**In Vitro** Whole-Virus Binding of a Norovirus Genogroup II Genotype 4 Strain to Cells of the Lamina Propria and Brunner’s Glands in the Human Duodenum

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Received 3 May 2011/Accepted 1 June 2011

Human norovirus (hNoV) remains refractory to propagation in cell culture systems. We believe that knowing the exact cell type that hNoV targets would provide important insights into culturing the virus. By the use of an *in vitro* whole-virus binding assay, the hNoV genogroup II genotype 4 Sakai variant was found to bind predominantly to cells of the lamina propria and Brunner’s glands, but not to those of the luminal epithelial surface, of human duodenum tissue. Our findings, together with accumulating evidence reported elsewhere, suggest that hNoV may display tropism to nonepithelial cells, which is distinct from observations of other human enteric pathogens.

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† Supplemental material for this article may be found at http://jvi.asm.org/.
‡ Published ahead of print on 15 June 2011.
FIG. 1. Characterization of formalin-inactivated stool filtrates used in in vitro whole-virus binding assays. A quantitative real-time PCR assay and an enzyme-linked immunosorbent assay (ELISA) showed high levels of norovirus RNA (A) and protein (B, left), respectively, in the virus-containing stool filtrate but not in the other 2 stool filtrate controls. Immunoblotting revealed the presence of the 58-kDa noroviral major capsid protein (B, right). Quantitative PCR targeting the polymerase/capsid gene junction was performed as we previously described (4). Viral antigen detection (with an input stool filtrate volume of 1.8 \( \mu \)l) was performed using an ELISA-based Ridascreen Norovirus 3rd generation kit (r-biopharm) per the manufacturer’s instructions. The major viral capsid protein (58 kDa) was detected using the same antibody cocktail as was used in the ELISA. A total of 7 \( \mu \)g of protein was loaded per lane in the immunoblot.

Our findings are summarized in Table 1 and Fig. 2. All tissue donors examined were ethnic Chinese individuals; the donors included both strong and weak secretors, as determined by saliva phenotyping and genotyping assays (see Methods in the supplemental materials). In the human duodenum samples, the hNoV GII.4 Sakai variant attached predominantly to cells of the lamina propria independently of donors’ secretor status (Fig. 2C and Table 1). The specificity of the staining was verified by the absence of signal in the isotype-matched immunoglobulin control (Fig. 2E) and both stool filtrate controls (Fig. 2G and I). In addition, antibody blocking abolished in vitro whole-virus binding in a dose-dependent manner (Fig. 3). Our findings agree with the limited experimental hNoV infection data from studies of humans (10) and chimpanzees (2), which describe viral capsid protein expression in cells of the small-intestinal lamina propria. However, they are in contrast to data from nonprimate pig and calf models of hNoV infection indicating that the viral capsid antigen was commonly detected in villous enterocytes and was virtually absent from the lamina propria along animal small intestines (5, 17). Inter-

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\(^a\) +, positive test result or presence of positive signals; −, negative test result or absence of positive signals.

\(^b\) Le\(^a\), Lewis a antigen; Le\(^b\), Lewis b antigen.
Interestingly, whereas positive PAS staining of brush borders showed that the epithelial lining of the sections was intact (Fig. 2A), we observed no virus binding to luminal and crypt epithelial surfaces. This disagrees with an earlier in vitro binding study by Marionneau et al. performed using virus-like particles (VLPs) of Norwalk virus (NV; hNoV GI.1), the prototype of hNoV (12). In their study, baculovirus-expressed recombinant NV VLPs were found to bind exclusively to gastroduodenal epithelial cells. This discrepancy may be attributable to the nature of the stool filtrate or the virus strain used. It has long been recognized that hNoVs exhibit diverse patterns of binding to histo-blood group antigens (HBGAs) (7, 11, 16). However, staining using lectin isolated from Ulex europaeus showed that cells of the lamina propria to which the Sakai variant attached did not express histo-blood group H antigen (Fig. 2M). This is not surprising, given recent findings that, unlike NV VLPs, Sakai variant VLPs exhibit virtually no binding to HBGAs (11), although conflicting binding data exist from studies when P-domain protein was used instead of VLPs (22). Other host surface molecules may be involved in the whole-virus binding, as proposed by others (3). In addition to the binding to cells of the lamina propria, we also observed that the virus attached to submucosal structures resembling Brunner’s glands (Fig. 2D). The identity of Brunner’s glands was confirmed by coexpression of the cell marker mucin 6 on neighboring sections (Fig. 2L). The specificity of the staining was supported by the absence or abolishment of signal in all negative controls (Fig. 2F, H, and J and Fig. 3).

FIG. 2. In vitro whole-virus binding of the human norovirus (hNoV) genogroup II genotype 4 Sakai variant to nonepithelial cells of human duodenum. (A and B) Carbohydrate content was visualized with periodic acid-Schiff (PAS) stain (pink). Immunoperoxidase staining (brown) revealed binding of hNoV to cells of the lamina propria (C) and Brunner’s glands (D). Insets denote magnified views of squared areas. hNoV was identified by the use of P7G3 monoclonal antibody (mAb) against virus surface antigen. Specificity of the staining was verified by the absence of signal in an isotype-matched immunoglobulin control (E and F) and both stool filtrate controls (G to J). Brunner’s glands were identified by the use of the mucin 6 cell marker (K and L). Expression of histo-blood group H antigen was determined by histochemical staining using lectin isolated from Ulex europaeus (M and N). Formalin-fixed, paraffin-embedded tissues were used. Cell nuclei were counterstained with Gill’s hematoxylin (blue). Mucosa and Brunner’s glands views were taken from sections of separate individuals. Reproducible staining patterns were observed on sections of 3 individuals. Scale bar, 30 μm. RV, rotavirus.

FIG. 3. Inhibition of in vitro whole-virus binding of the human norovirus genogroup II genotype 4 Sakai variant to human duodenal tissues. An antibody-blocking assay was performed by preincubation of hNoV stool filtrate with different dilutions (1:500 and 1:100) of NS14 hNoV GII cross-reactive monoclonal antibody (mAb) as indicated for 2 h under room conditions prior to binding to sections. Immunohistochemical virus detection was performed as described in the main text. Scale bar, 30 μm.
wai agent (hNoV GI(1)) indeed failed to detect virus particles in small-intestinal epithelial cells by the use of electron microscopy even when gastroenteritis symptoms and histopathological changes, such as blunted villi and shortened microvilli, were observed in the infected individuals (6). Third, the latest evidence from a chimpanzee model of hNoV infection indicates that the virus displays tissue tropism to cells of the small-intestinal lamina propria (2). Collectively, the general belief that hNoV targets the intestinal epithelium, as in other enteric viruses such as rotavirus, may need to be revisited, and our findings support this thought. If it is true that hNoV targets nonepithelial cells, an immediate important issue is that of determining how the virus penetrates the intact epithelial lining. Our observation that hNoV also attached to Brunner’s glands may provide some hints. Submucosal Brunner’s glands, found primarily in the proximal duodenum between the pyloroduodenal junction and the entrance of the pancreatic duct, are open to crypts and intestinal lumen through an extensive network of ducts (19). We speculate that the virus may explore luminal openings of submucosal glands as the portal of entry into the lamina propria of the human small intestine.

Our study has limitations. We examined only virus binding to human duodenal tissues. Further investigation using other parts of the small intestine—the jejunum and the ileum—is needed. Moreover, considering that the HBGA binding patterns of hNoV differ between virus genogroups, genotypes, and even strains, the question of whether other hNoVs (non-Sakai) share similar tissue tropism characteristics remains to be answered.

In summary, we provide evidence that the hNoV GI(4) Sakai variant recognizes cells of the lamina propria and Brunner’s glands, but not those of the luminal epithelial surface, of human duodenum tissue in vitro. hNoV may display tropism to nonepithelial cells, with implications for the future direction of hNoV cultivation efforts.

We thank Mary K. Estes of Baylor College of Medicine for kind provision of NS14 human norovirus monoclonal antibody.

The project team is jointly supported by the Research Fund for the Control of Infectious Diseases (grants 08070422 to J.J.-Y.S. and CU-09-02-05 to M.C.-W.C.) of the Food and Health Bureau of the Hong Kong SAR government, People’s Republic of China, and the Institute of Digestive Disease (to J.J.-Y.S.), The Chinese University of Hong Kong.

REFERENCES