Norovirus devours human milk oligosaccharides rich in α-fucose

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Human norovirus binding to histo-blood group antigens (HBGAs) is thought to direct their entry into host cells. However, the glycan epitopes characteristic of HBGAs are also present on oligosaccharides abundant in human milk. In this issue of JBC, Hanisch *et al*. compared norovirus binding to human gastric mucins and human milk oligosaccharides, finding those bound most avidly are rich in α-fucose. Mimicry of these epitopes with α-fucose multivalently displayed on other carbohydrate scaffolds successfully scavenged this prevalent virus, providing new insights into norovirus biology and clues for future therapeutic development.

Norovirus is the most common cause of acute gastroenteritis outbreaks, with infections characterized by diarrhea, vomiting, and stomach cramps. This "winter vomiting bug" replicates quickly, remains active in biological waste for weeks, and is easily transmitted, leading to infections and epidemics worldwide. The most recent outbreak was at the 2018 Olympics in South Korea, affecting almost 200 athletes. Moreover, norovirus encases its single-stranded RNA genome in a protein capsid without a lipid envelope, making it more resistant to disinfectants. No antiviral treatment is available at present, due to the difficulty in cultivating human noroviruses and the fact that their infection mechanisms are poorly understood. However, we do know that norovirus binds to histo-blood group antigens (HBGAs)[3] as it transits the digestive tract. The glycan modifications characteristic of HBGAs vary per individual, meaning some people will be more susceptible to different norovirus strains than others. In this issue of JBC, Hanisch *et al.* (1) decipher the HBGA specificity of the most clinically relevant norovirus GII.4 subfamily and its preferences for human milk oligosaccharides (HMOs), a source of natural inhibitor glycans. The structural insights gained can explain at the molecular level why individuals with certain blood groups are at increased risk of infection and how these infections may be prevented and treated.

HBGAs are present not only on red blood cells, but also on epithelial cells, lipids, and secreted proteins such as mucins. The core glycan can be type 1 or type 2, with type 1 cores found mainly on secreted molecules; these differ in the connectivity of the carbohydrate core. The number and location of fucose residues also differ, as regulated by the expression of fucosyltransferases FUT3 and FUT2 from the Lewis (*Le*) and Secretor (*Se*) genes, respectively. The terminal sugar defines the A, B, and H blood group. 80% of the general population is a carrier of the Secretor gene and 90% the Lewis gene, explaining the prevalence of H type 1 and Le-b antigens in healthy human beings (Fig. 1*A*) (2). Norovirus strains are classified into two genogroups, GI and GII. GI strains bind to Lewis-positive type 1 antigens (Le-a and Le-b), whereas GII strains display more diversity in their binding preferences, making it hard to predict the specificity of uncharacterized strains. For example, the GII.10 norovirus (AF504571) that first hit Vietnam in 2004 prefers Le-a, whereas the more recent GII.17 noroviruses bind a greater panel of HBGA types (3). This functional diversity can be traced to structural diversity in P domains protruding from the capsid shell (Fig. 1*B*) (4). An insertion in the P domain called the P2 subdomain displays the largest sequence variation among the GI Norwalk-like human caliciviruses and contains the determinants of strain specificity (4). Virus-like particles (VLPs), consisting only of the capsid proteins, can be used to study strain specificity, as in prior investigations of the dependence of noroviral strain infectivity on the blood group of the patient (5). HMOs compete with HBGAs for norovirus binding. These HMO glycans contain complex backbones that can reach more than 30 saccharides in size and that can be further modified by the addition of fucose and/or sialic acid. They can function as a first line of defense in newborns that have not yet acquired an adapted immune response (2).

Hanisch *et al.* (1) focused their efforts to identify glycan epitope specificity on the JX459908 strain of the GII.4 subfamily, which was responsible for the 2012 outbreak in Sydney and has been the dominant cause of outbreaks worldwide since. The authors developed a neoglycolipid array of HMO fractions to screen VLPs against complex glycan mixtures and separately examined VLP preferences for gastric mucin *O*-glycans from blood group Lewis-defined individuals. The mucin screening revealed that Sydney VLPs bind nonsialylated type 1 A, B, and H blood group and Le-b antigens, indicative of FUT2 expression (Fig. 1). No binding to type 2 glycans or to Le-a was observed. Mass spectrometric analysis of the strongest binding HMOs identified

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[3] The abbreviations used are: HBGA, human blood group antigen; HMO, human milk oligosaccharide; VLP, virus-like particle.

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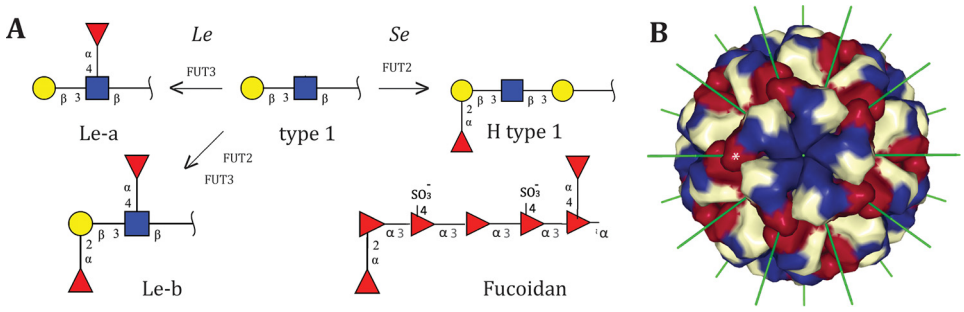

Figure 1. Oligosaccharide epitopes and their viral partner. *A*, humans infected by the prevalent norovirus GII.4 strain have an active FUT2 enzyme that transfers fucose to form the H-type 1 blood group antigen and perhaps the FUT3 enzyme that forms the Le-b antigen. *Yellow circle*, galactose; *blue square*, GlcNAc; *red triangle*, fucose. Fucoidan, from *Fucus vesiculosus*, contains α-fucoside branches that serve as a structural decoy for norovirus binding. *B*, the Norwalk virus capsid has icosahedral symmetry (*green lines*, in Protein Data Bank entry 1IHM (4)) displaying 180 copies of the major viral protein (3). The HBGA-binding P2 subdomain is a small β-barrel (*white*) that can be seen emanating from the protruding P domain surface.

showed that the preferred glycan structures were like those of the norovirus-interactive mucin glycans, with H type 1 or Le-b antigens at the end of branches of hepta- to decasaccharides (Fig. 1*A*). As in the gastric mucins, type 2 glycans were not detected in high-mass HMOs, perhaps indicating why HMOs are so effective as decoys for the continuously evolving viral capsids (Fig. 1*B*).

The authors noted that the high-affinity, high-mass HMOs present a multivalent display of 1 to 4 α-fucosides and proposed that the degree of fucosylation and their proper stereochemical presentation with respect to the HBGA-binding P domains on the noroviral capsid would influence binding affinity. Interestingly, it has been shown that attachment of an α-fucose to a polyacrylamide backbone to yield multivalent ligands improved norovirus binding by up to one millionfold over a univalent ligand (6). The authors tested for these effects using synthetic and natural oligovalent fucose conjugates (Fig. 1*A*), finding that these compounds strongly inhibited norovirus binding to HBGAs. The increases in avidity are sufficiently high to suggest that they could possibly overcome the noroviral strain's dependence on HBGAs.

The binding profile of the prevalent GII.4 norovirus is congruent with the secretion of type 1-glycosylated mucins in the oral route (7), and the known efficiency by which FUT2 modifies these core structures (7) explains why so much of the population is susceptible to this strain. Interestingly, piglets that display the same fucose modification on blood group antigens are susceptible to the mortal edema disease caused by infection with enterotoxigenic *Escherichia coli* if they are deprived of their mother's milk (8). H type 1 antigens and Le-b HBGAs might therefore serve as a selection factor for not only norovirus and *E. coli* but multiple gastrointestinal infectious agents, including rotavirus and *Helicobacter pylori*. Interestingly, recent molecular epidemiological studies have speculated that GII.17 noroviruses are replacing GII.4 in some regions in Asia, perhaps due to their broadened specificity. It has also been observed that H type 1 antigen is present in normal colon tissues while H type 2 and other types are aberrantly expressed in colon cancer tissues (8). Could the rise in GII.17 reflect its adaptation to a niche linked to cancerous or other aberrant physiology? These results provide intriguing new clues and directions for future research in this exciting field.

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