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Comparison of the sialic acid binding activity of transmissible gastroenteritis coronavirus and *E. coli* K99

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Abstract

Transmissible gastroenteritis coronavirus (TGEV) and *Escherichia coli* K99 are both enteropathogenic for pigs with infections being most severe in neonate animals. For both microorganisms, a sialic acid binding activity has been shown to be an essential pathogenicity factor. Here we demonstrate with haemagglutination and haemagglutination-inhibition assays that TGEV and *E. coli* K99 differ in their sialic acid binding activities with respect to the type and amount of sialic acid residues required on the erythrocytes surface as well as with respect to the type of sialoglycoconjugate preferentially recognized. Intestinal mucins from piglets (12–14 days old) and adult animals were shown to inhibit TGEV to the same extent. From our results we conclude that *E. coli* K99 and TGEV interact with different sialoglycoconjugates to establish an intestinal infection. The implications for the enteropathogenicity of TGEV are discussed.

Keywords: TGEV; *E. coli* K99; Sialic acid; Mucins; Glycolipids

Transmissible gastroenteritis virus (TGEV) is an enteropathogenic coronavirus causing diarrhea in pigs. Infections are most severe in newborn animals (up to 2 weeks old) where lethality approaches 100% (Pensaert et al., 1993). TGEV is a positive-stranded RNA virus surrounded by a lipid envelope. There are three proteins inserted into the viral membrane: the S (220 kDa), the M (29–36 kDa), and a minor E (10 kDa) protein. The S protein mediates the binding of the virus to the cell surface and the subsequent fusion between the viral and cellular membranes. Two different ligands have been shown to interact with the S protein. Binding to porcine aminopeptidase *N*, the cellular receptor for TGEV, is essential for infection of cells (Delmas et al., 1992). TGEV is also able to recognize sialic acid residues and attach to sialylated macromolecules (Schultze et al., 1996). Because of the latter binding activity, TGEV can agglutinate erythrocytes (Noda et al., 1987, 1988).

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The binding sites for sialic acid are located on different domains of the S protein (Schultze et al., 1996). Studies with mutants indicated that the sialic acid binding activity is dispensable for infection of cultured cells. However, mutants that had lost their haemagglutinating activity because of a single point mutation in the sialic acid binding site had also lost enteropathogenicity (Krempl et al., 1997). These results indicated that the sialic acid binding activity is a pathogenicity factor of TGEV. Other factors may also be required to render TGEV enteropathogenic, but they have not been identified in terms of molecular interaction. Interestingly, binding to sialic acid is also a pathogenicity factor for *E. coli* K99. Fimbriae on the surface of this microorganism attach to sialoglycoconjugates on the cell surface and allow colonization of the intestinal tract (Seignole et al., 1991; Teneberg et al., 1994). *E. coli* K99 causes diarrhea in piglets and some other young animals and, therefore, like TGEV has an age-dependent pathogenicity (Gaastra and de Graaf, 1982). Because of this similarity we compared the sialic acid binding activity of TGEV and *E. coli* K99.

TGEV was grown in ST (swine testicular) cells and sedimented by ultracentrifugation as described previously (Schultze et al., 1996). To obtain virus with full haemagglutinating activity, the virions were treated with neuraminidase from *Vibrio cholerae* and purified by sucrose gradient centrifugation according to published procedures (Schultze et al., 1996). *E. coli* K99 was grown overnight in Minca-medium (Mouricout et al., 1990). Bacteria were sedimented at $2700 \times g$ for 15 min at 4°C. The pellet was washed once with PBS containing 1% mannose and resuspended in the same buffer (Teneberg et al., 1994).

Haemagglutination assays were performed according to established methods (Schultze et al., 1996). As shown in Table 1, *E. coli* K99 was able to agglutinate porcine, bovine, and equine erythrocytes. No haemagglutinating activity or only a trace amount of it was measurable when chicken erythrocytes were used for the assay. This result is consistent with previous reports that *E. coli* K99 specifically recognizes *N*-glycolylneuraminic acid (Ono et al., 1989; Teneberg et al., 1990). This type of sialic acid accounts for 90% or more of the sialic acids on porcine, bovine, and equine erythrocytes (Reuter et al., 1988). It is absent, however, from chicken erythrocytes which mainly contain *N*-acetylneuraminic acid. In contrast to *E. coli* K99, TGEV was able to agglutinate erythrocytes from all four species. This result indicates that the sialic acid binding activity of TGEV is less restricted than that of *E. coli* K99.

The receptors for TGEV and *E. coli* K99 on erythrocytes were analyzed for their sensitivity to enzymatic inactivation. For this purpose bovine erythrocytes were treated for 30 min with various amounts of neuraminidase from *Vibrio cholerae*. After removal of the enzyme by washing the cells with PBS containing 1% BSA, the erythrocytes were suspended in PBS and used for haemagglutination assays with TGEV and *E. coli* K99, respectively. As shown in Fig. 1, treatment with 20 mU/ml rendered bovine erythrocytes resistant to agglutination by *E. coli* K99. When these cells were used for HA-assays with TGEV, no reduction of the titer was observed. In order to abolish agglutination by TGEV, neuraminidase had to be applied at a concentration as high as 3 U/ml. This result indicates either that the two organisms use different receptors for binding to erythrocytes or that *E. coli* K99 requires a higher number of surface-bound sialic acid residues for a haemagglutination reaction.

In order to get further information about the sialic acid binding activities of TGEV and *E. coli* K99, we compared the two microorganisms with respect to their sensitivity to haemagglutination-

Table 1
Comparison of the haemagglutinating activity of TGEV and *E. coli* K99 determined with erythrocytes from different species

Erythrocytes from	Haemagglutinating activity (HA units/ml)	
	TGEV	*E. coli* K99
Chicken	128	≤ 2
Cow	64	32
Horse	128	128
Pig	256	64

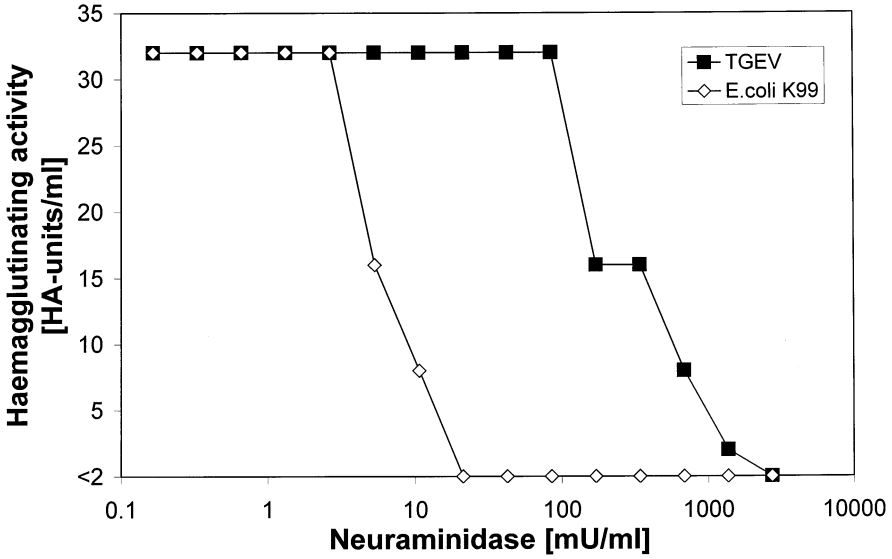

Fig. 1. Comparison of the neuraminidase sensitivity of the erythrocytes receptors for TGEV and *E. coli* K99. Bovine erythrocytes were treated for 30 min at 37°C with neuraminidase from *Vibrio cholerae* at the amounts indicated. After two washes with PBS containing 1% BSA, the cells were used as a 1% suspension in PBS to determine the haemagglutinating activity of TGEV and *E. coli* K99.

inhibitors. Intestinal mucins are highly sialylated glycoconjugates and, therefore, potential ligands for microorganisms such as *E. coli* K99 and TGEV that enter the organism via the gastrointestinal route. As infections by both TGEV and *E. coli* K99 are most severe in piglets, mucins were isolated from two piglets (12–14 days old) and from two pigs (10–15 weeks old) as described previously (Enss et al., 1996). Freeze-dried preparations of the mucins were suspended in water to determine the sialic acid content by HPLC-analysis (Krempl et al., 2000). For haemagglutination-inhibition assays, the mucins were suspended in PBS to give a concentration of 10 μg of sialic acids per milliliter. All four mucin samples inhibited the haemagglutination by neuraminidase-treated TGEV with a titer of 64 HIU/ml (Table 2). In the case of *E. coli* K99, the haemagglutination-inhibition titers determined with the mucins were 2- to 10-fold lower. As *E. coli* K99 has been reported to have an affinity for gangliosides expressed on intestinal cells (Teneberg et al., 1994), we analyzed two types of gangliosides that were available to us for their inhibitory activity. As shown in Table 2, both bovine brain gangliosides and hematoside isolated from equine erythrocytes (Hakomori and Saito, 1969) prevented *E. coli* K99 from agglutinating erythrocytes. However, they were unable to inhibit the haemagglutination

Table 2
Comparison of porcine intestinal mucins and glycolipids with respect to their ability to prevent TGEV and *E. coli* K99 from agglutinating bovine erythrocytes[a]

	Haemagglutination-inhibition activity (HI-units/ml)	
	TGEV	*E. coli* K99
Intestinal mucins		
Piglet A	64	16
Piglet B	64	8
Pig C	64	8
Pig D	64	32
Glycolipids		
Bovine brain gangliosides	<2	16
Hematoside	<2	4

[a] The inhibitors were used at a concentration of 10 μg of sialic acid per ml. The sialic acid content was determined by HPLC analysis.

by TGEV. Thus, with respect to mucins TGEV is more sensitive than is *E. coli* K99, whereas in the case of gangliosides, the bacterial microorganism is more sensitive than the virus. This result is in agreement with the report that gangliosides on the surface of intestinal epithelial cells serve as colonization factors for *E. coli* K99 (Teneberg et al., 1994). Taken together, our results indicate that TGEV and *E. coli* K99 differ in the specificities of their sialic acid binding activity. Therefore, it is likely that the two microorganisms use different sialoglycoconjugates as ligands to establish an intestinal infection. One way how the sialic acid binding activity may promote enteropathogenicity of TGEV is by increasing virus stability. Sialoglycoconjugates such as mucins may bind to the virion surface and protect the virus from the detrimental effects encountered during the gastrointestinal passage, i.e. the low pH, the proteolytic enzymes and the detergent-like bile salts. This view explains why TGEV variants lacking a sialic acid binding activity are unable to cause intestinal infections. As mucins from piglets and older pigs did not differ from each other in their haemagglutination-inhibition activity towards TGEV, our results do not support the notion that mucins from piglets are more protective for TGEV than are mucins from adult animals. Therefore, interactions with mucins are unlikely to explain why TGEV infections are more severe in piglets than in adult pigs. However, the sialic acid binding activity may promote enteropathogenicity of TGEV not only by increasing virus stability but also by increasing the efficiency of attachment to enterocytes. Viruses which are able to recognize sialoglycoproteins in addition to aminopeptidase *N* may have a better chance to infect their target cells compared to viruses that have to rely only on the binding to aminopeptidase *N*. If sialoglycoconjugates on the surface of intestinal cells are involved in the replication and pathogenicity of TGEV, from our results it is likely that glycoproteins rather than glycolipids are attachment sites for the virus. Attempts are in progress to isolate surface proteins from enterocytes and to analyze them for their ability to interact with TGEV.

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References

Delmas, B., Gelfi, J., L'Haridon, R., Vogel, L.K., Sjöström, H., Noren, O., Laude, H., 1992. Aminopeptidase N is a major receptor for the entero-pathogenic coronavirus TGEV. Nature 357, 417–420.

Enss, M.-L., Schmidt-Wittig, U., Müller, H., Mai, U.E.H., Coenen, M., Hedrich, H.J., 1996. Response of germfree rat colonic mucous cells to peroral endotoxin application. Eur. J. Cell Biol. 71, 99–104.

Gaastra, W., de Graaf, F.K., 1982. Host-specific fimbrial adhesins of non-invasive enterotoxigenic Escherichia coli strains. Microbiol. Rev. 46, 129–161.

Hakomori, S., Saito, T., 1969. Isolation and characterization of a glycosphingolipid having a new sialic acid. Biochemistry 8, 5082–5088.

Krempl, C., Schultze, B., Laude, H., Herrler, G., 1997. Point mutations in the S protein connect the sialic acid binding activity with the enteropathogenicity of transmissible gastroenteritis coronavirus. J. Virol. 71, 3285–3287.

Krempl, C., Ballesteros, M.L., Zimmer, G., Enjuanes, L., Klenk, H.-D., Herrler, G., 2000. Characterization of the sialic acid binding activity of transmissible gastroenteritis coronavirus by analysis of haemagglutination-deficient mutants. J. Gen. Virol. 81, 489–496.

Mouricout, M., Petit, J.M., Carias, J.R., Julien, R., 1990. Glycoprotein glycans that inhibit adhesion of Escherichia coli mediated by K99 fimbriae: treatment of experimental colibacillosis. Infection and Immunity 58, 98–106.

Noda, M., Yamashita, H., Koide, F., Kadoi, K., Omori, T., Asagi, M., Inaba, Y., 1987. Hemagglutination with transmissible gastroenteritis virus. Arch. Virol. 96, 109–115.

Noda, M., Koide, F., Asagi, M., Inaba, Y., 1988. Physicochemical properties of transmissible gastroenteritis virus hemagglutinin. Arch. Virol. 99, 163–172.

Ono, E., Abe, K., Nakazawa, M., Naiki, M., 1989. Ganglioside epitope recognized by K99 fimbriae from enterotoxigenic Escherichia coli. Infect. Immun. 57, 907–911.

Pensaert, M., Callebaut, P., Cox, E., 1993. Enteric coronaviruses of animals. In: Kapikian, A.Z. (Ed.), Viral Infections of the Gastrointestinal Tract. Marcel Dekker, New York, pp. 627–696.

Reuter, G., Stoll, S., Kamerling, J.P., Vliegenthart, J.F.G., Schauer, R., 1988. Sialic acids on erythrocytes and in blood plasma of mammals. In: Sialic Acids 1988: Schauer,

R., Yamakawa, T. (Eds,), Proceedings of the Japanese-German Symposium on Sialic Acids. Kieler Verlag Wissenschaft und Bildung, Kiel, Germany, pp. 88–89.

Schultze, B., Krempl, C., Ballesteros, M.L., Shaw, L., Schauer, R., Enjuanes, L., Herrler, G., 1996. Transmissible gastroenteritis coronavirus, but not the related porcine respiratory coronavirus, has a sialic acid (*N*-glycolylneuraminic acid) binding activity. J. Virol. 70, 5634–5637.

Seignole, D., Mouricout, M., Duval-Iflah, Y., Quintard, B., Julien, R., 1991. Adhesion of K99 fimbriated Escherichia coli to pig intestinal epithelium: correlation of adhesive and non-adhesive phenotypes with the sialoglycolipid content. J. Gen. Microbiol. 137, 1591–1601.

Teneberg, S., Willemsen, P., de Graaf, F.K., Karlsson, K.A., 1990. Receptor-active glycolipids of epithelial cells of the small intestine of young and adult pigs in relation to susceptibility to infection with Escherichia coli K99. FEBS Lett. 263, 10–14.

Teneberg, S., Willemsen, P.T., de Graaf, F.K., Stenhagen, G., Pimlott, W., Jovall, P.A., Angstrom, J., Karlsson, K.A., 1994. Characterization of gangliosides of epithelial cells of calf small intestine, with special reference to receptor-active sequences for enteropathogenic Escherichia coli K99. J. Biochem. (Tokyo) 116, 560–574.