae was reduced 2.2-fold by isolating the fimbriae from the intact organisms. Smit et al. (31) reported that the receptor of the equine erythrocyte is NeuGcLacCer. We used various glycosphingolipids, a glycoprotein, and sugar compounds (Table 1) that have NeuGc at the carbohydrate terminal for the inhibition test. NeuGcLacCer showed the strongest inhibition among all substances used (Table 2). NeuAcLacCer showed no inhibition at a concentration of 789 μM (4,000 times as much as the NeuGc type for isolated fimbriae and 16 times that for intact organisms), indicating that NeuGc is the most essential epitope for recognition. $NeuGcnLc_4Cer$ and $NeuGcnLc_6Cer$, which possess the same disaccharide sequence (NeuGc-α,2-3Gal) at the terminal, had lesser activities, and 4-*O*-Ac-NeuGc LacCer also had weaker potency. Cleavage of the hydrophobic ceramide from NeuGcLacCer reduced inhibitory potency 16 times in HA with intact organisms and 2,000 times in HA with fimbrial fractions. The terminal monosaccharide NeuGc showed a much more reduced inhibitory potency. Bovine erythrocyte major glycoprotein, which was expected to have a similar disaccharide moiety at the carbohydrate-terminal end, had activity similar to that of $NeuGcnLc_4Cer$. The fimbrial preparation showed 250 times higher affinity for NeuGcLacCer than did intact organisms, although it showed only 2 times higher reactivity with the oligosaccharide and the terminal monosaccharide moieties, indicating that some conformational change of the binding site (adhesin) occurred on the native membrane surfaces of the bacteria (Fig. 1).

It has been well known that human uropathogenic *E. coli* P fimbriae occur in association with a limited number of *E. coli* O serogroups (O2, O4, O6, O16, and O18) (33) and that there are several P fimbrial variants restricted by their O serotypes, which share common antigenic determinants but recognize different receptor structures (33). Therefore, we examined the difference in the recognition epitope by using 12 different strains, with a common K99 fimbrial antigen but different O antigens, which were isolated from calves (10

TABLE 2. Inhibition of HA with bacterial suspension and fimbrial preparation by various glycoconjugates and carbohydrates

Inhibitor	Lowest concn (μM) causing complete inhibition of HA with:	
	Bacterial suspension	Fimbrial fraction
NeuGcLacCer	48	0.19
NeuAcLacCer[a]	>789	>789
4-*O*-Ac-NeuGcLacCer	>376	94
$NeuGcnLc_4Cer$	76	4.7
$NeuGcnLc_6Cer$	248	31
NeuGcLac	770	385
NeuAcLac	>1605	>1605
NeuGc	3077	1538
NeuAc	>3236	>3236
Bovine erythrocyte major glycoprotein	80[b]	39

[a] Inhibition was not observed at lower concentrations.

[b] The concentration of the glycoprotein was calculated as sialic acid molarity from its content (10%) as previously determined (19).

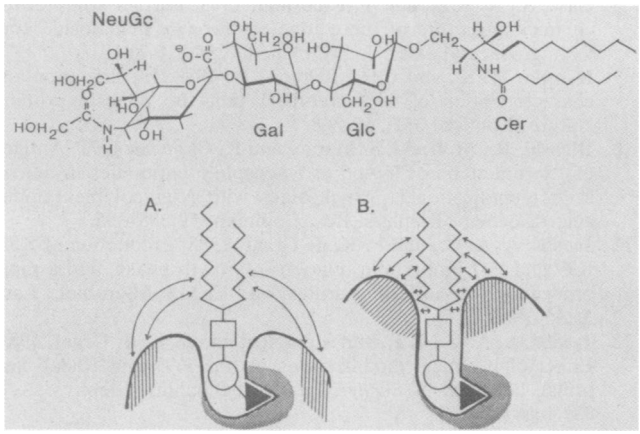

FIG. 1. Chemical structure of NeuGcLacCer and a model of its interaction with fimbrial protein. (A) Fimbrial adhesin on bacteria can recognize the carbohydrate moieties of the receptor ganglioside by the specific recognition domain (shaded area) and has a weak interaction (↔) with the ceramide moiety by the hydrophobic domain (hatched area). (B) The same adhesin subunits change in conformation during isolation of fimbriae from organisms, recognize the carbohydrate moieties, and have a strong non-specific interaction with the ceramide moiety. Symbols: ▼, NeuGc; ○, Gal; □, Glc.

strains) and piglets (2 strains) (Table 3). H1763, H1764, and NAS10K$^-$, belonging to serotype O9, hemagglutinated equine erythrocytes with the lowest titers. The other strains showed similar HA titers. HA in all strains were inhibited with NeuGcLacCer and bovine erythrocyte major glycoprotein, except for one strain (H1763). NeuGcnLc$_4$Cer did not inhibit all HA at concentrations of less than the limited amount used and showed lower HA activity than did Neu GcLacCer.

DISCUSSION

It was reported that *E. coli* possessing K99 fimbriae hemagglutinate sheep erythrocytes in the presence of D-mannose and that the HA is inhibited by GM_2 ganglioside, which has *N*-acetylgalactosamine (GalNAc) and NeuGc at the nonreducing terminal of the carbohydrate chain (5). Similarly, Lindahl and Wadström found that human erythrocyte HA with K99-fimbriated organisms can be inhibited by glycophorin, bovine submaxillary mucin, GalNAc, and NeuAc (15). On the other hand, Gaastra et al. (7) found that equine erythrocyte HA with fimbriated bacteria is a genuine HA. It was very sensitive and was specifically inhibited by a major glycosphingolipid of equine erythrocytes (NeuGcLac Cer), while sheep and human erythrocyte HA required 10 times the number of organisms and were influenced by the ionic strength of the physiological saline concentration (7). Recently, Teneberg et al. (submitted) found that [^{35}S]methionine-labeled K99 bacteria bind to a major ganglioside, NeuGcLacCer, of glycosphingolipid components from newborn pig intestinal mucosa and that no ganglioside from adult pig intestine shows binding.

In our study, therefore, we used an equine erythrocyte HA system, in which NeuGcLacCer was found to be the strongest inhibitor as described by Smit et al. (31). NeuAc LacCer, an analog of NeuGcLacCer, which has an *N*-acetyl group substitution for the *N*-glycolyl group of the terminal sialic acid, induced no response. 4-*O*-Ac-NeuGcLacCer, another analog of NeuGcLacCer, which has an *O*-acetyl group substitution for the C4-linked hydroxyl group of the terminal sialic acid, had 500-fold-reduced reactivity. This means that the recognition of the terminal NeuGc moiety by the fimbrial protein is very critical, as with human heterophilic Hanganutziu-Deicher antibody (19) or bacterial neuraminidase (30).

The terminal NeuGc moiety is important for recognition; however, the monosaccharide NeuGc or the oligosaccharide NeuGcLacCer showed very weak activity. NeuGcnLc$_4$Cer and NeuGcnLc$_6$Cer, both of which have an identical disaccharide (NeuGc-α,2-3Gal) at the carbohydrate terminal and a longer carbohydrate chain, also showed weaker activity. The exact distance between the specific epitope of the terminal NeuGc and the hydrophobic ceramide moiety may be critical for achieving the best recognition (Fig. 1). In a recent paper, Lindahl et al. compared the inhibitory potencies of various chemical derivatives of sialic acid in the human erythrocyte HA system and found that the presence of a hydrophobic region close to the carbohydrate epitope enhanced the binding affinity (14).

TABLE 3. Similarity in HA recognition sites of various strains of *E. coli* with K99 fimbriae

Strain	O serotype	Source	Minimum concn showing HA (10^6/ml)	Lowest concn (μM) of the following compounds showing HA inhibition		
				NeuGcLacCer	NeuGcnLc$_4$Cer	Bovine erythrocyte glycoprotein[a]
S-32	141	Bovine	48	48	303	80
B41	101	Bovine	80	48	76	80
H1914	101	Bovine	47	390	>779[b]	150
880K−	101	Bovine	63	195	>779	150
431K−	101	Swine	33	48	>779	310
EmiliK−	101	Swine	74	195	152	80
I-1	20	Bovine	66	48	76	80
I-11	20	Bovine	50	48	76	80
H1763	9	Bovine	210	195	>779	>310
H1764	9	Bovine	120	195	>779	150
NAS10K−	9	Bovine	90	195	779	80
H1915	8	Bovine	24	390	>779	150
H1918	8	Bovine	50	97	779	150
B117	8	Bovine	25	48	152	150

[a] Concentration of the glycoprotein was calculated as sialic acid molarity from its content (10%) as previously determined (19).
[b] Inhibition was not observed at lower concentrations.

Recently, it was suggested that fimbriae are composed of several different subunits, each of which has a different function. A fimbrial subunit called adhesin, which is localized on the tips of fimbriae, has an adhesive function. It was isolated from P fimbriae (16), S fimbriae (17), and type I fimbriae (9), while its presence is still uncertain in K99 fimbriae (12, 13, 25–27). Native fimbriae on bacteria are 0.5 μm in length, but isolated fimbrial preparations contain shorter fimbriae (25 nm in length). The deletion of large numbers of fimbrillin subunits may influence the conformation of the adhesin recognition site. Indeed, the K99 fimbrial preparation reacted with NeuGcLacCer 250 times more strongly than with the fimbriated organisms, indicating that a conformational change, which enhances the interaction with the hydrophobic ceramide moiety, occurs in the adhesin recognition site during the isolation of fimbriae (Fig. 1).

The specific recognition of NeuGcLacCer by K99-fimbriated *E. coli* might contribute to host specificity. It has been reported that the mucosa at the surface of the small intestine contains NeuAcLacCer or NeuGcLacCer ganglioside (2; Teneberg et al., submitted). Enterotoxigenic *E. coli* which possess K99 fimbriae usually adhere to the small intestine and have a higher affinity to small intestines from neonatal calves than to those from adult calves (28). Data presented by Teneberg et al. (submitted) showed identical phenomena in pigs. Their results suggest that the affinity and localization of toxigenic *E. coli* to the small intestine are related to the quantity and location of the ganglioside. Gangliosides of calf small intestine require further study.

ACKNOWLEDGMENTS

We thank P. A. M. Guinée, the National Institute of Public Health, Bilthoven, the Netherlands, I. Ørskov, World Health Organization Collaborative Centre for Reference and Research on *Escherichia*, Statens Seruminstitut, Copenhagen, Denmark, and K. Toriyabe, Kyoritsu Ltd. Co., Japan, for supplying us with various bacterial strains.

This work was supported by Grant-in-Aid for Scientific Research 61480081 and Grant-in-Aid for Cancer Research 63010027 from the Ministry of Education, Science and Culture, Japan.

LITERATURE CITED

1. **Awad-Masalmeh, M., H. W. Moon, P. L. Runnels, and R. A. Schneider.** 1982. Pilus production, hemagglutination, and adhesion by porcine strains of enterotoxigenic *Escherichia coli* lacking K88, K99, and 987P antigens. Infect. Immun. **35:** 305–313.
2. **Bouhours, J., and R. M. Glickman.** 1976. Rat intestinal glycolipid. II. Distribution and biosynthesis of glycolipids and ceramide in villus and crypt cells. Biochim. Biophys. Acta **441:**123–133.
3. **Chien, J., S. Li, R. A. Laine, and Y. Li.** 1977. Characterization of gangliosides from bovine erythrocyte membrane. J. Biol. Chem. **253:**4031–4035.
4. **Duguid, J. P., and D. C. Old.** 1980. Adhesive properties of Enterobacteriaceae. *In* E. H. Beachey (ed.), Bacterial adhesion, receptors and recognition, ser. B, vol. 6. Hapman and Hall, London.
5. **Faris, A., M. Lindahl, and T. Wadström.** 1980. GM_2-like glycoconjugate as possible erythrocyte receptor for the CFA/I and K99 hemagglutinins of enterotoxigenic *Escherichia coli*. FEMS Microbiol. Lett. **7:**265–269.
6. **Fujii, Y., H. Higashi, K. Ikuta, S. Kato, and M. Naiki.** 1982. Specificities of human heterophilic Hanganutziu and Deicher (H-D) antibodies and avian antisera against H-D antigen-active glycosphyngolipid. Mol. Immunol. **19:**87–94.
7. **Gaastra, W., and F. K. de Graaf.** 1982. Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol. Rev. **46:**129–161.
8. **Gasa, S., A. Kita, and Y. Kinoshita.** 1983. Further study of the chemical structure of the equine erythrocyte hematoside containing *O*-acetyl ester. J. Biol. Chem. **258:**876–881.
9. **Hanson, M. S., and C. C. Brinton, Jr.** 1988. Identification and characterization of *E. coli* type-1 pilus tip adhesin protein. Nature (London) **332:**265–268.
10. **Higashi, H., M. Naiki, S. Matuo, and K. Okouchi.** 1977. Antigen of "serum sickness" type of heterophile antibodies in human sera: identification as gangliosides with *N*-glycolylneuraminic acid. Biochem. Biophys. Res. Commun. **79:**388–395.
11. **Jacobs, A. A. C., and F. K. de Graaf.** 1985. Production of K88, K99 and F41 fimbriae in relation to growth phase, and a rapid procedure for adhesin purification. FEMS Microbiol. Lett. **26:**15–19.
12. **Jacobs, A. A. C., J. R. van Mechelen, and F. K. de Graaf.** 1985. Effect of chemical modifications on the K99 and K88ab fimbrillae adhesins of *Escherichia coli*. Biochim. Biophys. Acta **832:**148–155.
13. **Jacobs, A. A. C., P. A. van den Berg, H. J. Bak, and F. K. de Graaf.** 1986. Localization of lysine residues in the binding domain of the K99 fimbrillar subunit of enterotoxigenic *Escherichia coli*. Biochim. Biophys. Acta **872:**92–97.
14. **Lindahl, M., R. Brossmer, and T. Wadström.** 1987. Carbohydrate receptor specificity of K99 fimbriae of enterotoxigenic *Escherichia coli*. Glycoconjugate J. **4:**51–58.
15. **Lindahl, M., and T. Wadström.** 1981. K99 surface hemagglutinin of enterotoxigenic *Escherichia coli* recognizes terminal N-acetylgalactosamine and other complex glycoconjugates. Vet. Microbiol. **9:**294–257.
16. **Lindberg, F., B. Lund, L. Johansson, and S. Normark.** 1987. Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. Nature (London) **328:**84–87.
17. **Moch, T., H. Hoschutzky, J. Hacker, K.-D. Kroncke, and K. Jann.** 1987. Isolation and characterization of the α-sialyl-β-2,3-galactosyl-specific adhesin from fimbriated *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84:**3462–3466.
18. **Moon, H. W., B. Nagy, R. E. Isaacson, and I. Ørskov.** 1977. Occurrence of K99 antigen on *Escherichia coli* isolated from pigs and colonization of pig ileum by $K99^+$ enterotoxigenic *Escherichia coli* from calves and pigs. Infect. Immun. **15:** 614–620.
19. **Mukuria, C. J., Y. Fujii, S. Kato, and M. Naiki.** 1986. Specificities of human heterophile Hanganutziu and Deicher (HD) antibodies to glycosphingolipids and glycoprotein. J. Biochem. **100:**469–475.
20. **Nakazawa, M., H. Nemoto, H. Ueda, and T. Maruyama.** 1981. Enterotoxigenic *Escherichia coli* in calves with diarrhea. Jpn. J. Vet. Sci. **43:**89–91.
21. **Nakazawa, M., C. Sugimoto, Y. Isayama, and M. Kashiwazaki.** 1987. Virulence factors in *Escherichia coli* isolated from piglets with neonatal and post-weaning diarrhea in Japan. Vet. Microbiol. **13:**291–300.
22. **Newman, R. A., and G. G. Uhlenbruck.** 1977. Investigation into the occurrence and structure of lectin receptors on human and bovine erythrocyte milk-fat blobule and lymphocyte plasmamembrane glycoproteins. Eur. J. Biochem. **76:**149–155.
23. **Olsson, E., and O. Soderlind.** 1980. Comparison of different assays for definition of heat-stable enterotoxigenicity of *Escherichia coli* porcine strains. J. Clin. Microbiol. **11:**6–15.
24. **Ørskov, I., F. Ørskov, W. Smith, and W. J. Sojka.** 1975. The establishment of K99, a thermolabile, transmissible *Escherichia coli* K antigen, previously called "Kco", possessed by calf and lamb enteropathogenic *Escherichia coli*. Acta Pathol. Microbiol. Scand. Sect. B **83:**31–36.
25. **Roosendaal, E., M. Boots, and F. K. de Graaf.** 1987. Two novel genes, fanA and fanB, involved in the biogenesis of K99 fimbriae. Nucleic Acids Res. **15:**5973–5984.
26. **Roosendaal, E., A. A. C. Jacobs, P. Rathman, C. Sondermeyer, F. Stegehuis, B. Oudega, and F. K. de Graaf.** 1987. Primary structure and subcellular localization of two fimbrial subunit-like proteins involved in the biosynthesis of K99 fimbriae. Mol. Microbiol. **1:**211–217.
27. **Roosendaal, E., W. Gaastra, and F. K. de Graaf.** 1984. The

nucleotide sequence of the gene encoding the K99 subunit of enterotoxigenic *Escherichia coli*. FEMS Microbiol. Lett. **22:** 253–258.

28. **Runnels, P. L., H. W. Moon, and R. A. Schneider.** 1980. Development of resistance with host age to adhesion of K99$^+$ *Escherichia coli* to isolated intestinal epithelial cells. Infect. Immun. **28:**298–300.
29. **Sack, R. B.** 1978. The epidemiology of diarrhea due to enterotoxigenic *Escherichia coli*. J. Infect. Dis. **137:**639–640.
30. **Schauer, R.** 1982. Chemistry, metabolism, and biological functions of sialic acids. Adv. Carbohydr. Chem. Biochem. **40:** 132–234.
31. **Smit, H., W. Gaastra, J. P. Kamerling, J. F. G. Vliegenthart, and F. K. de Graaf.** 1984. Isolation and structural characterization of the equine erythrocyte receptor for enterotoxigenic *Escherichia coli* K99 fimbrial adhesin. Infect. Immun. **46:**578–583.
32. **Ueda, H., Y. Isayama, K. Sakurai, and M. Nakazawa.** 1981. Etiologic studies on enteropathogenic *Escherichia coli* among isolates from domestic animals in Japan. Natl. Inst. Anim. Health Q. **21:**108–109.
33. **Väisänen-Rhen, V., E. Elo, E. Väisänen, A. Siitonen, I. Ørskov, F. Ørskov, S. B. Svenson, P. H. Mäkelä, and T. K. Korhonen.** 1984. P-fimbriate clones among uropathogenic *Escherichia coli* strains. Infect. Immun. **43:**149–155.
34. **Wiegandt, H., and G. Baschang.** 1964. Die Gewinnung des Zuckeranteiles der Glykosphingolipide durch Ozonolyse und Fragmentierung. Z. Naturforsch. Teil B **20:**164–166.
35. **Yamakawa, T., and S. Suzuki.** 1951. The chemistry of the lipids of posthemolytic residue or stroma of erythrocytes. I. Concerning the ester-insoluble lipids of lyophilized horse blood stroma. J. Biochem. **38:**199–212.