Are Intestinal Mucins Involved in the Pathogenicity of Transmissible Gastroenteritis Coronavirus?

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1. INTRODUCTION

Transmissible gastroenteritis virus (TGEV) is a prototype of enteropathogenic coronaviruses. The virus causes diarrhea in pigs of all ages. Infections are most severe in newborn piglets where letality can be as high as 100% (Pensaert *et al* 1993). The determinants of the enterotropism of TGEV are not known. A crucial factor appears to be the sialic acid binding activity of this virus (Schultze *et al* 1996). The ability of TGEV to attach to sialoglycoconjugates allows the virus to bind to erythrocytes. This interaction results in an agglutination reaction that probably has no physiological importance. However, hemagglutination provides a convenient assay for the sialic acid binding activity and allows quantitation of the virus. Mutants of TGEV that have lost their haemagglutinating activity because of a single point mutation in the S protein also had lost enteropathogenicity (Krempl *et al* 1997). Porcine respiratory coronavirus (PRCV), a respiratory variant of TGEV also lacks hemagglutinating activity. Both PRCV and the hemagglutination-deficient mutants are still able to grow in cell culture. Therefore, the sialic acid binding activity appears to be essential for enteropathogenicity but dispensible for growth in cultured cells. The sialic acid binding activity is located on the viral surface protein S. Another binding activity of this glycoprotein is the ability to interact with aminopeptidase N, the cellular receptor for TGEV (Delmas *et*

The Nidoviruses (Coronaviruses and Arteriviruses).
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al 1992). PRCV and the hemagglutination-deficient mutants have retained the ability to bind to aminopeptidase N. Therefore, the interaction with aminopeptidase N – though essential for the infection of cells – does not explain the enteropathogenicity of TGEV.

How the sialic acid binding activity contributes to the enteropathogenicity of TGEV is not known. One possibility is that it may facilitate the binding to and infection of enterocytes. Another possibility is that the sialic acid binding activity may protect the virus from detrimental effects encountered during passage through the gastrointestinal tract, such as the action of detergent-like bile salts. The latter view is supported by the finding that a concentration of the detergent octylglucoside that completely inactivates PRCV and hemagglutination-deficient mutants, only results in a partial reduction of the infectivity of TGEV (Krempl *et al* 2000). This protection may be achieved by binding of sialoglycoconjugates to the viral surface. Potential ligands encountered in the gastrointestinal tract are mucins because of their high content of sialic acids.

As there is an age-dependent difference in the severity of the disease caused by TGEV, we wondered whether the sialic acid binding activity may explain these differences. Therefore, it was of interest whether intestinal mucins obtained from piglets and pigs differ in the amount and in the type of sialic acid. To answer this question we isolated mucins from the small intestine and analyzed the sialic acids by high performance liquid chromatography (HPLC).

2. METHODS

Mucins were isolated from two piglets (12 to 14 days old) and from two pigs (10 to 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).

3. RESULTS

Intestinal mucins were obtained form two piglets that were in the age group where TGEV infections are most severe. These samples were compared to mucins from two older animals. Mucin preparation involved Sepharose chromatography. The sialic acid of the mucin fractions were analyzed by HPLC. The result is shown in Fig. 1. The two samples derived from piglets, both showed two sialic acid peaks, one representing N-glycolylneuraminic acid (Neu5Gc) and the other representing N-

acetylneuraminic acid (Neu5Ac). The samples resembled each other in that the Neu5Gc peak was more prominent than the Neu5Ac peak. They differed in the total amount of sialic acid. This difference is most likely explained by the presence of nonsialylated substances in one of the samples. One of the samples derived from adult pigs was found to have a sialic acid profile similar to one of the piglet samples both in the Neu5Gc/Neu5Ac ratio and in the total amount of sialic acid. The other sample was quite different with the Neu5Ac peak being much more prominent than the Neu5Gc peak. This result indicates that there is a variation in the distribution of sialic acids present in the mucin samples and that there are no clearcut differences between the samples of young and adult animals.

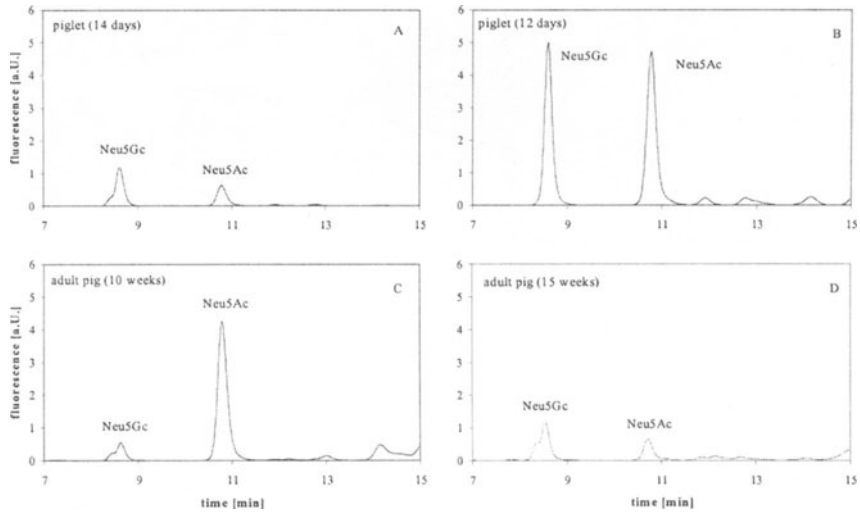

Figure 1. HPLC-analysis of the sialic acid content of mucins from two piglets and two pigs.

4. CONCLUSION

Most enteropathogenic viruses that enter the host via the gastrointestinal tract are nonenveloped viruses. Coronaviruses are unique among this group of viruses because they contain a lipid envelope. The predominance of nonenveloped viruses has been explained by the ability of nonenveloped viruses to resist the harsh conditions encountered during gastrointestinal passage, e.g. the low pH and the proteases. Enveloped viruses might be expected to be sensitive to such environmental factors, especially to the action of detergent-like bile salts. How coronaviruses survive under these

conditions is unclear. An attractive idea is the binding of protective substances to the viral surface. Sialoglycoconjugates are potential candidates for ligands that may help coronaviruses to survive. Several coronaviruses have been shown to contain a sialic acid binding activity. Whereas bovine coronavirus and serologically related viruses use this binding activity in the process of infection for attachment to cellular surface receptors (Schultze *et al* 1992), TGEV does not require it to infect cultured cells. For several TGEV mutants as well as for the respiratory variant PRCV loss of the enteropathogenicity has been shown to be associated with the loss of the sialic acid binding activity. So, TGEV is ideal for analyzing whether the binding of protective ligands is required to survive in the gastrointestinal tract.

Mucins are prime candidates for sialoglycoconjugates that may bind to the surface of TGEV. Mucins are abundantly found in the gastrointestinal tract and they are rich in sialic acids. If intestinal mucins play a role in the enteropathogenicity of TGEV, one might expect age-dependent differences, because TGEV infections are much more severe in piglets than they are in adult animals. Our analysis showed that the mucins from piglets contain both Neu5Gc and Neu5Ac. The former type of sialic acid was present in higher amounts in the two samples analyzed. As TGEV has a preference for Neu5Gc over Neu5Ac – at least in the recognition of low amounts of sialic acid-, the sialic profile found in the piglet samples is in accordance with the expected profile of a ligand for the sialic acid binding activity of TGEV. In the mucin sample of one of the two adult pigs, the sialic acid profile was similar to that found in the two piglet samples. In the other sample of adult pigs, a predominance of Neu5Ac was detected. Our data do not indicate that there are differences between young and adult animals in the sialic acid content of intestinal mucins, that can be interpreted such that piglet mucins are less protective ligands. Therefore, other explanations have to be considered. One possibility that the sialic acid binding activity is required for binding to intestinal epithelial cells and that the sialoglycoconjugates expressed on the surface of piglet cells allow a more efficient binding compared to glycoconjugates of cells from adult animals. Work is in progress to find out whether this concept is correct.

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REFERENCES

Delmas, B., Gelfi, J., L'Haridon, R., Vogel, L.K., Sjöström, H., Noren, O. and 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. and Hedrich, H.J., 1996, Response of germfree rat colonic mucous cells to peroral endotoxin application. *European Journal of Cell Biology* **71**: 99-104.

Krempl, C., Schultze, B., Laude, H. and Herrler, G., 1997, Point mutations in the S protein connect the sialic acid binding activity with the enteropathogenicity of transmissible gastroenteritis coronavirus. *Journal of Virology* **71**: 3285-3287.

Krempl, C., Ballesteros, M.L., Zimmer, G., Enjuanes, L., Klenk, H.-D. and Herrler, G., 2000, Characterization of the sialic acid binding activity of transmissible gastroenteritis coronavirus by analysis of haemagglutination-deficient mutants. *Jounal of General Virology* **81**: 489-96.

Pensaert, M., Callebaut, P. and Cox, E., 1993, Enteric coronaviruses of animals. In *Viral Infections of the Gastrointestinal Tract.* Edited by A.Z. Kapikian. New York, Marcel Dekker, pp. 627-696.

Schultze, B. and Herrler, G., 1992, Bovine coronavirus uses N-acetyl-9-O-acetylneuraminic acid as a receptor determinant to initiate the infection of cultured cells. *Journal of General Virology* **73**:901-906.

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