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Norovirus disease: changing epidemiology and host susceptibility factors

Anne M. Hutson[1], Robert L. Atmar[1,2] and Mary K. Estes[1,2]

[1]Department of Molecular Virology & Microbiology, Baylor College of Medicine, One Baylor Plaza BCM-385, Houston, TX 77030, USA
[2]Department of Medicine, Baylor College of Medicine, Houston, TX 77030, USA

Noroviruses cause the majority of acute viral gastroenteritis cases that occur worldwide. The increased recognition of noroviruses as the cause of outbreaks and sporadic disease is due to the recent availability of improved norovirus-specific diagnostics. Transmission of these viruses is facilitated by their high prevalence in the community, shedding of infectious virus particles from asymptomatic individuals and the high stability of the virus in the environment. Currently, the spectrum of clinical disease and the understanding of host susceptibility factors are changing. Cases of chronic norovirus gastroenteritis have been observed in transplant recipients and unusual clinical presentations have been recognized in otherwise healthy adults that are under physical stress. Recently, noroviruses were found to bind to gut-expressed carbohydrates, leading to a correlation between a person's genetically determined carbohydrate expression and their susceptibility to Norwalk virus infection. Greater community surveillance and further investigation of carbohydrate receptor-binding properties could provide further insights into norovirus transmission, susceptibility and pathogenesis, and should aid in developing vaccines and antiviral therapies for this common viral disease.

A bout of acute gastroenteritis can be debilitating, inconvenient and sometimes embarrassing. People of all ages and every walk of life suffer from infectious diarrheal disease. Viruses, bacteria and parasites can cause these acute gastrointestinal infections. One type of virus that causes acute gastroenteritis is a norovirus, previously called a 'Norwalk-like virus' or small round structured virus. These viruses are in the genus *Norovirus* within the family *Caliciviridae*. Noroviruses infect humans and cause symptoms of severe vomiting, watery diarrhea, nausea, abdominal cramps, fever and general malaise [1]. The onset of symptoms is generally 15–48 hours after exposure and illness usually lasts 12–60 hours. The first recognized norovirus, Norwalk virus (NV), gained its name from an outbreak of 'winter vomiting disease' in 1968 at an elementary school in Norwalk, Ohio, in the USA [2]. At that time, there was no conclusive evidence that viruses were agents of acute gastroenteritis. But in 1972, Kapikian *et al.* [3] demonstrated that the unidentified Norwalk agent was indeed a virus. From this time, additional viruses from other families have been recognized as causing gastroenteritis in humans, including rotaviruses, astroviruses, adenoviruses, Aichi virus (a picornavirus) and sapoviruses (from another genus in the *Caliciviridae* family).

During the past 30 years, norovirus investigation has been fraught with challenges. The amount of virus in infected stool samples is so low that purification of native virus has not yet been achieved. These viruses do not grow in cell or organ culture and there is no small animal model for norovirus infection and gastrointestinal disease. Without an infection model, our current knowledge of NV infection and disease is derived from outbreaks and volunteer challenge studies. Data from early studies have been reviewed elsewhere [4], therefore this review focuses on new information from recent studies that have used molecular approaches to detect and study noroviruses.

Noroviruses redefined by molecular approaches

The molecular era of norovirus studies began with the successful cloning of the NV genome from stool samples [5]. This allowed the virus to be characterized as a calicivirus, containing a characteristic positive-stranded, polyadenylated RNA genome of 7.7 kilobases that is protected from the environment by a protein capsid but lacking a lipid envelope. The capsid is composed of the major capsid protein, known as viral protein 1 (VP1), and a few copies of a second small basic structural protein known as VP2 (Figure 1) [5–7]. Genome sequence information and expression of norovirus proteins VP1 and VP2 to produce virus-like particles (VLPs), which are similar to virion capsids, has led to the development of genome-specific assays, such as reverse transcription-polymerase chain reaction (RT–PCR) and sensitive protein antigen solid phase immunoassays, including enzyme-linked immunosorbent assays (ELISAs). These sensitive assays are now being used to identify noroviruses as the cause of acute gastroenteritis in sporadic cases and also in outbreak settings. Before these sensitive norovirus-specific molecular assays were available, the cause of the majority of acute gastroenteritis cases was unknown. Because of these newer assays, disease surveillance has increased

Corresponding author: Mary K. Estes (mestes@bcm.tmc.edu).

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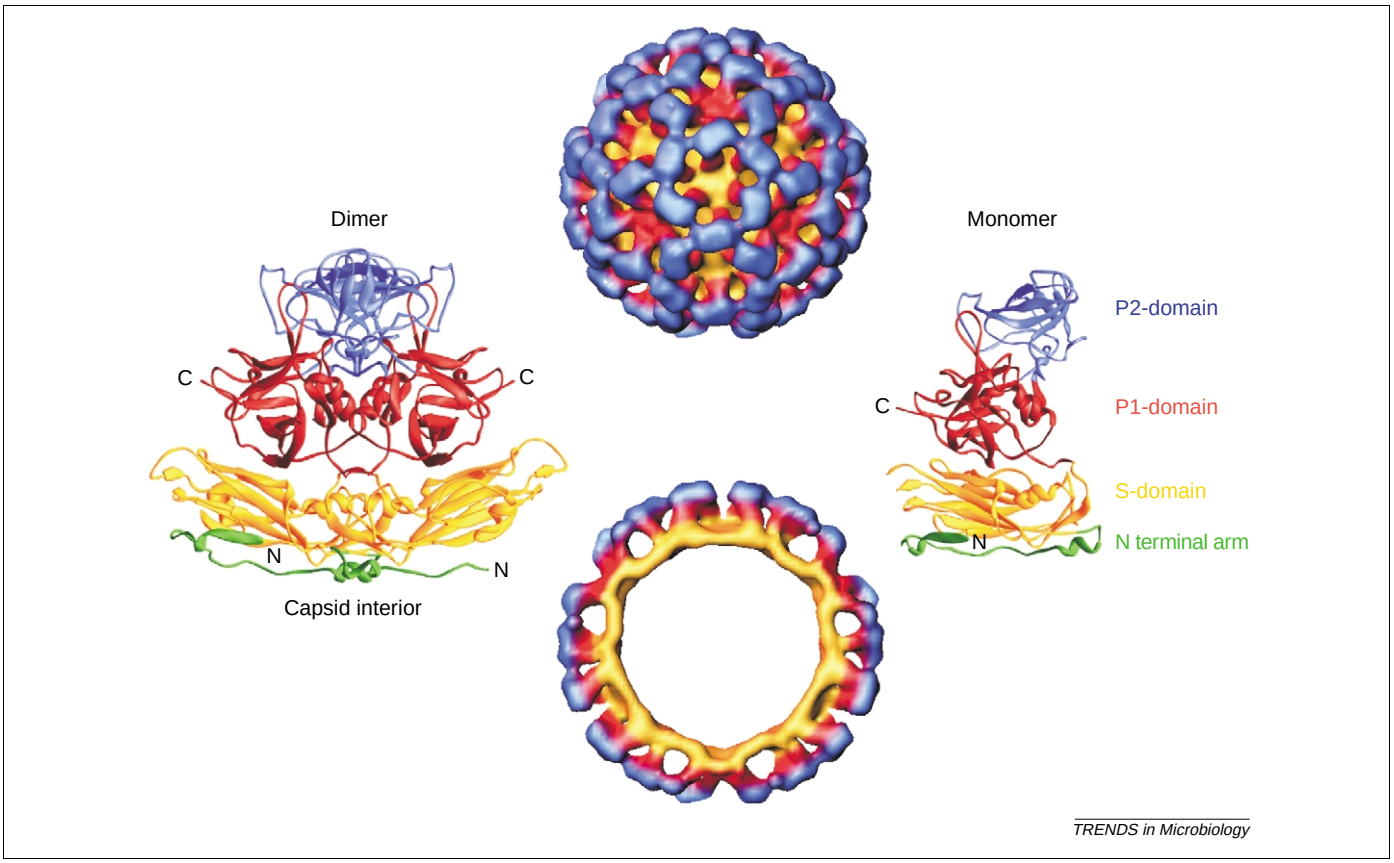

Figure 1. The Norwalk virus-like particle (NV VLP) structure has been solved by cryo-electron microscopic reconstruction to 22 Å (top, surface representation; bottom, cross-section) and by x-ray crystallography to 3.4 Å. The NV VLPs have 90 dimers of capsid protein (left, ribbon diagram) assembled in T = 3 icosahedral symmetry. Each monomeric capsid protein (right, ribbon diagram) is divided into an N-terminal arm region (green) facing the interior of the VLP, a shell domain (S-domain, yellow) that forms the continuous surface of the VLP, and a protruding domain (P-domain) that emanates from the S-domain surface. The P-domain is further divided into subdomains, P1 and P2 (red and blue, respectively) with the P2-subdomain at the most distal surface of the VLPs. Adapted, with permission, from Refs. [6,72].

and the majority of gastroenteritis cases of 'unknown etiology' are now attributed to noroviruses. These viruses are currently recognized as the cause of almost all (>96%) outbreaks of non-bacterial gastroenteritis [8], particularly in Europe and Australia where there is active surveillance. Noroviruses also cause an estimated 23 million cases of gastroenteritis per year in the USA [8–12].

To date, RT–PCR technology has allowed many strains of noroviruses to be cloned and sequenced from stool and emesis samples. Sequence comparisons indicate that noroviruses can be classified into at least five genetic groups on the basis of similarity across highly conserved regions of the genome, such as the RNA-dependent RNA polymerase (RdRp) and the shell domain of the VP1 capsid protein [4,13]. Two of these genetic groups, called genogroups I and II (GI and GII), contain the majority of the human noroviruses. By phylogenetic analysis, other caliciviruses that infect cattle, pigs and mice also fall within the *Norovirus* genus [14–18]. The bovine caliciviruses cluster into a proposed GIII, the members of which are most closely related to GI noroviruses [14–16]. Phylogenetic analysis places at least two human noroviruses within a proposed GIV: strains Alphatron (Genbank accession number AF195847) and Ft. Lauderdale (Genbank accession number AF414426) [19]. The porcine noroviruses fall within GII [17], and the recently described murine norovirus forms a proposed GV, the members of which are closer to GII noroviruses than those of GI by sequence comparison [4,18]. In the major capsid protein VP1, human norovirus strains within the same genogroup share at least 60% amino acid sequence identity, whereas most GI strains share less than 50% amino acid identity with those in GII [4]. Noroviruses in GI and GII can be further subdivided into genetic clusters, designated I.1 to I.7 and II.1 to II.7 (or II.8), respectively (Table 1). For example, the prototype 8FIIa Norwalk virus is in genogroup I, genetic cluster 1 (GI.1). Norovirus strains within a genetic cluster share at least 80% VP1 amino acid sequence identity with the cluster's reference strain [4]. Although strains can circulate simultaneously and different strains can circulate in distinct geographic regions at different times, the GII.4 noroviruses have been the predominant circulating strains detected in the population from the 1990s [19–21]. Additional epidemiological studies are needed to determine if the predominant GII.4 noroviruses have characteristics of infection that increase person-to-person transmissibility, for instance, by having an increased tendency to cause acute vomiting.

New insights into norovirus infection and disease

Reports of unusual norovirus outbreaks are becoming more frequent. For example, recent popular press reports have highlighted the rapid spread of norovirus illness among vacationing passengers and the crew aboard cruise ships, and the difficulty that has arisen during attempts to decontaminate ships following outbreaks [20]. In addition,

Table 1. Noroviruses, original outbreaks and carbohydrate-binding[a]

Virus Name	Accession Number	G	gc	Outbreak Site	Year	Carbohydrate-binding[b]									
						Le[c]	H1	Le[a]	Le[b]	H2	Le[x]	Le[y]	H3	A	B
Norwalk	M87661	I	1	Norwalk, OH, USA	1968	o	+	o	+	+	o	+	+	+	o
Hawaii	U07611	II	1	Hawaii, USA	1971	nd	o	o	o	nd	nd	nd	o	o	o
Snow Mountain	AY134748	II	2	Snow Mountain, CO, USA	1976	nd	o	o	o	nd	nd	nd	o	o	+s
Mexico	U22498	II	3	Mexico City, Mexico	1988	nd	+s	nd	+s	nd	nd	nd	nd	+s	+s
Grimsby	AJ004864	II	4	Grimsby, UK	1995	o	+	o	+	+	o	+	nd	+sr	+sr
VA387	AY038600	II	4	Virginia, USA	1998	nd	+s	o	+s	nd	o	+s	nd	+	+
MOH	AF397156	II	5	Three cities in Hungary	1999	nd	+s	o	+s	nd	o	n	nd	+	+
VA207	AY038599	II	na	Virginia, USA	1997	nd	o	+s	o	nd	+s	n	nd	o	o

[a]Abbreviations: G, genogroup; gc, genetic cluster; Le, Lewis; na, not assigned [4] or nominally assigned gc 8 [19]; nd, not done; o, no binding; +, binding; +sr, binding inferred by saliva and red blood cell binding assays; +s, binding inferred by saliva binding assays.
[b]Carbohydrates listed below.
Le[c]: H type 1 precursor, Galβ1,3GlcNAcβ-
H1: H type 1, Le[d], Fucα1,2Galβ1,3GlcNAcβ-
Le[a]: Galβ1,3(Fucα1,4)GlcNAcβ-
Le[b]: Fucα1,2Galβ1,3(Fucα1,4)GlcNAcβ-
H2: H type 2, Fucα1,2Galβ1,4GlcNAcβ-
Le[x]: Galβ1,4(Fucα1,3)GlcNAcβ-
Le[y]: Fucα1,2Galβ1,4(Fucα1,3)GlcNAcβ-
H3: H type 3, Fucα1,2Galβ1,3GalNAcα-
A: GalNAcα1,3(Fucα1,2)Galβ-
B: Galα1,3(Fucα1,2)Galβ-

noroviruses are now recognized as the most common cause of sporadic cases of diarrhea in the community [22]. They are the cause of acute gastroenteritis in people of all ages and in everyday settings: day care centers, schools, hospitals, hotels, nursing homes, the military, catered events and recreational camps [23–32]. Interestingly, symptomatically ill children are more likely to have episodes of vomiting, whereas adults who are ill tend to have diarrhea [33]. One of the reasons these viruses are so prevalent is their low infectious dose; less than 10 virions could be enough to infect a healthy adult (C.L. Moe *et al.* abstract P4–6, International Workshop on Human Caliciviruses. Atlanta, Georgia, USA, 1999). Transmission has occurred following direct person-to-person contact (from the defensive team to the opposing offense during a college football game), after consumption of contaminated food (raw oysters, bakery products, fresh fruit and vegetables), after intake of water (from ice, well or bottled water and during swimming), and following exposure to contaminated environmental surfaces and to airborne vomitus droplets that contain virus [34–41]. Because of the debilitating nature of the disease, the explosiveness of outbreaks, the high infectivity and stability of the virus, which is more resistant to disinfection techniques than most bacteria and other viral pathogens [42], the noroviruses have been classified as class B biological pathogens.

Noroviruses usually cause a short-term, self-limiting disease that can be treated with rest, oral rehydration and, if needed, intravenous replacement of electrolytes. Complications from norovirus infection are usually observed in infants and the elderly because they are generally more sensitive to volume depletion. However, new data suggest that unusual clinical presentations and complications from norovirus infection can occur in immunocompromised and physically stressed individuals. Recently, cases of chronic diarrhea in transplant recipients undergoing immunosuppressive therapy have been attributed to norovirus infection. An infant who received an intestinal transplant developed persistent diarrhea from norovirus infection, which resolved only after a reduction in immunosuppressive therapy [43]. Similarly, an adult transplant recipient suffered from a persistent norovirus infection with chronic diarrhea for more than two years [44]. For otherwise healthy adults, high stress situations could contribute to more severe norovirus disease. An unusual outbreak occurred in a military field hospital in Afghanistan, with four infected soldiers displaying unusual symptoms, such as neck stiffness, light sensitivity, confusion and in one case disseminated intravascular coagulation [45]. Dehydration and stress due to work and environmental conditions, in addition to infection-related volume loss, might have contributed to the more severe disease presentation in these soldiers.

Why are noroviruses transmitted so easily? In addition to viral stability, clinical challenge studies in the 1990s discovered the existence of a group of individuals who are asymptomatically infected with NV [46]. Asymptomatic individuals shed virus and mount a NV-specific antibody response, however they have no symptoms of disease. These asymptomatic individuals, as well as those who recover from the acute symptomatic form of the disease, can shed virus particles for up to three weeks after exposure, much longer than previously realized, and these virus carriers can transmit the disease unknowingly [22,46,47].

Host-susceptibility factors related to carbohydrate-binding

More than 25 years ago, the initial NV challenge studies conducted in volunteers found that a subset of individuals was repeatedly susceptible to NV infection, whereas a second subset was repeatedly resistant to infection [48]. It was hypothesized that a genetic factor, possibly a receptor, could affect a person's susceptibility to NV infection. Recently, a mechanism that explains susceptibility or resistance to NV infection has been identified; noroviruses attach to potential host cells in the gut only if the individual expresses specific, genetically determined

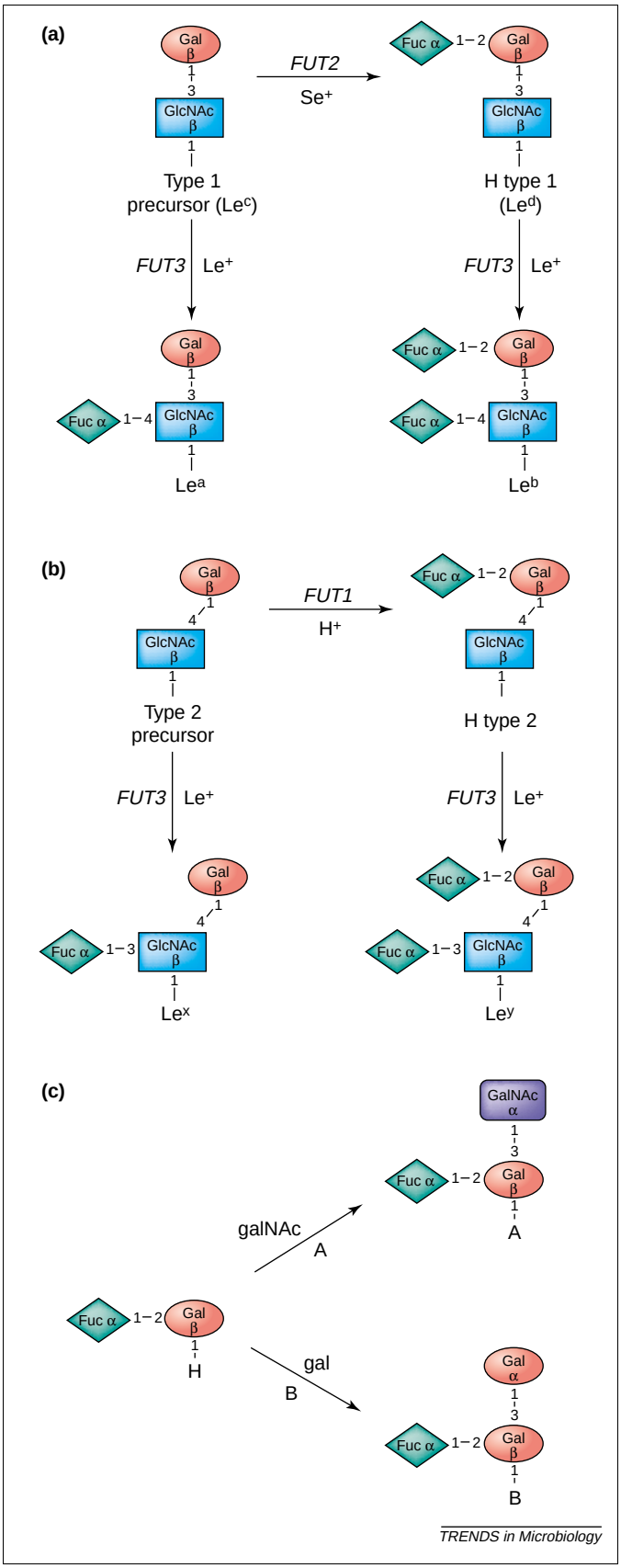

Figure 2. ABH and Lewis antigens are synthesized by sequential enzymatic transfer of single carbohydrate residues to specific precursor carbohydrate substrates. **(a,b)** H antigens are made by enzymatic addition of a fucose (Fuc) residue to the terminal galactose (Gal) residue in α1,2 linkage. **(a)** Secretor positive (Se^+) individuals express the *FUT2* gene product, a fucosyltransferase that adds Fuc to a type 1 precursor to make H type 1 (also known as Lewis(d) or Le^d). Eighty percent of Northern Europeans and Caucasian Americans are Se^+. **(b)** The *FUT1* fucosyltransferase adds Fuc to a type 2 precursor to make H type 2. Less than 0.002% of people throughout the world lack *FUT1* expression; they also do not express H antigen on their red blood cells (histo-blood group type Bombay), which is normally expressed on type O red blood cells. Type 1 and 2 precursor substrates have different Gal to N-acetylglucosamine (GlcNAc) linkages, **(a)** Galβ1,3-GlcNAcβ- and **(b)** Galβ1,4-GlcNAcβ-, respectively. The Lewis carbohydrate antigens are made when the *FUT3* enzyme is expressed in Lewis positive (Le^+) individuals. Eighty percent of Northern Europeans and Caucasian Americans individuals are Le^+, independent of secretor status. **(a,b)** *FUT3* transfers Fuc to the GlcNAc of type 1 and 2 precursors and H types 1 and 2 in α1,4 and α1,3 linkages, respectively. **(c)** H types 1 and 2 are the terminal moieties expressed in histo-blood group type O individuals, but in types A, B and AB individuals the H antigens are further modified by enzymes that transfer N-acetylgalactosamine (GalNAc, type A), Gal (type B), or either carbohydrate (type AB) to the terminal Gal residue of an H antigen in α1,3 linkage. ABH, Lewis and secretor phenotypes and enzymatic pathways are described in greater detail in other reviews [55,56].

Table 2. Microorganisms that use cellular carbohydrates for attachment

Microorganism	Carbohydrate
Viruses	
Orthomyxoviruses	Sialic acid
Polyomaviruses	Sialic acid
Reoviruses	Sialic acid
Coronaviruses	Sialic acid
Paramyxoviruses	Sialic acid
Parvoviruses (murine, canine)	Sialic acid
Adenovirus-associated virus 2	Heparan sulfate
Herpes viruses	Heparan sulfate
Flaviviruses	Heparan sulfate
B19 human parvovirus	P antigen
Caliciviruses	ABH and Lewis antigens
Bacteria	
Escherichia coli, pyelonephritogenic	P antigen
Bacteroides thetaiotaomicron	H antigen
Pseudomonas aeruginosa	ABH and P antigens
Staphyloccocus aureus	Le^a
Helicobacter pylori	Le^b
Mycoplasma	
Mycoplasma pneumoniae	I or i antigen
Protozoa	
Plasmodium falciparum	Sialic acid

carbohydrates [49]. This recent discovery is a breakthrough in understanding NV host-susceptibility factors. Here we summarize the existing evidence that NV carbohydrate binding is biologically relevant to NV infection and disease. Studies to date have used VLPs generated from insect or mammalian cell cultures as virus models to study potential host cell–virus interactions and to identify a cellular receptor for this virus [50,51].

Carbohydrate binding is a common method many viruses and other microorganisms use to attach to their host cells (Table 2). Commonly, viruses use the negatively charged sialic acid and heparan sulfate carbohydrates as cellular receptors, which are expressed on many types of cells and tissues. But the Norwalk virus VLPs bind to a different group of structurally related carbohydrates: the H, Lewis and A histo-blood group antigens (Table 1; Figure 2) [50,52–54]. These carbohydrates are synthetically related tri- and tetra-saccharide moieties that are located at the distal ends of carbohydrate chains on cellular glycolipids and glycoproteins found on the exterior cell surface. Unique varieties of carbohydrates are expressed by different cell types. This variety is determined by the presence or absence of specific glycotransferase enzymes as a result of a person's genetics

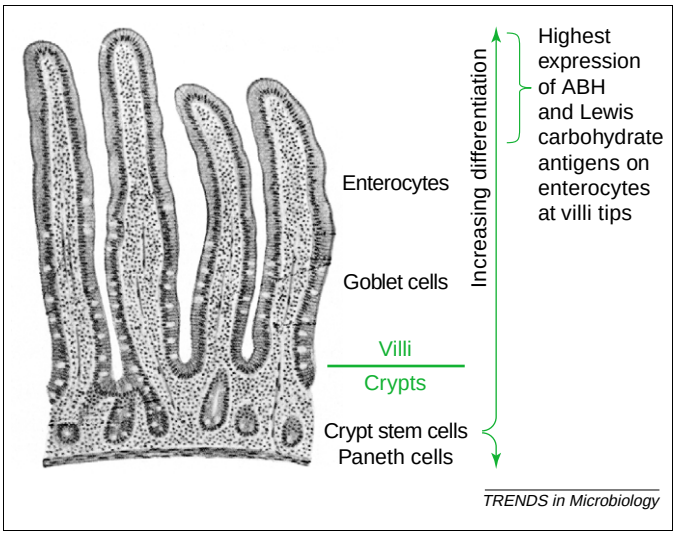

Figure 3. The small intestinal gut section shows the villi projecting into the lumen (top) with the crypts beneath the villi. Within the crypts are the progenitor stem cells of the small intestine. Crypt stem cell division supplies cells that differentiate as they are pushed down into the base of the crypt, becoming paneth cells, or up into the villus, becoming goblet cells or enterocytes. Paneth cells at the base of the crypt secrete lysozyme, goblet cells secrete mucins, undifferentiated enterocytes secrete chloride ions, and differentiated enterocytes absorb nutrients from the gut lumen until they undergo apoptosis and are sloughed off the villus. As enterocytes differentiate and travel up the villus, they express higher amounts and a greater variety of carbohydrates, including ABH and Lewis histo-blood group antigens [55]. Further description of these and other specialized intestinal cells can be found elsewhere [73].

and the developmental stage and differentiation state of the cells (Figures 2 and 3). These carbohydrates function in cell-to-cell interactions, self and non-self identification, and protection from the environment and pathogens. The relationship between the glycotransferases and carbohydrates discussed in this review are found in Figure 2 and reviewed elsewhere [55,56].

NV VLPs show the strongest level of binding when interacting with the H type 1 [also know as Lewis(d) or Le^d] synthetic carbohydrate antigen (Table 1; Figure 2a) [50,52]. H type 1 oligosaccharides are also best at inhibiting NV VLP binding to enterocytes, red blood cells (RBCs) and synthetic carbohydrate [50,53]. The Le^b antigen differs from H type 1 only by the presence of a fucose in α1–4 linkage to the most distal N-acetylglucosamine (GlcNAc) of a carbohydrate chain (Figure 2). The binding of NV VLPs appears to be minimally affected by this additional fucose, because Le^b closely follows H type 1 in strength of binding to synthetic carbohydrates and hemagglutination inhibition [50,52]. Both H type 1 and Le^b carbohydrates are found in saliva and other mucosal secretions from individuals who are Lewis-positive secretors (Figure 2). NV VLPs bind to saliva from all secretor-positive individuals, and their saliva can inhibit VLPs from binding to enterocytes and saliva [52–54]. Hemagglutination and synthetic carbohydrate-binding show that H type 2 and 3 carbohydrates also bind to NV VLPs and can inhibit homologous and heterologous carbohydrate-binding [50,52,53]. Furthermore, the heterologous inhibition between H and Lewis antigens suggests that these carbohydrates bind NV VLPs at the same site [50]. NV VLPs also bind type A and AB RBCs, and type A saliva and synthetic carbohydrate, but they do not bind B antigen synthetic carbohydrate nor do they bind the majority of type B RBCs and saliva [49,50,52,54]. The A carbohydrate antigen is dissimilar to the H and Lewis antigens. To date, it is unclear if the NV VLPs bind the H antigen segment of the A antigen (Figure 2) or if the A antigen interacts at a distinct site on the NV VLP. The A, but not B, trisaccharide antigens bind the VLPs, however the H disaccharide does not bind VLPs well compared with that of the H and A trisaccharides [54]. This suggests that there might be a unique A antigen-binding site on the NV VLPs.

To date, the carbohydrate-binding of one GI and seven GII norovirus VLPs have been reported, with the single GI NV VLPs binding to carbohydrates being the most thoroughly studied interaction (Table 1). The GII VLPs bind to the same group of carbohydrate antigens as NV VLPs, but their binding specificities are different. In addition, the carbohydrate-binding properties of GII VLPs are varied. For example, VLPs from VA387 norovirus (GII.4, clustered with the current predominant circulating norovirus strains) binds saliva and synthetic carbohydrates of all secretors regardless of their ABO type, whereas the VLPs from Snow Mountain virus (GII.2) bind the saliva of secretors of types B and AB, but not of types O and A [52,54]. It remains to be determined if the carbohydrate-binding profiles correlate with genetic clusters within a norovirus genogroup, or if the carbohydrate-binding properties are more strain-specific. Finding the carbohydrate-binding sites on noroviruses and identifying residues that are important for binding should help predict the carbohydrate-binding characteristics of a norovirus on the basis of its amino acid sequence. However, if noroviruses are consistent with many carbohydrate-binding proteins, the three-dimensional nature of the binding pocket could complicate binding predictions based on linear sequence and antigenic properties [57,58].

From previous volunteer challenge studies, there is strong evidence that carbohydrate-binding is essential for NV infection. Individuals who are non-secretors (Se^-), who do not express the *FUT2* fucosyltransferase and consequently do not make H type 1 or Le^b (Figure 2a), do not become infected after challenge with NV [49]. Furthermore, individuals expressing the B antigen are less likely to be infected by NV; however, when type B individuals are infected they are asymptomatic [59]. This strongly supports the hypothesis that carbohydrate-binding is crucial for NV replication, because NV VLPs show strongest binding to H type 1 and Le^b carbohydrates, and minimal binding to type B RBCs and saliva [50,52]. Even though the number of people in each secretor and ABO phenotype is low, especially for type B, these associations have been subsequently confirmed by other volunteer challenge studies (A.M. Hutson *et al.*, unpublished).

Therefore, we predict that secretors of types O and A are at greatest risk of NV infection and disease. Because other noroviruses display different ABH and Lewis carbohydrate-binding profiles [52,54], individuals resistant to NV infection could be susceptible to other norovirus strains. If carbohydrate-binding is conserved within genetic clusters, this could help explain the wide prevalence of GII.4 noroviruses, where the broad range of

carbohydrates to which the VA387 virus binds increases the proportion of susceptible individuals within the population. These data also help to explain why the absence of antibodies to a norovirus strain does not necessarily predict susceptibility to infection with that virus strain. Persons without NV-specific antibodies could be completely resistant to infection because of the lack of expression of carbohydrates necessary for virus attachment to host cells. Conversely, the presence of norovirus-specific antibodies could indicate previous infection and therefore susceptibility at the virus–receptor binding level. As with many mucosal infections, a single norovirus infection does not appear to provide long-term protection [60]. Without re-exposure to a particular norovirus strain, protection wanes after six months and it has been reported that susceptible individuals have been reinfected two years after prior exposure [48]. However, multiple exposures to the virus can result in induction of protective immunity and establishes a rationale for vaccine development [60,61]. A recent study has suggested that production of NV-specific salivary IgA early in infection might be associated with protection against more severe disease [49]. These data support the development of a norovirus vaccine that generates a strong mucosal immune response.

Further evidence supporting the biological relevance of NV VLP–carbohydrate interaction comes from the presence of antibodies in convalescent sera that specifically block the binding of VLP to carbohydrate. For influenza A virus, the capacity of antibodies to specifically block the virus binding to its cellular receptor (sialic acid) shows better correlation with levels of protection from influenza A challenge than the total amount of antibodies that can recognize the virus but that might not interfere with receptor-binding [62]. Therefore, if binding of NV to H antigen is essential for virus infection, then antibodies that inhibit NV receptor interactions will probably develop in response to infection. Notably, hemagglutination inhibition and antiserum blockade assays demonstrated that the convalescent phase sera of NV-infected individuals have more NV–H antigen blocking antibodies than pre-challenge serum samples [50,52]. The presence of antibodies that inhibit this binding could be associated with protection from NV infection or disease.

ABH and Lewis carbohydrates are used by other mucosal pathogens. For example, the bacteria *Helicobacter pylori* and *Staphylococcus aureus* attach to cells through H and Lewis carbohydrates (Table 2). However, the ABH and Lewis carbohydrate-binding specificity has only been described for viruses in the *Caliciviridae* family. Rabbit hemorrhagic disease virus (RHDV) was the first calicivirus shown to bind H type 2 carbohydrate [63]. RHDV is distantly related to noroviruses and causes a respiratory infection in rabbits that spreads systemically with high replication in the liver, causing internal hemorrhaging and death. The H type 2 carbohydrate is expressed on rabbit lung epithelia, but its role in RHDV infection remains to be determined. Binding of H and Lewis carbohydrates could be a common theme in calicivirus–cell interactions, but such binding interactions have not been shown for viruses in the *Vesivirus* or *Sapovirus* genera. RHDV, feline calicivirus (vesivirus) and porcine enteric calicivirus (sapovirus) are systemic pathogens [64–66]. For human noroviruses, replication and pathogenesis have only been described in the gut and norovirus has not been identified in the sera of infected individuals. However, because NV VLPs bind H type 2 and Lewis antigens, which are found on erythrocytes and other circulating and vascular cells, the possibility of systemic spread of NV should be reexamined with more sensitive molecular tests.

In the human gut, the developmental and differentiation-dependent nature of H and Lewis antigen expression on enterocytes, combined with the uniqueness of this virus–carbohydrate interaction, suggests that noroviruses are very selective in their binding to cells and that virus replication might be cell-type dependent. This could help explain the difficulty in adapting noroviruses to growth in cell culture. Potential host cells could be transfected with glycotransferases to enable the expression of norovirus-binding carbohydrates. This has been achieved with Chinese hamster ovary (CHO) cells, which normally do not express H antigen carbohydrates and show little binding of NV VLPs [53]. When the CHO cells were transfected with the rat *FTB* gene, the rat homolog to human *FUT2*, they expressed H type 1 and were able to bind NV VLPs [53]. However, the lack of carbohydrate expression does not explain the block to NV replication. Caco-2 cells, a human cell line that expresses markers of differentiated enterocytes that are characteristic of small intestinal cells, do express H types 1 and 2 [67]. These cells bind to rNV VLPs more efficiently than other cell lines that have been tested, but they do not support NV replication [51]. This suggests that a co-receptor could be required for sufficient norovirus entry, or that the block in viral replication in Caco-2 cells occurs at a post-binding step.

In secretor-positive individuals, ABH and Lewis antigens are found on epithelial cell surfaces and secreted mucins (heavily glycosylated glycoproteins) in saliva, milk and other mucous secretions. Because NV VLPs can bind free oligosaccharides, the role of these secreted antigens, which could bind NV and potentially inhibit infection, is uncertain. Even with secreted carbohydrates potentially binding NV VLPs, secretor-positive individuals are reported to be more susceptible to NV infection [49]. One explanation for this apparent contradiction is related to the multivalent nature of carbohydrate expression on cells that interact with multiple binding sites on VLPs. As with other carbohydrate-binding proteins, the affinity of NV VLPs for free monomeric oligosaccharides appears to be low [53]. This suggests that the avidity of binding of VLP to H and Lewis carbohydrates on a cell surface is a more biologically relevant event. Furthermore, for a multivalent viral or bacterial protein the monomeric affinity between its carbohydrate ligand and the monomeric protein is generally low [68]. Too high an affinity between a virus and its cellular receptor can inhibit the dissemination and consequently the viability of the virus [69]. Therefore, the strength or weakness of the binding of a norovirus strain to cellular carbohydrates could affect susceptibility within a

population, virulence within an infected person and transmissibility to others.

Questions for future norovirus research

This new information on norovirus epidemiology and host susceptibility factors leads to many new questions about infection with these viruses. What is the nature of the norovirus carbohydrate-binding site? To date, it is known that the carbohydrate-binding epitope on NV can be found in the P domain and is conformation-dependent [50]. Additionally, all carbohydrate-binding studies have been done using VLPs. Therefore, even though the NV volunteer challenge studies indicate that similarities exist between the binding of VLP and native virus, it is unknown whether norovirus virions have the same carbohydrate-binding properties as their VLP counterparts. Knowledge of the receptor-binding site for these viruses could lead to the development of antiviral carbohydrate analogs for prophylactic use in especially sensitive situations, such as in military, hospital or nursing home settings, or for treatment of individuals who are frail or immunocompromised.

What blocks norovirus replication in cultured cells? Finding potential receptor(s) for NV could aid in identifying cells or tissues that are susceptible to NV infection and could help explain one level of host tropism for this non-cultivatable virus. Because H antigens are expressed on Caco-2 cells, a lack of carbohydrate receptors is not the block in NV replication in these cells. There could be a co-receptor missing on Caco-2 and other cells, the presence of which increases NV internalization. Alternatively, other factors downstream of viral entry might be missing, or factors that interfere in NV replication could be expressed in cultured cells. Recently, a murine calicivirus was identified that is closely related to noroviruses by sequence analysis [18]. This virus infects mice orally, causing a systemic infection without gastroenteritis. Studies with this small animal model of infection are expected to provide new information about essential host and viral factors that are important for pathogenesis by making use of knockout and transgenic mice, and possibly recombinant viruses. Initial studies have indicated that immunocompromised mice become persistently infected and that the innate immune response plays an important role in the outcome of murine norovirus infection. The role of the innate immune response in human norovirus replication and pathogenesis also needs to be examined.

Do cross-species norovirus infections occur naturally? GI and GII noroviruses were initially only isolated from humans. Recently, phylogenetic analysis has indicated that other caliciviruses that infect cattle, pigs and mice also fall within the *Norovirus* genus [14–18]. These viruses form their own genogroups or genetic clusters within GI and GII, but the number of animal noroviruses that have been characterized remains relatively small compared with the number of human norovirus strains. The classification of animal caliciviruses within the *Norovirus* genus raises the question of whether zoonotic infections occur. Answering this question is important, because cross-species infections would affect the epidemiology and evolution of these viruses and complicate our ability to block transmission by vaccination or other therapy [14].

How can norovirus outbreaks and sporadic infections be prevented? Currently, good personal hygiene is the best preventative action against norovirus infection. Washing hands frequently and thoroughly and disinfecting contaminated surfaces with a 5–10% solution of household bleach in water, especially after contact with persons showing norovirus symptoms, is the best defense. People who are ill should refrain from preparing food for others, and once symptoms clear, they should be aware that they could still shed virus for up to three weeks. Furthermore, steps should be taken to keep water and food supplies free from noroviruses.

Currently, candidate vaccines are being tested for use in preventing norovirus disease [70,71]. Also, progress is being made to determine the location and structure of the NV-carbohydrate binding site for developing possible antivirals. These studies will help in the development of therapies to reduce norovirus illness and transmission, especially in nursing home, hospital and military settings.

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