RESEARCH PAPER

Rotavirus-specific Salivary and Fecal IgA in Indian Children and Adults

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Objective: To compare serum, salivary and fecal IgA responses in infants and adults following rotavirus vaccination.

Study design: Laboratory testing of samples from clinical trials.

Setting: Medical College Hospital.

Participants: 13 healthy adult volunteers not given vaccine, 20 healthy adult volunteers given one dose of bovine rotavirus tetravalent vaccine (Shantha Biotechnics), and 88 infants given 3 or 5 doses of Rotarix.

Outcome measures: Serum, salivary and fecal IgA at one or more time points.

Methods: IgA antibodies were estimated in serum, saliva and fecal samples by enzyme-linked immunosorbent assay, and normalized to total IgA in saliva.

Results: In naturally infected adult volunteers, comparing serum and salivary IgA showed significant positive correlation (r=0.759; *P*=0.003). Of 20 vaccinated adults, complete samples showing change were available for 10; among them there was a significant positive correlation (*P*<0.05) between pre-vaccination serum and pre-vaccination salivary IgA but not between post-vaccination serum and post-vaccination salivary IgA. Of 88 infants given 3 or 5 doses of vaccine, 13 had more than 4-fold IgA response in serum, saliva and fecal samples, 6 had a 2-4 fold increases in all specimens. There was weak correlation between seroconversion rates measured by serum and salivary antibody responses. Salivary and stool assays were able to detect seroconversion in a few children in whom there was no detectable response in serum.

Conclusions: Evaluation of multiple samples is useful for intensive experimental study designs and may help improve our understanding of the induction and dynamics of immune responses to rotavirus vaccination.

Keywords: *Antibody, Immunity, Serum, Vaccine*.

All live attenuated rotavirus vaccines have been developed based on evidence that natural rotavirus infections elicit protective immune responses, particularly against severe rotavirus disease [1]. There are two currently available oral rotavirus vaccines licensed in over 100 countries [2-4], as well as nationally licensed vaccines in India, Vietnam and China. For a period after rotavirus vaccination, much of the serum IgA is expected to be rotavirus-specific as in natural rotavirus infections [5]. Although there is no defined correlate of protection, serum IgA is the most widely available measure of seroconversion so far, and has been used to measure immunogenicity of all candidate rotavirus vaccines [6,7]. As rotavirus is a mucosal pathogen infecting the epithelial cells of small intestinal villi, mucosal gut antibodies could be expected to be reliable indicators of immune response following natural rotavirus infection or rotavirus vaccination [8].

Measuring local immunity in the small intestine is considered the most sensitive marker of rotavirus infection; although obtaining duodenal fluid from children by intubation is inappropriate, and therefore other surrogate markers that accurately reflect intestinal immune responses are necessary. Detecting rotavirus-specific antibodies in feces, saliva and other suitable body fluids has been proposed as a non-invasive alternative [5,8-11]. Secretory IgA responses in secretions from sublingual glands (non-parotid glands) may better reflect B cell induction in gut-associated lymphoid tissue (GALT) than the parotid response, which may be more strongly linked to immune induction in nasal-associated lymphoid tissue (NALT) [12]. Saliva collection is easy, rapid, requires less training and eliminates the need for blood draws. Further, previous studies suggest that in settings with low vaccine immunogenicity, measuring salivary antibody responses to vaccination might add to the apparent vaccine 'take' rate determined by detection of serum antibodies alone [13,14].

METHODS

Rotavirus-specific serum and salivary IgA antibody levels were measured in healthy adult volunteers and infants and adults vaccinated with either Rotarix or Bovine-Human

Reassortant Rotavirus Vaccine (BRV-TV). Fecal IgA was measured in infants given Rotarix in previously published studies [15,16] conducted at our institute after approval by the Institutional Review Board and with written informed consent from participants or from their parents.

Healthy adults, presumed to be previously exposed who did not receive rotavirus vaccine: A pilot study with collection of serum and saliva was carried out in 13 healthy adult (6 female) volunteers in order to assess the use of a Phosphate buffered saline (PBS)-based antibody transport medium to extract and process saliva which could be stored at -20°C and later be used to measure rotavirus - specific IgA antibody levels. One saliva sample was collected from each healthy adult volunteer using Oracol swabs (Malvern Medical Developments Ltd, Worcester, UK). The swab was placed between the lower cheek and gums of the volunteers and gently rubbed back and forth for about 1 minute until the absorbent sponge was moist. Salivary and serum IgA antibody levels were compared.

Healthy adult volunteers who received one dose of rotavirus vaccine: Rotavirus-specific serum and salivary IgA antibody responses before and after vaccination with a BRV-TV were measured in 20 healthy Indian adults who were given a single dose of BRV-TV as previously reported [16]. From the 20 adult volunteers in the Phase 1 BRV-TV study, parotid and sublingual salivary secretions and whole saliva were collected prior to vaccination and the second sample set 28-30 days post-vaccination. Prior to collection, all volunteers filled a questionnaire, which documented time of last beverage intake and absence of dental/gum bleeding. They then rinsed their mouths thoroughly with water. Two sorbettes (Salimetrics Item No. 5029.00) were placed together under the tongue or between gums and cheek for 60 seconds in each site, to obtain the secretions from sub lingual and parotid glands, respectively; the sorbettes were stored as described by the manufacturer. Volunteers were also instructed to pool saliva in their mouths for about 90 seconds before collecting the whole saliva into a sterile storage tube. The storage tubes were centrifuged for 15 minutes at 3000-3500 rpm to extract the saliva. The processed saliva was recovered; protease inhibitors were added and mixed thoroughly. All processed oral fluid specimens were stored at -20°C until testing.

Healthy infants who received 3 or 5 doses of rotavirus vaccine: Comparison of rotavirus specific serum, salivary and fecal IgA antibody responses before and after vaccination with 3 or 5 doses of Rotarix was studied in 90 infants recruited at 6 weeks of age and enrolled in a Phase 4 randomized, parallel group comparison study as described previously [15]. Two saliva samples were collected from all vaccinated infants, one prior to administration of the first dose of Rotarix and the second was collected 28 days after the last (3rd or 5th) dose of vaccine administration. Saliva was collected using Salimetric Infant swabs (Item no: 5001.08) and storage tubes (Item no: 5001.05). Specimens were transported to the laboratory in ice packs. A total of 88 infants (44 in each arm) had a complete set of serum and saliva samples available. Salivary IgA levels were also compared with fecal IgA levels in a subset of vaccinated infants, from whom stool samples were collected to study vaccine shedding.

Sample collection and Processing

Blood: Approximately 5 mL and 3.5 mL of venous blood were obtained by trained phlebotomists prior to and 28-30 days after oral rotavirus vaccine immunization from adult volunteers and infants, respectively. Serum was separated and stored at -20 ºC till testing.

Saliva: To each tube containing either an Oracol or a Salimetrics swab, 1 mL of antibody transport medium (containing 0.2% Tween 20, 10% fetal bovine serum (FBS) and 0.7% Antibiotic/antimycotic, in phosphate buffered saline, pH 7.2) was added, vortexed for 20 seconds and centrifuged at 3000 rpm for about 10 minutes. After centrifugation, the processed saliva was recovered and the oral fluid was stored at -20°C until testing.

Stool: Following the first immunization, mothers/ guardians of immunized infants were requested to collect approximately 5 g of stool, at 0, 3 and 7 days after each dose of immunization, except the first where there was no 0 day sample. These stool samples were stored at -70°C until tested. Fecal IgA was measured in a subset of stool specimens obtained from infants who were either baseline sero-negative and responded to vaccination (n=12), or infants who were baseline sero-positive but had a >4 fold increase in serum IgA from baseline (n=11).

Measuring Rotavirus-specific IgA

All processed serum, salivary and fecal samples were tested in an antibody-sandwich, enzyme linked immunosorbent assay (ELISA) using the bovine G6P [5] WC3 strain or the human G1P[8] rotavirus antigen. The procedure for measurement of rotavirus specific IgA expressed as was used [16,17], and specimen processing modified for saliva and fecal specimens. Undiluted neat processed saliva in antibody transport medium (supernatants and stool samples diluted in 1% bovine serum albumin (supernatants from 10% homogenized suspensions centrifuged at 3000 rpm for 15 min) were assayed to measure rotavirus-specific salivary and fecal IgA respectively. Total salivary IgA was also measured in

all saliva samples so that the amount of rotavirus-specific IgA could be normalized to 1 mg of total IgA present in the processed specimen. The results for the rotavirus-specific salivary IgA measurement were expressed as rotavirus IgA U/mL saliva in antibody transport medium. The final concentrations of stool rotavirus IgA was calculated by normalizing the total IgA concentration present in one milligram of stool.

Among vaccinated infants, seroconversion or a positive response was considered as the development of detectable (*i.e.* ≥20 U/mL) rotavirus-specific serum anti-rotavirus IgA antibodies in the serum 28 days after immunization, from a baseline of negative (<20 U/mL) or no detectable antibody levels. Sero-response, as a measure of response to the vaccine not meeting criteria for seroconversion, was indicated as a two- or greater-fold increase in antibody titres in serum or body fluids as compared to baseline.

Measuring Total IgA

To measure total salivary IgA concentrations, wells of microtiter plates were coated overnight with either rabbit anti-human IgA (Sigma, St Louis, MO) or normal rabbit serum (DakoCytomation, Glostrup, Denmark) as negative control. The next day known concentrations of serially diluted purified human IgA (Sigma, St Louis, MO.) standards and doubling dilutions of salivary secretions diluted in 1% skimmed milk or supernatants from the 10% stool suspensions was added and incubated for an hour. This was followed by washing and the addition of biotinylated rabbit anti-human IgA. After incubating for an hour, plates were washed and peroxidase-conjugated avidin-biotin was added and incubated. The substrate (orthophenylenediamine/ H_2O_2) was added after washing plates and incubated in dark. After 30 min, the reaction was stopped with 1M H_2SO_4 and absorbance OD at 492 nm was measured. Total salivary IgA concentrations were determined from the plotted values for standard IgA concentrations. The final concentrations of salivary or fecal rotavirus IgA were calculated and expressed by normalizing to the total IgA concentration present in one milliliter of saliva in antibody transport medium or relative rotavirus IgA unit per milligram of total IgA [17], respectively.

Statistical analysis: Statistical analyses were done using GraphPad Prism, Version 4.0. Fold changes were calculated as a ratio between the post- and the pre-immunization sample. The rotavirus-specific serum, salivary and fecal IgA units derived from the optical density measurements were compared. Pearson's correlation coefficients were computed after log transformation and assessed for significance. Where IgA was undetectable, a constant number (1) was added to all the IgA values to make them non-zero values for calculation of geometric mean concentrations (GMC) and prior to log transformations, if required. $P<0.05$ was considered statistically significant.

RESULTS

Eleven of the 13 adult volunteers were positive for serum rotavirus IgA while 10 had detectable levels of rotavirus specific salivary IgA. The GMC of rotavirus specific serum IgA was 98.9 whereas that of salivary IgA was 1.4. There was a significant positive correlation (r=0.759; $P=0.003$) between the serum and salivary IgA units.

From 20 volunteers who received BRV-TV vaccine/ placebo, 89 salivary secretions were tested for rotavirus-specific IgA and compared with the corresponding serum rotavirus IgA levels (***Table* I**), with all samples available for only 10 volunteers. Of the 10 sets of pre-post parotid secretions that were tested, >2-fold response was seen in 5/10 sublingual and 4/10 whole saliva samples, but only 2/ 10 parotid responses. There was a significant positive correlation ($P<0.05$) between pre-vaccination serum and pre-vaccination saliva, but not between post-vaccination serum and post-vaccination salivary IgA. Pre- and post-vaccination rotavirus specific IgA was significantly correlated (r= 0.9, $P<0.01$) between parotid and non-parotid secretions. For whole saliva, the post-vaccination samples alone had a positive correlation of IgA levels in parotid and non-parotid secretions (r=0.8, $P<0.05$).

Of the 176 saliva specimens collected from 88 infants, total salivary IgA was detected in all samples and rotavirus-specific salivary IgA was detected in 124 (70.4%) saliva specimens, as compared to 151 (85.7%) rotavirus-specific IgA in paired serum samples. In the 3 and 5 dose arm together, 25 infants pre-vaccination and 27 infants post-vaccination had no detectable levels of

TABLE I ROTAVIRUS-SPECIFIC SALIVARY IGA IN PAROTID, SUB-LINGUAL AND WHOLE SALIVA SECRETIONS IN ADULT VOLUNTEERS (*N*=20) GIVEN A SINGLE DOSE OF BOVINE ROTAVIRUS TETRAVALENT VACCINE

Pre-Post vaccination salivary IgA response	*Number of volunteers showing rotavirus -specific salivary IgA response*		
	Parotid secretions	*Sub-lingual secretion*	*Whole saliva*
<2 - fold	8	5	15
2- 4 fold	1	4	3
>4 - fold	1	1	1
NA	10	10	1

NA - Sample not available for testing.

rotavirus-specific salivary IgA.

The rotavirus-specific salivary IgA and the salivary IgA normalized to 1 mg of total IgA both correlated with serum IgA pre- and post-vaccination in both arms of the infant vaccination study (***Fig.* 1**). Despite a significant positive correlation, when seroconversion or seroresponse were examined at the individual level, there was low correlation between serum and salivary antibody responses. Among the 43% infants with a >4-fold increase in either salivary or serum IgA, 29 (33%) had a serum response and 17 (19%) had a salivary response. When rotavirus salivary IgA was not normalized with total IgA, 4 (5%) more infants had a ≥4-fold increase in rotavirus-specific salivary IgA.

***Web Table* I** provides the GMC of serum, salivary and fecal IgA stratified by serum response. Of the 88 infants, 13 had >4-fold IgA response in serum, saliva and fecal samples, 6 had a 2-4 fold increases in all specimens. Of the 35 infants who had no serum immune response after vaccination, 15 infants showed increases in rotavirus-specific salivary IgA levels and 13 infants also showed measurable levels of fecal IgA. In this group of infants, the levels of rotavirus-specific fecal IgA measured at 3 days post 1st dose of vaccination showed a strong positive correlation with rotavirus specific salivary IgA measured in secretions collected prior to vaccination (r=0.96, P<0.0001). However, the fecal IgA measured at 3 days post 3 or 5 dose of vaccination did not correlate with post vaccination salivary IgA antibody levels.

DISCUSSION

In early studies, we attempted to measure rotavirus-specific IgA in parotid, sublingual secretions and whole saliva and did not get satisfactory results. We subsequently used improved collection devices along with a PBS-based oral-fluid transport medium, which contains antibacterial and anti-proteolytic substances. This oral fluid medium had previously been successfully used to study salivary immune responses of rubella and pertussis vaccination and infections [18,19]. We documented that rotavirus salivary IgA units among adult volunteers were low but showed a significant positive correlation with titres of rotavirus-specific serum IgA units. The limited results from the adult vaccination study did not result in a clear distinction of responses in parotid and non-parotid secretion. We also documented that RV-salivary IgA/mg total IgA could be detected in more than half of samples from infants who were vaccinated with Rotavirus vaccine, but had negative results for serum antibodies. There was a significant correlation between rotavirus-specific salivary and serum IgA units, but seroresponse rates as fold changes between the pre- and post-vaccination serum and salivary IgA of the infants did not correlate perfectly, with salivary responses being generally lower than serum.

Grimwood, *et al*. [14], showed that anti-rotavirus IgA in saliva had a high predictive accuracy of almost 86% for specific IgA immune response in duodenal fluid of children at 4 weeks after rotavirus infection [5]. A report from Delhi [14] showed that salivary IgA antibodies were a better indicator of asymptomatic rotavirus infection in neonates than serum antibodies. The results from this study suggest that measurement of salivary rotavirus IgA titers may add to information on rotavirus infections and vaccine response. However, it is important to note that previous studies [20,21] have shown that both salivary and intestinal IgA levels rise and fall quickly at about 2 weeks post infection or vaccination unlike serum antibodies

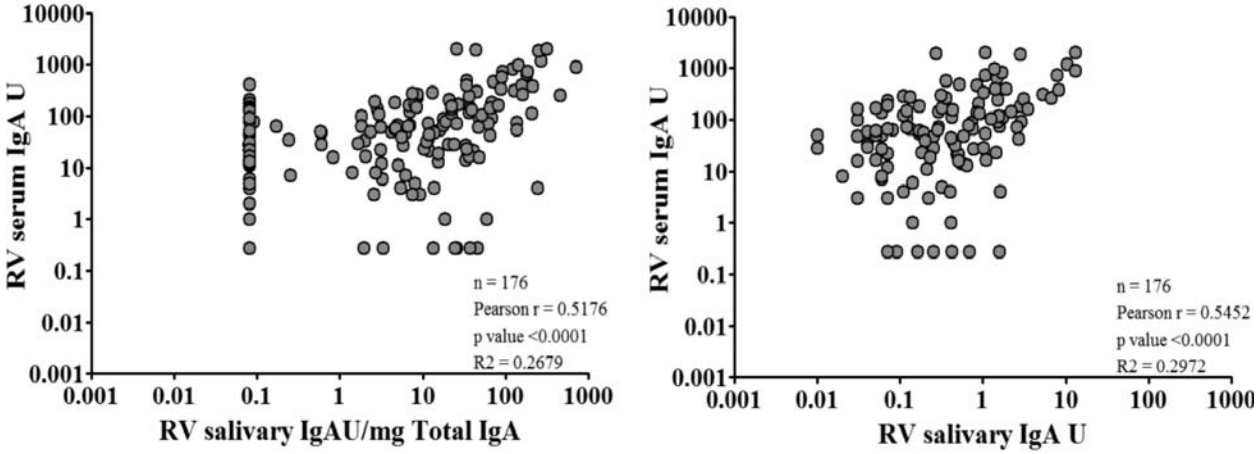

FIG. 1 *Correlation between serum anti-rotavirus IgA and salivary anti-rotavirus IgA antibodies.*

WHAT THIS STUDY ADDS?

- This study reports on evaluation of rotavirus-specific IgA in serum and saliva in naturally exposed and vaccinated Indian adults and infants and the first study to report rotavirus specific fecal IgA in vaccinated Indian infants.

which peak at about day 27 post infection. Hence, collection of salivary specimens at 2-week intervals might provide more valuable information to answer whether salivary antibody levels actually reflect intestinal antibody levels.

In our study, there was considerable variability in the RV-fecal IgA responses of individual subjects. Bishop, *et al*. [22], observed that among young infants, levels of fecal antibodies fluctuated widely during the first few weeks of life while breast-feeding was being established, possibly due to the amount of breast milk ingested and intervals between evacuation of feces. Another study speculated that the fluctuations in anti-rotavirus fecal antibody levels reflects the fluctuating production in the small intestine, as a response to a recurrent asymptomatic infection or to a persisting infection [23].

Limitations of this study include a small sample size, lack of more frequent sample collections and the absence of data on factors such as stress and feeding that might affect salivary and fecal IgA. Nonetheless, this is the first study to report evaluation of IgA in serum and saliva in naturally exposed and vaccinated Indian adults and infants and fecal IgA in vaccinated Indian infants. Given that fecal IgA in particular has been described as a correlate of protection in naturally infected children, such studies in vaccinated children particularly in developing countries are needed. The results indicate that evaluation of multiple samples is useful for intensive experimental study designs and may help improve our understanding of the induction and dynamics of immune responses to rotavirus vaccination.

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REFERENCES

1. Soares-Weiser K, Maclehose H, Bergman H, Ben-Aharon I, Nagpal S, Goldberg E, *et al*. Vaccines for preventing rotavirus diarrhoea: vaccines in use. Cochrane Database Syst Rev. 2012;11:CD008521.
2. de Oliveira LH, Camacho LA, Coutinho ES, Ruiz-Matus C, Leite JP. Rotavirus vaccine effectiveness in Latin American and Caribbean countries: A systematic review and meta-analysis. Vaccine. 2015;33:A248-54.
3. Karafillakis E, Hassounah S, Atchison C. Effectiveness and impact of rotavirus vaccines in Europe, 2006-2014. Vaccine. 2015;33:2097-107.
4. Vashishtha VM, Choudhury P, Kalra A, Bose A, Thacker N, Yewale VN, *et al*. Indian Academy of Pediatrics (IAP) recommended immunization schedule for children aged 0 through 18 years–India, 2014 and updates on immunization. Indian Pediatr. 2014;51:785-800.
5. Grimwood K, Lund JC, Coulson BS, Hudson IL, Bishop RF, Barnes GL. Comparison of serum and mucosal antibody responses following severe acute rotavirus gastroenteritis in young children. J Clin Microbiol. 1988;26:732-8.
6. Franco MA, Angel J, Greenberg HB. Immunity and correlates of protection for rotavirus vaccines. Vaccine. 2006;24:2718-31.
7. Jiang B, Gentsch JR, Glass RI. The role of serum antibodies in the protection against rotavirus disease: an overview. Clin Infect Dis. 2002;34:1351-61.
8. Davidson GP, Hogg RJ, Kirubakaran CP. Serum and intestinal immune response to rotavirus enteritis in children. Infect Immun. 1983;40:447-52.
9. Parry JV, Perry KR, Mortimer PP. Sensitive assays for viral antibodies in saliva: An alternative to tests on serum. Lancet. 1987;2:72-5.
10. Bishop R, Lund J, Cipriani E, Unicomb L, Barnes G. Clinical serological and intestinal immune responses to rotavirus infection of humans. Med Virol. 1990;9:85-109.
11. Riepenhoff-Talty M, Bogger-Goren S, Li P, Carmody PJ, Barrett HJ, Ogra PL. Development of serum and intestinal antibody response to rotavirus after naturally acquired rotavirus infection in man. J Med Virol. 1981;8:215-22.
12. Brandtzaeg P. Do salivary antibodies reliably reflect both mucosal and systemic immunity? Ann NY Acad Sci. 2007;1098:288-311.
13. Friedman M, Segal B, Zedaka R, Sarov B, Margalith M, Bishop R, *et al*. Serum and salivary responses to oral tetravalent reassortant rotavirus vaccine in newborns. Clin Exp Immunol. 1993;92:194-9.
14. Jayashree S, Bhan M, Kumar R, Raj P, Glass R, Bhandari N. Serum and salivary antibodies as indicators of rotavirus infection in neonates. J Infect Dis. 1988:1117-20.
15. Kompithra RZ, Paul A, Manoharan D, Babji S, Sarkar R, Mathew LG, *et al*. Immunogenicity of a three dose and five

dose oral human rotavirus vaccine (RIX4414) schedule in south Indian infants. Vaccine. 2014;32:A129-A33.
16. Paul A, Babji S, Sowmyanarayanan T, Dhingra MS, Ramani S, Kattula D, *et al*. Human and bovine rotavirus strain antigens for evaluation of immunogenicity in a randomized, double-blind, placebo-controlled trial of a single dose live attenuated tetravalent, bovine-human-reassortant, oral rotavirus vaccine in Indian adults. Vaccine. 2014;32:3094-100.
17. Ward RL, Bernstein DI, Smith VE, Sander DS, Shaw A, Eiden JJ, *et al*. Rotavirus immunoglobulin a responses stimulated by each of 3 doses of a quadrivalent human/bovine reassortant rotavirus vaccine. J Infect Dis. 2004;189:2290-3.
18. Litt DJ, Samuel D, Duncan J, Harnden A, George RC, Harrison TG. Detection of anti-pertussis toxin IgG in oral fluids for use in diagnosis and surveillance of Bordetella pertussis infection in children and young adults. J Medical Microbiol. 2006;55:1223-8.
19. Nokes D, Enquselassie F, Vyse A, Nigatu W, Cutts F, Brown D. An evaluation of oral-fluid collection devices for the determination of rubella antibody status in a rural Ethiopian community. Trans Royal SocTropMedHyg. 1998;92:679-85.
21. Kerr AC. The physiological regulation of salivary secretions in man: A study of the response of human salivary glands to reflex stimulation: Pergamon Press; 1961.
20. Bernstein DI, McNeal MM, Schiff GM, Ward RL. Induction and persistence of local rotavirus antibodies in relation to serum antibodies. J Med Virol. 1989;28:90-5.
21. Ward RL, Pax KA, Sherwood JR, Young EC, Schiff GM, Bernstein DI. Salivary antibody titers in adults challenged with a human rotavirus. J Med Virol. 1992;36:222-5.
22. Bishop RF, Bugg HC, Masendyez PJ, Lund JS, Gorrell RJ, Barnes GL. Serum, fecal, and breast milk rotavirus antibodies as indices of infection in mother-infant pairs. J Infect Dis. 1996;17:S22-S9.
23. Matson DO, O'Ryan ML, Herrera I, Pickering LK, Estes MK. Fecal antibody responses to symptomatic and asymptomatic rotavirus infections. J Infect Dis. 1993;167:577-83.

WEBTABLE I GEOMETRIC MEAN CONCENTRATIONS (GMC) OF ROTAVIRUS-SPECIFIC FECAL, SALIVARY AND SERUM IGA IN INFANTS GIVEN THREE OR FIVE DOSES OF ROTAVIRUS VACCINES

	Rotavirus-specific fecal IgA U						*Rotavirus-specific serum IgA*			*Rotavirus-specific salivary IgA*		
Serum IgA response categorization	*3 dpv Dose 1 (n=42)*	*28 dpv Dose1 (n=42)*	*3 dpv Dose2 (n=25)*	*28 dpv Dose2/4 (n=25)*	*3-7 dpv Dose3/5 (n=41)*	*pre-post fold change*	*Pre vaccination (n=42)*	*28 dpv (n=42)*	*pre-post fold change*	*Pre vaccination (n=42)*	*28 dpv (n=42)*	*pre-post fold change*
No response (*n*=13)	38.7	27.8	24.7	20.9	18.3	0.5	1	2	1.5	7.5	1.6	0.2
<2 fold (*n*=4)	74.1	71.6	ND*	ND*	315.9	4.3	56.4	93.9	1.7	27.5	1.8	0.1
2-4 fold (*n*=6)	35.1	16.4	11.5	15.5	40.7	1.2	22.6	63.9	2.8	7.7	9.6	1.3
≥4 fold (*n*=19)	56.8	56.0	17.6	34.5	116.3	2.0	19.9	265.5	13.4	1.4	8.2	5.6

**ND: Fecal IgA measurement was not done in samples from this group; dpv: days post vaccination.*