Norovirus Gastroenteritis—New Strategies of Control and Prevention

a report by

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The major symptoms of norovirus gastroenteritis include a sudden onset of watery diarrhea, vomiting, abdominal pain, nausea, and/or fever, which last for two to three days.[1–3] Noroviruses were also called Norwalk-like viruses from the prototype Norwalk virus that was discovered in an outbreak of acute gastroenteritis in an elementary school in Norwalk, OH, in 1968.[1,4–6] Due to a lack of proper diagnosis, many of the early discovered Norwalk-like viruses were named by their locations of discovery and generally were referred to as small round structured viruses by their common appearance under electron microscopy. The cloning of Norwalk virus in 1990[7] and subsequently of many other Norwalk-like viruses greatly facilitated the research of noroviruses, including the development of molecular diagnostic methods and application of the methods in epidemiology and molecular virology of noroviruses, which significantly emphasized the importance of noroviruses as a pathogen causing epidemics of acute gastroenteritis. Noroviruses now are classified as one of the four genera in the family *Caliciviridae* (http://www.ncbi.nlm.nih.gov/ICTVdb/-ICTVdB). Although noroviruses remain non-cultivatable in laboratories, the recent finding of the host range and receptors of noroviruses provides new approaches in studying and development of strategies in control and prevention of norovirus gastroenteritis.

Virology

Noroviruses are small (Φ=~38nm), non-enveloped viruses containing a positive sense RNA genome of ~7.5 kilobases (kb). The genome is encapsulated by an icosahedral protein capsid that is composed of 180 copies of a single major structural protein.[8,9] The norovirus genome is organized into three open reading frames (ORFs).[7,10] ORF1 encodes several non-structural proteins, including an RNA-dependent RNA polymerase, a 3C-like proteinase, a helicase, and a genome-linked viral protein, that are important for viral replication.[11] ORF2 encodes the capsid protein, while ORF3 encodes a minor structural protein with an as-yet unknown function.[7,12–14] *In vitro* expression of ORF2 produces virus-like particles (VLPs),[15,16] while expression of the protruding (P) domain of the capsid protein leads to formation of a subviral P particle.[17,18] Both VLPs and subviral P particles are valuable reagents in development of diagnostic assays and studying host–pathogen interaction between norovirus and human host.[17–24]

Classification

Noroviruses are genetically and antigenically diverse and have been classified into five genogroups (GI to GV) based on the genomic sequence similarity,[25] but only GI, GII, and GIV viruses infect humans and these viruses are also called human caliciviruses. Phylogenetic analysis of the capsid protein or the RNA polymerase sequences further divided the noroviruses into at least 25 genetic clusters.[26–28] The GII-4 cluster, also called the Lordsdale-like viruses, has been found dominant worldwide,[29,30] possibly due to its broad spectrum of hosts.[19,20,31] The serological classification of noroviruses remains impossible due to a lack of cell culture or an animal infection model for noroviruses.

Diagnosis

Reverse transcription polymerase chain reaction (RT-PCR) is the most commonly used approach to detect noroviruses.[3,32,33] Several degenerate primers based on the conserved regions in both RNA polymerase and capsid protein encoding genes have been designed to amplify the corresponding regions of the norovirus genomes.[32–34] Further validation of the RT-PCR detection includes sequencing of the PCR products and a comparison of the sequence with the known norovirus sequences in public databases. This sequence information has been found to be a useful tool for molecular epidemiology of noroviruses. Although the currently available primer pairs are able to detect the majority of norovirus strains,[34] continual improvement of the assays by updating primer pairs based on genomic sequences of new strains is necessary.

Other diagnostic methods include electron microscopy, enzyme immune assay (EIA), and serologic assays. Electron microscopy allows direct detection of virus particle in stool samples but is less sensitive.[35] The sensitivity may be enhanced by using specific antibody to aggregate the virus. Antigen-detection EIAs based on antibodies against recombinant norovirus VLPs is a

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choice of method but still faces problems of broad detection of wide antigenic types.[32,33,36] Cross-reactive monoclonal antibodies recognizing conserved epitopes among strains within GI and GII, respectively, have been found,[37] which may help to increase the broad reactivity of the EIA assay. Alternately, pooled hyperimmune antibodies against strains representing different antigenic types of noroviruses also have been developed for broad detection of wild types. Several diagnostic kits based on these different antibodies have been and will be developed.[35,38] Diagnosis based on seroconversion against norovirus using VLP-based EIAs also has been described but these assays are mainly used for epidemiology studies in research laboratories.

Epidemiology

Noroviruses have been recognized as the most important cause of non-bacterial epidemic acute gastroenteritis, affecting individuals of all ages, with particularly severe illness occurring in infants, the elderly, patients with chronic diseases,[39] and immunocompromised individuals.[40–43] Noroviruses are highly contagious and can be spread quickly by person-to-person transmission, through contact with contaminated environmental surfaces, or by contaminated food and water, which are common sources of large outbreaks in a variety of settings including childcare centers, schools, restaurants, summer camps, hospitals, nursing homes, cruise ships, battle ships, and military troops.[44–49] In the US it has been estimated that there are over 23 million norovirus infections annually[50] and norovirus causes US$350–750 millions in losses each year due to care for illness and lost revenue from recalled foods.

Host Susceptibility

Increasing data indicate that noroviruses recognize human histo-blood group antigens (HBGAs) as receptors,[19,20,53–59] although controversy remains.[20,60] HBGAs are complex carbohydrates linked to glycoproteins or glycolipids presenting on mucosal epithelium as the first line of host defense against microbial pathogens. HBGAs also appear as free antigens in biological fluids, such as saliva, milk, and intestinal contents.[61,62] Human HBGAs are highly polymorphic and three major blood group systems, controlled by the ABO, Lewis, and secretor gene families, are involved in norovirus host range.[2,31] The recognition of HBGAs by noroviruses is strain specific, and so far eight distinct receptor-binding patterns of noroviruses have been identified.[19,20,31,58] The linkage of norovirus binding to HBGAs with clinical infection has been demonstrated by human volunteer studies on the prototype Norwalk virus[53–55] and by outbreak investigations of norovirus-associated gastroenteritis on a Lordsdale-like virus.[63] However, direct evidence for other receptor binding patterns is still lacking. Soluble HBGAs in human milk also have been found to be able to block noroviruses binding to HBGA receptors, suggesting these soluble antigens may serve as decoy receptors in protection of breast-fed infants from norovirus infection.[64,65]

Immunology

In addition to natural resistance related to the host HBGAs, the acquired immunity also plays a role in protection of host against norovirus infection. Volunteer challenge studies showed that the immune protection following a norovirus infection is short-lived (several weeks to months) and highly strain specific,[42,66–68] which explains why norovirus gastroenteritis is so widely spread even among adults. The understanding of host range related with HBGAs also explains the controversy of norovirus immunology observed in the 1970s to 1980s, in which some individuals with a higher level of antibodies against noroviruses were found to be more susceptible to norovirus infection than other individuals without or with low levels of antibodies. The detailed explanation of this controversy has been discussed in a recent review article.[31]

Disease Control and Prevention

The high disease burden of norovirus indicates a need for effective control and prevention. Development of a vaccine based on recombinant VLPs has been a major effort due to the lack of cultivation methods for noroviruses. The recent finding of noroviruses recognizing HBGAs as receptors has provided a new strategy to develop antivirals against the viruses by designing or screening inhibitors to block the virus–receptor attachment in the intestinal tract. However, before the vaccines and antivirals are available, proper public health measurements for outbreak control and prevention are necessary. These measurements include protection of food and water sources in the community from contamination of noroviruses and public education on personal hygiene, which have been outlined in the CDC webpage (http://www.cdc.gov/foodnet/) and a recent review article.[3]

Vaccine Development

Several studies have demonstrated that recombinant norovirus VLPs are capable of inducing immune response following oral or intranasal administration in laboratory animals and human volunteers.[69–76] However, the fact that noroviruses are antigenically diverse could be a challenge for the VLP vaccine for broad protection against wild types. A recent report could be a solution, in which multivalent norovirus VLP vaccines induced strong mucosal and systemic antibodies that blocked the attachment of multiple norovirus stains to their HBGA receptors.[77] Another challenge for norovirus vaccine is that the viruses may not induce a long-lasting immunity following a natural infection.[66,67] To this end, multiple dosages of the non-replicable sub-viral VLP vaccine may be a choice for boosting the immune protection. One study has demonstrated a simple and effective way by feeding mice with raw recombinant yeast extract containing norovirus VLPs,[78] which could be suitable for the multiple immunization strategy. Other options include production of the recombinant VLPs in transgenic plants, such as potatoes, as an 'edible' vaccine.[70,79] Finally, compared with VLPs, whose production is time consuming, the sub-viral P particle, which forms from the protruding domain of the capsid protein, may serve as a substitution for VLP because the P particle contains all exterior portions of the virus and retains the authentic receptor binding function.[17,18] Most importantly, the P particle is more stable and easily produced in a bacterial system, which is particularly suitable for large-scale production and multiple dosage delivery. All these vaccine approaches are still in the stages of investigation and none are available to public yet.

Antivirals

The hypothesis of antivirals based on the inhibition of norovirus/HBGA interaction is novel and studies are at an early stage. One study used a saliva-based blocking assay to screen a small compound library and identified a dozen high-affinity compounds that inhibited the capsid/HBGA interaction with a 50% effective concentration value less than 15μM.[80] Significant emphasis also has been put on understanding the structure and function of the noroviral capsid involvement in the receptor binding interface,[8,22,81] which may result in rational design of antivirals based on these structures. Another study used hydrogels pre-linked with HBGA analogs as a 'cage' to trap noroviruses in gastrointestinal tract,[82] which could serve as a potential therapeutic or preventive treatment for norovirus disease. The study of HBGAs in human milk is also highly significant in identification of high-affinity milk glycans against norovirus infection, because human milk is a natural gift resulting from a long evolution with the HBGA recognizing micro-organisms and thus is likely to contain high-affinity molecules to prevent norovirus infection.[64] Finally, since the norovirus P particles are easily produced and contain authentic receptor binding activities, they also have been proposed to be developed into an antiviral against noroviruses.[17] ■

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