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Serological Correlates of Protection against a GII.4 Norovirus

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Noroviruses are the leading cause of acute gastroenteritis worldwide, and norovirus vaccine prevention strategies are under evaluation. The immunogenicity of two doses of bivalent genogroup 1 genotype 1 (GI.1)/GII.4 (50 μg of virus-like particles [VLPs] of each strain adjuvanted with aluminum hydroxide and 3-*O*-desacyl-4′monophosphoryl lipid A [MPL]) norovirus vaccine administered to healthy adults in a phase 1/2 double-blind placebo-controlled trial was determined using virus-specific serum total antibody enzyme-linked immunosorbent assay (ELISA), IgG, IgA, and histoblood group antigen (HBGA)-blocking assays. Trial participants subsequently received an oral live virus challenge with a GII.4 strain, and the vaccine efficacy results were reported previously (D. I. Bernstein et al., J Infect Dis 211:870–878, 2014, doi:10.1093/infdis/jiu497). This report assesses the impact of prechallenge serum antibody levels on infection and illness outcomes. Serum antibody responses were observed in vaccine recipients by all antibody assays, with first-dose seroresponse frequencies ranging from 88 to 100% for the GI.1 antigen and from 69 to 84% for the GII.4 antigen. There was little increase in antibody levels after the second vaccine dose. Among the subjects receiving the placebo, higher prechallenge serum anti-GII.4 HBGA-blocking and IgA antibody levels, but not IgG or total antibody levels, were associated with a lower frequency of virus infection and associated illness. Notably, some placebo subjects without measurable serum antibody levels prechallenge did not become infected after norovirus challenge. In vaccinees, anti-GII.4 HBGA-blocking antibody levels of >1:500 were associated with a lower frequency of moderate-to-severe vomiting or diarrheal illness. In this study, prechallenge serum HBGA antibody titers correlated with protection in subjects receiving the placebo; however, other factors may impact the likelihood of infection and illness after virus exposure. (This study is registered at ClinicalTrials.gov under registration number NCT1609257.)

Noroviruses are the most common cause of infectious gastroenteritis worldwide and cause both endemic and epidemic disease (1). In the United States alone, they are estimated to cause 19 to 21 million illnesses each year, leading to 56,000 to 71,000 hospitalizations and 570 to 800 deaths (2). Since the introduction of rotavirus vaccines for children, noroviruses have become the most common cause of medically attended acute gastroenteritis illness in U.S. children (3). The annual economic burden of norovirus-related disease in the United States has been estimated to be approximately $5.5 billion (4).

Noroviruses are genetically and antigenically diverse. They are divided into at least six genogroups based upon phylogenetic analysis of the major capsid protein VP1, and they are further subdivided into genotypes (5, 6). Human infection is caused by strains belonging to genogroups I (GI), II (GII), and IV (GIV), with most infections caused by GII strains, followed by GI strains. For more than a decade, GII genotype 4 (GII.4) strains have caused the majority of norovirus outbreaks, and GII.4-associated disease has been reported to be more severe than that caused by other genotypes (1, 7, 8). The emergence of new GII.4 variants through antigenic drift has likely contributed to the continued impact of this genotype (9, 10).

Higher levels of serum antibody that block virus binding to histoblood group antigens (HBGAs) are associated with a lower risk of illness and infection following oral inoculation with a GI.1 strain (Norwalk virus), but similar findings have not been reported for other norovirus genotypes (11–13). We conducted a vaccination and challenge study to evaluate whether intramuscular administration of a bivalent norovirus candidate vaccine reduces gastroenteritis symptoms (14). In this analysis, we determined the serological responses to vaccination and then measured the association of prechallenge serum antibody levels with the development of infection and illness following oral challenge with a GII.4 norovirus.

MATERIALS AND METHODS

Clinical study design. The details on the study are as previously described (14). In summary, this study (registered at ClinicalTrials.gov under registration no. NCT1609257) was a randomized, double-blind, and placebo-controlled phase 1/2 trial conducted between May 2012 and July 2013 at five sites in the United States; the study was approved by an institutional review board for each of the study sites. Eligible participants were healthy

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18- to 50-year-old volunteers who were available for all study visits and provided informed consent. Exclusion criteria included secretor-negative subjects, as determined by salivary HBGA expression, serum antibody levels to the P domain of the challenge strain of >1:1,600, and factors that may have increased risks of participation to the subject or his/her contacts (14). Study participants were randomized (1:1) to receive two 0.5-ml intramuscular injections of placebo (normal saline) or bivalent candidate vaccine containing 50 μg each of baculovirus-expressed GI.1 and GII.4 virus-like particles (VLPs) adjuvanted with 50 μg of 3-*O*-desacyl-4′monophosphoryl lipid A (MPL) and 0.5 mg of aluminum hydroxide on study days 0 and 28. The GI.1 VLP was derived from the 1968 Norwalk strain, and the GII.4 VLP was designed as a VP1 consensus sequence obtained by aligning the major capsid protein sequences from three GII.4 variants (15). Twenty-eight days or later after the second dose, participants who remained eligible were admitted to an inpatient facility and orally administered 4.4×10^3 reverse transcription-quantitative PCR (RT-qPCR) units of a GII.4 strain (Hu/GII.4/Cin-1/2003/US), a 2002 Farmington Hills-like variant. Over the next 4 days, subjects were monitored for signs and symptoms of gastroenteritis, and symptoms were graded as mild, moderate, or severe. Acute viral gastroenteritis was defined as norovirus infection and the presence of diarrhea (≥3 loose or liquid stools or >400 g of loose or liquid stools within a 24-h period), vomiting (≥2 episodes within a 24-h period) or a single vomiting episode associated with at least two of the following during a 24-h period: nausea, oral temperature of ≥99.7°F, abdominal cramps or pain, abdominal gurgling or bloating, or myalgia. Norovirus infection was defined as fecal virus excretion, as measured by RT-qPCR, or a ≥4-fold rise in antibody to the P particle of the challenge strain between pre- and postchallenge serum samples.

Serology. Serum samples were collected before administration of the first and second doses of vaccine or placebo, 4 weeks (day 56) after the second dose, and 30 days following norovirus challenge. An additional prechallenge serum sample was also collected if the challenge occurred >2 weeks after the day-56 visit. Antibody assays for immunogenicity used VLPs from strains contained in the vaccine as the target antigen. The following assays were performed as previously described: total genotype-specific enzyme-linked immunosorbent assay (ELISA) antibody (16, 17), IgA genotype-specific antibody (17, 18), IgG genotype-specific antibody (17, 18), and GI.1 HBGA-blocking antibody (11, 17).

The GII.4 HBGA-blocking antibody assay was conducted using a modification of previously described methods (17). First, 96-well microtiter plates were coated with pig gastric mucin (PGM) (5 μg/ml, 100 μl per well) and incubated at room temperature for 2 h. The plates were washed with phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBST) and then blocked with PBST with 5% nonfat dried milk. Serum samples were diluted 30-fold into PBST with 5% nonfat dried milk, added to column 1 of a separate 96-well polypropylene microtiter plate, and serially diluted 2-fold across the plate. An equal volume of consensus GII.4 VLPs (at 1.4 μg/ml) was added to each well containing the diluted serum samples and incubated for 2 h at room temperature. Each row of the microtiter plate containing diluted serum samples and VLPs was transferred to the 96-well microtiter plate previously coated with PGM. The plate was incubated at room temperature for 2 h. Bound VLPs were detected with rabbit anti-VLP antibodies, followed by goat anti-rabbit IgG and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) substrate. The absorbances were plotted against the VLP concentration and the curve fit to a four-parameter logistic. The 50% blocking titer (BT_{50}) was defined as the c-parameter from the four-parameter curve fit (19). The lower limit of antibody detection assay was a titer of 1:184, and values <1:184 were assigned a value of 1:92.

Statistical methods. Geometric mean antibody titers (GMT), geometric mean fold rises (GMFR), and percent seroresponse (≥4-fold increase in serum antibody level) frequencies were determined following vaccination, along with their respective 95% confidence intervals (CIs). Per-protocol and modified intention-to-treat analyses were performed, and as these had similar results, only the per-protocol analyses are shown. Prechallenge serum antibody levels among subjects receiving the placebo only (to remove the influence of vaccination on antibody levels and subsequent infection or illness risk) were compared by Wilcoxon rank sum tests.

RESULTS

One hundred twenty-seven (63 vaccine and 64 placebo) of 132 enrolled subjects received both study injections. One hundred nine subjects participated in the norovirus challenge portion of the study, and 98 (50 vaccine and 48 placebo) were in the per-protocol analysis group. The demographics for the respective treatment groups were similar. As reported previously, the primary endpoint of the study was not met (14), but as discussed in that report, vaccine recipients were significantly less likely to develop vomiting or diarrhea (VorD) graded as mild or greater in severity ($P = 0.028$). The frequencies of protocol-defined illness (PDI) (26% versus 33%) and norovirus infection (54% versus 62.5%) were not significantly different between the vaccine and placebo groups, respectively (14).

Vaccine immunogenicity. Serum antibody levels measured using a total antibody ELISA were reported previously (14) and are shown again in Tables 1 and 2. The serum antibody levels measured prior to the first vaccination were similar between the vaccine groups for each of the assays and each of the antigens tested (Tables 1 and 2 and Fig. 1). In general, serum antibody levels were lower prevaccination for the GI.1 antigen than for the GII.4 antigen; therefore, the seroresponse frequency and GMFR were higher for the GI.1 antigen than for the GII.4 antigen (Tables 1 and 2). Seroresponse frequencies ranged from 88 to 100% for the GI.1 antigen and from 69 to 84% for the GII.4 antigen. Persons who had a seroresponse to the GII.4 antigen had significantly lower prevaccination titers than those of the nonresponders for each of the four assays ($P \leq 0.006$). There was little increase in antibody levels after the second vaccine dose (Fig. 1).

Correlates of protection. Prechallenge, GII.4-specific serum antibody levels among subjects receiving placebo only were compared for those achieving several different study endpoints (cases) with those not achieving study endpoints (controls) for norovirus infection, PDI, and VorD (Table 3). These analyses serve to establish functional antibody (Ab) levels as correlates of protection in a natural history setting in the absence of any vaccine effect. Neither total serum GII.4-specific antibody as measured by ELISA nor serum GII.4-specific IgG geometric mean titers were significantly different between the cases and controls for each of the study endpoints examined in the placebo group. On the other hand, serum IgA and HBGA-blocking antibody levels were significantly lower among subjects who developed GII.4 infection, GII.4 protocol-defined illness (PDI), and GII.4-associated vomiting or diarrhea (VorD) of any severity than those in subjects who did not meet these endpoints (Table 3). A similar pattern was observed when GII.4-specific antibody seropositivity was determined by the different serological assays, with the exception that prechallenge serum IgA seropositivity was no longer significantly different between groups that met or did not meet the study endpoints. We found that 1/16 (PDI) and 2/18 (VorD) placebo recipients who developed illness had detectable HBGA-blocking antibody at the time of virus inoculation compared with ~44% who did not become ill. The presence of detectable serum HBGA-blocking an-

TABLE 1 Immune responses to GI.1 antigen before and after each vaccination

Response by antibody type[a]	Data (95% CI) by day in group receiving[b]:					
	Vaccine			Placebo		
	Day 0 ($n = 50$)	Day 28 ($n = 49$)	Day 56 ($n = 49$)	Day 0 ($n = 48$)	Day 28 ($n = 47$)	Day 56 ($n = 48$)
Total antibody						
GMT	2,023 (1,400, 2,923)	128,819 (100,421, 165,248)	120,023 (95,697, 150,533)	1,759 (1,186, 2,607)	1,550 (1,009, 2,384)	1,590 (1,045, 2,418)
GMFR		62.2 (42.8, 90.5)	58 (41.2, 81.6)		0.9 (0.8, 1)	0.9 (0.8, 1)
% seroresponse		100.0 (92.7, 100.0)	100.0 (92.7, 100.0)		0.0 (0.0, 7.5)	2.1 (0.1, 11.1)
IgG						
GMT	4.2 (3.1, 5.5)	114.2 (83, 157.1)	105.5 (79.7, 139.8)	4.6 (3.5, 6.1)	4.4 (3.3, 5.9)	4.4 (3.3, 5.9)
GMFR		27.8 (19.2, 40.2)	24.9 (18.3, 34.1)		1 (0.8, 1.2)	1 (0.8, 1.1)
% seroresponse		95.9 (86.0, 99.5)	93.9 (83.1, 98.7)		0.0 (0.0, 7.5)	0.0 (0.0, 7.4)
IgA						
GMT	3.7 (3.2, 4.3)	64.7 (44.2, 94.7)	44 (30.5, 63.4)	4 (3.4, 4.7)	4 (3.4, 4.8)	4.1 (3.4, 4.8)
GMFR		17.5 (11.9, 25.8)	11.9 (8.2, 17.1)		1 (1, 1.1)	1 (1, 1.1)
% seroresponse		87.8 (75.2, 95.4)	81.6 (68.0, 91.2)		0.0 (0.0, 7.5)	0.0 (0.0, 7.4)
HBGA blocking						
GMT	17 (13.7, 20.9)	435.9 (297.3, 639.3)	538.8 (416.4, 697.2)	16.2 (13.4, 19.6)	16.5 (13.8, 19.7)	15.7 (13.3, 18.6)
GMFR		25.6 (18, 36.3)	31.6 (24.6, 40.5)		1 (0.9, 1.2)	1 (0.9, 1.1)
% seroresponse		95.9 (86.0, 99.5)	100.0 (92.7, 100.0)		0.0 (0.0, 7.5)	0.0 (0.0, 7.4)

[a] GMT, geometric mean titer; GMFR, geometric mean fold rise; % seroresponse, % with ≥4-fold increase in antibody level; HBGA, histoblood group antigen.
[b] 95% CI, 95% confidence interval.

tibody was associated with a 65% lower risk of infection, 85% lower risk of PDI, and a 73% lower risk of VorD, while the presence of measurable serum GII.4-specific IgA was associated with 38%, 61%, and 55% lower risks of infection, PDI, and VorD, respectively.

We next examined whether prechallenge serum antibody levels among vaccine recipients were associated with a decreased risk of infection or illness (Table 4). Overall, serum antibody levels were similar between groups that were or were not infected, had or did not have PDI, and those who had or did not have VorD. However, vaccinees with a prechallenge serum HBGA-blocking antibody level of >1:500 were significantly less likely to develop moderate or severe VorD than were those with lower antibody levels (0/35 versus 3/15; $P = 0.023$, Fisher's exact test).

TABLE 2 Immune response to GII.4 antigen before and after each vaccination

Response by antibody type[a]	Data (95% CI) by day in group receiving[b]:					
	Vaccine			Placebo		
	Day 0 ($n = 50$)	Day 28 ($n = 49$)	Day 56 ($n = 49$)	Day 0 ($n = 48$)	Day 28 ($n = 47$)	Day 56 ($n = 48$)
Total antibody						
GMT	5,412 (3,928, 7,456)	60,011 (45,410, 79,307)	54,354 (43,360, 68,135)	4,496 (3,254, 6,212)	4,686 (3,323, 6,609)	5,120 (3,524, 7,440)
GMFR		10.8 (7.3, 15.8)	9.9 (7.1, 13.8)		1.1 (0.9, 1.2)	1.1 (1, 1.4)
% seroresponse		83.7 (70.3, 92.7)	89.8 (77.8, 96.6)		2.1 (0.1, 11.3)	2.1 (0.1, 11.1)
IgG						
GMT	7.3 (5.7, 9.4)	65.3 (52, 82.1)	52.9 (44.4, 62.9)	6.7 (5.2, 8.7)	7 (5.2, 9.3)	7 (5.1, 9.6)
GMFR		8.8 (6.5, 11.9)	7.2 (5.5, 9.4)		1 (0.9, 1.2)	1 (0.9, 1.2)
% seroresponse		73.5 (58.9, 85.1)	71.4 (56.7, 83.4)		0.0 (0.0, 7.5)	2.1 (0.1, 11.1)
IgA						
GMT	5.6 (4.4, 7.2)	52.9 (41.1, 68)	43.7 (35.4, 54)	5.3 (4.3, 6.5)	5.5 (4.4, 6.8)	5.8 (4.6, 7.3)
GMFR		9.3 (6.7, 12.7)	7.7 (5.8, 10.1)		1 (0.9, 1.1)	1.1 (0.9, 1.3)
% seroresponse		75.5 (61.1, 86.7)	75.5 (61.1, 86.7)		2.1 (0.1, 11.3)	2.1 (0.1, 11.1)
HBGA blocking						
GMT	115.6 (101.4, 131.7)	902.2 (714.1, 1,139.8)	767.3 (631, 933.1)	126.3 (104.9, 152.1)	128 (104.7, 156.4)	134.7 (108.3, 167.5)
GMFR		7.8 (5.9, 10.2)	6.6 (5.2, 8.3)		1 (0.9, 1.1)	1.1 (0.9, 1.2)
% seroresponse		69.4 (54.6, 81.7)	77.6 (63.4, 88.2)		0.0 (0.0, 7.5)	2.1 (0.1, 11.1)

[a] GMT, geometric mean titer; GMFR, geometric mean fold rise; % seroresponse, % with ≥4-fold increase in antibody level; HBGA, histoblood group antigen.
[b] 95% CI, 95% confidence interval.

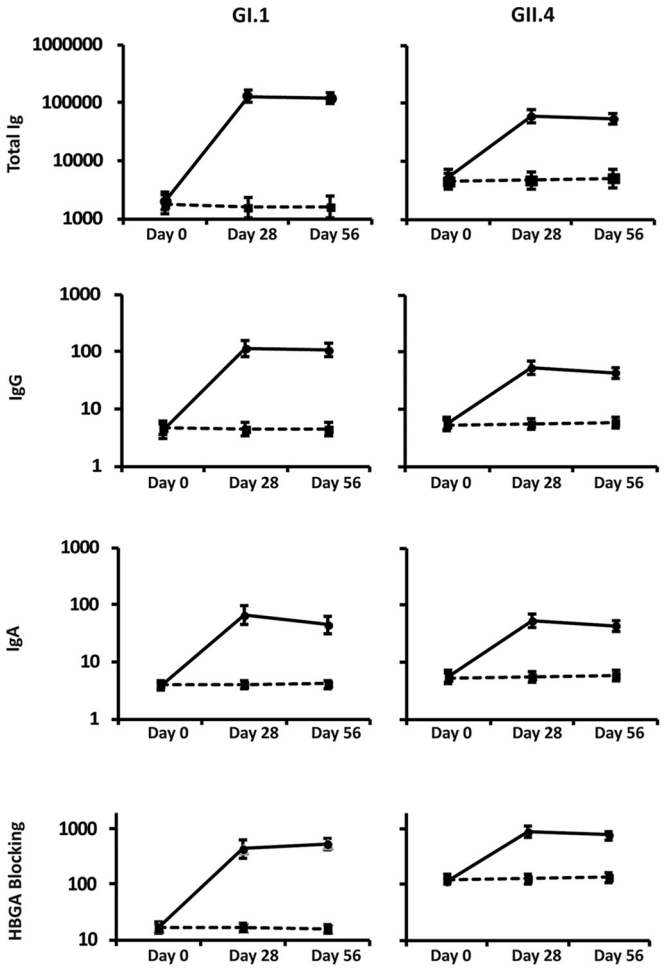

FIG 1 Geometric mean serum antibody levels pre- and postvaccination for virus-specific total ELISA antibody (total Ig), IgG antibody, IgA antibody, and HBGA-blocking antibody. The circles represent vaccinated persons, and the squares represent placebo recipients. The error bars represent 95% confidence intervals.

DISCUSSION

Histoblood group antigens have been identified as putative attachment factors involved in the establishment of infection for several different norovirus genotypes (20–22). This requirement was first demonstrated for Norwalk virus, a GI.1 strain, based upon the inability of secretor-negative subjects to become infected following experimental virus challenge (23, 24). The secretor phenotype is determined by the presence of at least one functional fucosyltransferase 2 (*FUT2*) gene, and the active FUT2 enzyme modifies glycan structures expressed on the gut epithelium. Subsequent epidemiological studies and a human challenge study also demonstrated the importance of secretor status on the susceptibility to infection with GII.4 noroviruses (25–27). One mechanism of virus neutralization is to block virus attachment to susceptible cells. Human noroviruses currently cannot be propagated serially using standard *in vitro* cultivation systems (28–30), so it is not possible to test antibody neutralization functionality. In addition, some norovirus genotypes do not bind to the HBGAs that have been tested (31, 32), so it is not possible to measure HBGA-blocking antibody levels against those viruses. Nevertheless, an antibody level that causes blocking of norovirus binding to HBGAs has been proposed as a surrogate for neutralization for HBGA-binding strains and potentially a correlate of protection (11, 12, 33). The current study, following experimental virus inoculation in placebo subjects, demonstrated that even when screening for low antibody levels, higher levels of HBGA-blocking serum antibody to a GII.4 norovirus are associated with a lower frequency of infection and illness.

The same pattern of protection was not observed among vaccine recipients. Prechallenge serum HBGA-blocking antibody levels postvaccination were significantly higher than those of the placebo recipients, but higher antibody levels were not associated with corresponding decreases in infection, PDI, or mild VorD illness frequency, as seen in the placebo group. However, vaccinees with higher levels of HBGA-blocking antibody (>1:500) were significantly less likely to develop moderate-to-severe VorD. This suggests that serum HBGA-blocking antibody titers may be a possible correlate of protection for vaccine recipients, although at a higher level than that observed for placebo subjects. However, HBGA-blocking antibody titers may not be a candidate for a level 1 surrogate of protection (as defined by Qin et al. [34]) following vaccination, due to the apparent differences in thresholds in vaccinees and nonvaccinees. A discordance between apparent protective serum antibody levels among vaccinated and placebo subjects has also been described for the hemagglutination-inhibition assay in influenza virus infection (35). In that study, subjects who developed laboratory-confirmed influenza illness after receiving an inactivated influenza virus vaccine had a significantly lower serum hemagglutination-inhibition (HAI) antibody GMT than that of vaccinated subjects without illness, but the GMT was higher than that of unvaccinated subjects who did not become ill and above the putative seroprotective level of 1:32. Field efficacy studies are needed to determine whether a similar phenomenon occurs following vaccination against norovirus.

The immunogenicity of the bivalent norovirus candidate vaccine in the current study was reported in an earlier study (17), and immune responses were similar in this study, even though the previous study included secretor-negative subjects and did not prescreen individuals for lower antibody levels. It is important to note that a different HBGA-blocking assay format (PGM) was used to measure the GII.4 antibody response in the current study due to the lack of availability of the H type 3 glycan used previously. Although PGM has been shown to bind norovirus VLPs (36), the PGM-based assay has differences compared to the HBGA assay in readouts because of the format, e.g., the threshold for detecting HBGA-blocking antibody was higher in the current study using PGM (1:184) than that with the previous assay that used H type 3 glycan (1:50), and the postvaccination geometric mean titers were also higher for the PGM-based assay. The assays measure similar binding properties, and the GMFRs at day 56 were similar (6.6 in the current study versus 6.9 in the previous study [17]).

Some subjects receiving the placebo who had no detectable virus-specific HBGA-blocking antibody were not infected after receipt of the norovirus challenge inoculum. The reasons for this finding are unclear at this time but might include one or more of the following: (i) a virus inoculum dosage was around the 50% human infectious dose (HID_{50}) of the strain, so some subjects were not infected due to chance; (ii) inadequate sensitivity of the serum HBGA-blocking assay, starting with an initial dilution of

TABLE 3 Association of prechallenge GII.4-specific serum antibody with achieving different study endpoints among placebo recipients[a]

Antibodies by study endpoint[b]	GMT (95% CI)			% seropositive prechallenge[d]		
	Study endpoint met	Study endpoint not met	*P* value[c]	Study endpoint met	Study endpoint not met	*P* value[e]
Infected						
Total	4,032 (2,736, 5,941)	8,533 (3,827, 19,024)	0.132	100	100	1.0
IgG	5.6 (4.2, 7.7)	10.2 (5.0, 21)	0.102	79	74	0.732
IgA	4.6 (3.6, 5.8)	9.2 (5.5, 15.3)	0.009	35	63	0.077
HBGA blocking	103.5 (91.1, 117.6)	220.3 (130.5, 371.8)	<0.001	14	58	0.003
PDI						
Total	3,467 (2,023, 5,943)	6,785 (4,022, 11,446)	0.108	100	100	1.0
IgG	5.6 (3.7, 8.4)	8.1 (5.1, 12.9)	0.296	81	75	0.729
IgA	4.0 (3.1, 5.0)	7.5 (5.2, 10.6)	0.024	25	56	0.065
HBGA blocking	97.6 (86, 110.9)	166.9 (119.2, 233.6)	0.01	6	44	0.009
VorD						
Total	4,064 (2,395, 6,895)	6,451 (3,708, 11,221)	0.355	100	100	1.0
IgG	6.2 (4.1, 9.2)	7.8 (4.8, 12.8)	0.615	83	73	0.499
IgA	4.4 (3.2, 6.1)	7.3 (5.1, 10.4)	0.048	28	57	0.074
HBGA blocking	105.2 (86.2, 128.4)	165.3 (116.2, 235.3)	0.027	11	43	0.026

[a] The numbers for the endpoint met and endpoint not met groups are: 29 and 19 for infected, 16 and 32 for PDI, and 18 and 30 for VorD, respectively.
[b] PDI, protocol-defined illness with infection; VorD, illness with infection and vomiting or diarrhea of mild or greater severity.
[c] Determined by Wilcoxon rank sum test.
[d] Seropositive is defined as above the limit of detection for each respective antibody assay.
[e] Chi-square or Fisher's exact test.

1:184; (iii) serum HBGA-blocking antibody is not a functional correlate of protection but is instead associated with a different functional immune response; (iv) the VLP used to measure HBGA-blocking antibody was from a different variant (most like GII.4/2006a) than the one used for challenge (GII.4,2002); or (v) there was an additional unrecognized innate mechanism of resistance to infection.

Another possible correlate suggested by the current study is virus-specific serum IgA, since higher GII.4-specific serum IgA antibody levels were associated with a decreased frequency of infection and illness among placebo subjects. However, virus-specific serum IgA levels were not associated with protection from symptomatic illness in a GI.1 norovirus challenge study (37). In that study, higher virus-specific salivary IgA antibody levels were

TABLE 4 Association of prechallenge GII.4-specific serum antibody with achieving different study endpoints among vaccine recipients[a]

Antibodies by study endpoint[b]	GMT (95% CI)			% seropositive prechallenge[d]		
	Study endpoint met	Study endpoint not met	*P* value[c]	Study endpoint met	Study endpoint not met	*P* value[e]
Infected						
Total	52,067 (37,230, 72,817)	51,606 (38,430, 69,300)	0.968	100	100	1.0
IgG	54.1 (42.0, 69.8)	50.6 (39.7, 64.5)	0.541	100	100	1.0
IgA	37.9 (26.7, 53.6)	45.6 (36.1, 57.7)	0.398	100	100	1.0
HBGA blocking	744.6 (563.8, 983.5)	702.6 (529.5, 932.4)	0.607	100	100	1.0
PDI						
Total	43,203 (27,081, 68,925)	55,276 (42,928, 71,176)	0.270	100	100	1.0
IgG	47.6 (32.1, 70.8)	54.2 (44.6, 65.8)	0.627	100	100	1.0
IgA	48.1 (29.7, 77.9)	39.3 (31.0, 49.7)	0.241	100	100	1.0
HBGA blocking	865.3 (591.3, 1,266)	680.3 (541.3, 854.8)	0.278	100	100	1.0
VorD						
Total	38,217 (23,341, 62,575)	55,953 (43,765, 71,535)	0.134	100	100	1.0
IgG	49.1 (31.1, 77.5)	53.2 (44.0, 64.4)	0.818	100	100	1.0
IgA	44.6 (26.8, 74.1)	40.6 (32.0, 51.5)	0.363	100	100	1.0
HBGA blocking	790.8 (509.4, 1,227.8)	708.4 (567.6, 884.1)	0.743	100	100	1.0

[a] The numbers for the endpoint met and endpoint not met groups are: 26 and 24 for infected, 13 and 37 for PDI, and 10 and 40 for VorD, respectively.
[b] PDI, protocol-defined illness with infection; VorD, illness with infection and vomiting or diarrhea of mild or greater severity.
[c] Determined by Wilcoxon rank sum test.
[d] Seropositive is defined as above the limit of detection for each respective antibody assay.
[e] Chi-square or Fisher's exact test.

associated with a decreased risk of norovirus gastroenteritis. In the current study, saliva was not collected after vaccination and prior to challenge, so we were not able to assess the association of virus-specific salivary IgA with infection and illness.

The immunogenicity of the vaccine to the GII.4 strain was lower than that to the GI.1 strain in the vaccine, similar to the findings from an earlier study (17). The imbalanced response between GI.1 and GII.4 strains was noted after the challenge study was already planned with this study vaccine formulation. Additional studies are being conducted to determine whether the immunogenicity to the GII.4 component can be improved by adjusting the ratios of the antigens and evaluating the contribution of adjuvant components. Additional studies to assess the impact of vaccines on other potential immune correlates, such as salivary IgA (37), are under way.

In summary, intramuscular administration of a bivalent GI.1/GII.4 norovirus candidate vaccine in a phase 1/2 study induced statistically significant increases in serum antibody levels (total, IgG, IgA, and HBGA blocking) to both vaccine strains. Placebo subjects with higher anti-GII.4 serum HBGA-blocking or IgA antibody levels were less likely to be infected or become ill after challenge with a GII.4 norovirus. In vaccinated subjects, however, the relevance of these serum antibodies to protection was less apparent in the current study, and different threshold levels of serum antibody titers may apply to vaccine protection. Some placebo subjects who were not infected had no measurable serum HBGA-blocking antibody prior to challenge, which raises the possibility that other immunological mechanisms may be related to protection and should be studied further. It is worthwhile to continue to identify and assess the value of potential correlates of protection from norovirus infection and illness as tools to aid vaccine development across the broad populations for whom vaccines are most needed.

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