Norovirus: The Disease and Vaccine Development

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Introduction

The viruses of the Norovirus genera belong to the *Caliciviridae* family, which also includes genetically-different viruses of similar structure, and composition from four other genera: Sapovirus, Lagovirus, Vesivirus, and Nebovirus. Norovirus, and sapovirus only infect human-beings, the others infect various animal species. The "Norwalk Virus" was the first virus to be identified from this family, in 1972, by immune electron microscopy in stools derived from an outbreak of gastroenteritis in Norwalk, Ohio, in 1968.[1] This outbreak affected a significant number of students and professors who experienced vomiting and acute diarrhea. Since then, researchers from different regions of the world have identified several viruses of comparable structure which cause similar outbreaks and were originally assigned nomenclature related to the location of the occurrence (Virus Snow Mountain, South Hampton, Mexico, etc.).[2] Years later these viruses were reclassified and grouped within the Norovirus genera. Currently, noroviruses cause the greatest number of human infections due to *Caliciviridae* followed by sapoviruses with prevalence rates only moderately lower to norovirus in some recent studies.[3,4]

Noroviruses are relatively small with a diameter of ~40nm and consist of a simple structure, a major capsid (VP1) protein and a capsid minor (VP2) protein where the single-stranded RNA is located. The virus has no lipid envelope.[5]

There is significant genetic diversity among noroviruses given specific mutations and genetic recombination.[2,6] Based on major and minor differences in the genetic sequences, seven genogroups (GI to GVII) are described with human viruses in the GI, GII, GIV genogroups. Within each genogroup there are minor sequence variations that determine the genotypes. The Norwalk virus, for example, is a GI.1 virus (serotype 1, belonging to the genogroup I); the viruses most frequently detected at present belong to genogroup II genotype 4 (GII.4). Moreover, there can be minimal variations within each genotype leading to "variants" of relevance as mentioned below.[2]

The potential relevance of this genetic diversity lies in its role with regard to certain viral virulence variability and/or antigenicity that may impact its susceptibility to infection. Most of the human infections have been caused by noroviruses belonging to serotype GII.4, possibly associated with higher hospitalization rates and death, as compared to other serotypes.[7,8] This epidemiological behavior may be due to the fact that when a genetic variation leads to a significantly relevant antigenic variation, the "new" virus avoids the existing herd immunity at a specific point in time and there is an increase of new cases, including more severe cases. For example, two GII.4 variants caused gastroenteritis outbreaks in Australia and New Zealand between 2005 and 2006 and one of the strains was associated with approximately 25% of the outbreaks reported in the United Kingdom in that period.[9] In 2012, the prevalent strain in the United States changed from the GII.4 "New Orleans" strain variant

to the "Sydney" variant.[10] In 2014, the GII.17 serotype emerged in Japan, spreading to the rest of the world.[11] In addition to the increase of cases in the population, genetic/antigenic variability may have individual relevance in connection with the likelihood for reinfection (with a circulating strain which is sufficiently different from an earlier infectious strain) and, thus, have impact on vaccine protection as will be addressed below. Recently, the concept of "static" versus "evolving" norovirus genotypes has been postulated, where GII.4 strains represent the genotype with the highest number of variants leading to periodic variant replacement (the predominantly "evolving" genotype).[12]

Sapoviruses are also divided into genogroups: GI to GV. To date, all of the genogroups, except for GIII, have been causes of human disease.[13]

Epidemiological Relevance and Disease Impact

In the 70's, the first nosological characterization associated with this virus family was as a "winter vomiting disease". It referred to outbreaks of various significance (from a few household cases to hundreds of cases in schools and communities) characterized by abrupt onset of vomiting, followed by watery diarrhea, with low-grade fever or no fever, lasting a few days. The most common infectious source was contaminated water or food. We currently know that noroviruses infect persons of all ages, from young infants to older adults. Most individuals, if not everyone globally, are infected at least once and typically several times throughout their lives. Most of the episodes of infection were moderate or asymptomatic, and even though only a fraction would develop moderate-to-severe clinical symptoms, with dehydration risk, the high frequency of the infection meant that this fraction is epidemiologically significant.[2,14,15] As seen later on, studies in cohorts of children are indicative of the low possibility of suffering from a second symptomatic infection from the same serogroup as the previous infection (this observation is not conclusive), creating the possibility of "natural" protection.

Acute norovirus-induced diarrhea is characterized by 4 to 8 instances of watery or semi-formed, non-bloody bowel movements. Acute onset vomiting before diarrhea is frequent, even as the only relevant symptom of the infection. Adults frequently report generalized myalgia, decreased energy and intense headaches. Fever is reported in almost half of the cases as mild, moderate or high (only in a low number of cases).[2,14,16]

Children <12 months, persons with some level of compromised immune system or who acquire the infection while hospitalized, and older adults comprise the highest risk groups for severe norovirus disease.[2,16-18] Post-infection sequelae include dyspepsia, constipation and/or gastroesophageal reflux.[19] In infants, seizures associated with the infection as well as encephalitis (less frequently) have been described.[20-22]

Noroviruses are associated with two typical clinical/epidemiological situations: Outbreaks caused by the consumption of contaminated water and/or food and acute endemic gastroenteritis in children.

Outbreaks have been described in several locations, including cruise liners and warships, schools, restaurants, nursing homes, summer camps, resorts, and communities, among many others where a contaminated product may infect several persons, and in a few hours may infect other persons through fecal-oral or oral-oral transmission.[2] Despite bivalve mollusks being specifically implicated in many of these outbreaks, vegetable and animal products, as well as water may be sources of contamination and induce outbreaks. Based on epidemiological studies conducted in countries with good surveillance systems, norovirus has been established

as the cause in approximately 50% or more of outbreaks due to water and/or food consumption in the middle-to-high income stratum.[23,24] A recent estimate concluded that in 2010 norovirus caused approximately 125 million cases of gastroenteritis associated with water and/or food consumption and resulted in 35,000 associated deaths.[25] In a norovirus outbreak, the individuals with greater exposure to the contaminated product, adults mostly but not exclusively, are the most affected. Outbreaks at hospitals and nursing homes are particularly harmful since the risk of suffering severe consequences (dehydration, hydroelectrolytic imbalance, and death) is higher given the vulnerability of the population.[26-28] In immunocompromised patients, there is significantly prolonged excretion of noroviruses in bowel movements, likely to last years, and occasionally difficult-to-manage cases of severe and/or prolonged diarrhea.[29] Among individuals with solid organ transplants, norovirus infection can cause significant morbidity due to prolonged diarrhea.[30,31]

In children <5 years of age who live in middle and upper-middle income countries, norovirus is currently the second most common cause of endemic acute gastroenteritis after rotavirus. In high-income countries that have implemented systemic rotavirus vaccination, such as the United States and Finland, norovirus has become the most common cause of hospitalizations and medical consultations due to acute diarrhea in children.[32,33] In low-income countries, such as Nicaragua, norovirus seems to be equally relevant, in particular after the introduction of the rotavirus vaccine.[4] In heavily-deprived countries in Africa and Asia, the Global Enteric Multicenter Study (GEMS) case-control study identified rotavirus, enterotoxigenic *Escherichia coli*, *Shigella spp,* and *Cryptosporidium* as the microorganisms most commonly associated with moderate-to-severe diarrhea,[34] and norovirus was relevant in only a few sites. The methodology of the GEMS study could have an impact on the outcome and findings of other studies conducted in similar regions that have previously suggested norovirus is a relevant pathogen.[35,36]

In a recent review of studies intended to measure the impact of norovirus infection in Latin America, it was concluded that 14–16% of acute gastroenteritis episodes in children (requiring hospitalization and/or consultation at a walk-in clinic) were caused by norovirus.[37] In other words, norovirus is associated with one out of six episodes of acute diarrhea in Latin American children.

In spite of the fact that norovirus-induced gastroenteritis is typically less severe than rotavirus, clinical manifestations of both may be undifferentiated, given that both lead to dehydration due to intense vomiting and frequent watery diarrhea.[14]

Prevention

The possibility of suffering from a norovirus infection emerges shortly after birth. Cohort studies demonstrate multiple exposures throughout the life span.[15,38,39] The mode of transmission is mainly fecal-oral, in particular for endemic gastroenteritis when a symptomatic person, or quite frequently, an asymptomatic excretor transmits the infection to a susceptible individual. Oral-oral transmission for individuals suffering from vomiting is also viable.[40,41] Contaminated food and water are the primary sources in outbreaks, followed by person-to-person transmission. The spread of the infection is facilitated by its low-infecting dose (between 20 and 1,000 viral particles to infect a person), prolonged excretion in stools (up to 2–4 weeks) and relative stability in the environment, food and water, as compared to other viruses.[2,42]

The possibility of vaccine prevention has long been considered a remote possibility for several reasons:

- Genotype diversity, which may or may not have cross-immunity.
- Repeated infections throughout life, which supports the concept that the virus may not develop long-lasting protective immunity.
- Studies conducted in adult volunteers in the 1970's–1990's, which concluded that immunity obtained through exposure to the viral inoculum (against similar viruses only) could be short lived (6–14 weeks).[43-45]

However, more recent studies are suggestive of the following:

- Immunity after natural infection may last several years.[46]
- In children, reinfection is frequent but symptomatic reinfection is not that frequent and exposure to a virus from a genogroup may confer a high level of protection against re-exposure to an intragroup virus.[15]

Moreover, it should be noted that, as is the case with other enteric microorganisms, some norovirus genogroups bind to the A, B, H, and Lewis antigens (of the histo-blood group oligosaccharides) on the intestinal surface and there are individuals who lack the above mentioned antigens (known as non-secreting antigens) and are thus naturally immune to the infection. Receptor binding is seemingly common intragenogroup and intergenogroup for the different "variants", since recent studies indicate that there would be cross-immunity among the variants when studying antibodies that block molecular binding of the viruses.[47,48] This finding is indicative of the possibility of cross immunity for viral binding across the intragenogroup and intergenogroup variants.

In 2011, a proof-of-concept study was designed to maximize the likelihood of developing effective vaccines with a GI.1 genotype intranasal prototype that conferred protection to adult volunteers exposed experimentally to the GI.1 virus.[49]

Vaccine Development: Where Do We Stand?

To date noroviruses are uncultivable, i.e. no system has been found to reproduce the virus, yet this situation may change in the near future as two culture systems for human noroviruses have shown promising results.[50,51] Uncultivability has hindered the development of live attenuated vaccines, such as the rotavirus vaccine. Currently available candidate vaccines are described in the table below (Table 1).

Table 1. Norovirus Candidate Vaccines

Antigens Included in the Vaccine	Current Clinical or Pre-Clinical Phase	References
VLPs GI.1, and GII.4	Phase I study in adults completed, progressed to Phase II studies in adults and children.	49,60,61
VLPs GI.3, and GII.4 plus rotavirus rVP6	Immunogenic in the murine BALB/c model; clinical studies in human-beings have not been started.	62
P Particle	Immunogenic in the murine BALB/c and gnotobiotic model; clinical studies in human-beings have not been started.	53,63,64
Particles replicating in vital vector	Immunogenic in the murine BALB/c model; one phase I clinical study has been reported although data presentation is pending.	65,66,67

The development of antigens against norovirus has been based mainly on the use of molecular tools, in particular, the expression of viral proteins through genetic expression systems, such as insect cells susceptible to infection with a so-called baculovirus that may recombine with norovirus genes.[52] In addition, other eukaryotic cells have also been used as expression systems, including yeast. These systems allow for the addition of specific norovirus genes, in particular the viral capsid genes for vaccine purposes. These genes are translated into self-assembly proteins, spontaneously creating a large amount of empty capsids resembling the viral capsid of the native virus, without the viral RNA. Therefore, these are non-infectious particles denominated "virus like particles" (VLPs).

Particles corresponding to a portion of the capsid, the P particle from the VP1 protein P domain, the protruding part of the capsid most exposed to the environment, have also been synthesized.[53] These particles may be synthesized in bacterial cells such as *Escherichia coli*, and simplify large-scale production. In addition, these particles may recombine with other antigens (rotavirus, influenza, hepatitis E, HIV) and create a platform to confer further protection against more than one virus.[54,55]

A third strategy has been the inclusion of norovirus capsid genes into viral vectors to use these recombinant plasmid-type structures to create human immunity. To date experiments have been conducted with vesicular stomatitis virus,[56] Newcastle virus,[57] adenovirus,[58] and Venezuelan equine encephalitis,[59] to induce "in vivo" (i.e. in the person rather than in an expression system as described above) VLP synthesis and protect vaccinated individuals. Out of the three strategies presented: VLPs, P particle, and viral vectors, only the first one is part of a clinical study phase and, therefore, a viable vaccine may be envisioned within the next 5 years. The use of viral vectors will have to overcome a series of perceptions on safety before human testing can take place. A recent press release reported completion of a Phase I study with a non-replicating adenovirus recombinant containing norovirus P particle in adults, but data remains to be presented.

To date, the most advanced vaccine candidate tested in three clinical studies is based on VLPs (see Table 2). The first study, a proof-of-concept study, demonstrated that adult volunteers vaccinated intranasally with the monovalent GI.1 vaccine adjuvanted with monophosphoryl lipid A (MPL) in addition to chitosan, had a 47% (95% CI: 15–67%) reduction of gastroenteritis occurrence after experimental infection with the GI.1 virus in adult volunteers. Local and systemic adverse events were reported in approximately 70% of participants vaccinated, not differing between vaccine or placebo recepients.[49] The vaccine development strategy shifted from an intranasal to an intramuscular vaccine because the latter demonstrated more efficient antigen delivery and better antibody responses in adults at a lower dosage level and with fewer doses than intranasal formulations. Low intergenogroup cross-reactogenicity and high frequency of GII norovirus infection led to the formulation of a bivalent GI.1 and GII.4 vaccine. The first study concluded that the 50ug (MPL-adjuvanted) dose per antigen conferred optimal immune response with an acceptable level of mostly mild adverse events in the injection site.[60] The second study with the GI.1/GII.4 vaccine in doses of 50ug (MPL-adjuvanted) dose per antigen, administered to adult volunteers had a protective effect against an experimental GII.4 norovirus infection. The study was not optimal because the level of infection and disease attained in the volunteers in general was below the expected level; nevertheless, protection was demonstrated.[61]

Table 2. Main Outcome of Human Clinical Studies Using VLP-Based Vaccines

Candidate Vaccine	Main Outcome	Reference
Intranasal adjuvanted VLP GI.1	Pivotal two-dose placebo-controlled study in 77 adults exposed to GI.1 norovirus demonstrated a reduction of norovirus-induced gastroenteritis by 47%.	[49]
Intramuscular adjuvanted VLP GI.1, and GII.4	Two-dose placebo-controlled study with various concentrations to determine safety and immunogenicity demonstrated good tolerance at various concentrations and rapid antibody response after the first dose.	[60]
Intramuscular adjuvanted VLP GI.1, and GII.4	Two-dose placebo-controlled study in 98 adults exposed afterwards to GII.4 norovirus demonstrated a reduction of severe (0% vs 8.3%, p=0.054), moderate-to-severe (6% vs 18.8%, p=0.68), mild-to-severe (20% vs 37.5%, p=0.74) norovirus-induced gastroenteritis and a reduction of severity (Score Vesikari 4.5 vs 7.3, p=0.002).	[61]
Intramuscular adjuvanted VLP GI.1, and GII.4	Two formulations including 15 or 50 µg of GI.1 combined with 50 µg of GII.2 were evaluated in 442 healthy adults aged 18–49 years. Reactions were mainly mild to moderate in 64% and 73% of vaccinated children, respectively, compared to 8% placebo controls. Immune responses to vaccination peaked by days 7–10 and persisted through day 28. GI.1 responses were highest with the 50/50 formulation, but GII.4 responses were higher with the 15/50 formulation. Authors conclude that the 15/50 formulation displayed the best balance of tolerability and immunogenicity.	[68]

How Will Norovirus Vaccines Be Used?

The first generation of norovirus vaccines for intramuscular administration in infants and adults will likely be available toward 2020–2022 absent any major setback. The use of the vaccine will depend on several factors to be understood from Phase II and III studies, and later trials. The protective efficacy against prevalent serogroups, in particular from the genogroup GII, as well as the interserogroup and intraserogroup cross-immunity spectrum, will be key for vaccine acceptance. A protective vaccine against 70–80% or more of the circulating noroviruses affecting individuals will likely interest decision makers in the field of vaccination. Duration of immunity will be equally important for administration in adults and infants. For adults, revaccination could be required within a few years based on the robustness of protection, which could be accepted by specific groups such as travelers or military personnel. On the contrary, the use of the vaccine during the first months of life to protect children, similarly to rotavirus vaccination, should not require revaccination at a later time so as not to diminish enthusiasm surrounding its application. Contrary to rotavirus, this is a norovirus candidate vaccine with non-replicating antigens. Therefore, although not ideal, two or more doses are likely to be required to attain a good level of protective immunity. Age at vaccination will be an important topic to define in infants considering the need to provide protection before 12 months of age since the vaccination schedule is quite prolific at 12 months. As new injectable vaccines to decrease morbidity and mortality in children, such as the norovirus vaccine, enter the market, the likelihood of vaccination at additional ages in addition to the current schedule (3, 5, and 7 months, for example) should be considered. A future significant challenge will be the development of new-generation combination vaccines that allow for a decrease of challenges while maintaining immunogenicity of the various components included in the combination vaccines. Combination of recombinant norovirus and rotavirus antigens seems to be an interesting alternative as well as the use of the P particle as platforms for other antigens. Nevertheless, these strategies require several years of analysis.

Conclusion

During the past 40 years much has been learned about norovirus structure, animal and human infectious mechanisms, immune responses, epidemiology and molecular epidemiology. Protective immunity in children and adults, in light of significant circulating virus variability, remains partially understood at the moment. Nevertheless vaccine efforts are underway and candidates are currently based on synthesized outer capsid particles, "virus-like" (VLP) or the protruding P particle. Proof of concept of vaccine protection has been obtained for an intranasal VLP in adults. The most advanced candidate is a GI.1/GII.4 bivalent VLP candidate for intramuscular use which has proven to be immunogenic in adults. Future Phase II and III studies in adults and children are projected for the near future.

Conflict of Interest Statement

Dr. Miguel O'Ryan has received research funding to conduct epidemiological studies on norovirus from Takeda Vaccines.

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