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Norovirus Vaccine against Experimental Human Norwalk Virus Illness

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Abstract

Background—Noroviruses cause epidemic and sporadic acute gastroenteritis. No vaccine is available to prevent norovirus illness or infection.

Methods—We conducted a randomized, double-blind, placebo-controlled, multicenter trial to assess the safety, immunogenicity, and efficacy of an investigational, intranasally delivered norovirus viruslike particle (VLP) vaccine (with chitosan and monophosphoryl lipid A as adjuvants) to prevent acute viral gastroenteritis after challenge with a homologous viral strain, Norwalk virus (genotype GI.1). Healthy adults 18 to 50 years of age received two doses of either vaccine or placebo and were subsequently inoculated with Norwalk virus and monitored for infection and gastroenteritis symptoms.

Results—Ninety-eight persons were enrolled and randomly assigned to receive vaccine (50 participants) or placebo (48 participants), and 90 received both doses (47 participants in the vaccine group and 43 in the placebo group). The most commonly reported symptoms after vaccination were nasal stuffiness, nasal discharge, and sneezing. Adverse events occurred with similar frequency among vaccine and placebo recipients. A Nor-walk virus–specific IgA seroresponse (defined as an increase by a factor of 4 in serum antibody levels) was detected in 70% of vaccine recipients. Seventy-seven of 84 participants inoculated with Norwalk virus were included in the per-protocol analysis. Vaccination significantly reduced the frequencies of Norwalk virus gastroenteritis (occurring in 69% of placebo recipients vs. 37% of vaccine recipients, P = 0.006) and Norwalk virus infection (82% of placebo recipients vs. 61% of vaccine recipients, P = 0.05).

Conclusions—This norovirus VLP vaccine provides protection against illness and infection after challenge with a homologous virus. (Funded by LigoCyte Pharmaceuticals and the National Institutes of Health; ClinicalTrials.gov number, NCT00973284.)

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Noroviruses are a leading cause of epidemic acute gastroenteritis and are also an important cause of sporadic cases of acute gastroenteritis.[1] Because human noroviruses have not been grown in cell culture and there are no convenient animal models in which to evaluate immunity and illness, much of our knowledge about these viruses comes from the study of outbreaks and experimental human infection. Norwalk virus (genotype GI.1), the prototype human norovirus, caused a school-based outbreak of epidemic gastroenteritis in 1968,[2] and it is the most extensively studied human norovirus.[3-5] Susceptibility to Norwalk virus infection is dependent on expression of a functional fucosyltransferase 2 (FUT2) gene; persons who have a nonfunctional FUT2 gene are genetically resistant to Norwalk virus infection.[6,7] The FUT2 gene is involved in expression of the histo-blood group antigen H type 1 on the surface of epithelium. H type 1 and other histo-blood group antigens serve as receptors or attachment factors for human noroviruses and thus influence host susceptibility.[1,8,9] Norwalk virus viruslike particles (VLPs) bind less to B histo-blood group antigens than to A or H histo-blood group antigens, and persons in whom the blood group B antigens are expressed are less likely to become ill if infected with Norwalk virus.[10,11] Similarly, persons with serum antibodies that block the binding of Norwalk virus to H type 1 histo-blood group antigen are less likely to become ill if infected with Norwalk virus.[12]

Currently, there is no vaccine to prevent human norovirus infection, and there is no specific therapy available to treat it. Expression of the capsid proteins in eukaryotic cells leads to the spontaneous formation of VLPs,[13] and these particles have been immunogenic in animal models, whether delivered parenterally, orally, or intranasally.[14,15] A monovalent Norwalk virus VLP formulation delivered intranasally induced virus-specific serum antibodies in the majority of vaccine recipients.[16] The purpose of the current study was to determine whether the vaccine provides protection against illness after a homologous norovirus challenge.

Methods

Study Design

We conducted this randomized, double-blind, placebo-controlled trial at four clinical sites. Enrollment began in September 2009 and was completed by January 2010. The study was sponsored by LigoCyte Pharmaceuticals and was designed by the academic authors in collaboration with employees of the sponsor. Data were collected with the use of Internet-based electronic case-report forms and were reported to the data management coordinating center (EMMES, Rockville, MD). All authors had free access to the data, wrote the manuscript, participated in the decision to submit it for publication, and vouch for the completeness and accuracy of the data and analyses presented and the fidelity of this report to the study protocol, which is available with the full text of this article at NEJM.org.

Enrollment, Randomization, and Follow-up

Eligible persons were healthy men and women between 18 and 50 years of age who were positive for the presence of fucosyltransferase 2 (i.e., they had a functional FUT2 gene) as determined phenotypically by means of identification of histo-blood group antigens in saliva.[12] Enrollment criteria are described in the Supplementary Appendix (available at NEJM.org) and the study protocol. The study was approved by the institutional review board for each of the four clinical sites conducting the study. Written informed consent was obtained from all participants before enrollment.

The study was conducted in two stages: the vaccination stage and the Norwalk virus challenge stage. Eligible participants were randomly assigned to receive the study vaccine or placebo in a 1:1 ratio, stratified according to clinical site. Vaccine and placebo were administered in two intranasal doses given 3 weeks apart. Reactogenicity data were

collected (as described in the Supplementary Appendix). Serum samples were collected before the first administration and 3 weeks after the second administration of vaccine or placebo.

Participants who completed the 3-week followup period after the second administration were eligible to participate in the Norwalk virus challenge. Participants who agreed to participate were admitted to an inpatient challenge facility on the day of or the day before the challenge, which consisted of oral administration of 48 reverse-transcriptase–polymerase-chain-reaction (RT-PCR) units of Norwalk virus (approximately 10 times the amount of inoculum required to infect 50% of persons to whom it is administered).[4,17] After virus inoculation, study participants were assessed for symptoms and signs of gastroenteritis at least twice daily until discharge (the minimum length of stay was 96 hours [4 days]), and stool samples were collected to identify Norwalk virus infection, as described in the Supplementary Appendix.

Vaccine and Placebo

The study vaccine contained 100 μg of Norwalk virus VLPs produced in a baculovirus expression system, monophosphoryl lipid A (GlaxoSmithKline) as an adjuvant, chitosan (ChiSys, Archimedes Development) as a mucoadhesive agent, and sucrose and mannitol excipients as bulking agents to stabilize the VLPs during lyophilization.[16] (This application of chitosan has been licensed from Archimedes Development.) The placebo contained only the sucrose and mannitol excipients. A single vaccine dose was defined as the delivery of the contents of two loaded intranasal devices (Bespak UniDose DP, Milton Keynes), one in each nostril, with each device containing 50 μg of Norwalk virus VLPs.

Assessment of Efficacy

The primary objective was to evaluate the efficacy of the vaccine in preventing the primary end point: viral gastroenteritis caused by the Norwalk virus. Definitions of infection and illness are provided in the Supplementary Appendix.

Laboratory Assays

Fecal samples were evaluated for the presence of Norwalk virus with the use of a real-time RT-PCR assay for the viral genome and with a sandwich enzyme-linked immunosorbent assay (ELISA) for viral antigen, as previously described.[17] Infection with other noroviruses was detected with the use of a standard RT-PCR assay and sequencing of viral amplicons.[18] Total and class-specific Norwalk virus serum antibodies and histo-blood group antigen-blocking antibodies were measured by means of previously described assays.[12,19]

Statistical Analysis

We calculated that a sample of approximately 40 participants per group was required for approximately 85% statistical power in comparing the rate of viral acute gastroenteritis between the two groups, assuming a rate in the placebo group of 40% and a vaccine efficacy rate of 70%. Frequencies of norovirus disease and infection were compared between the two groups by means of Fisher's exact test. A committee that was unaware of the study-group assignments determined the dates of onset and resolution of acute gastroenteritis by reviewing the participants' clinical signs and symptoms. The severity of norovirus disease was graded on the basis of a modification of the Vesikari scale (Table 1 in the Supplementary Appendix), originally used to assess the severity of rotavirus disease in children.[20] The scale ranges from 0 to 17, with higher scores indicating more severe disease. The severity of norovirus disease and the duration of viral gastroenteritis were compared

between the two groups with the use of the Wilcoxon rank-sum test, and the time to the onset of viral gastroenteritis was compared by means of the log-rank test.

Geometric mean antibody titers and factor increases in the geometric mean titers were compared between the two groups by means of the Wilcoxon rank-sum test, and response rates were compared with the use of Fisher's exact test. A logistic-regression model was used for evaluating prechallenge titers in relation to infection and illness, and post hoc 2-by-2 analyses were performed to evaluate the association of individual antibody levels with protection from viral illness or infection. All reported P values are two-sided. All data analyses and statistical computations were conducted with the use of SAS software (version 9.2).

Results

Participants

Of 454 persons screened, 98 were enrolled and randomly assigned to receive either vaccine or placebo (Fig. 1, and Table 2 in the Supplementary Appendix). The mean age of participants was 32.1 years, 41% were women, and 65% had blood type O (Table 3 in the Supplementary Appendix). Eight participants did not receive a second dose of vaccine or placebo: four were unavailable for followup visits, two declined to participate further, one had an intercurrent respiratory illness, and one had an intercurrent intestinal illness.

Of the 90 persons who received two doses of vaccine or placebo, 84 participated in the Norwalk virus challenge (of the remaining 6 participants, 4 were lost to follow-up, 1 withdrew, and 1 became pregnant) and constituted the intention-to-treat population for the efficacy evaluation. A total of 82 participants began the challenge phase 3 weeks after completing the vaccination phase; 1 in the vaccine group and 1 in the placebo group began the challenge 19 and 9 weeks, respectively, after the vaccination phase. Of the 84 challenge participants, 7 were eliminated from the per-protocol efficacy analysis for the following reasons: 5 owing to malfunction of at least one of the four dose-delivery devices, resulting in incomplete vaccine delivery (Table 4 in the Supplementary Appendix); 1 because of infection with another norovirus during the challenge period; and 1 owing to excessive eating followed by vomiting.

Vaccine Reactions

Local (nasal) and systemic adverse events after vaccination occurred with similar frequency in the vaccine and placebo groups after the first study dose was administered (Table 1, and Tables 5 and 6 in the Supplementary Appendix). Local symptoms were reported more frequently after the second dose in the vaccine group than in the control group. None of the participants reported severe nasal symptoms. One participant in the placebo group reported severe diarrhea (8 stools in 24 hours) for 1 day in the week after the first dose. Systemic symptoms were reported more frequently after the first dose than after the second in both the vaccine group (52% vs. 26%) and the placebo group (51% vs. 37%). Similarly, adverse events were reported most frequently on the day of vaccination and declined in frequency thereafter.

Clinical laboratory abnormalities were infrequent and occurred in similar proportions of participants in the vaccine and placebo groups. No vaccine-related severe adverse events occurred during the study, and no new medically significant conditions occurred. Two severe adverse events occurred in the placebo group during the followup period after Norwalk virus inoculation: one participant was hospitalized for treatment of appendicitis 76 days after inoculation, and another was hospitalized for psychosis 42 days after inoculation

(Table 7 in the Supplementary Appendix). Both episodes resolved and were judged to be unrelated to the study.

Vaccine Immunogenicity

Five Norwalk virus–specific serologic assays were used to measure vaccine immunogenicity (Table 2). Immune responses were observed most frequently with the use of the total serum antibody ELISA (assays of IgG, IgA, and IgM antibodies combined) and the serum IgA antibody ELISA. These two tests showed a seroresponse (defined as an increase by a factor of 4 in serum antibody levels) in 70% of participants in the per-protocol population after two doses of study vaccine. Sero-responses were observed less frequently with the IgG, IgM, and histo-blood group antigen–blocking antibody assays. The factor increases in the geometric mean antibody titers were also greatest after two doses of vaccine and were highest with the IgA antibody assay (an increase by a factor of 7.5) and the total antibody assay (an increase by a factor of 4.9). Among participants infected by Norwalk virus, the factor increase was lower after vaccination than after challenge (Table 8 in the Supplementary Appendix). For example, the factor increases in the geometric mean titers 1 month after challenge among infected recipients of vaccine and placebo were 62.1 (95% confidence interval [CI], 35.5 to 108.8) and 84.8 (95% CI, 52.8 to 136.3), respectively, for the total serum antibody ELISA; 11.6 (95% CI, 5.9 to 22.8) and 61.9 (95% CI, 33.6 to 114.0), respectively, for the IgA antibody ELISA; and 42.2 (95% CI, 28.0 to 63.6) and 33.2 (95% CI, 20.2 to 33.2), respectively, for the histo-blood group antigen-blocking antibody assay.

Vaccine Efficacy

Vaccine efficacy was similar in the per-protocol and intention-to-treat analyses (Table 3); only the per-protocol results are presented here. Norwalk virus-associated gastroenteritis occurred in 69% of the placebo recipients and 37% of vaccinees (Table 3), for an absolute reduction with the vaccine of 32 percentage points (P = 0.006) and a relative reduction of 47%. Vaccination also reduced the relative frequency of Norwalk virus infection after challenge with the virus, with infection occurring in 82% of placebo recipients and 61% of vaccine recipients, for an absolute reduction of 21 percentage points P = 0.05) and a relative reduction of 26%. The mean Vesikari score for the severity of disease was significantly reduced in the vaccine group (3.6, vs. 5.5 in the placebo group; P = 0.009), with a relative reduction of 35%. The reduction in the severity of disease was less apparent when analyses were restricted to the participants infected with Norwalk virus (mean score, 5.0 with vaccine and 6.1 with placebo; P = 0.14; relative reduction, 18%), and no significant reduction in disease severity was found among participants with Norwalk virus–associated viral gastroenteritis (mean score, 6.4 with vaccine and 6.7 with placebo; P = 0.72), most likely owing to the small numbers of participants in these subgroups.

The onset of illness was delayed among vaccinees, as compared with placebo recipients, by 2.1 hours (P = 0.12) in the per-protocol population and by 4.3 hours (P = 0.02) in the intention-to-treat population. Vaccination did not significantly reduce the duration of illness among those infected (Table 3).

Gastroenteritis developed within 19 minutes after inoculation with the Norwalk virus in one participant in the placebo group. The participant, who was infected with a GII.4 norovirus strain, was the only person who had an ELISA seroresponse to Norwalk virus and did not have shedding of Norwalk virus in the stool. This person did not have an increase in the titer of histo-blood group antigen–blocking antibodies specific to Norwalk virus.

Increased prechallenge levels of histo-blood group antigen–blocking antibodies were correlated with increased protection against viral gastroenteritis and infection in a univariate logistic-regression analysis. Examination of reverse cumulative distribution curves (Fig. 1 in the Supplementary Appendix) resulted in the post hoc evaluation of a histo-blood group antigen–blocking antibody titer of 200 or higher on illness and infection outcomes; a serum titer of at least 200, as compared with a titer under 200, was associated with a relative reduction of more than 50% in the frequency of viral illness and infection (Table 4).

Discussion

Noroviruses cause an estimated 21 million cases of gastroenteritis annually in the United States and are responsible for up to 1.1 million hospitalizations and 218,000 deaths annually among children in developing countries.[21,22] The increasing recognition of noroviruses as causes of disease and the limited success in preventing outbreaks of illness have led to the consideration of vaccines as a potential means for disease control.[1] Our proof-of-concept study was designed to maximize the likelihood of identifying a vaccine effect by enrolling persons in whom illness was likely to develop after Norwalk virus exposure: healthy adults with blood group O or A and a functional FUT2 gene, use of a challenge strain homologous to the vaccine antigen, use of a moderate challenge dose so as not to overwhelm the immunity induced by vaccination, and performance of the Norwalk virus challenge shortly after vaccination was completed. Previous studies of experimental human infection have shown that symptomatic infection provides short-term protection against recurrent illness after reexposure to the same virus.[5,23] Our study shows that symptomatic infection can be prevented by means of intranasal immunization with nonreplicating homologous Norwalk virus VLPs. As compared with placebo recipients, vaccine recipients were significantly less likely to have illness or infection; among those who did have illness, the overall severity was decreased, and the time to the onset of illness was delayed.

A Norwalk virus–specific serum IgA antibody response was observed in 70% of vaccinees, which is similar to the response rate of 79% reported by El-Kamary and colleagues.[16] For each of the assays that we used, the serum antibody response (measured as the factor increase in the geometric mean titer) was lower than the response after Norwalk virus infection but similar to that observed after oral administration of Norwalk virus VLPs.[24,25] The prechallenge serum levels of histoblood group antigen–blocking antibodies correlated with protection against both illness and infection. This observation confirms and extends an earlier finding that the levels of antibodies that block the binding of Norwalk virus VLPs to histo-blood group antigen H type 1 in serum before challenge with Norwalk virus is associated with a lower risk of virus-associated illness after the challenge.[12] Others have noted or suspected an association between the levels of serum antibodies and protection against disease for other mucosal viral infections, including influenza, rotavirus, and human papillomavirus.[26-29] The increased activity of histo-blood group antigen–blocking antibodies after vaccination and the associated protection against illness and infection are analogous to hemagglutination-inhibition antibody responses and protection after inactivated influenza vaccines.[26,29]

Our study had several limitations. First, the placebo did not include the monophosphoryl lipid A and chitosan adjuvants, raising the possibility that the observed protection was due to nonspecific effects. Monophosphoryl lipid A is a toll-like receptor 4 agonist, and its administration in animals is associated with local resistance to infection.[30,31] However, the short duration of nonspecific protection in these models (<1 week), the down-regulation of toll-like receptor 4 in intestinal epithelium leading to lipopolysaccharide hyporesponsiveness, and the association of protection with virus-specific antibodies all

support the hypothesis that the observed protection was due to virus-specific adaptive immune responses.[30,32]

Second, the dose-delivery device (Bespak) used in our study malfunctioned several times, adversely affecting vaccine delivery. Alternative intranasal delivery systems and parenteral delivery of vaccine antigens are being evaluated[33] to determine the optimal route of vaccine delivery.

Third, vaccine immunogenicity and protective efficacy must be determined in other populations, including young children and the elderly. Also, it is possible that norovirus vaccines, like rotavirus vaccines, will provide greater protection against severe disease than against milder illness.

The future success of vaccine development will be influenced by a number of challenges related to biologic characteristics of norovirus. The frequency and magnitude of serum antibody responses after vaccination were lower than those induced by infection. Immunity after natural infection is short-lived (<2 years), and the duration of protection after vaccination remains to be determined.[1]

Natural infection occurs after exposure to a range of virus levels, including those used in the current study.[34] Vaccine efficacy must also be determined in a natural setting, where exposure to higher virus levels may occur. Noroviruses are antigenically and genetically diverse, with at least 8 and 19 genotypes in the GI and GII genogroups, respectively.[1] Norwalk virus infection induces a histo-blood group antigen-blocking antibody response against other GI genotypes but not against a GII norovirus.[35] Thus, even if a cross-reactive antibody response induced by infection or vaccination is associated with protective immunity, a multivalent vaccine will most likely be required to provide protection against both GI and GII strains. Furthermore, GII.4 strains, the most prevalent genotype, and other GII strains undergo genetic drift analogous to that seen among influenza viruses, raising the possibility of a need for periodic changes in vaccine composition.[36-38]

In summary, a two-dose, intranasally administered, VLP vaccine with adjuvants provided homologous protection against both Norwalk virus–associated viral gastroenteritis and infection. This study shows that it may be possible to use a vaccination strategy to prevent norovirus disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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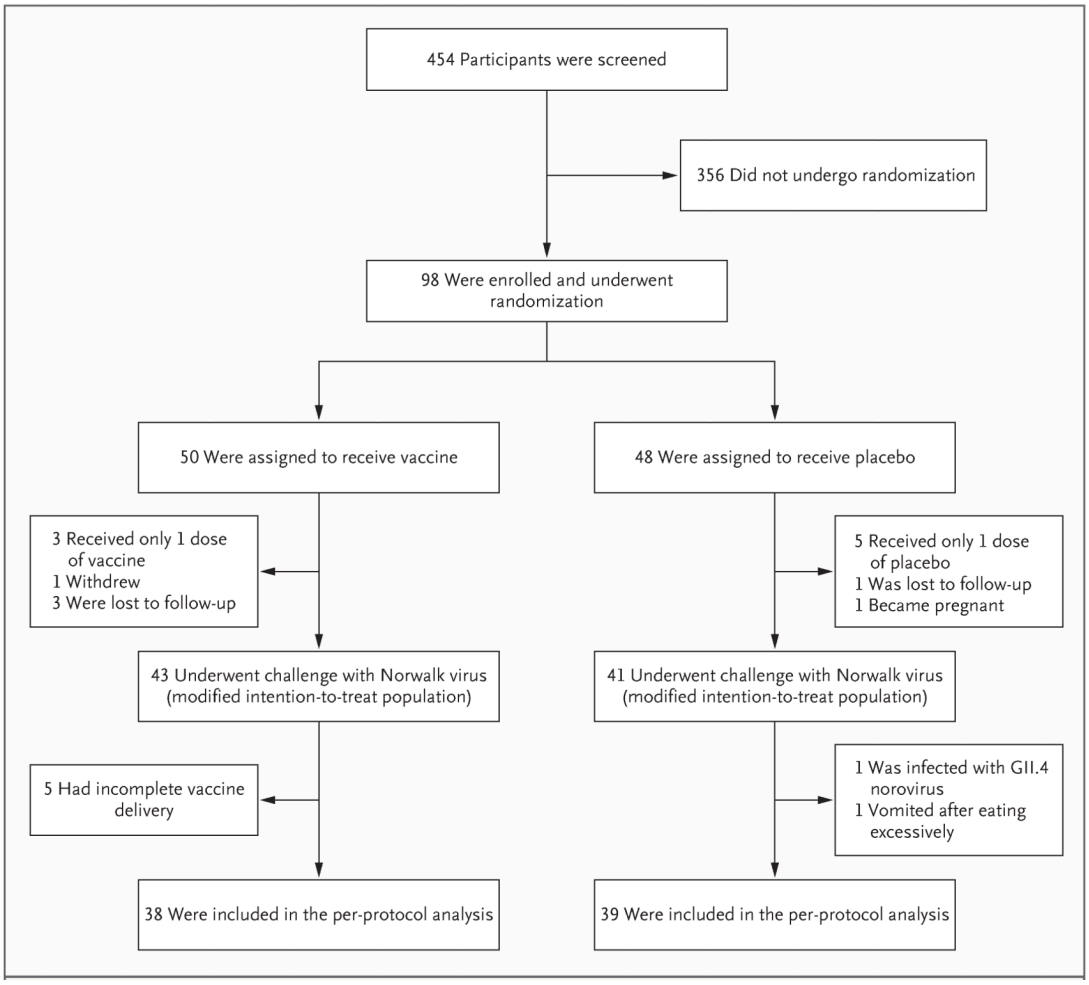

Figure 1. Enrollment, Randomization, and Follow-up of the Study Participants

Table 1

Frequency of Solicited Reports of Adverse Events during the First 7 Days after Vaccine Administration, According to Study Group*

Event	Vaccine				Placebo†			
	First Dose (N = 50)		Second Dose (N = 47)		First Dose (N = 47)		Second Dose (N = 43)	
	no.	*% (95% CI)*	*no.*	*% (95% CI)*	*no.*	*% (95% CI)*	*no.*	*% (95% CI)*
Local symptoms								
Nasal discomfort	7	14 (6–27)	5	11 (4–23)	2	4 (1–15)	2	5 (1–16)
Nasal discharge	15	30 (18–45)	15	32 (19–47)	16	34 (21–49)	10	23 (12–39)
Nasal stuffiness	15	30 (18–45)	11	23 (12–38)	12	26 (14–40)	8	19 (8–33)
Nasal itching	7	14 (6–27)	10	21 (11–36)	10	21 (11–36)	5	12 (4–25)
Sneezing	11	22 (12–36)	16	34 (21–49)	11	23 (12–38)	9	21 (10–36)
Blood-tinged mucus	1	2 (0–11)	0	0 (0–8)	1	2 (0–11)	0	0 (0–8)
Nasal bleeding	0	0 (0–7)	0	0 (0–8)	0	0 (0–8)	0	0 (0–8)
Any local symptom	22	44 (30–59)	22	47 (32–62)	23	49 (34–64)	13	30 (17–46)
Systemic symptoms								
Fever	0	0 (0–7)	0	0 (0–8)	2	4(1–15)	0	0 (0–8)
Fatigue or malaise	9	18 (9–31)	1	2 (0–11)	7	15 (6–28)	2	5 (1–16)
Headache	16	32 (20–47)	8	17 (8–31)	14	30 (17–45)	9	21 (10–36)
Nausea	7	14 (6–27)	2	4 (1–15)	6	13 (5–26)	3	7 (2–19)
Loss of appetite	5	10 (3–22)	1	2 (0–11)	5	11 (4–23)	2	5 (1–16)
Abdominal cramps	6	12 (5–24)	3	6 (1–18)	7	15 (6–28)	4	9 (3–22)
Vomiting (≥1 episode)	0	0 (0–7)	0	0 (0–8)	2	4(1–15)	0	0 (0–8)
Diarrhea								
Any	1	2 (0–11)	0	0 (0–8)	1	2 (0–11)	0	0 (0–8)
≥2 loose or liquid stools	5	10 (3–22)	6	13 (5–26)	10	21 (11–36)	10	23 (12–39)
Any systemic symptom	26	52 (37–66)	12	26 (14–40)	24	51 (36–66)	16	37 (23–53)
Any systemic or local symptom	33	66 (51–79)	29	62 (46–76)	33	70 (55–83)	21	49 (33–65)

*CI denotes confidence interval.

†Data were not available during the follow-up period for one participant in the placebo group.

Table 2

Results of Serum Antibody Studies before and 3 Weeks after Each Dose in the Per-Protocol Population, According to Study Group*

ELISA Result	Vaccine†			Placebo		
	Day 0 (N =37)	Day 21 (N=38)	Day 42 (N=38)	Day 0 (N =39)	Day 21 (N=39)	Day 42 (N=39)
Total serum antibody (IgG, IgA, and IgM) ELISA						
GMT (95% CI)	3500 (2100–5700)	6900 (4200–11,100)	16,500 (10,800–25,100)	4700 (3000–7300)	5000 (3100–8000)	4900 (3100–7800)
Seroresponse — % (95% CI)	NA	30 (16–47)	70 (53–84)	NA	3 (0–14)	3 (0–14)
GMFR (95% CI)	NA	2.0 (1.6–2.6)	4.9 (3.6–6.8)	NA	1.1 (0.9–1.2)	1.1 (0.9–1.2)
IgG ELISA						
GMT (95% CI)	3.2 (1.8–5.6)	6.5 (4.1–10.4)	14.1 (9.5–21.1)	4.6 (2.9–7.3)	4.5 (2.8–7.0)	4.8 (3.1–7.4)
Seroresponse — % (95% CI)	NA	16 (6–32)	49 (32–66)	NA	0 (0–9)	0 (0–9)
GMFR (95% CI)	NA	2.0 (1.6–2.6)	4.5 (3.1–6.5)	NA	1.0 (0.9–1.0)	1.0 (1.0–1.1)
IgA ELISA						
GMT (95% CI)	1.0 (0.5–1.9)	3.4 (1.9–6.0)	7.4 (4.2–13.1)	1.2 (0.7–2.1)	1.1 (0.7–2.0)	1.2 (0.7–2.2)
Seroresponse — % (95% CI)	NA	43 (27–61)	70 (53–84)	NA	0 (0–9)	0 (0–9)
GMFR (95% CI)	NA	3.4 (1.9–6.0)	7.5 (4.6–12.2)	NA	0.9 (0.9–1.0)	1.0 (0.9–1.1)
IgM ELISA						
GMT (95% CI)	28.5 (22.6–35.9)	30 (22.3–40.4)	91.3 (47.7–174.7)	29.9 (23.2–38.4)	29.9 (23.2–38.4)	29.9 (23.2–38.4)
Seroresponse — % (95% CI)	NA	3 (0–14)	32 (18–50)	NA	0 (0–9)	0 (0–9)
GMFR (95% CI)	NA	1.1 (1.0–1.2)	3.3 (1.8–6.2)	NA	1.0‡	1.0‡
Histo-blood group antigen–blocking activity						
GMT (95% CI)	32.1 (22.9–44.9)	59.0 (40.6–85.8)	109.0 (71.7–165.6)	39.3 (28.5–54.0)	45.1 (32.1–63.4)	47.1 (32.5–68.4)
Seroresponse — % (95% CI)	NA	16 (6–32)	32 (18–50)	NA	5 (1–17)	3 (0–14)
GMFR (95% CI)	NA	1.9 (1.5–2.4)	3.6 (2.8–4.7)	NA	1.1 (1.0–1.4)	1.2 (1.0–1.5)

*GMFR is the factor increase in the geometric mean titer (GMT) from baseline. A seroresponse was defined as an increase by a factor of 4 or more. ELISA denotes enzyme-linked immunosorbent assay, and NA not applicable.

†Data for day 21 and day 42 in the vaccine group were available for 38 participants for GMT and 37 participants for all other values.

‡The 95% CI could not be calculated.

Table 3

Vaccine Efficacy Measurements, According to Analysis and Study Group*

Outcome	Intention-to-Treat Analysis			Per-Protocol Analysis		
	Vaccine (N = 43)	Placebo (N = 41)	Relative Reduction	Vaccine (N = 38)	Placebo (N = 39)	Relative Reduction
	no. (%)		*% (95% CI)*	*no. (%)*		*% (95% CI)*
Norwalk virus infection and illness	17 (40)	29 (71)	44 (15.0 to 63.2)	14 (37)	27 (69)	47 (15 to 67)
Norwalk virus infection						
Positive RT-PCR assay	28 (65)	33 (80)	19 (−6 to 38)	23 (61)	32 (82)	26 (1 to 45)
Positive for antigen	25 (58)	24 (59)	1 (−43 to 31)	20 (53)	23 (59)	11 (−33 to 40)
Seroconversion	27 (63)	34 (83)	24 (1 to 42)	23 (61)	32 (82)	26 (1 to 45)
Positive on any assay	28 (65)	34 (83)	22 (−2 to 39)	23 (61)	32 (82)	26 (1 to 45)
	Vaccine (N = 43)	Placebo (N = 41)	P Value†	Vaccine (N = 38)	Placebo (N = 39)	P Value†
Vesikari score for disease severity‡						
All participants	3.8±2.8	5.5±2.6	0.008	3.6±2.9	5.5±2.7	0.009
Norwalk virus infection	5.0±2.4	6.1±2.4	0.07	5.0±2.6	6.1±2.4	0.14
Norwalk virus infection and illness	6.2±1.9	6.7±2.1	0.39	6.4±2.1	6.7±2.2	0.72
Duration of Norwalk virus illness — hr						
Norwalk virus infection, all			0.28			0.47
Median	18.4	30.2		14.5	29.4	
Range	0.0 to 85.5	0.0 to 108.0		0.0 to 85.5	0.0 to 108.0	
Norwalk virus infection and illness			0.44			0.45
Median	45.4	36.2		44.0	34.7	
Range	8.6 to 85.5	5.2 to 108.0		8.6 to 85.5	5.2 to 108.0	
Time to illness onset — hr	37.1±7.0	32.8±9.0	0.02	35.9±7.1	33.8±6.7	0.12

*Plus–minus values are means ±SD. Illness was defined as viral gastroenteritis. RT-PCR denotes reverse transcriptase–polymerase chain reaction.

†P values were calculated with the use of the Wilcoxon test, except for comparisons of the time to onset of illness, for which the log-rank test was used.

‡The severity of norovirus disease was assessed on the basis of the modified Vesikari score, with possible scores ranging from 0 to 17 and higher scores indicating more severe disease.

Table 4

Odds Ratios for Viral Gastroenteritis and Norwalk Virus Infection, According to Prechallenge Histo-Blood Group Antigen–Blocking Antibody Titer and Analysis*

Outcome	Intention-to-Treat Analysis				Per-Protocol Analysis			
	Antibody Titer <200	Antibody Titer ≥200	Odds Ratio (95% CI)	Relative Reduction (95% CI)	Antibody Titer <200	Antibody Titer ≥200	Odds Ratio (95% CI)	Relative Reduction (95% CI)
	no./total no. (%)				*no./total no. (%)*			
Viral gastroenteritis	43/66 (65)	3/18 (17)	9.4 (2.5-35.7)	74.4 (27.1-91.0)	38/60 (63)	3/17 (18)	8.1 (2.1-31.2)	72.1 (20.8-90.2)
Norwalk virus infection	55/66 (83)	7/18 (39)	7.9 (2.5-24.8)	53.3 (15.9-74.1)	49/60 (82)	6/17 (35)	8.2 (2.5-26.9)	56.8 (16.8-77.5)

* Odds ratios were calculated with the use of a 2-by-2 analysis.