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Original article

Vaccines against norovirus: state of the art trials in children and adults

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ABSTRACT

Noroviruses (NoVs), a group of nonenveloped, single-stranded RNA viruses belonging to the *Caliciviridae* family, are the leading cause worldwide of acute infectious gastroenteritis. Serious and eventual fatal outcomes may be observed in at-risk populations such as the very young or older adults, especially in those with underlying diseases. NoVs are highly infectious, with a low number of virus particles causing infection, and they are highly resistant to environmental conditions. NoVs have multiple routes of transmission including faecal–oral, aerosolized vomitus, person to person and *via* contaminated surfaces or food and water. NoVs can cause frequent and dramatic outbreaks where people congregate in close quarters such as hospitals, long-term care facilities, cruise liners and military barracks and ships. Of the seven NoV genogroups, human disease is most frequently caused by genogroups I and II, although genogroup IV has also been associated with illness. The absence of reliable, high-yield cell culture systems or animal models has steered the development of vaccines towards nonreplicating recombinant capsid proteins including viruslike particles and the sub-virus-sized P particles. Takeda Vaccines is developing a candidate NoV vaccine formulation based on adjuvanted viruslike particles from the GI.1 genotype and a consensus GII.4 sequence derived from three natural GII.4 variants. Early clinical trial results show good tolerability and robust immune responses to both components. This approach is designed to induce broad protective immune responses in adults and children. **F. Baehner, CMI 2016;22:S136**

Introduction

Norovirus (NoV)-related disease regularly hits the news headlines, often as dramatic and repeated outbreaks aboard cruise ships as well as in restaurants, schools, hospitals and institutions for care of the elderly. However, the global sporadic disease burden in the community is much more important than the headlines cases may suggest. While still often underdiagnosed and underreported [1] (http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/), NoV is the leading cause of acute gastroenteritis in the world, especially in countries where vaccination against rotavirus was successfully introduced [2–4].

Transmitted *via* the faecal–oral route, through contaminated food or water or as aerosolized vomitus or fomites, NoV infection typically results in acute nausea, uncontrolled and often projectile vomiting and diarrhoea for up to 72 hours. Dehydration is the greatest risk for complicated disease and may require hospitalization for intravenous fluid therapy. While in developing countries most of the severe disease burden is in the very young; in industrialized countries a substantial part of the NoV-related morbidity and mortality occurs in older adults [5,6]. Older adults, young children and immunocompromised individuals may experience prolonged clinical disease and virus shedding. Patients with underlying conditions such as chronic renal or chronic cardiac disease may have poor outcomes after a NoV gastroenteritis episode [7]. Only 5–10% of individuals with symptoms seek medical attention, and in the United Kingdom it is estimated that there are about 300 additional cases in the community for each case reported to the national health authority [1]. Not surprisingly this disease burden also results in significant direct (medical) and indirect (societal) healthcare costs: an estimated $5.5 billion in the United States, as well as economic costs due to work loss [8,9].

The first identification of the causative agent was from an outbreak of "winter vomiting disease" in Norwalk, Ohio (1968) [10]. The later visualization by Kapikian *et al.* [11] and then the genetic characterization of the pathogen in the 1990s allowed for its classification as a member of the *Caliciviridae* family [12]. They are

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nonenveloped, positive-sense, single-strand RNA viruses divided into seven genogroups (GI to GVII) and further subdivided into genotypes. GI and GII NoVs are the genogroups primarily responsible for human illness, although GIV has also been detected in humans [13]. Natural immunity after NoV exposure appears to be strain or genotype specific, with little or no protection conferred across genogroups, and the duration of natural immunity has been estimated to range 4–9 years [14]. Genogroup II type 4 (GII.4) currently accounts for approximately 60–90% of all NoV outbreaks, making it the dominant type worldwide [15]. This genotype has been shown to undergo genetic evolution, and new GII.4 strains emerge every 2–4 years. RNA recombination is one of the major driving forces of virus evolution and has been identified for a number of different genotypes, including GII.4 [16]. NoVs undergo genetic evolution, and new genotypes and subtypes emerge every 2–4 years with limited natural cross-protection between genogroups. In 2012 a new GII.4 strain emerged (Sydney), and recent data show increased numbers of GII.17 cases in Asia [17].

As a "perfect human pathogen" [5] that is difficult to avoid, that is highly infectious and for which there is no etiologic treatment, NoV represents a good candidate for potential prevention through vaccination. A serologic correlate of protection against NoV infection or disease has not been definitively established, and assessment of neutralizing activity is not possible because of the lack of an *in vitro* culture model for NoV. However, since NoVs most likely initiate infection by attachment to human histo–blood group antigens (HBGAs) [18], assessment of the ability to block this interaction may be a surrogate for neutralizing activity and has been identified as a potential serologic correlate of protection in human challenge studies [19].

The search for NoV vaccines has been hampered by the absence of reliable, efficient systems to propagate the virus and the paucity of small animal models to study (vaccine-induced) protective responses. Therefore, one focus has been on expression systems for nonreplicating recombinant proteins either as viruslike particles (VLPs) or a P particle. NoVs are covered by a protein capsid that is formed by a major (VP1) and a minor (VP2) structural protein. The major capsid protein VP1, when expressed in cell culture, assembles spontaneously into 180-unit VLPs that are morphologically and antigenically similar to native virus capsids [20]. VLPs can be expressed in systems such as recombinant viral vectors (e.g. baculovirus, Venezuelan equine encephalitis virus, vesicular stomatitis virus) insect cells, mammalian cells, plasmids, and yeast as well as in transgenic plants (tomato, potato, tobacco). The NoV P particle is an octahedral sub-virus-sized nanoparticle formed by 24 copies of the protrusion (P) domain of the NoV capsid protein [21]. P particles can be produced in *Escherichia coli* and are very stable and strongly immunogenic.

Vaccine Candidates

Several NoV vaccine candidates have been evaluated in preclinical trials, including bivalent and trivalent VLPs, and truncated VP1 P particles from the Universities of Arizona, Tampere and the Cincinnati Children's Hospital Medical Center, respectively. Following from early work conducted with orally administered VLP antigens [22–25], Velasquez *et al.* [26] from Arizona State University, in an attempt to improve the immunogenicity of a bivalent NoV VLP GI.1, GII.4 formulation for intranasal use, were able to demonstrate robust systemic and mucosal immunity in various lab animals with a mucoadhesive (GelSite)-based preparation [27]. Tamminen *et al.* [28] from the University of Tampere used an intramuscular combined vaccine candidate consisting of a bivalent NoV (GI.3, GII.4) component with a rotavirus (rVP6) component in BALB/c mice to show high-level vaccine-type-specific serum and intestinal immunoglobulin G (IgG) as well as cross-reactive IgG antibodies against heterologous NoV VLPs (GII.4, GII.12, GI.1). Fang *et al.* [29] from the Cincinnati Children's Hospital were able to show that NoV P domain complexes injected intramuscularly in BALB/c mice were efficiently presented by dendritic cells to elicit humoral and cellular immune responses. Chimeras with both the NoV P particle and the rotavirus VP-8 may serve as a future dual vaccine against both pathogens.

The VLP-based approach from Takeda Vaccines, using a baculovirus/insect cell expression system, has resulted in several investigational intramuscular aluminium hydroxide ($Al(OH)_3$)-based vaccine formulations being pursued, which are currently undergoing testing in clinical trials. The vaccine candidates are designed to elicit a high antibody response to both the component GI.1 and GII.4 antigens, representing the two major genogroups infecting humans, as well as broad cross-protective responses against other genotypes. The GI.1 component is based on the prototypic Norwalk virus, while the GII.4 component is based on a consensus sequence between three GII.4 viruses: 2006a (Yerseke), 2006b (Den Haag) and 2002 (Houston) [30]. Initial preclinical studies in animals had shown that this consensus GII.4 VLP elicits a broad antibody response against multiple GII.4 strains isolated over years of antigen drift [29]. Proof of concept was demonstrated in humans using an early formulation administered intranasally [31], but subsequent formulations developed for intramuscular injection have been associated with higher seroresponse rates after administration of fewer doses.

Takeda Vaccines Candidate Studies

Candidate intramuscular vaccine formulations with doses ranging 5–150 μg of each of the two VLPs, with and without 3-O-desacyl-4′-monophosphoryl lipid A (MPL; GlaxoSmithKline) as an adjuvant in addition to $Al(OH)_3$, have been evaluated in phase 1 and 2 trials in adults [32,33]. All formulations were generally well tolerated with acceptable safety profiles, but as expected with an MPL-adjuvanted vaccine, there were higher rates of pain (46.8% vs. 12.9%) and tenderness (66.1% vs. 24.2%) at the injection site in the vaccine subjects compared to the placebo subjects. These injection-site reactions resolved quickly. Data on all other solicited events (systemic or local) suggested no difference between these two groups.

Robust and high immune responses to the vaccine GI.1 VLP and GII.4 cVLP have been observed when assessed as total Ig, IgG and IgA, and functional antibody responses as measured by the ability to block binding to HBGA, the purported ligand for NoV attachment [32–34].

In a double-blind, randomized, placebo-controlled phase 1 study, Treanor *et al.* [32] evaluated escalating VLP doses (5, 15, 50 and 150 μg of each VLP) in different age strata of healthy adults aged 18–85 years. Vaccinees received two intramuscular doses 28 days apart. All formulations were well tolerated with no dose-related effects or increased reactogenicity after the second dose. Solicited adverse events were primarily mild or moderate in severity, with headache the most frequent systemic, and tenderness and pain the most frequent local adverse events. Serum immune responses (total Ig, IgG and IgA) to each of the vaccine VLPs peaked at day 7 after the first dose with no evidence of boosting after the second dose. It should be noted, however, that all of these adult subjects had preexisting anti-NoV serum antibodies at baseline, indicating that they had probably been primed by earlier natural exposure. Responses were similar by age group. Interestingly, the highest immune responses were observed for the GI.1 VLP antigen in the 150/150 μg dose, whereas the highest immune responses to the GII.4 VLP antigen were observed with the 50/50 μg dose. The 50/50 μg vaccine was therefore selected for further evaluation in the