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Norovirus Vaccine Development –Next Steps

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Noroviruses (NoVs) are a common cause of acute gastroenteritis in humans, with an estimated 21 million cases occurring in the United States annually [1]. Although the disease is often self-limited and recovery is the rule, NoV infection is estimated to cause up to 10% of hospitalizations for gastroenteritis in the United States [2], up to 200,000 deaths in children under 5 years of age in developing countries [3], and mortality in elderly [4]. The morbidity and mortality associated with infection highlights the need for the development of strategies to prevent NoV infection. Our recent report describing the efficacy of a NoV vaccine candidate made of virus-like particles (VLPs) is a step in that direction [5].

Norovirus is a genus in the family *Caliciviridae*. The genus is divided into five genogroups (I-V), of which three (I, II and IV) contain human viruses [6]. Each genogroup is further divided into genotypes based upon analysis of the complete amino acid sequence of the major viral capsid protein, VP1. Norwalk virus, the prototype human NoV, is classified as a genogroup I, genotype 1 (or GI.1) virus. The human NoV strains cannot be propagated *in vitro*, so it has not been possible to determine whether the genetic classification into genotypes has biological significance such as through the use of neutralization assays for serotyping.

Histoblood group antigens (HBGA) are glycans that are found on the surface of epithelium, and their expression influences susceptibility to infection with some NoVs in that failure to express certain HBGAs due to a nonfunctional glycosylase has been associated with absolute resistance to infection (e.g., Norwalk virus and fucosyl transferase 2) [7]. HBGAs have been proposed to be an attachment factor or receptor for these NoVs. Adaptive immunity also influences susceptibility to infection in that the presence of serum antibodies that bind to VLP particles and then inhibit binding of VLPs, a virus surrogate, to the HBGAs have been associated with a decreased risk of infection and illness following exposure to the virus [5,8,9]. This serum antibody blocking assay is proposed to represent a surrogate for virus neutralization. The observation that the presence of serum antibody that blocks VLPs binding to HBGA reduces the risk of illness contrasts with the lack of correlation of pre-existing serum antibody as measured by ELISA with protection from infection [9,10]. The duration of immunity following infection has been up to six months but less than two years based upon relatively small human experimental infection studies [10,11].

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NoV VLPs have been suggested as a vaccine candidate since their initial description 20 years ago. The VLPs are non-replicating particles that lack the viral genome. Preclinical studies showed the VLPs are immunogenic by parenteral, oral or intranasal routes and serum and mucosal antibodies were induced, the latter response being more vigorous if the VLPs were administered with a mucosal adjuvant [12]. Administration of Norwalk virus VLPs to people by the oral [13,14] or intranasal [15] route led to measurable serum antibody responses. The latter vaccine was then examined in a proof-of-concept efficacy trial in which healthy adult study participants received either two 100-mcg doses of adjuvanted Norwalk virus VLP vaccine or placebo intranasally 3 weeks apart [5]. All eligible participants were then administered ~10 human infectious dose 50% of live Norwalk virus. The vaccine induced a four-fold or greater serum IgA antibody response in 70% of recipients, although four-fold increases in antibody that blocked VLP binding to HBGA occurred in 32% of vaccinees. Vaccine recipients were significantly less likely to become ill if infected than placebo recipients (37% vs. 69%, respectively, P=0.006) and were also less likely to be infected (82% vs. 61%, respectively, P=0.05). Higher levels of serum antibody that blocked binding of VLPs to HBGA (titer >200) were associated with greater relative reductions in risk of illness and infection (72% and 57%, respectively) [5].

The clinical study results demonstrate that it is possible to prevent NoV-associated illness and infection via vaccination. It specifically showed homotypic protection since the VLPs and the challenge virus were from the same strain. However, there are a number of questions that still need to be addressed in order to demonstrate the feasibility on a large scale of preventing NoV-associated disease through vaccination. One of the first is to determine whether vaccine immunogenicity can be improved and, by doing so, can vaccine efficacy be improved. In dose escalation studies of the intranasal vaccine, the highest vaccine dose tested (100-mcg) was associated with the most frequent immune seroresponses, but only 15 of 19 (79%) vaccinees had four-fold or greater increases in IgA antibody levels (a frequency similar to that observed in the vaccine efficacy study) [15]. Fourteen of 19 (74%) had four-fold or greater responses measured by hemagglutination inhibition, with a geometric mean fold rise of 9.1 at the 100-mcg dosage level. Administration of VLPs by the oral route generated a similar magnitude of response (geometric mean fold rise = 6.5) at a dosage level of 250-mcg, and responses were not increased further by the administration of dosage levels up to 2-mg [13,14]. Parenteral administration may improve seroresponse frequencies and the magnitude of the antibody response, a concept that is currently being evaluated in a clinical trial (NCT01168401, www.clinicaltrials.gov). If the frequency and magnitude of response improves following intramuscular vaccination, the protective efficacy of this alternative route of administration will then need to be determined.

As noted earlier, NoVs have extensive antigenic and genetic diversity, with more than 25 genotypes recognized among the 3 genogroups containing human viruses. In addition, there is evidence that NoVs in some genotypes undergo antigenic drift in a fashion similar to that observed for influenza. This phenomenon has been best described for the GII.4 NoVs, the most prevalent circulating NoV genotype, and has possible implications for the development of successful vaccines [16,17]. The diversity of NoV genotypes and the antigenic drift within a genotype raise questions as to how many different strains will need to be included in a broadly effective vaccine and whether and how often the vaccine will need to be updated. Serological studies of persons infected with Norwalk virus have shown the occurrence of cross-reactive antibody responses that block binding of other genogroup I (GI. 2, GI.3, GI.4) NoVs to HBGAs, suggesting that infection with Norwalk virus may lead to increases in ‘protective antibody’ levels against other genogroup I strains [18]. Similar evaluations of vaccine responses are needed. The NoV capsids do contain epitopes that can elicit monoclonal antibodies that are cross-reactive and that bind to many different genotypes of VLPs [19]. In fact, such monoclonal antibodies are used in diagnostic assays to

detect infections with a range of NoV strains. Thus, cross-reactive antibody may be induced following vaccination. On the other hand, cross-protection studies conducted in the 1970s found that infection with a NoV from one genogroup (Norwalk virus, a GI.1 strain) did not prevent infection with a NoV of a different genogroup (Hawaii agent, a GII.1 strain) [20]. These results suggest that a broadly effective vaccine will need to include a minimum of two virus strains representing the two major human genogroups (I and II).

Another important next step in NoV vaccine development will be to extending the findings observed in the controlled study of young healthy adults to other populations. All adults in the first successful trial had pre-existing antibodies from previous infections [5]. Whether immunological priming from previous NoV infection is important for successful immunization with the nonreplicating VLPs will need to be assessed when evaluating vaccine immune responses among pediatric subjects. The studies by El-Kamary et al. [15] demonstrated that adults immunized intranasally with a NoV VLP vaccine develop circulating NoV-specific, antibody secreting cells (ASCs) that express the integrin α4/β7, a gut mucosal homing marker. Whether immunologically naïve pediatric patients and persons immunized parenterally also will produce ASCs that express gut mucosal homing signals following intranasal vaccination and whether this is important in protective immunity must be determined. Similarly, aging and the presence of chronic diseases may adversely influence immune responses at the other extreme of life. The impact of NoVs in these populations makes both important targets for vaccination [2,3,6].

Immunity following infection is not long-lived based upon observations that persons who have been experimentally infected can be re-infected and develop disease with the same virus 2 to 3 years after initial infection. The vaccine efficacy trial was designed to minimize the effect of waning immunity in that most participants were challenged with live virus three to five weeks following receipt of their second vaccine dose. The duration of vaccine-induced immunity must be determined, and for a vaccine to be practical immune protection probably needs to persist up to one year.

Field efficacy trials will address many of the questions raised here, including the duration of vaccine-induced immunity, the impact of NoV antigenic diversity and antigenic drift on protection, and the importance of host-related factors on immune responses. The outcomes of such studies will guide the further development of prospective norovirus vaccines and will help determine whether the development of an effective norovirus vaccine is achievable. Hopefully, the success achieved in the initial proof-of-concept efficacy study will be the first of many along the development pathway to a viable norovirus vaccine.

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Reference List

1. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States--major pathogens. Emerg Infect Dis. 2011; 17(1):7–15. [PubMed: 21192848]
2. Lopman BA, Hall AJ, Curns AT, Parashar UD. Increasing rates of gastroenteritis hospital discharges in US adults and the contribution of norovirus, 1996-2007. Clin Infect Dis. 2011; 52(4): 466–474. [PubMed: 21258098]
3. Patel MM, Widdowson MA, Glass RI, et al. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis. 2008; 14(8):1224–1231. [PubMed: 18680645]

4. Hall AJ, Curns AT, McDonald LC, Parashar UD, Lopman BA. The roles of Clostridium difficile and norovirus among gastroenteritis-associated deaths in the United States, 1999-2007. Clin Infect Dis. 2012
5. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness. N Engl J Med. 2011; 365(23):2178–2187. [PubMed: 22150036]
6. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. N Engl J Med. 2009; 361(18):50–59.
7. Rydell GE, Kindberg E, Larson G, Svensson L. Susceptibility to winter vomiting disease: a sweet matter. Rev Med Virol. 2011; 21(6):370–382. [PubMed: 22025362]
8. Czako R, Atmar RL, Opekun AR, et al. Serum hemagglutination inhibition activity correlates with protection from gastroenteritis in persons infected with Norwalk virus. Clin Vaccine Immunol. 2012; 19(2):284–287. [PubMed: 22190401]
9. Reeck A, Kavanagh O, Estes MK, et al. Serological correlate of protection against norovirus-induced gastroenteritis. J Infect Dis. 2010; 202(8):1212–1218. [PubMed: 20815703]
10. Johnson PC, Mathewson JJ, Dupont HL, Greenberg HB. Multiple-challenge study of host susceptibility to Norwalk gastroenteritis in US adults. J Infect Dis. 1990; 161(1):18–21. [PubMed: 2153184]
11. Parrino TA, Schreiber DS, Trier JS, Kapikian AZ, Blacklow NR. Clinical immunity in acute gastroenteritis caused by Norwalk agent. N Engl J Med. 1977; 297(2):86–89. [PubMed: 405590]
12. Estes MK, Ball JM, Guerrero RA, et al. Norwalk virus vaccines: challenges and progress. J Infect Dis. 2000; 181(2):S367–S373. [PubMed: 10804150]
13. Ball JM, Graham DY, Opekun AR, et al. Recombinant Norwalk virus-like particles given orally to volunteers: phase I study. Gastroenterology. 1999; 117(1):40–48. [PubMed: 10381908]
14. Tacket CO, Sztein MB, Losonsky GA, Wasserman SS, Estes MK. Humoral, mucosal, and cellular immune responses to oral Norwalk virus-like particles in volunteers. Clin Immunol. 2003; 108(3): 241–247. [PubMed: 14499247]
15. El Kamary SS, Pasetti MF, Mendelman PM, et al. Adjuvanted intranasal Norwalk virus-like particle vaccine elicits antibodies and antibody-secreting cells that express homing receptors for mucosal and peripheral lymphoid tissues. J Infect Dis. 2010; 202(11)
16. Siebenga JJ, Vennema H, Renckens B, et al. Epochal evolution of GGII.4 norovirus capsid proteins from 1995 to 2006. J Virol. 2007; 81(18):9932–9941. [PubMed: 17609280]
17. Lindesmith LC, Beltramello M, Donaldson EF, et al. Immunogenetic mechanisms driving norovirus GII.4 antigenic variation. PLoS Pathog. 2012; 8(5):e1002705. [PubMed: 22615565]
18. Lindesmith LC, Donaldson E, Leon J, et al. Heterotypic humoral and cellular immune responses following Norwalk virus infection. J Virol. 2010; 84(4):1800–1815. [PubMed: 20007270]
19. Kitamoto N, Tanaka T, Natori K, et al. Cross-reactivity among several recombinant calicivirus virus-like particles (VLPs) with monoclonal antibodies obtained from mice immunized orally with one type of VLP. J Clin Microbiol. 2002; 40(7):2459–2465. [PubMed: 12089262]
20. Wyatt RG, Dolin R, Blacklow NR, et al. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. J Infect Dis. 1974; 129(6):709–714. [PubMed: 4209723]