



Safety and immunogenicity of a parenterally administered rotavirus VP8 subunit vaccine in healthy adults



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ARTICLE INFO

Article history:

Received 22 January 2015

Received in revised form 9 April 2015

Accepted 12 May 2015

Available online 8 June 2015

Keywords:

Rotavirus

Vaccine

Diarrhea

Subunit

Parenteral

Non-replicating

ABSTRACT

Background: The P2-VP8 subunit vaccine for the prevention of rotavirus gastroenteritis is comprised of a truncated VP8 subunit protein from the rotavirus Wa strain (G1[P8]) fused to the tetanus toxin P2 epitope, and adsorbed on aluminum hydroxide for intramuscular administration.

Methods: Three groups of 16 adults were randomized to receive three injections of P2-VP8 (12) or placebo (4) at doses of 10, 30 or 60 µg of vaccine. IgG and IgA antibodies to P2-VP8 were assessed by ELISA in serum and lymphocyte supernatant (ALS). Serum samples were tested for neutralizing antibodies to homologous and heterologous strains of rotavirus.

Results: The vaccine was well-tolerated. All vaccine recipients demonstrated significant IgA responses and all but one demonstrated IgG responses; in the 60 µg cohort, geometric mean titers (GMTs) rose 70- and 80-fold for IgA and IgG, respectively. Homologous neutralizing antibody responses were observed in about half of participants in all three dose cohorts; in the 60 µg cohort, GMTs against Wa rose from 128 to 992. Neutralizing antibody responses were robust to P[8] strains, moderate to P[4] strains and negligible to P[6] strains. ALS IgA responses were dose dependent.

Conclusions: The P2-VP8 subunit vaccine was well tolerated and evoked promising immune responses.

Clinical trials registration: NCT01764256

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1. Introduction

Live oral rotavirus vaccines are of great benefit to infants and young children in developing-country populations. However, their efficacy in these populations is inferior to that demonstrated in the developed world [1,2]. Such discrepancy in performance is not a unique feature of rotavirus vaccines; it has been demonstrated with other oral, live attenuated enteric vaccines such as those against cholera and poliomyelitis [3]. Among the purported

reasons for the diminished efficacy are inhibition of the vaccine virus growth by transplacentally acquired or breast-milk antibodies, interference by other pathogens in the gut and gut-associated factors, such as enteropathy, all commonly observed in developing countries. This impediment to higher vaccine efficacy for live oral vaccines might be overcome by strategies involving parenterally administered rotavirus vaccines [4]. Such an approach is supported by the success of parenteral vaccines for several diseases caused by orally transmitted pathogens including poliovirus, hepatitis A virus, *Vibrio cholera* and *Salmonella typhi*. Jiang has summarized indirect observations in humans that support the possibility that parenterally-administered, non-replicating rotavirus vaccines may be successful, including observations derived from natural rotavirus infection studies in infants, rotavirus challenge studies in adult volunteers, and human rotavirus vaccine trials [5]. Further, promising direct observations have been made in animal studies [6–9]. The induction of high antibody titers by adjuvanted

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parenteral injection of non-replicating vaccines may result in mucosal protection mediated by the induction and transduction of antibodies into the gut. The high titers of circulating anti-rotavirus antibodies transmitted transplacentally in humans appear to both modulate the take of live oral vaccines and be protective against early rotavirus disease [10–13]. Furthermore, passively administered antibody may prevent or diminish rotavirus disease [6,14]. In limited human challenge studies, the presence of high concentrations of neutralizing antibodies appears to be correlated with resistance to infection and disease [15].

Truncated VP8 subunit proteins containing most of the neutralizing epitopes expressed by VP4 have been shown to elicit rotavirus neutralizing antibody responses in animals [16]. The immunogenicity of these VP8 proteins could be significantly enhanced when they were fused with the P2 epitope of tetanus toxin, which exerts a strong T cell helper function [17]. Immunization of neonatal piglets with a P2-VP8-P[8] chimeric protein conferred significant protection against experimental rotavirus gastroenteritis [17]. In the present study we describe a first in human clinical trial to evaluate the safety and immunogenicity of this vaccine administered by the intramuscular route with the aim to elicit sufficient antibody responses to afford protection in the mucosa and circumvent many of the purported mechanisms interfering with the efficacy of orally administered, live rotavirus vaccines in developing world populations.

2. Materials and methods

2.1. P2-VP8 vaccine

The gene sequence for the P2-VP8 protein was supplied by T. Hoshino, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD [16]. A synthetic gene, codon optimized for *Escherichia coli*, was inserted into the pJ411 expression plasmid, which also confers kanamycin resistance, under control of a T7 promoter (optEc2) by DNA 2.0, Menlo Park, CA. The plasmid was transfected into *E. coli* BI21 (DE3), Novagen, Billerica, MA. Master and working seed lots, as well as the final vaccine, were produced at the Walter Reed Army Institute of Research (WRAIR), Pilot Bioproduction Facility, Silver Springs, MD.

The P2-VP8 protein was purified from physically disrupted cells cultured in APS Superbroth (Becton Dickinson, Franklin Lakes, NJ) plus kanamycin, induced with isopropyl- β -D1-thiogalactopyranoside (0.5 mM). The cell lysate was clarified by centrifugation, the supernatant collected and passed over a Q Sepharose FF column (GE Healthcare, Wilmington, MA) equilibrated in Q buffer (50 mM Tris, pH 7.5) at a ratio of 1 gram of initial cell paste/2.5 ml of resin. The flow through which contained the P2-VP8 protein was collected, adjusted to 1 M $(\text{NH}_4)_2\text{SO}_4$ and applied to a 500 ml Butyl 650 column (Tosoh Bioscience, Grove City, OH) equilibrated in HIC buffer (50 mM Tris, pH 7.5, 1 M $(\text{NH}_4)_2\text{SO}_4$). The column was washed with one column volume of HIC buffer and the p2-VP8 protein eluted in a step-wise fashion by washing to baseline with HIC buffer containing 0.6 M $(\text{NH}_4)_2\text{SO}_4$ followed by HIC buffer with 0.4 M $(\text{NH}_4)_2\text{SO}_4$. P2-VP8 rich fractions were collected and buffer exchanged against PBS pH 7.1, concentrated five-fold against a membrane with a 10 Kdal cutoff, and brought back to the original volume in PBS, pH 7.1. This process was repeated four times in total. The protein concentration was determined by measuring the calculated extinction coefficient ($1 \text{ mg/ml} = A_{280}$ of 1.74). To remove small molecular weight components, the P2-VP8 containing solution was extensively diafiltered and ultrafiltered against a 5 Kdal membrane using PBS pH 7.1. This material was recirculated for 18 h at 4 °C over a Q Sepharose FF column (1 ml of resin per 5 ml of protein solution), the unbound material collected and the protein

content determined by absorbance at A_{280} . This solution was sterile filtered using a Millipack-60 0.22 μm filtration unit (Millipore Corp., Billerica, MA), and aseptically filled into 3 ml glass vials.

The vaccine in final vial form contained 428 μg protein per ml (purity of 98% as determined by size exclusion HPLC), was non-pyrogenic ($<2.4 \text{ EU/ml}$, by LAL testing), contained less than 100 pg of *E. coli* DNA per ml, and was sterile.

Repeated intramuscular injections of the vaccine in rabbits did not lead to any unexpected local or system toxicity. The vaccine was found to be stable for 18 months when stored at 4 °C.

2.2. Vaccine formulation

The vaccine was adsorbed onto aluminum hydroxide adjuvant (Alhydrogel, Brenntag Biosector, Frederikssund, Denmark) just prior to administration as follows. A sterile, non-pyrogenic aluminum hydroxide stock solution was diluted to 2.6 mg of aluminum per ml using sterile saline, pH 7. The vaccine was diluted to either, 40, 120 or 240 $\mu\text{g/ml}$ in sterile saline and equal volumes of vaccine and aluminum hydroxide were mixed in labeled, sterile glass vials to yield a final protein concentration of 10, 30 or 60 μg per 0.5 ml containing 0.56 mg of aluminum as aluminum hydroxide.

2.3. Study design

A first in human, randomized, double-blind, placebo-controlled, dose-escalation study was performed in healthy, 18–45 year old adults in Baltimore, MD, between December 2012 and October 2013.

Human experimentation guidelines of the United States Department of Health and Human Services were followed in the conduct of the clinical research. Prior to initiation of the study, the protocol was reviewed and approved by an institutional review board for the Johns Hopkins School of Public Health and PATH. Participants were enrolled after providing written informed consent.

2.4. Procedures

Three dose-levels of the P2-VP8 subunit vaccine were sequentially tested: 10 μg , 30 μg and 60 μg in 0.5 ml. Cohorts of 16 individuals (12 vaccine and 4 placebo recipients) per dose level were randomly assigned to receive three intramuscular injections of vaccine or placebo, four weeks apart. Vaccine was admixed with 0.56 mg of aluminum hydroxide on the day of vaccination, as noted above.

To evaluate vaccine tolerability, participants were provided with memory aids to assess and record local and systemic reactivity during the 7 days after each vaccination, and participants were seen 7 and 28 days after each vaccination for safety evaluation. In addition, participants were contacted six months after the final vaccination to inquire about new chronic health conditions, serious health events or hospitalizations since the last visit. Monitoring laboratory assessments were performed at baseline and 7 days after each injection. A safety review committee assessed clinical and laboratory safety data for the week after the first injection of the first four participants in the lowest dose cohort before proceeding to enrolling the balance of that cohort. Similar assessment was performed for the safety data from the week after the first injection of the two lower dose cohorts before proceeding to the next higher dose level, and before proceeding to subsequent injections within each dose cohort.

Serum samples were obtained at baseline and 28 days after each injection to assess IgG and IgA responses to the P2-VP8 antigen by ELISA and neutralizing antibody to homologous and heterologous rotavirus strains, and at baseline and 7 days after the first

Table 1
Maximum reactogenicity per participant^a

	Parameter		None N (%)	Mild N (%)	Moderate N (%)	
Injection site	Pain	Placebo	10(83.3)	1(8.3)	1(8.3)	
		10 µg	6 (50.0)	5 (41.7)	1 (8.3)	
		30 µg	10 (83.3)	2 (16.7)	0	
	Tenderness	60 µg	8 (66.7)	3 (25.0)	1 (8.3)	
		Placebo	8(66.7)	3(25.0)	1(8.3)	
		10 µg	5 (41.7)	6 (50.0)	1 (8.3)	
	Itching	30 µg	7 (58.3)	5 (41.7)	0	
		60 µg	4 (33.3)	8 (66.7)	0	
		Placebo	9(75.0)	3(25.0)	0	
	Redness	10 µg	11 (91.7)	1 (8.3)	0	
		30 µg	11 (91.7)	1 (8.3)	0	
		60 µg	11 (91.7)	1 (8.3)	0	
	Swelling	Placebo	11(91.7)	0	1(8.3)	
		10 µg	10 (83.3)	2(16.7)	0	
		30 µg	10 (83.3)	2 (16.7)	0	
	Systemic	Fever	60 µg	10 (83.3)	2 (16.7)	0
			Placebo	12(100.0)	0	0
			10 µg	12 (100.0)	0	0
Nausea		30 µg	12 (100.0)	0	0	
		60 µg	12 (100.0)	0	0	
		Placebo	10(83.3)	2(16.7)	0	
Vomiting		10 µg	11 (91.7)	1 (8.3)	0	
		30 µg	12 (100.0)	0	0	
		60 µg	10 (83.3)	2 (16.7)	0	
Arthralgia		Placebo	12(100.0)	0	0	
		10 µg	11 (91.7)	1 (8.3)	0	
		30 µg	11 (91.7)	1 (8.3)	0	
Chills		60 µg	9 (75.0)	3 (25.0)	0	
		Placebo	11(91.7)	1(8.3)	0	
		10 µg	11 (91.7)	1 (8.3)	0	
Fatigue		30 µg	11 (91.7)	1 (8.3)	0	
		60 µg	11 (91.7)	1 (8.3)	0	
		Placebo	10(83.3)	1(8.3)	1(8.3)	
Headache	10 µg	9 (75.0)	2 (16.7)	1 (8.3)		
	30 µg	11 (91.7)	1 (8.3)	0		
	60 µg	10 (83.3)	1 (8.3)	1 (8.3)		
Myalgia	Placebo	10(83.3)	2(16.7)	0		
	10 µg	10 (83.3)	2 (16.7)	0		
	30 µg	10 (83.3)	2 (16.7)	0		
		60 µg	9 (75.0)	3 (25.0)	0	

^a No severe reactogenicity was observed in any participants.

injection to assess antibody to the P2-VP8 antigen in lymphocyte supernatant (ALS).

2.5. Laboratory methods

Anti-P2-VP8 immunoglobulin G (IgG) and IgA antibody were quantitated using standard ELISA assay techniques. Although the antigen used for the IgA and IgG assays is the P2-VP8 vaccine itself, not VP8 alone, competitive binding assays indicated that the P2 component does not contribute to the binding responses (data not shown).

A reference standard containing pooled sera from subjects known to have had a natural rotavirus infection was used for both anti-P2-VP8 IgG and anti-P2-VP8 IgA. The reference standard was assigned a value of 10,000 units/mL of anti-P2-VP8 specific IgG or IgA. The standard curve was modeled using a four parameter

logistic regression function in the SoftMax software for the ELISA reader. The concentration of anti-P2-VP8 specific IgG or IgA in a sample was derived by extrapolation from the reference standard curve. The lower limit of detection was determined during qualification of the assay to be 8.72 and 15.15 units per mL for anti-P2-VP8 IgA and IgG, respectively.

Neutralizing antibody to Wa (G1P[8]), 89-12 (G1P[8]), DS-1 (G2P[4]), P (G3P[8]), ST3 (G4P[6]), WI61 (G9P[8]), SC2 G2 P[6]) and BrB (G4P[6]) was determined using previously described methods [18]. Titer is defined as the reciprocal of the serum dilution that resulted in a 60% reduction in virus.

ALS were obtained by culturing peripheral blood mononuclear cells (PBMCs) for 72 h in the absence of P2-VP8 antigen as described [19]. The supernatant of the culture was collected after low speed centrifugation and tested in the same ELISA assay used for serological evaluations.

Table 2
Unsolicited adverse events (maximal severity per participant).

Study product	Mild	Moderate	Severe	Any
Placebo	5 (41.7%)	1 (8.3%)	0	6 (50.0%)
10 µg	6 (50.0%)	3 (25.0%)	0	9 (75.0%)
30 µg	2 (16.7%)	3 (25.0%)	0	5 (41.7%)
60 µg	6 (50.0%)	0	1 (8.3%)	7 (58.3%)

2.6. Statistical analyses

As a first-in-human study, the trial was designed to provide preliminary safety and immunogenicity data to support subsequent testing in age-descending studies. The sample size allowed for recognition of unacceptable toxicity rates occurring at a frequency of 10% or higher. The probability of observing at least one event among the twelve subjects in each dose cohort was 72% if the true attack rate of that event were 10%.

To investigate the fold-rise in IgG, IgA and neutralizing antibody responses from baseline to one month post-3rd injection in each treatment group, the non-parametric signed-rank test was used [20], setting the null hypothesis to be H_0 : Ratio = 1 versus the alternative H_1 : Ratio \neq 1. To investigate whether there was a significant trend in the proportions of subjects with ALS responses with increasing dose, the Cochran–Armitage test was used [21].

3. Results

3.1. Study population

A total of 48 participants were enrolled; 25 female and 23 male. Median age was 34 years (range 19–44 years). Twelve participants each were randomized to receive placebo or 10 µg, 30 µg or 60 µg of the P2-VP8 subunit vaccine. All participants in the vaccine arms received at least two study injections, and all 12 participants in the 60 µg arm received all three study injections. Ten participants in the placebo arm received all three study injections, one received two injections and another received one injection. Injections in one placebo recipient were discontinued after the first vaccination due to moderate induration at the injection site, as required by the protocol. The reasons for incomplete injection series for the two participants in the vaccination arms who received only two injections were assessed unrelated to study injections.

3.2. Safety

Overall, the vaccine was well-tolerated at all three dose levels, and no safety signals were identified.

Only one participant experienced an objective site reaction greater than mild, and that volunteer received placebo (Table 1). One vaccine recipient each in the 10 and 60 µg cohorts complained

of greater than mild pain/tenderness; no participant in the 30 µg cohort reported greater than mild pain/tenderness.

Similarly, systemic reactions were relatively uncommon and generally restricted to mild (Table 1). No volunteers experienced severe reactions, and moderate reactions were limited to fatigue in one volunteer in each of the groups receiving placebo, 10 µg or 60 µg. None of the volunteers experienced fever during the 7 days following study injections.

The adverse event data through 28 days after completion of study injections are summarized in Table 2. No volunteers experienced a serious adverse event through 28 days following completion of study injections, and the only severe adverse event was assessed as unrelated to receipt of vaccine (back pain secondary to trauma). There were no discernible trends in adverse events or safety laboratory values, which included basic clinical hematology and chemistry to evaluate liver and renal function.

3.3. IgG and IgA antibody responses

Sera were assessed for IgG and IgA responses to the P2-VP8 antigen by ELISA (Figs. 1 and 2, respectively) at baseline and one month after each of the three study injections. Almost all vaccine recipients demonstrated greater than four-fold rise in IgG and IgA response to P2-VP8 antigen by ELISA after three vaccinations: only one vaccine recipient did not demonstrate an IgG response (in the 30 µg group) and all vaccine recipients demonstrated IgA responses. There was a steady rise in both IgA and IgG geometric mean titers (GMTs) after each vaccination, with a statistically significant increase ($p=0.0005$) over baseline one month after the third vaccination in all treated groups. No changes in IgG and IgA GMTs were observed in the placebo group

3.4. Neutralizing antibody responses

Sera were assessed for neutralizing antibodies to the homologous Wa strain (Fig. 3) at baseline and one month after each of the three study injections, as well as several VP4 homologous and heterologous strains at baseline and after the third dose (Day 84, Table 3). About half of vaccine recipients demonstrated a four-fold or greater neutralizing antibody response to Wa, with clear increases in GMTs for all three dose levels at one month post-third study injection (Day 84) compared to pre-vaccination levels ($p<0.002$). Of particular interest, of the 12 vaccine recipients (at any dose level) with low baseline titers (<100), 10 (83%) demonstrated a >four-fold increase in neutralizing antibody; five of these 12 were in the 60 µg group, all of whom demonstrated a >four-fold increase. Among the 19 participants with intermediate baseline titers (100–500) and the five participants with high baseline titers (>500), 12 (63%) and none, respectively, mounted a four-fold response.

Table 3
Neutralizing antibody response rate to rotavirus strains (>four-fold increase 28 days after 3rd dose) and geometric mean titer (GMT) 28 days after third study injection.

Strain	P type	10 µg		30 µg		60 µg	
		Response rate % (95% CI)	GMT (95% CI)	Response Rate % (95% CI)	GMT (95% CI)	Response rate % (95% CI)	GMT (95% CI)
Wa	8	67 (35, 90)	670 (335, 1341)	42 (15, 72)	695 (402, 1201)	58 (28, 85)	828 (493, 1393)
89–12	8	83 (52, 98)	1792 (869, 3697)	67 (35, 90)	2040 (1065, 3907)	83 (52, 98)	2906 (1661, 5082)
P	8	58 (28, 85)	751 (438, 1282)	67 (35, 90)	886 (429, 1831)	83 (52, 98)	1509 (738, 3086)
WI61	8	42 (15, 72)	1344 (740–2441)	67 (35, 90)	1498 (862–2605)	83 (52, 98)	2507 (1149–5469)
DS1	4	0 (0, 26)	212 (140, 321)	50 (21, 79)	202 (141, 291)	58 (28, 85)	407 (219, 755)
SC2	6	8 (0, 38)	258 (166–401)	17 (2, 48)	390 (282–537)	25 (6, 57)	201 (136–298)
ST3	6	0 (0, 26)	97 (54, 177)	8 (0, 38)	88 (59, 131)	17 (2, 48)	65 (43, 99)
BrB	6	0 (0, 26)	331 (181–605)	25 (6, 57)	262 (177–387)	25 (6, 57)	117 (71–192)

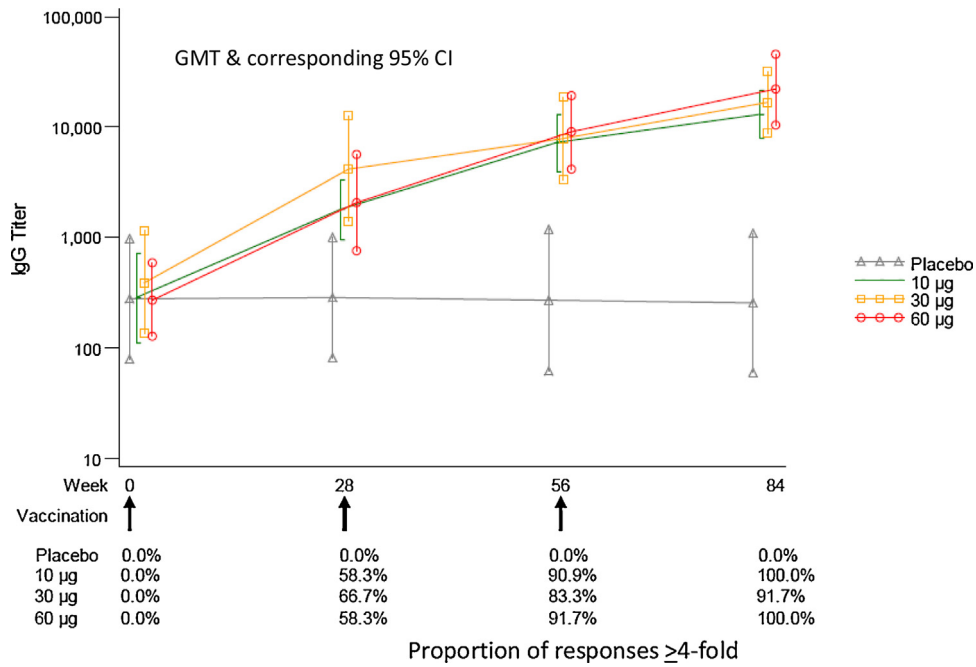


Fig. 1. Anti-P2-VP8 IgG EIA titers and response rates.

Neutralizing antibody responses to other P[8] rotavirus strains tested were robust and comparable to the vaccine homologous Wa strain. However responses were moderate to P[4] strains and fairly limited to P[6] strains, which correlates well with the level of antigenic relatedness among these antigens [16,22] (Table 3).

3.5. Antibody in lymphocyte supernatant responses

Antibodies were assessed to P2-VP8 in ALS at baseline and 7 days after the first study injection (Fig. 4). None of the participants had detectable ALS at baseline. There was a steady rise in proportion of participants with detectable IgA responses 7 days after the first dose of vaccine from lowest (33.3%) to highest (66.7%) dose level, though the trend was not statistically significant.

4. Discussion

Live oral rotavirus vaccines have recently been recommended for use in developing countries by the World Health Organization even though their efficacy against severe disease in these populations (~50%) is lower than in developed countries [1,2,23]. This finding is neither rotavirus vaccine strain specific (it is found with both globally licensed vaccines), nor unique to rotavirus vaccines [3]. The diminished efficacy of both viral and bacterial live oral vaccines among developing country populations is believed to be multifactorial and not easily overcome. Parenteral vaccines may circumvent the factors associated with diminished protection in these populations and potentially offer an enhanced level of protection. Additional benefits of this approach include the ability

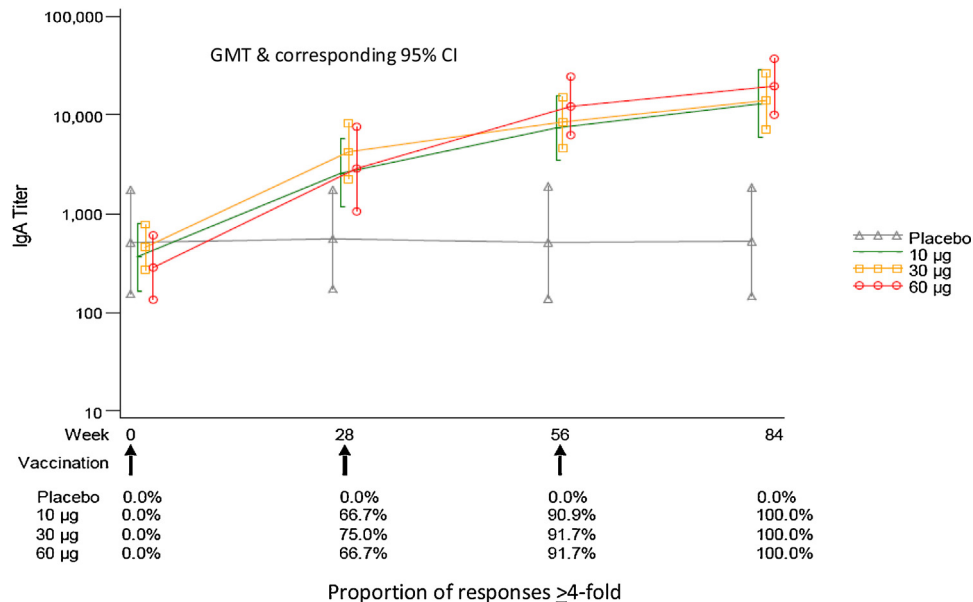


Fig. 2. Anti-P2-VP8 IgA EIA titers and response rates.

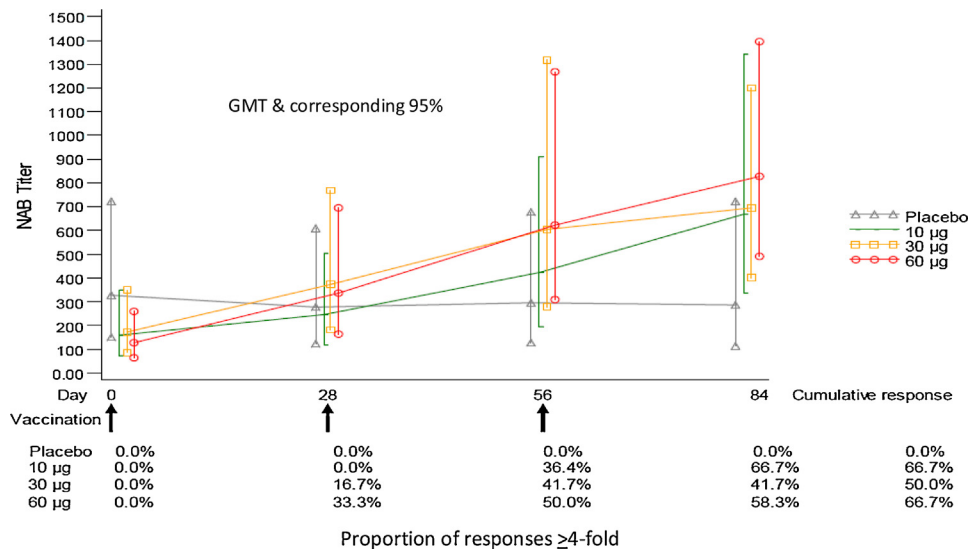


Fig. 3. Neutralizing antibody titers and responses to Wa strain.

to combine parenteral vaccines, such as the P2-VP8 vaccine, with existing Expanded Programme on Immunization (EPI) vaccines to facilitate vaccine uptake and reduce costs [24].

Our efforts to develop a non-replicating rotavirus vaccine focused on utilizing a truncated protein that contains most, if not all, of the neutralizing epitopes expressed in the virus VP8, a cleavage product of the VP4 structural protein [25]. Selectively deleting portions of this protein rendered it soluble, thereby greatly enhancing yield and simplifying purification. The yields obtained from the streamlined manufacturing process (approximately 200 mg/liter) promise to result in a vaccine with a very low cost of goods. The P2

universal helper T cell epitope was fused to the shortened VP8 protein to enhance immunogenicity, which has also been tested with other vaccine constructs [26–28].

The P2-VP8 subunit vaccine was very well tolerated and demonstrated promising immunogenicity. Binding antibody responses were almost universally induced, and IgA responses to the P2-VP8 were demonstrated in all vaccinees, at all dose levels. It is important to note that previous assessment of IgA responses as a possible correlate of protection has been based on IgA assays using antigens from whole virus lysates, presumed to be predominantly VP6 [29], and no inferences about whether VP8-specific responses are meaningful in terms of protection can be made at this stage. The ALS IgA responses demonstrated in the majority of participants receiving the highest vaccine dose are also encouraging.

Immunization induced respectable concentrations of neutralizing antibody, albeit not as ubiquitous as the binding antibody responses. Response rates were greatly dependent on baseline titers, with those participants possessing low baseline titers demonstrating much higher response rates compared to subjects with intermediate or high titers. As expected, the most robust responses were to the strains that express the P[8] antigen, including the Wa strain from which the vaccine is derived, 89–12 (also a G1P[8] strain), and the P and WI61 strains, which are heterologous with respect to the VP7 G glycoprotein antigen. The less robust responses to the P[4] strains and meager responses to the P[6] strains raise the question of whether a multivalent vaccine, including subunits from these other strains would be necessary for a vaccine to be efficacious in diverse geographic areas. Although P[8] strains constitute the majority of strains identified globally, strains expressing P[4] constitute an appreciable proportion of strains identified in both Africa and Asia (up to 20%), while P[6] strains represent as much as 30% of clinical isolates in Africa [30–32].

Whether or how the induced antibodies reach the intestinal mucosal at protecting concentrations will be difficult to assess. There is circumstantial evidence from human and animal research to support that possibility. To address this indirectly, we are planning a test of concept to assess whether immunization with the P2-VP8 subunit vaccine, which induces respectable concentrations of neutralizing antibody to the 89–12, the progenitor strain of Rotarix, impacts shedding of Rotarix, as a proxy of efficacy.

Undoubtedly, the participants in this study had previously experienced repeated exposure to rotavirus, and the promising

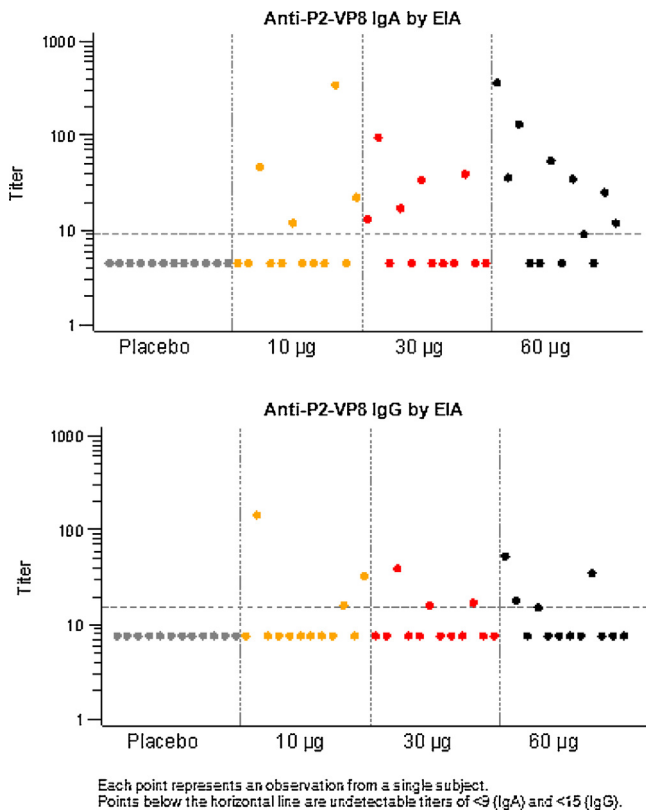


Fig. 4. Antibody to P2-VP8 in lymphocyte supernatant 7 days after the first vaccination.

responses could be driven by anamnestic responses following natural exposure to live virus. However, participants generally had relatively low binding antibody concentrations at baseline, and the kinetics of the responses, for both binding and neutralizing antibodies, are not characteristic of anamnestic responses. The critical next test for the vaccine will be to evaluate its safety and immunogenicity in the target population, which is immunologically naïve infants residing in a developing country. To accomplish this goal, a phase I/II descending age, dose-escalation study in South African toddlers and infants has been initiated.

Funding

The authors would like to acknowledge the contributions of the Bill & Melinda Gates Foundation, which provides financial support to PATH's Non-replicating Rotavirus Vaccine Project. The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

Conflicts of interest

J.W.B is a shareholder in Merck & Co., Inc. (Whitehouse Station, NJ). J.W.B. is currently employed at Takeda Vaccines, Inc., but during the conduct of the trial and drafting of this manuscript was employed at PATH.

The Laboratory for Specialized Clinical Studies, Division of Infectious Diseases at the Cincinnati Children's Hospital Medical Center has laboratory service agreements with Merck & Co, GlaxoSmithKline and Sanofi to provide testing for rotavirus vaccines.

A.D.F., C.H., L.D., J.F., G.R., and S.C.—No conflict.

Acknowledgments

We thank Barbara Deneari and Alicia Cage for leading the clinical research staff at the study clinic. We also thank Allison Stanfill and Sophie Haralson for critical administrative support for the trial.

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