Transmissible gastroenteritis coronavirus (TGEV) is a porcine pathogen causing enteric infections that are lethal for suckling piglets. The enterotropism of TGEV is connected with the sialic acid binding activity of the viral surface protein S. Here we show that, among porcine intestinal brush border membrane proteins, TGEV recognizes a mucin-type glycoprotein designated MGP in a sialic acid-dependent fashion. Virus binding assays with cryosections of the small intestine from a suckling piglet revealed the binding of TGEV to mucin-producing goblet cells. A nonenteropathogenic mutant virus that lacked a sialic acid binding activity was unable to bind to MGP and to attach to goblet cells. Our results suggest a role of MGP in the enteropathogenicity of TGEV.

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recognized by TGEV is more common in piglets than it is in
older animals.

To analyze the carbohydrate content of the BBM proteins,
samples from the 3-day-old piglet used for the experiment
shown in Fig. 1 were treated with neuraminidase or mock
treated (−) or treated with VCNA (+). Following electrophoretic
separation under nonreducing conditions, the proteins were trans-
ferred to nitrocellulose. The immobilized proteins were incubated with
purified virus, and bound virus was detected by an enzyme-linked
immunoassay. Lanes 5 and 6. Western blot with the anti-pAPN anti-
body. On the left the positions of molecular mass markers are indi-
cated.

FIG. 1. Binding of TGEV and the mutant m10 to BBM. BBM were
isolated from the small intestine of a 3-day-old piglet and either mock
treated (−) or treated with VCNA (+). Following electrophoretic
separation under nonreducing conditions, the proteins were trans-
ferred to polyvinylidene difluoride membrane. The carbohydrates were oxidized
with periodate as described by Zimmer et al. (14). After label-
ing of oxidized residues with biotin hydrazide and incubation
with peroxidase-conjugated streptavidin, the bands were de-
tected by chemiluminescence. As shown in Fig. 2, lane 1, the
major band labeled by this procedure is MGP. A mild meta-
periodate oxidation on ice selectively labels sialic acid residues.
The major protein detectable by this procedure again is MGP.
A second prominent band appeared at 200 kDa (Fig. 2, lane 3);
this sialoglycoprotein, whose identity is not known, is not rec-
ognized by TGEV (Fig. 1), maybe because of a lower sialic acid
content. After sialidase treatment, MGP changed its electro-
phoretic mobility and shifted to a position closer to the top of
the gel (Fig. 2, lanes 2 and 4). This is a typical behavior of
mucin-type sialoglycoproteins. After enzymatic release of sialic
acids there still are some residues left for mild periodate oxida-
tion (Fig. 2, lane 4). Obviously the enzymatic treatment with
sialidase did not result in a complete release of sialic acids.
Taken together, these results indicate that the BBM protein
MGP that is recognized by TGEV is a carbohydrate-rich sia-
loglycoprotein, most likely a mucin-type glycoprotein. There-
fore it is designated MGP. As mucins are highly O glycosy-
lated, we analyzed whether MGP is recognized by jacalin (data
not shown). This lectin binds to galactose-β(1-3)-N-acetyl-
galactosamine, a disaccharide present in O-glycosylated pro-
teins. MGP was readily recognized by jacalin, suggesting that
the sialoglycoprotein MGP is highly O glycosylated. The high
carbohydrate content renders mucins rather resistant to deg-
radation by proteolytic enzymes. For this reason, we have been

FIG. 2. Detection of major BBM glycoproteins. BBM from a 3-day-
old piglet were either mock treated (−) or treated with VCNA (+). After electrophoretic
separation, the proteins were transferred to a polyvinylidene difluoride membrane. Carbohydrate residues (lanes 1
and 2) and sialic acid residues (lanes 3 and 4) were detected by metaperiodate oxidation.

FIG. 3. Binding of TGEV and the mutant m10 to cryosections of
the small intestine from a 3-day-old piglet. The cryosections were
incubated with purified virions and with monoclonal antibody 6A.C3
(for detection of bound virus). The sections were examined by immu-
nofluorescence microscopy (B, D, F, and H). The same parts of the
sections are shown in phase contrast on the left (A, C, E, and G). The
binding of TGEV is shown in an overview (B, arrows) and at a higher
magnification (D, arrows). The binding of TGEV after VCNA treat-
ment of the section (F) and the binding of the mutant m10 to the
mock-treated intestine (H) are shown in an overview.
unable so far to determine the amino acid sequence of this mucin-type glycoprotein.

We were interested in knowing whether the binding of TGEV to BBM proteins in a virus overlay binding assay reflects virus binding to jejunal tissue. For this purpose, cryosections were prepared from the jejunal tissue of a suckling piglet (3 days old). After fixation the tissue was incubated with VCNA-treated purified TGEV or TGEV mutant m10. Bound virions were detected by incubation with a monoclonal antibody (6A,C3) against the viral S protein (4) and fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Amersham Pharmacia). To see if virus binding is sialic acid dependent, some of the cryosections were incubated with VCNA or mock treated prior to incubation with TGEV virions. As shown in Fig. 3, TGEV bound to certain regions of the jejunal goblet cells (Fig. 3A to D). Goblet cells are specialized epithelial cells which synthesize and secrete mucins. In VCNA-treated sections no binding of TGEV to goblet cells was detectable (Fig. 3F). Sections which were incubated with the nonenteropathogenic mutant m10 instead of TGEV also did not show any fluorescence in the area of goblet cells (Fig. 3H). These results indicate that TGEV attaches to mucin-producing goblet cells in a sialic acid-dependent fashion. As MGP is a mucin-type glycoprotein and is the only BBM component that interacts with TGEV in an overlay binding assay in a sialic acid-dependent fashion, it is likely that MGP mediates the binding of TGEV to goblet cells.

In our view, MGP may be involved in a TGEV infection as follows. After passage through the stomach, the virions reach the small intestine. There they bind via their sialic binding sites to mucins, such as MGP, synthesized in the jejunal goblet cells. In VCNA-treated purified TGEV or TGEV mutant m10. Bound virions were detected by incubation with a monoclonal antibody (6A,C3) against the viral S protein (4) and fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Amersham Pharmacia). To see if virus binding is sialic acid dependent, some of the cryosections were incubated with VCNA or mock treated prior to incubation with TGEV virions. As shown in Fig. 3, TGEV bound to certain regions of the jejunal goblet cells (Fig. 3A to D). Goblet cells are specialized epithelial cells which synthesize and secrete mucins. In VCNA-treated sections no binding of TGEV to goblet cells was detectable (Fig. 3F). Sections which were incubated with the nonenteropathogenic mutant m10 instead of TGEV also did not show any fluorescence in the area of goblet cells (Fig. 3H). These results indicate that TGEV attaches to mucin-producing goblet cells in a sialic acid-dependent fashion. As MGP is a mucin-type glycoprotein and is the only BBM component that interacts with TGEV in an overlay binding assay in a sialic acid-dependent fashion, it is likely that MGP mediates the binding of TGEV to goblet cells.

In our view, MGP may be involved in a TGEV infection as follows. After passage through the stomach, the virions reach the small intestine. There they bind via their sialic binding sites to mucins, such as MGP, synthesized in the jejunal goblet cells and present in the mucus layer. This binding prevents the loss of virions by the intestinal peristalsis. The interaction of TGEV with sialic acids is a dynamic process. The virus may detach from some sialic acids and attach to others. In this way the virus may pass the mucus layer, which is as thick as 100 μm (reviewed in reference 1), and reach the glycocalyx covering the apical membrane of the intestinal cells (about 100 nm thick). Here the virus particles can again bind to mucin-type glycoproteins such as MGP. Finally the virions reach the cellular receptor pAPN present in the apical membranes of the epithelial cells. Binding to MGP may allow the virus to stay longer in the intestine and make it easier to find the pAPN receptor for initiating intestinal infection.

Taken together, our results indicate that the sialic acid binding activity of TGEV may help the virus to overcome the mucus barrier.

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REFERENCES