Norovirus Vaccine: One Step Closer

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(See the major articles by Bernstein et al on pages 870–8 and Sakon et al on pages 879–88.)

The need for a norovirus vaccine may be best described by the figure 200,000, which in round figures is the estimated number of annual deaths attributable to norovirus worldwide [1], mostly in children. As cited in the report of Bernstein et al in this issue of The Journal of Infectious Diseases, in the United States there are 800 deaths and 71,000 hospitalizations each year. Although children form a sizeable proportion of the hospitalizations (about 18,000), most of the deaths and costs are among the elderly [2]. Residents of nursing homes and more limited target groups, such as cruise ship passengers or naval recruits, are appealing target populations for norovirus vaccine developers as relatively low-hanging fruit for their return on investment. However, after successful elimination of most of rotavirus gastroenteritis through vaccination in the United States and many other countries, elimination of norovirus gastroenteritis in children is also receiving increasing attention [3].

The norovirus story goes back to 1971, when Kapikian et al detected virus-like particles (VLPs) by electron microscopy in stool specimens collected during a 1968 outbreak of gastroenteritis in schoolchildren in Norwalk, Ohio [4]. "Norwalk-like viruses" were later renamed as noroviruses.

For >40 years, noroviruses have defied attempts to propagate them in cell culture and, thus, development of a live attenuated or killed norovirus vaccine is not an option. In contrast, norovirus VLPs were developed relatively early by the Estes group [5]. A private company (LigoCyte) has been working on norovirus VLP vaccine development with slow but steady progress over the years.

The first candidate norovirus vaccine by LigoCyte was a genogroup GI.1 norovirus VLP (actually derived from the original Norwalk virus) produced in a baculovirus–insect cell (SF9) system. The vaccine contained topical adjuvant or mucoadherent chitosan, and was given intranasally [6]. In a challenge study in volunteers, the vaccine induced a moderate level of protection against Norwalk virus [7]. Whereas the route of administration may not have been optimal and the vaccine caused a stuffy nose, the study nevertheless was an important milestone, as it showed the proof of principle that a norovirus VLP vaccine can induce clinical protection, at least against homologous challenge.

The current study by Bernstein et al takes the proof of principle several steps further. First, the vaccine is a bivalent norovirus VLP combination representing GI.1 and GII.4 viruses and possibly protecting against GI and GII viruses. Second, the vaccine was given intramuscularly, which not only is more practical but also showed that parenterally induced immunity can protect against oral (intestinal) challenge. Third, the challenge study was done with a GII.4 VLP, which is a much more important causative agent of human disease than GI. Fourth, the challenge virus, while also a GII.4 virus, was not homologous to the vaccine. This is particularly encouraging because it would be unrealistic to consider development of norovirus vaccine against each genotype (29 in GII and 11 in GI) and all variants of GII.4.

As expected, the conditions of the challenge study reported by Bernstein et al were designed as favorable for detection of maximal protection. The subjects were selected on the basis of having low prevaccination antibody levels against the challenge strain (with a chance of high immune response after vaccination) and having the presence of salivary histo-blood group antigens that generally correlate with susceptibility to norovirus infection. Moreover, most of the subjects were challenged on day 42 after the second vaccination, at a time when protection might be assumed to be at a high level.

Protection was, indeed, detected against challenge with a dose that might be equal to about a thousand live virus particles. However, the protection was only partial. The vaccine did not protect against infection but protected against symptomatic illness, and the protection was greater against the more severe symptoms. Protection was 100% against severe vomiting or diarrhea, 68% against moderate to severe vomiting and diarrhea, and 47% against vomiting and diarrhea of any...
severity. On a much-used severity scale, the mean score of gastroenteritis symptoms was reduced from 7.3 in the placebo group to 4.5 in the vaccinees (P = .002). In other words, the vaccine significantly ameliorated the clinical illness that was induced by the challenge virus. A score of 4.5 is actually mild illness with little clinical significance.

Analogous to the performance of rotavirus vaccine is not far-fetched. Live attenuated oral rotavirus vaccines induce a high level of protection against severe rotavirus gastroenteritis, but a lower degree of protection against any (including mild) gastroenteritis [8]. The comparison with rotavirus may help to set the expectations for performance of norovirus vaccine at a realistic level. When the concept of rotavirus vaccination was first introduced, many found the performance of the vaccine to be inadequate, but now it is widely accepted that vaccine-induced protection against severe gastroenteritis is what really matters. It now seems that protection against severe gastroenteritis should be regarded as the primary target of norovirus vaccination as well.

It is commonly thought that a vaccine cannot do better than nature—in other words, that the limit of vaccination is as good as protection that follows after natural infection. In the case of norovirus, natural protection is not very good. The bottom line of findings from early challenge studies was that immunity from norovirus is type-specific and not long lasting. This point is also highlighted in the article of Sakon et al, also published in this issue of JID.

This large epidemiological study from Japan illustrates the complexity of norovirus epidemiology and, even though immunity was not directly measured, demonstrates the establishment of herd immunity and immune evasion of noroviruses by emergence of new variants. In young children, documented symptomatic reinfections were common, but usually associated with another genotype, suggesting presence of genotype-specific protective immunity from the first infection. Overall, Sakon et al observed development of protection over years, as episodes associated with genogroup GI.4 became fewer and the clinical picture milder. In the elderly, GI.4 was rampant again, with successive annual epidemics caused by the same serotype. An explanation for this might be, as pointed out by the authors, that the population of adults and elderly is large and not everyone gets infected in one particular year and, therefore, the same genotype and variant of GI.4 can cause infections again the following year.

New variants of GI.4 appear at approximately 2-year intervals, in both children and in in adults [9]. Therefore, the duration of type-specific immunity can be estimated to last for 1–2 years, with very little effect on herd immunity. If this were the whole truth of norovirus protective immunity, then a new vaccine would have to be developed and applied at least every 2 years, not much different from annual influenza vaccinations. This is neither realistic nor attractive.

Thus, a norovirus vaccine should perform better than nature. Although a natural norovirus infection in children induces a good genotype-specific response, this is of narrow spectrum only [10]. The challenge experiment conducted by Bernstein et al gives a hint that a VLP vaccine can induce significant cross-protection against at least severe norovirus gastroenteritis, which, as noted above, may be a reasonable and sufficient goal for vaccine development. In this case, the vaccine, which is an “artificial” VLP based on a consensus sequence from 3 GI.4 strains, was different from the challenge strain GI.4 Farmington by 19 amino acids in the hypervariable domain of the norovirus capsid protein. This is of a similar order of magnitude as a difference between naturally occurring norovirus GI.4 variants, giving hope that a single composition of GI.4 VLPs may be sufficient to induce a broadly protective immune response within GI. Genogroup GI is another matter, as cross-reactivity between the 2 genogroups is low. Therefore, a bivalent composition of GI.4 and a representative of GI VLPs appears as a reasonable candidate for a successful norovirus vaccine.

Our group has also worked on a candidate norovirus VLP vaccine for several years [11]. Our composition has been a GI.4 VLP based on the natural sequence from the year 1999 and a GI.3 VLP, with both sequences originating from isolates derived from Finnish patients. In preclinical studies, both VLPs induce strong homologous responses and good blocking antibody responses against other variants of GI.4 and at least 1 variant of GI (GI.1) [12]. Our vaccine also contains rotavirus VP6 protein (hence the name “trivalent”). VP6 in the composition seems to serve as a natural adjuvant that enhances the cross-reactive blocking antibody response to norovirus. It is possible that the monophosphoryl lipid A (MPL)-aluminum adjuvant in the LigoCyte vaccine does the same, although this has not yet been specifically shown.

An adjuvant may well be needed for a broad and durable immune response, particularly in the elderly and the very young. However, it remains to be determined if an adjuvanted vaccine such as the combination of MPL and aluminum would be approved by regulatory agencies and accepted by the public for use in young children. For the latter group, it certainly would be better if a protective immune response could be induced without an adjuvant.

The components of protective immune response against severe norovirus illness are not known. A high level of overall antibody response containing various levels of cross-reacting blocking antibodies is certainly important. But VLP-based vaccines also induce cell-mediated immune (CMI) responses and it is reasonable to assume that CMI plays a role in the protection against norovirus disease. Altogether, in comparison with natural infection, vaccination may induce not only a different but also better immunity.

The next step should be demonstration of protection against natural challenge. In
2012, LigoCyte was acquired by Takeda, a large pharmaceutical company. The acquisition indicates that a major company sees norovirus vaccine as a lucrative target for development.

Although there is no doubt that Takeda has a significant and lead role in norovirus VLP vaccine development and sufficient resources to carry out an extensive clinical development program, there are other important players in the field. Nanotherapeutics is working on development of a dry-powder norovirus VLP vaccine for intranasal delivery [13]. A slightly different approach has been undertaken by researchers at the Cincinnati Children’s Hospital Medical Center, using a P particle produced in Escherichia coli as a norovirus vaccine candidate [14].

Our own concept has been to develop a combined norovirus VLP–rotavirus VP6 vaccine for universal protection against childhood gastroenteritis. There is at least 1 other similar candidate with a combination of norovirus P-particle and rotavirus VP8 antigen [15]. The combination vaccines are primarily targeted for pediatric use, whereas the “pure” norovirus vaccines may be more suited for the elderly and several special groups such as the military or cruise ship passengers. In any case, the norovirus vaccines under development will have to be both scientifically and financially feasible for successful implementation.

**Note**

**Potential conflicts of interest.** The authors are co-holders in Finland and co-applicants in other countries of a patent on combined norovirus VLP–rotavirus VP6 vaccine; the authors also have received funding from UMN Pharma, Yokohama, Japan, outside the submitted work.

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**References**