MAJOR ARTICLE

A Systematic Review of Anti-Rotavirus Serum IgA Antibody Titer as a Potential Correlate of Rotavirus Vaccine Efficacy

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Background. Identifying an immunological correlate of protection for rotavirus vaccines (Rotarix [RV1] and RotaTeq [RV5]) would substantially facilitate testing of interventions for improving efficacy in developing countries and evaluating additional candidate rotavirus vaccines.

Methods. We accessed PubMed and ClinicalTrials.gov to identify immunogenicity and efficacy trials for RV1 and RV5 to correlate anti-rotavirus serum immunoglobulin A (IgA) antibody titers vs efficacy in regions stratified by all-cause under-5 mortality rates (u5MR). We established a cutoff point for IgA geometric mean concentration or titer (GMC) that predicted lower efficacy and calculated pooled vaccine efficacy among countries with high vs low IgA titers.

Findings. We observed an inverse correlation between u5MR and IgA titers for RV1 ($r^2 = 0.72$; $P < .001$ and RV5 ($r^2 = 0.66$; $P < .001$) and between efficacy and IgA titers for both vaccines ($r^2 = 0.56$; $P = .005$). Postimmunization anti-rotavirus IgA GMC <90 were associated with decline in vaccine efficacy. Efficacy during first 2 years of life was significantly lower among countries with IgA GMC < 90 (44%; 95% confidence interval [CI], 30–55) compared to countries with GMC > 90 (85%; 95% CI, 82–88).

Interpretation. We observed a significant correlation between IgA titers and rotavirus vaccine efficacy and hypothesize that a critical level of IgA antibody titer is associated with a sufficient level of sustained protection after rotavirus vaccination.

Keywords. rotavirus; vaccines; diarrhea; efficacy; antibody; immunity; protection.

Two live, oral rotavirus vaccines (Rotarix [RV1], GSK Biologicals; and RotaTeq [RV5], Merck) have been extensively evaluated and have demonstrated their efficacy in preventing severe disease and death in children [1, 2]. Additional candidate vaccines are currently undergoing efficacy and safety testing [3, 4], but, for both financial and ethical reasons, it may be difficult to continue to evaluate each of these vaccine candidates by conducting large randomized controlled trials using clinical endpoints to determine efficacy. Also, trials of both RV1 and RV5 have shown that vaccine efficacy against severe rotavirus disease is greater in richer countries with low child mortality (approximately 85%–98%) than in poorer settings with high child mortality (approximately 50%) [1]. This has spurred interest in testing interventions (eg, alternative vaccination schedules, supplementation with probiotics and/or micronutrients) to improve vaccine performance in high-mortality settings. Identifying correlates of protection after rotavirus vaccination could facilitate licensing new candidate vaccines and identification of strategies to improve the performance of current vaccines.

Immune correlates have been identified for protection induced by and against natural rotavirus infection [5–9] and oral rotavirus vaccines [10–13], but none have been conclusive. These correlates have been detected in samples of serum, stool, and duodenal fluid

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after natural rotavirus infection and reflect adaptive mucosal immune responses, likely secretory immunoglobulin A (IgA), and humoral responses such as total serum IgA, total serum immunoglobulin G (IgG), and neutralizing activity [5–9]. However, identifying correlates of protection after rotavirus vaccination has yielded conflicting findings partly due to differences in laboratory assays, vaccine type, number of doses, vaccine titer, age at immunization, variation in preimmunization titers between regions, and timing of vaccination in relation to natural exposure (eg, prior to, during, or after a rotavirus epidemic).

In the past several years, the efficacy of RV1 and RV5 has been reported from trials conducted in a number of countries that span the range of economies [1]. In each of the trials, rotavirus-specific serum IgA antibody titers before and after vaccination were measured in a subset of vaccinees allowing for a direct comparison of antibody titers and efficacy. These studies employed generally comparable protocols and assays, with a high inoculum of antigen administered at an early age before most infants are naturally infected, along with routine childhood immunizations. The standard application of these procedures in a wide range of settings worldwide allows for data to be compared across regions stratified by all-cause under-5 mortality rate (u5MR). We conducted a systematic review of these studies to correlate immune response with u5MR and efficacy of rotavirus vaccines among infants receiving the World Health Organization (WHO) approved formulations of RV1 and RV5 at routine vaccination schedules by the WHO Expanded Programme on Immunization [14].

METHODS

Study Selection

We followed PRISMA guidelines for this systematic review. We included all electronically available clinical trial results from peer-reviewed articles and unpublished reports submitted to ClinicalTrials.gov registry and regulatory agencies for licensure approval. We included those studies that measured immunogenicity in infants who received the WHO recommended formulations of either RV5 (aggregate viral titer of approximately 6.7×10^7 to 12.4×10^7 infectious units per dose of pentavalent WC3 vaccine strain) or RV1 ($10^{6.5}$ median cell-culture infective doses of the RIX4414 vaccine strain) [14]. We included studies that measured both immunogenicity and efficacy (even if presented in separate papers), with efficacy being measured during the first two years of life. Studies were excluded if sample size was <20. We excluded all studies where immunogenicity data were not presented. We also excluded studies of specialized outcomes that were subsets of larger studies in a population, focusing on specialized outcomes not generalizable to the population (eg, malnourished populations, human immunodeficiency virus [HIV] positive populations, assessing interference with other vaccines, modification of vaccine formulation).

Search Methods and Study Selection

To identify studies documenting the immune response and efficacy of rotavirus vaccines, we searched PubMed with the keywords "RIX4414" or "WC3" or "RotaTeq" or "Rotarix" that were published until 30 October 2012. We also searched 3 internet sites for unpublished clinical trial data submitted for regulatory purposes to the US Food and Drug Administration (FDA) [15] and the European Medicines Agency (EMEA) [16] and ClinicalTrials.gov, an international registry of clinical trials [17], to identify additional studies using the same search term "rotavirus vaccine(s)." We cross-referenced these search results to identify data on the immunogenicity from clinical trials of both vaccines that were unpublished as well as data that were pooled for publication but were reported stratified by country in the ClinicalTrials.gov database. Where there were multiple sources of data from the same study population, we relied on the data from the most recent peer-reviewed publication. Whenever possible, we considered data from individual countries as separate data points. We also checked the reference lists of all studies identified by the above methods to identify additional sources of data.

From this search, we identified 59 full-text peer-reviewed articles, 89 full study reports from ClinicalTrials.gov, and 1 report from FDA.gov (Supplementary Figure 1). From these, we identified 25 peer-reviewed publications and 7 reports that provided immunogenicity data from 39 countries and efficacy and immunogenicity data from 15 countries. We dropped immunogenicity data from 1 country because sample size was <20 (Nicaragua). We excluded efficacy data from 1 country (Mali) [18] because authors reported logistical challenges in disease surveillance leading to unreliable estimates; however, we did include the immunogenicity data from Mali when assessing immune response to vaccine by u5MR.

Data Extraction and Management

Data from included studies were extracted onto a standardized table by M. P. and verified by R. G. and U. P. Reviewers were not blinded to study authors, affiliations, or journal name. Variables recorded from each article included study location (country), vaccine formulation, sample size, age at immunization, and number of vaccine doses, vaccine efficacy against severe rotavirus disease (defined as Vesikari severity score ≥11 or severity score >16 on the 24-point scale) and duration of protection separately for each of the first 2 years postvaccination when available. We also extracted data on rotavirus specific serum IgA antibodies after vaccination expressed as geometric mean concentration in U/mL (GMC) for RV1 and geometric mean titer (GMT) for RV5 and the proportion of infants who "seroconverted." IgA seroconversion was defined by the vaccine

manufacturers as an IgA titer or neutralizing antibody rise of 3-fold or greater for RV5 or a postvaccination IgA titer ≥20 U/mL for RV1. Data on neutralizing antibody titers were not reviewed because these titers were collected primarily in studies with RV5 and had not previously correlated well with efficacy overall or against individual serotypes included in the vaccine [19].

Data Analysis

Immune responses were stratified in groups of countries representing the 3 different WHO mortality strata, very low child mortality (WHO region A; 1st quintile); low child mortality (WHO regions B&C; 2nd and 3rd quintiles); and high child mortality (WHO regions D&E; 4th and 5th quintiles) [20, 21].

Table 1. Immune Response (Geometric Mean Concentration) of Anti-Rotavirus Serum IgA and Seroconversion) Following Vaccination With Rotarix (RV1), Grouped by Under-5 Child Mortality

Study Location by u5MR	WHO Stratum	2009 u5MR[a]	Sample Size[b]	IgA GMC 1–2 mo after Last Dose (95% CL)	% Seroconversion (95% CL)	Ref
Low u5MR (mean)				**236 (174, 329)**	**87 (78, 92)**	
Hong Kong	A	2	40	315 (215, 460)	98 (87, 100)	17, 37
Finland (liq)	A	3	746	375 (329, 427)	89 (86, 91)	40
Finland (lyoph)	A	3	252	332 (265, 415)	91 (86, 94)	40
Finland	A	3	167	412 (326, 521)	95 (90, 98)	40
Japan	A	3	34	217 (110, 460)	85 (69, 95)	32
Singapore	A	3	40	369 (231, 588)	98 (87, 100)	17, 37
Czech Republic	A	4	182	153 (119, 195)	85 (79, 90)	17, 41
France	A	4	83	182 (126, 262)	84 (75, 91)	17, 41
Germany	A	4	156	166 (126, 219)	82 (75, 88)	17, 41
Korea	A	5	48	73 (45, 120)	67 (52, 80)	17
Korea	A	5	318	209 174, 250	84 (84, 91)	34
Spain	A	5	186	156 (123, 198)	86 (80, 90)	17, 41
Taiwan	A	5	35	106 (67, 166)	86 (70, 95)	17, 37
Medium u5MR (mean)				**101 (66, 157)**	**74 (61, 84)**	
Argentina	B	14	46	142 (84, 241)	76 (61, 87)	17, 26, 38
Thailand	B	14	157	134 (105, 173)	85 (78, 90)	33
Mexico	B	18	48	175 (107, 288)	90 (77, 97)	17, 26, 38
Venezuela	B	19	40	64 (35, 116)	63 (46, 77)	17, 26, 38
Colombia	B	20	40	89 (53, 148)	75 (59, 87)	17, 26, 38
Brazil	B	21	35	117 (69, 200)	86 (70, 95)	17, 26, 38
Panama	B	21	434	112 (94, 133)	74 (69, 78)	17, 26, 38
Peru	B	21	45	66 (38, 113)	67 (51, 80)	17, 26, 38
Vietnam[c]	B	23	128	77 (55, 109)	63 (54, 72)	31
Honduras	B	25	38	122 (72, 210)	84 (69, 94)	17, 26, 38
Dominican Republic	B	28	40	42 (27, 66)	63 (46, 77)	17, 26, 38
Philippines	B	30	76	90 (62, 129)	79 (68, 88)	17
Philippines[c]	B	30	120	76 (52, 109)	59 (50, 68)	31
High u5MR (mean)				**47 (31, 74)**	**53 (41, 64)**	
Bangladesh[d]	D	51	66	47 (30, 72)	57 (44, 68)	47
South Africa (2 do.)	E	61	131	29 (23, 37)	44 (36, 53)	39
South Africa (2 do.)[e]	E	61	69	59 (38, 94)	57 (45, 69)	24, 35
India	D	63	115	49 (36, 67)	58 (49, 67)	36
Malawi (2 do.)[e]	E	98	67	52 (26, 102)	47 (30, 64)	23, 35

Serum rotavirus IgA antibody concentrations 1–2 months after dose 2 for Rotarix; seroconversion defined as GMC >20 U/mL after Rotarix.

Abbreviations: CL, confidence limit;GMC, geometric mean concentration; IgA, immunoglublin A; u5MR,under-5 mortality rate; WHO, World Health Organization.

[a] Excludes studies with N < 20.

[b] u5MR (Under-5 child mortality) per 1000 live births (21).

[c] This study evaluated immune response after vaccine was administered at 9 and 13 weeks of age in Vietnam and at 6 and 15 weeks of age in Philippines; an alternative age strategy was also evaluated but is not reported here to maintain comparability with age regimens in other studies in the table.

[d] To maintain consistency with other studies, data are presented for Rotarix given concomitantly with oral polio vaccine (OPV); immunogenicity data for Rotarix given at 12 and 16 weeks, 2 weeks after OPV, were not considered in this analysis.

[e] Excludes studies with 3 doses to allow for comparability with other studies in this table following the WHO 2-dose Rotarix regimen.

These strata are referred to hereafter as low, medium, and high, respectively.

We compared the average of the GMC/GMT and seroconversion rates and 95% confidence intervals between the different WHO mortality strata and also assessed the correlation between these 2 measures of immune response.

We fit linear regression models to examine the association between seroconversion rates and IgA GMC/GMT with vaccine efficacy, controlling for vaccine type. The likelihood ratio test was used to determine if the association changed significantly between the first and second year postvaccination.

Previous reviews of rotavirus vaccine efficacy trials had demonstrated a gradient in vaccine efficacy that correlated with level of child mortality and with decline of efficacy during the second year of life in high mortality settings of Africa [1, 22–24]. Thus, we were interested in establishing a cutoff point between high and low IgA GMC/GMT that would identify 1 of 2 outcomes of "suboptimal vaccine performance": (1) lower efficacy, defined as efficacy <50; or (2) decline in efficacy, defined as a 25% relative reduction in efficacy during year 2 vs year 1 postvaccination. This definition of suboptimal vaccine performance was based on the finding of efficacy <50% and waning after both vaccines in most low-income settings but not in any of the high-income countries (Supplementary Table 1) [1, 19, 22, 24–28]. The cutoff values of antibody titers of IgA were assessed in 12 classes grouped by increments of 30 U/mL: 0–30, 31–60, 61–90, 91–120, 121–150, 151–180, 181–210, 211–240, 241–270, 271–300, 301–330, 331–360 (Supplementary Table 2). We assessed the sensitivity and specificity of each IgA cutoff value in predicting the presence or absence of either low efficacy or waning [29]. The IgA GMC/GMT value with the highest sensitivity and specificity for suboptimal vaccine performance was established as the cutoff point. Using a random effects model, we subsequently calculated pooled estimates of efficacy and 95% confidence limits for each of the 2 groups with high and low IgA titers [30].

Analyses were conducted using SAS v9.3 and StatsDirect v2.5.7.

RESULTS

In total, we identified 39 data points to assess immunogenicity of the vaccines stratified by u5MR (Tables 1 and 2) and 15 data points to correlate immunogenicity with vaccine efficacy (Table 3) [17, 19, 22–24, 26, 28, 31–49]. The immunogenicity data represented studies in 33 different countries—12 that evaluated RV5 and 26 that evaluated RV1—representing all 3 WHO child mortality strata.

Immunogenicity After RV1 and RV5

For RV1, we observed a significant inverse relation between u5MR and both IgA titers ($r^2 = 0.72$; $P < .001$) and seroconversion rates ($r^2 = 0.66$; $P < .001$; Table 1; Figure 1). Average IgA GMC in countries with low u5MR (236; 95% CI, 174–329) were 2-fold higher than those in countries with medium u5MR (101; 95%CI, 66–157) and 4-fold higher than those in countries

Table 2. Immune Response (Geometric Mean Titers) of Anti-Rotavirus Serum IgA and Seroconversion)[a] Following Vaccination With RotaTeq (RV5) in Countries Where Studies Were Conducted, Grouped by Levels of Under-5 Child Mortality

Study Location by u5MR	WHO Stratum	2009 u5MR[b]	Sample Size	IgA GMT 1–2 months after Last Dose (95% CL)	% Seroconversion (95% CL)	Ref
Low u5MR (mean)				**322 (225, 467)**	**95 (87, 98)**	
Taiwan	A	5	49	306 (185, 504)	94 (83, 99)	43
US and Finland[c]	A	6	197	338 (266, 429)	95 (91, 98)	19, 42
Medium u5MR (mean)				**157 (117, 212)**	**95 (90, 100)**	
Mexico, Costa Rica, Guatemala, Brazil[d]	B	21	372	155 (126, 190)	93(. . ., . . .)	45
Viet Nam	B	23	67	159 (107, 235)	97 (90, 100)	28, 46
High u5MR (mean)				**39 (25, 60)**	**79 (66, 88)**	
Bangladesh	D	51	64	29 (19, 46)	78 (66, 88)	28, 46
India	D	63	102	80 (55, 116)	82 (. . ., . . .)	17
Ghana	D	77	71	24 (16, 37)	79 (68, 88)	22, 48
Kenya	E	87	64	31 (18, 51)	74 (61, 84)	22, 48
Mali	D	182	57	31 (19, 52)	83 (70, 91)	22, 48

Abbreviations: CL, confidence limit;GMT, geometric mean titer; IgA, immunoglublin A; u5MR,under-5 mortality rate; WHO, World Health Organization.

[a] Serum rotavirus IgA antibody concentrations 1–2 months after dose 3 for RotaTeq; seroconversion defined as >3-fold rise in GMT titers after RotaTeq.

[b] u5MR per 1000 live births (21).

[c] Average of under-5 mortality for US and Finland was used because data for immunogenicity were not stratified by country.

[d] Country specific data were not presented; mean under-5 mortality for the 4 countries is presented; vaccine given concomitantly with oral polio vaccine; confidence limits for seroconversion not reported by authors.

Table 3. Vaccine Efficacy During First Two Years of Life After Vaccination With RV5 and RV1 Among Countries With Low (<90) and High (>90) Anti-Rotavirus Serum IgA Titers

Location	u5MR	Vac.	IgA Titer (95% CL)	Vaccine Efficacy over 2 y (95% CL)	Ref
IgA titer > 90					
US and Europe	Low	RV5	338 (266–429)	98 (88–100)	19, 42
Singapore, Taiwan, Hong Kong	Low	RV1	239 (183–310)	97 (88–100)	37
Japan	Low	RV1	217 (110–122)	92 (62–99)	32
Europe	Low	RV1	197 (175–222)	90 (85–94)	17, 41, 49
Vietnam	Med.	RV5	159 (107–235)	64 (8–91)	28, 46
Latin America	Med.	RV1	103 (86–122)	80 (71–87)	26, 38
South Africa (3-dose)	High	RV1	94 (56–157)	85 (35–98)	24
POOLED[a]			**192 (140–228)**	**85 (80–90)**	
IgA titer <90					
Malawi (3-dose)	High	RV1	63 (36–109)	42 (9–64)	23
South Africa (2-dose)	High	RV1	59 (38–94)	32 (–71 to 75)	24
Malawi (2-dose)	High	RV1	52 (26–102)	34 (– to 58)	23
Kenya	High	RV5	31 (18–51)	64 (– to 89)	22, 44, 48
Bangladesh	High	RV5	29 (19–46)	43 (10–64)	22, 44, 48
Ghana	High	RV5	24 (16–37)	56 (28–73)	22, 44, 48
POOLED[a]			**41 (25–70)**	**44 (30–55)**	

Immunoglobulin A (IgA) titer denotes geometric mean titers (or concentrations in U/mL) of anti-rotavirus IgA antibodies 1–2 months after the full series of the vaccine. Abbreviations: CL, confidence limit; RV1, Rotarix; RV5, RotaTeq; u5MR, under-5 mortality rate.

[a] Pooled estimates for IgA titers were averaged; vaccine efficacy estimates and 95% CL were pooled using random effects model.

with high u5MR (47; 95% CI, 31–74). A similar gradient was also observed for seroconversion, with higher rates among infants in low u5MR countries (87%; 95% CI, 78–92) compared to those in medium u5MR (74%: 95% CI, 61–84) and high u5MR countries (53%; 95% CI, 41–64).

Similar to RV1, we observed a significant inverse relationship between u5MR and IgA titers ($r^2 = 0.84$; $P < .001$) after RV5 administration (Table 2; Figure 1). The average GMTs in countries with low u5MR (322; 95% CI, 225–467) were 2-fold higher than those in countries with medium u5MR (157; 95% CI, 117–212) and 8-fold higher than those in countries with high u5MR (39; 95% CI, 25–60). Child mortality also correlated with seroconversion rates ($r^2 = 0.63$; $P = .01$) after RV5. However, although IgA titers were similar for both vaccines, seroconversion rates were greater for RV5 than those after RV1, a difference possibly explained by the distinct endpoints chosen by the companies (ie, IgA titer rise of ≥3-fold for RV5 vs a final titer ≥20 for RV1) or possibly due to the difference in the dosing regimen (3 doses for RV5 vs 2 doses for RV1).

Overall Efficacy and Immunogenicity

Among studies evaluating both vaccine efficacy and immunogenicity, we observed a significant trend ($r^2 = 0.56$; $P = .005$) where efficacy was lower in countries with lower postvaccination IgA GMC/GMT (Figure 2). All studies, with titers <94 had vaccine efficacy <50% or >25% decline in efficacy during year 2 after vaccination. We identified that IgA GMC/GMT ≤90 predicted suboptimal vaccine performance with sensitivity and specificity of 100% (Supplementary Table 2). Among countries with GMC/GMT ≤90, 2-year efficacy was significantly lower ($P < .001$; 44%; 95% CI, 30–55) compared to countries with GMC/GMT >90 (85%; 95% CI, 82–90; Table 3). Although the assays were performed in 2 different laboratories for the 2 vaccines, the association between titers and efficacy was similar for both RV1 and RV5.

Decline in Efficacy and Immunogenicity

In all studies, efficacy declined in year 2 compared to year 1 but confidence limits were wide, particularly in countries with high u5MR (Table 4; Figure 3). This average relative decline in efficacy between year 2 vs 1 was substantially greater (66%; range: 15%–100%) among countries with postvaccination GMC/GMT ≤90 compared to those with titers >90 (7%; range: 4%–10%). Likewise, the association between efficacy and IgA titers was stronger in year 2 ($r^2 = 0.67$; $P < .001$; 0.29% increase in efficacy for every 1 unit increase in GMC/GMT) compared to year 1 ($r^2 = 0.49$; $P = .016$; 0.18% increase in efficacy for every 1 unit increase in GMC/GMT; P value comparing year 2 to year 1 = .011).

DISCUSSION

The recent publication of clinical trials of RV1 and RV5 from high u5MR settings fills in the matrix of vaccine immunogenicity

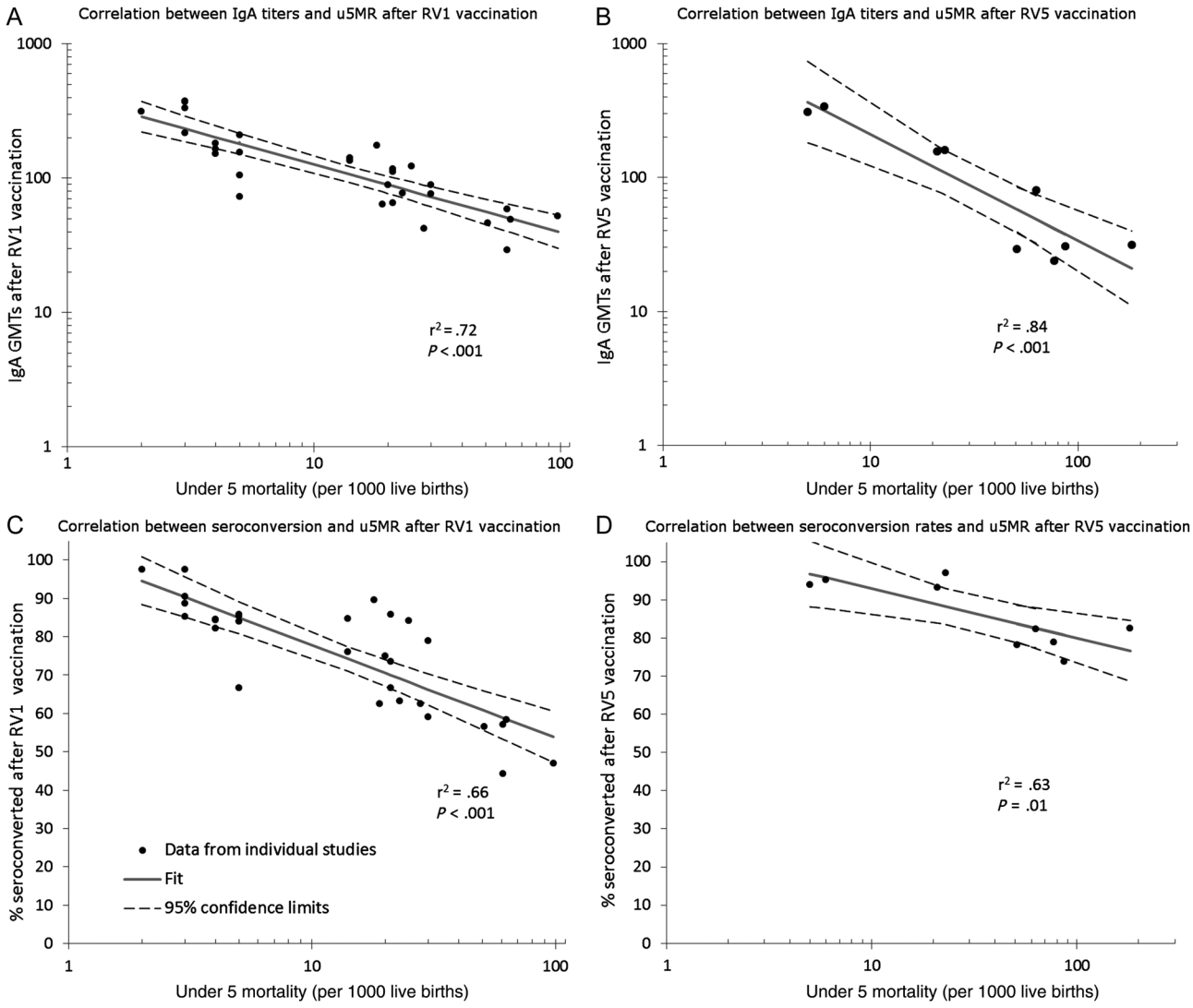

Figure 1. Relationship between under-5 child mortality and immune response to rotavirus vaccination.

and efficacy results from trials in regions of the world with different levels of child mortality [1]. Our analysis has identified several critical findings that could facilitate the interpretation of results from clinical testing of future rotavirus vaccines and the discovery of approaches to improve the efficacy of rotavirus vaccines in countries with the highest child mortality. First, we observed a significant inverse relationship between IgA titers and the level of child mortality. Second, these aggregated antibody titers were highly predictive of vaccine efficacy, with significant increase in efficacy when IgA titer was >90. Third, lower IgA titers were associated with lesser duration of protection after rotavirus vaccination. Fourth, seroconversion rates generally correlated with antibody titers for RV1 but less so for RV5, likely related to the different and arbitrary cutoffs used by the manufacturers of the 2 vaccines. The consistent relationship between titers of IgA and efficacy for both vaccines supports the contention that IgA titers may be a useful predictor of vaccine performance.

The lack of discrete data on immune response and protection in individual vaccinees prohibited us from assessing whether IgA titers or seroconversion predicted protection. Individual data on immune response and efficacy would facilitate the identification of a more accurate cutoff point for suboptimal vaccine performance. The strong correlation, however, between serum IgA antibody titers and efficacy after rotavirus vaccination indicates that serum IgA levels are an important and measureable indicator of vaccine "take," even if serum IgA itself is not the sole immunological effector of this protection. Much work remains to delineate the full profile of immune response and identify the true effectors of protection after rotavirus

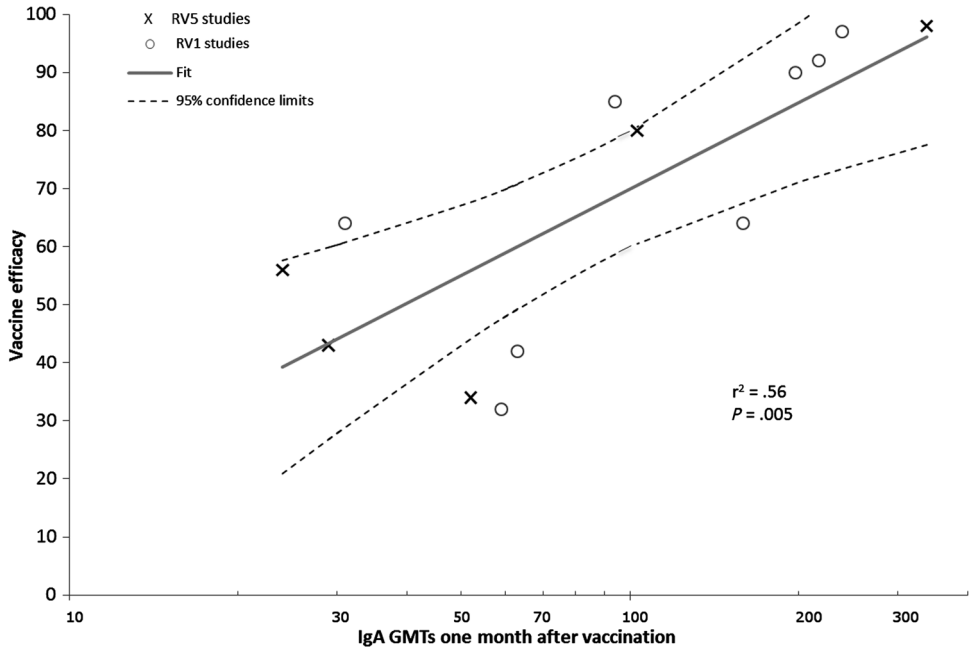

Figure 2. Relationship between anti-rotavirus serum IgA geometric mean titers/concentrations and rotavirus vaccine efficacy. Abbreviation: IgA, immunoglobulin A.

illness and vaccination. That said, demonstrating a correlation between serum antibodies and protection at a population level is valuable for testing new vaccines and identifying solutions to improve vaccine efficacy.

Table 4. Duration of Vaccine Protection Following Vaccination With RV5 and RV1 Among Countries With Low (<90) and High (>90) Anti-Rotavirus Serum IgA Titers

Location	Vac.	IgA titer[a]	Vaccine Efficacy (95% CL) Year 1	Vaccine Efficacy (95% CL) Year 2	Relative decline in efficacy year 2 vs1[b]	Ref
IgA titer >90						
US and Europe	RV5	338	99 (88–100)	95 (88–100)	4%	19, 42
Singapore, Taiwan, Hong Kong	RV1	239	100 (72–100)	94 (78–99)	6%	37
Europe	RV1	197	96 (90–99)	86 (76–92)	10%	17, 41, 49
Vietnam	RV5	159	72 (–45 to 97)	65 (–48 to 94)	10%	28, 46
Latin America	RV1	103	83 (67–92)	79 (66–87)	5%	26, 38
South Africa (3-dose)	RV1	94	82 ((55–94)	76 (–143 to 100)	7%	24
POOLED[c]			**88 (75–94)**	**83 (76–88)**	**7%**	
IgA titer <90						
Malawi (3-dose)	RV1	63	50 (11–72)	33 (–9 to 71)	34%	23
South Africa (2-dose)	RV1	59	72 (40–88)	3 (–43 to 82)	96%	24
Malawi (2-dose)	RV1	52	49 (11–72)	3 (–101 to 53)	94%	23
Kenya	RV5	31	83 (26–98)	0 (–1752 to 82)	100%	22, 44, 48
Bangladesh	RV5	29	46 (–1 to 72)	39 (–18 to 70)	15%	36, 37, 40
Ghana	RV5	24	65 (36–82)	29 (–65 to 71)	55%	22, 44, 48
POOLED[c]			**57 (43–67)**	**17 (–10 to 37)**	**66%**	

Abbreviations: CL, confidence limit; IgA, immunoglobulin A; RV1, Rotarix; RV5, RotaTeq.

[a] IgA titer denotes geometric mean titers or concentrations of anti-rotavirus IgA antibodies 1–2 months after the full series of the vaccine.

[b] Calculated as 1-(year 2 efficacy/year 1 efficacy) × 100%.

[c] Vaccine efficacy estimates and 95% CL were pooled using random effects model, and relative decline in efficacy in year 2 vs 1 was averaged.

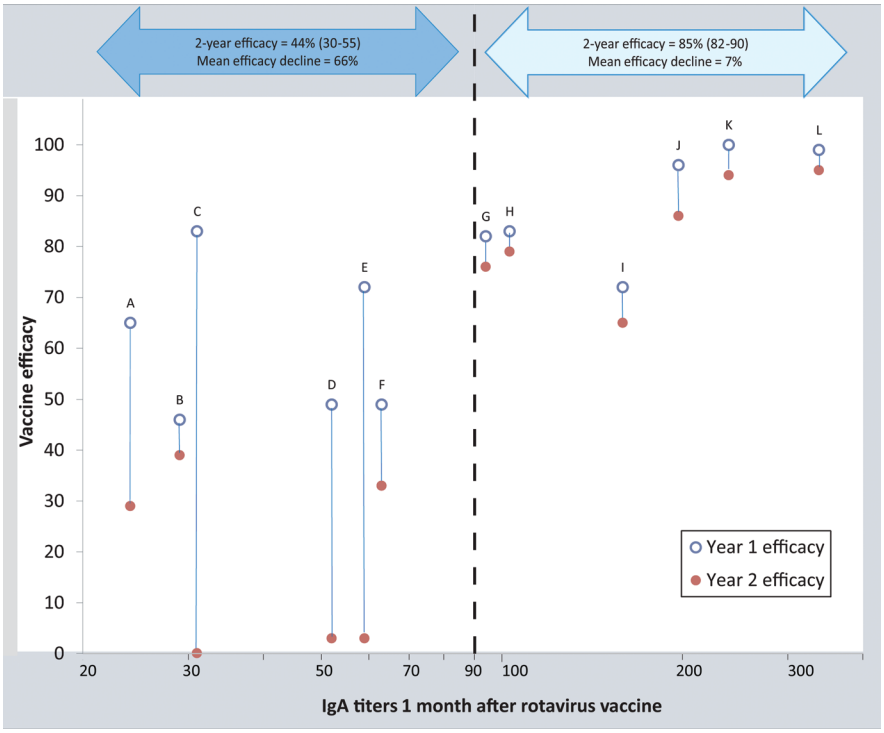

Figure 3. Decline in vaccine efficacy between year 1 (*open circles*) and year 2 (*solid circles*) after rotavirus vaccination, by location and titers rotavirus IgA geometric mean titers/concentrations. *A*, Data from Japan are not included because efficacy stratified by year 1 and year 2 after vaccination was not reported. *B*, Dashed black line depicts IgA titer of 90, below which efficacy was significantly lower and waned between years 1 and 2 after vaccine. Abbreviation: IgA, immunoglobulin A.

A variety of factors could decrease the efficacy of live oral rotavirus vaccines for infants in low-income settings, including the presence of high titers of transplacental IgG antibody against rotavirus from the mother, neutralizing activity against rotavirus in breast milk, and gut factors such as coinfections, competing microflora, or low levels of zinc and other micronutrients [50]. Lower performance in low-income settings has also been a hurdle for oral vaccines for other diseases such as cholera [51], typhoid [52], and polio [53]. Identifying a critical titer of IgA antibody that is associated with adequate vaccine efficacy has important implications for future trials of candidate rotavirus vaccines and testing interventions such as zinc, probiotics, withholding of breastfeeding shortly before and after vaccination, and optimizing the age schedule for rotavirus immunization. We identified that pooled 2-year efficacy was significantly higher in trials where IgA GMC or GMT exceeded 90 (85%) compared to those with rotavirus IgA GMC or GMT below 90 (44%) [28, 38]. Importantly, a substantial decline in efficacy was observed in the lower titer settings compared to higher titer settings, raising the hypothesis that IgA titers exceeding 90 in a vaccinated population could serve as a potential "threshold" for attaining a reasonable level of sustained vaccine efficacy.

Data from South Africa [24] and Malawi [23] were particularly illuminating because the standard 2-dose regimen of RV1 given at 10 and 14 weeks of life was directly compared against a 3-dose regimen given at 6, 10, and 14 weeks of life and could serve as proof of concept for such a threshold value of IgA titers (ie, 90) needed for sustained vaccine efficacy. In South Africa, 3 doses of RV1 stimulated IgA antibodies exceeding this potential threshold value (94; 95% CI, 54–67) with overall efficacy during the first 2 years of life to 85% (95% CI, 35–98). In contrast, 2 doses in the same setting led to titers below the threshold (59; 95% CI, 38–94) and a 2-year efficacy of 32% (95% CI,−71–75). The higher titers after the 3-dose regimen was also associated with a substantially lower 7% relative decline in efficacy during the second year, compared to 96% decline after the 2-dose regimen. In the Malawian population, which has a higher u5MR compared to South Africa, the 3-dose regimen improved immune response and efficacy somewhat compared to 2 doses, but GMCs failed to exceed 63 and was associated with lower efficacy than in South Africa.

Interpretation of these data should be in the context of several caveats. While a trend in antibody levels and efficacy clearly exists, serum IgA antibody was not a perfect correlate of protection. As discussed, other effectors likely contribute to

host defense, and these certainly could differ between animal strain derived RV5 and the human strain derived RV1 [54–56]. We have evaluated group data for diverse populations, but data on individual infants enrolled in these field studies could provide greater insight into how well these population-based hypotheses correlate with individual protection from disease. Confidence limits of efficacy in individual clinical trials were wide, particularly when assessing duration of protection. However, the general trend that vaccine efficacy declines markedly in those countries with lower IgA titers but not in those with higher titers provides support that this effect is real. Because these studies provided pooled titers from vaccinated groups, they included children who failed to mount any antibody response along with those having a robust antibody response. Finally, immune responses to both RV1 and RV5 correlated with u5MR and efficacy, prompting us to pool data for the 2 vaccines. An individual cutoff for each vaccine could not be estimated due to the limited number of data points. Specifically, the cutoff point might be less accurate for RV5, where IgA titers were either ≤31 or ≥159, partly because paired immunogenicity and efficacy data from medium u5MR settings were limited for this vaccine. In addition, while the relationship between titers of IgA and efficacy for both vaccines is consistent, the assays used by the vaccine manufacturers for measuring immune response might differ for RV1 and RV5 and have not undergone external standardization. Thus, while the consistent relationship between IgA GMT and RV5 efficacy and IgA GMC and RV1 efficacy suggests the assays should be reasonably comparable, standardization of the assays would be useful for facilitating the identification of an accurate cutoff value for a titer that predicts vaccine efficacy.

In summary, a review of the extensive clinical trial data for RV1 and RV5 clearly demonstrates the usefulness of serum IgA antibodies to examine the efficacy of rotavirus vaccines and supports the hypothesis that titers of IgA antibodies are at least an important predictor of the host defense mechanism that protects children from rotavirus diarrhea. IgA titers <90 were associated with lower efficacy and waning during the second year after vaccination. As future consideration is given to assessing the effect of additional vaccine doses, such as a third RV1 dose, optimizing age schedules, and adding a potential booster dose later in infancy, IgA antibody titers could serve as a potential marker to assess vaccine performance. Attention should be given to validate this threshold value through a closer analysis of unpublished data from these trials, examination of data for individual vaccinees, and assessment of the emerging results from efficacy trials for candidate rotavirus vaccines. Confirmation of an antibody level that correlates with protective efficacy at a population level would enhance feasibility of vaccine assessments to find the most suitable vaccine for a given region, facilitate identification of solutions to improve efficacy in high mortality settings, and reduce expenses and potential ethical challenges with conducting efficacy trials. Indeed, they might in the future eliminate the need for large-scale clinical trials of efficacy for new vaccines when we gain confidence in the predictive value of the serum immune response.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Contributors. M. P., R. G., B. J., B. L., M. S., and U. P. created and designed the study. M. P., R. G., and U. P. collected the data. M. P. and B. L. did the data analysis. M. P., R. G., B. J., B. L., M.S., and U.P. interpreted the data. M.P. and R.G. drafted the report. M.P., R.G., B.J., B.L., M. S., and U. P. critically revised the report.

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