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Comparison of Immunoglobulin A (IgA), IgG, and IgM Enzyme-Linked Immunosorbent Assays, Plaque Reduction Neutralization Assay, and Complement Fixation in Detecting Seroresponses to Rotavirus Vaccine Candidates

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In a phase 1 study to evaluate human-rhesus rotavirus reassortant vaccines, 116 infants 1 to 5 months of age received one of the following five preparations: the serotype 1 reassortant, the serotype 2 reassortant, rhesus rotavirus (serotype 3), a bivalent preparation (serotypes 1 and 3), or a placebo. Seroresponses to the different vaccines were measured by plaque reduction neutralization assay (PRNA); rotavirus-specific immunoglobulin A (IgA), IgG, and IgM enzyme-linked immunosorbent assays (ELISAs); and complement fixation (CF). The seroresponse rate, calculated by using a fourfold or greater antibody rise by any assay, was similar in the four vaccine groups (83 to 96%). When the data from all the vaccinees were pooled, IgA ELISA, IgG ELISA, and PRNA were comparable in detecting seroresponses (67, 62, and 70%, respectively) and more efficient than IgM ELISA (53%) and CF (44%). When the vaccinees were analyzed by age, the overall seroresponse rates were the same for infants 1 to 2 and 3 to 5 months old (90%). The IgA ELISA and PRNA were the most efficient for detecting antibody rises in both age groups. IgG ELISA was among the least efficient methods for detecting antibody rises in the younger age group but among the most efficient in the older age group (44 versus 78%). CF was among the least efficient methods in both age groups but was significantly better in the older age group than in the younger age group (54 versus 21%). Our findings show that ELISA, in particular rotavirus-specific IgA ELISA, is a sensitive indicator of vaccine takes in 1- to 5-month-old infants, the target population for vaccination. The ELISA should also be very useful in demonstrating natural rotavirus infections in field studies in which a stool specimen from a diarrheal episode is not always available. The ELISA has the advantages of being easier and quicker and requiring less serum than PRNA, but it does not give serotype-specific information about the immune response.

Rotavirus is the single most important etiologic agent of severe diarrhea in infants and young children worldwide (19, 20). The development of an effective vaccine has become a public health priority, and several different vaccine candidates have been evaluated in efficacy trials. The most experience to date has been with bovine rotavirus and rhesus rotavirus (RRV) strains. Bovine rotavirus (strain RIT 4237, serotype 6) had a protection rate of 82 to 88% against clinically significant rotavirus diarrhea in two separate trials in Finland but failed to provide efficacy in several trials in Africa (4, 12, 31–33). RRV (strain MMU 18006, serotype 3) demonstrated a protection rate of 90% against severe rotavirus diarrhea in Venezuela but failed to protect in several trials in the United States (7, 20). Analysis of the serotypes of rotavirus causing illness suggested that RRV protected against homologous (serotype 3) but not heterologous serotypes of rotavirus. Because these results suggested the need for a multivalent rotavirus vaccine, trials with human-RRV reassortants representing serotypes 1, 2, and 4, administered individually or in combination with each other and RRV, were initiated (6, 11, 20, 25, 26). These reassortants derive the gene that codes for the major neutralization protein, VP7, from their human rotavirus parent. The remaining 10 genes, including the fourth gene, which encodes the other major neutralization protein, VP4, are derived from RRV. Thus, the VP7 proteins of the four epidemiologically important human rotavirus serotypes are represented by the reassortants (serotypes 1, 2, and 4) and RRV (serotype 3). An integral part of early trials with these different rotavirus vaccine candidates is the evaluation of their immunogenicity. In this study, serologic responses in 1- to 5-month-old Venezuelan infants after rotavirus vaccination were measured by rotavirus-specific immunoglobulin A (IgA), IgG, and IgM enzyme-linked immunosorbent assays (ELISAs), plaque reduction neutralization assay (PRNA), and complement fixation (CF), and the efficiencies of these different methods for detecting seroresponses were compared.

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MATERIALS AND METHODS

Serum specimens. Paired serum specimens were available from 110 of 116 1- to 5-month-old Venezuelan infants who had participated in a phase 1 rotavirus vaccine trial in March 1987 (6). The serum specimens had been collected 1 to 3 days before and 4 weeks after administration of (i) placebo, (ii) human-RRV reassortant D × RRV (serotype 1), (iii) human-RRV reassortant DS-1 × RRV (serotype 2), (iv) RRV (serotype 3), or (v) D × RRV and RRV (6). Each reassortant derived the VP7 gene from the human rotavirus parent and the remaining 10 genes from RRV (25). The vaccine had been given orally at a dose of 10^4 PFU. For the bivalent preparation, 0.5×10^4 PFU of each vaccine strain had been administered.

Serology. Paired serum specimens were tested for antibody to rotavirus by PRNA, CF, and IgA, IgG, and IgM

TABLE 1. Rate of seroresponse to different rotavirus vaccines in 1- to 5-month-old infants

Vaccine (serotype)	No. of infants	% of infants with seroresponse[a]
Placebo	21	14
D × RRV (1)	23	83
DS1 × RRV (2)	24	96
RRV (3)	20	85
D × RRV and RRV (1,3)	22	95

[a] Defined as a fourfold or greater antibody rise by any assay (PRNA; IgA, IgG, or IgM ELISA; or CF).

ELISAs. Human rotavirus strains bearing a VP7 protein belonging to the same serotype as the vaccine strains were used in PRNA to detect rises in levels of neutralizing antibody directed to the VP7 protein of the vaccine strains. Specifically, the Wa (serotype 1), DS-1 (serotype 2), and P (serotype 1) strains were used in testing the sera from recipients of the D × RRV (serotype 1), DS-1 × RRV (serotype 2), and RRV (serotype 3) vaccines, respectively. The paired sera from the four vaccine cells were additionally tested in PRNA with RRV. This assay allowed for the detection of rises in neutralizing antibody directed to either VP4 of RRV (contained in all the vaccine strains in this trial) or VP7 of RRV (contained in the RRV vaccine only). The O agent was used in the CF test. The common group antigen located on the major inner capsid protein, VP6, of all group A rotaviruses is the viral antigen of primary importance in the CF test. Previous studies have shown that the O agent, human rotavirus, and SA11 (a simian strain) are of comparable efficiency in detecting antibody responses in children after wild-type infection (17). Both the PRNA and CF test were performed as previously described (17, 18, 35). The performance of the rotavirus-specific IgA, IgG, and IgM ELISAs was based on a previously described method, with certain modifications (23, 36). Polyvinyl chloride microdilution plates were coated with hyperimmune goat antiserum at a dilution of 1:10,000 in carbonate buffer at 4°C overnight. The plates were then coated with supernatant of RRV-infected or uninfected MA104 cell culture, incubated at 4°C overnight, and then blocked with 5% fetal bovine serum in phosphate-buffered saline-Tween (20% fetal bovine serum for the IgM ELISA). Twofold serial dilutions of paired serum specimens were placed in duplicate wells and incubated at 4°C overnight. The sera were diluted in 1% fetal bovine serum in phosphate-buffered saline (10% fetal bovine serum for the IgM ELISA), starting at dilutions of 1:50, 1:100, and 1:200 for the IgA, IgM, and IgG ELISAs, respectively. Alkaline phosphatase-conjugated goat antibody to human α, μ, or γ chains (Kirkegaard-Perry) was used to detect human IgA, IgM, or IgG, respectively. The plates were incubated with the conjugate for 1 h at 37°C and were then developed with a substrate solution containing 1 mg of *p*-nitrophenyl phosphate per ml of diethanolamine buffer for 30 min at room temperature. The antibody titer was expressed as the reciprocal of the highest dilution of serum that yielded an optical density at 405 nm greater than or equal to 0.40 (sum of duplicate wells) and twice that of the corresponding serum dilution in the uninfected cell culture control wells. For all of these assays, a significant seroresponse was defined as a fourfold or greater antibody rise between the pre- and postvaccination sera.

Statistical methods. The chi-square test, Student's *t* test, and the Mann-Whitney statistic were used in analysis.

RESULTS

The seroresponse rates measured by an antibody rise in any one of the assays (PRNA, CF, or IgA, IgG, or IgM ELISA) were similar in the four vaccine cells and ranged from 83 to 96%. These values were significantly different from the seroconversion rate of 14% in the placebo cell. (Table 1). In order to compare the efficiencies of different assays in detecting a seroresponse, the results of the four vaccine cells were pooled. The rate of detecting seroresponses among vaccinees was comparable with PRNA (70%), IgA ELISA (67%), and IgG ELISA (62%), and PRNA and IgA ELISA were significantly more efficient than IgM ELISA (53%; $P < 0.05$) and CF (40%; $P < 0.0005$) (Table 2).

When the vaccinees were analyzed by age, the overall seroresponse rates for the infants 1 to 2 and 3 to 5 months old were the same (90%) (Table 3), and PRNA and IgA ELISA were the most efficient at detecting antibody rises in both age groups. However, the seroresponse rate measured by PRNA with human rotavirus strains differed from that with RRV in that both were of comparable efficiencies in the older age group but PRNA with human rotavirus strains was significantly less efficient in the younger age group. A similar finding was noted with the IgG ELISA, which was among the most efficient methods for detecting seroresponses in the older age group (78%) and among the least efficient in the younger age group (44%; $P < 0.001$). CF was least efficient in both age groups but was significantly better in the older group than in the younger age group (54 versus 21%; $P < 0.005$). The prevaccination geometric mean titers (GMTs) in vaccinees to rotavirus as measured by IgA and IgM ELISA were similar in the two age groups, whereas the GMT by IgG ELISA was higher in the younger age group than in the older age group. Although the difference in GMT by IgG ELISA was not significant between infants 1 to 2 and 3 to 5 months old (Mann-Whitney statistic, $P = 0.06$), a significant difference could be shown between 1- and 5-month-old infants (Mann-Whitney statistic, $P = 0.028$). As expected, the GMT by IgG ELISA among the vaccinees as a whole was signif-

TABLE 2. Comparison of efficiencies of various assays in detecting seroresponses in 1- to 5-month-old infants after rotavirus vaccination

Treatment	Seroresponse[a] as determined by:					
	PRNA	ELISA			CF	Any test[b]
		IgA	IgG	IgM		
Placebo	1/19 (5)	2/21 (10)	1/21 (5)	0/21 (0)	0/21 (0)	3/21 (14)
Vaccination	61/87 (70)	60/89 (67)[c]	56/89 (62)	47/89 (53)	32/80 (40)	80/89 (90)

[a] Number of infants with a fourfold or greater antibody rise/number of infants tested; percentage of seroresponses is given in parentheses.
[b] Indicates a seroresponse determined by PRNA; IgA, IgG, or IgM ELISA; or CF.
[c] Significantly different from seroresponse rate by IgM ($\chi^2 = 3.96$; $P < 0.05$) or CF ($\chi^2 = 12.77$; $P < 0.0005$).

TABLE 3. Effect of age on development of seroresponse, as determined by various assays

Age of vaccinees (mo)	Seroresponse[a] as determined by:							
	PRNA			ELISA			CF	Any test[b]
	HRV[c]	RRV[d]	Either[e]	IgA	IgG	IgM		
1–2	12/38 (32)	19/36 (53)	23/38 (61)	23/39 (59)	17/39 (44)[f]	23/39 (56)	7/33 (21)[f]	35/39 (90)
3–5	27/46 (59)	31/49 (63)	38/49 (78)	37/50 (74)[g]	39/50 (78)	25/50 (50)	25/46 (54)	45/50 (90)

[a] Number of infants with a fourfold or greater antibody rise/number of infants tested; percentage of seroresponses is given in parentheses.
[b] Indicates a seroresponse determined by PRNA; IgA, IgG, or IgM ELISA; or CF.
[c] Human rotavirus; the serotype 1, 2, and 3 vaccine cells were tested against Wa (serotype 1), DS-1 (serotype 2), and P (serotype 3), respectively, and the bivalent cell (serotype 1 and 3) was tested against Wa (serotype 1) and P (serotype 3).
[d] All of the subjects from the four different vaccine cells were tested against RRV.
[e] Indicates a seroresponse determined by PRNA with HRV or RRV.
[f] Significantly different from seroresponse rate by same assay in 3- to 5-month-olds (for IgG, $\chi^2 = 11.12$, $P < 0.001$; for CF, $\chi^2 = 8.75$, $P < 0.005$).
[g] Significantly different from seroresponse rate by IgM ($\chi^2 = 6.11$, $P < 0.02$) and CF ($\chi^2 = 4.05$, $P < 0.05$) assays in 3- to 5-month-olds.

icantly greater than the GMT by IgA or IgM ELISA (Table 4). The efficiency of detecting seroresponses in vaccinees by combining various assays was also examined (Table 5). The seroresponse rate measured by combining any two of the three ELISAs (IgA, IgG, or IgM) was similar to that measured by combining all three ELISAs or any two ELISAs and PRNA, although there was a trend favoring the use of three assays over two assays. The seroresponse rate measured by combining the three ELISAs and PRNA was more efficient than combining only two ELISAs (90 versus 78 to 79%; $\chi^2 = 4.24$; $P < 0.05$).

DISCUSSION

Different methods have been used to measure seroresponses after natural infection or vaccination with rotavirus. In studies of natural infection, the CF test, ELISA, and neutralization assay have all been sensitive in detecting seroresponses to rotavirus. The CF test detected antibody rises in 75 to 85% of children and adults with rotavirus diarrhea but appeared to be an insensitive method of detecting seroresponses in infants under 6 months of age, on the basis of small numbers (10, 29).

Several studies have measured rotavirus-specific IgA, IgG, and IgM in serum after natural infection and have found that the IgM level is usually elevated during the acute phase of illness, whereas IgG and IgA levels rise during convalescence (3, 9, 13–15, 28). However, some investigators have noted that (i) the level of IgA in serum rises more rapidly than that of IgG, often becoming elevated during the first 2 weeks after the onset of rotavirus diarrhea; (ii) serum and fecal IgA levels parallel each other; and (iii) IgA in serum protects against rotavirus infection and more severe rotavirus illness (13–15). Most studies have found that antibody rises are detected in 75 to 100% of rotavirus diarrhea cases by the IgG assay (3, 8, 9, 24, 29, 37). However, few children in these studies were 5 months of age or younger, and one study specifically noted that although the IgG ELISA detected rises in 40 of 44 (91%) cases, only 1 of 4 patients 3 months of age or younger had an IgG rise. Some studies have found that the IgA assay is comparable in sensitivity to the IgG assay, although one study found that IgG in serum was a more sensitive indicator of immune response (91 versus 68%) (9). Most studies have found that detectable levels of IgM were present in acute-phase sera and then decreased with convalescence (3, 9, 28). The convalescent-phase sera were drawn approximately 1 month after illness in these studies. However, in a study of 30 5- to 21-month-old infants hospitalized with rotavirus diarrhea, paired serum specimens were collected within 1 to 6 days of the onset of illness and 5 to 12 days after the resolution of illness. The IgM assay detected fourfold or greater antibody rises in 57% of children, compared with 93 and 100% for the IgG and neutralization assays, respectively (37). A recent study of asymptomatically infected neonates showed a humoral antibody response quite different from that observed in older infants and children. In neonates with rotavirus-positive stool specimens, rises in antibody levels in serum were detected in 3 of 37 by IgG, 2 of 37 by IgM, and 0 of 37 by IgA ELISAs. In contrast, 23 of 37 neonates developed detectable rotavirus-specific IgA in their saliva (16).

Different methods have been used to evaluate the immunogenicity observed in several RRV vaccine studies in which sera were collected immediately prior to and 3 to 6 weeks after vaccination. The seroresponse rates measured in 31 adults by neutralization and CF and/or immune adherence hemagglutination were similar, at 77 and 84%, respectively (21). In a study of 50 children 4 months to 12 years of age (including 8 children under 6 months of age), the neutralization, CF, and IgG assays had similar efficiencies in detecting serum antibody rises (61, 53, and 51%, respectively) (34). Likewise, a study of 14 children 5 to 20 months of age showed seroresponse rates of 91 to 100% and 86% by neutralization assays and the CF test, respectively (22). In contrast, a study that included younger infants 1 to 4 months of age showed that the neutralization assay appeared to be more efficient than CF (71 versus 45%) (27). A study of neonates showed that the IgA assay was more efficient than the neutralization assay and the CF test (64 versus 10 and 0%, respectively) (5). A study of 34 children (3 to 20 months old, including 12 under 6 months) found the following rates

TABLE 4. Effect of age on prevaccination GMTs (reciprocal) to rotavirus by IgA, IgG, or IgM ELISA in vaccinees

Age (mo)	GMT by ELISA		
	IgA	IgG	IgM
1–2	63	745[a]	102
3–5	79	506	109
1–5	72	600[b]	106

[a] GMT by IgG ELISA in 1- to 2-month-olds is not significantly different from that in 3- to 5-month-olds (Mann-Whitney test, Z statistic = 1.86, $P = 0.06$). However, a difference could be shown between 1-month-olds (GMT = 1,000) and 5-month-olds (GMT = 302) (Mann-Whitney test, Z statistic = 2.19, $P = 0.028$).
[b] Significantly different from GMT by IgA (t statistic = −14.4, df = 88, $P < 0.00001$) and IgM (t statistic = 12.3, df = 88, $P < 0.00001$) ELISAs in 1- to 5-month-olds.

TABLE 5. Comparison of efficiencies of combinations of assays in detecting seroresponses in 1- to 5-month-old infants after rotavirus vaccination

Age (mo)	Seroresponse[a] as determined by:							
	IgA + IgM	IgA + IgG	IgG + IgM	IgA + IgG + IgM	IgA + IgM + PRNA	IgA + IgG + PRNA	IgG + IgM + PRNA	IgA + IgG, IgM + PRNA
1–2	30/39 (77)	26/39 (67)	28/39 (72)	32/39 (82)	34/39 (87)	32/39 (82)	33/39 (85)	35/39 (90)
3–5	40/50 (80)	43/50 (86)	42/50 (84)	43/50 (86)	43/50 (86)	45/50 (90)	44/50 (88)	45/50 (90)
1–5	70/89 (79)	69/89 (78)	70/89 (79)	75/89 (84)	77/89 (87)	77/89 (87)	77/89 (87)	80/89 (90)

[a] Number of infants with a fourfold or greater antibody rise/number of infants tested; percentage of seroresponses is given in parentheses.

of seroresponses: 91% by PRNA, 68% by IgG ELISA (82% in children ≥6 months old, 41% in children <6 months old), 50% by IgA ELISA, and 24% by IgM ELISA (23).

Although several studies with bovine rotavirus vaccine strains (RIT 4237 and WC3) have been done, most have used only one assay to test for seroresponses (1, 2, 31, 33). One exception is a study in which 25 2-year-old children were given the RIT 4237 bovine strain (32). The seroresponse rate in these children was the same when measured by neutralization assay or by IgG ELISA. In summary, most studies of rotavirus diarrhea or vaccination suggest that neutralization assay, CF, and IgG ELISA are sensitive or efficient means of detecting rises in serum antibody to rotavirus. However, the applicability and relative efficiencies of these different methods of detecting antibody rises in a large group of infants under 6 months of age, the target population for vaccination, have not been evaluated.

The results of our study show that rotavirus-specific IgA ELISA is the single best test for detecting seroresponses in infants 1 to 5 months of age. CF and IgG ELISA were the least efficient means of detecting seroresponses in the 1- to 2-month-olds but improved significantly in 3- to 5-month-olds. The IgG ELISA was actually among the most efficient methods in the older infants, comparable to IgA ELISA and PRNA, whereas CF was still relatively inefficient. PRNA was efficient at detecting seroresponses in both age groups, although the efficiency depended on the rotavirus strain used in the neutralization assay. Rises in levels of neutralizing antibodies to human rotavirus strains containing the same or similar VP7 genes as the vaccine strain were significantly lower in the younger age group, whereas rises in antibodies to RRV were similar in the two groups. This result may be because PRNA with the RRV strain, but not with human rotavirus strains, detects neutralizing antibody to the RRV VP4 protein, which is present in the vaccine strains tested in this study. A detailed analysis of the PRNA with the different strains of rotavirus has been made elsewhere (6). The IgM ELISA was one of the less efficient methods but was comparable for the two age groups. The age-related change in the efficiency of the IgG ELISA, the PRNA with human rotavirus strains, and the CF test is probably related to the waning of maternally acquired antibody with increasing age, as reflected in the decrease in rotavirus-specific prevaccination GMT by IgG ELISA. In contrast to IgG, IgA and IgM do not cross the placenta, and the rotavirus-specific prevaccination GMT by IgA and IgM ELISAs remained stable in the two age groups. It should be noted that the seroresponse rate of 14% in the placebo recipients is similar to the rate of 15% found in the placebo recipients in a previous study in Venezuela (27). Rotavirus infection is endemic throughout the year in Venezuela. Our finding that the IgA and IgG assays are of comparable efficiencies in detecting seroresponses in 1- to 5-month-olds, with a trend favoring the IgA over the IgG assay in the 1- to 2-month-olds, is in contrast to the results of one of the natural-infection studies (9). This study found the IgG assay to be significantly more sensitive than the IgA assay. The reasons for this finding are probably a difference in the age group and the times at which serum samples were obtained. In the natural-infection study, the mean age of the children was 17.5 months, and only four children were under the age of 3 months. Only 1 of these 4 children had a rise in antibodies as determined by IgG assay, compared with 39 of 40 children older than 3 months. This finding of insensitivity of the IgG assay in young infants is consistent with our results. The IgA ELISA may have been less sensitive in this study of natural infection because the first serum sample was drawn during the acute phase of illness, whereas in our study, the first serum sample was obtained prior to vaccination. It is also possible that the IgA ELISA is less sensitive than the IgG ELISA in detecting antibody rises in older infants and children, although several other studies have found the IgA and IgG assays to be comparable in children and adults. The efficiency of the IgM assay in detecting seroresponses in our vaccinees is similar to its sensitivity in detecting seroresponses in a study of 5- to 20-month-old children with rotavirus diarrhea (37). It is likely that our IgM assay would have been more efficient if the postvaccination serum had been drawn within 1 to 2 weeks instead of a month after vaccination. The insensitivity of the serum IgA, IgG, and IgM assays in a group of asymptomatically infected neonates is in contrast to our findings in 1- to 5-month-old vaccinees and a previous study of neonates vaccinated with RRV (5, 16). Larger numbers of neonates will have to be studied to determine the best parameters of vaccine take in this age group.

In conclusion, the rotavirus-specific IgA ELISA is the single best test for detecting seroresponses among 1- to 5-month-old vaccinees. Furthermore, the majority of seroresponses detected by combining all of the assays (PRNA, CF, and IgA, IgG, and IgM ELISAs) can be detected by combining the results of any two of the IgA, IgG, and IgM ELISAs. The IgA ELISA should be useful in field studies in which a diarrheal stool specimen is not always available with which to make an etiologic diagnosis. The ELISA is also much easier and quicker to perform than PRNA but does not provide serotype-specific information about the immune response. PRNA or an epitope-blocking assay with serotype-specific monoclonal antibodies must be performed in order to specifically determine the seroresponses to the major neutralization proteins (VP7 and VP4) of the different vaccine strains (30).

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LITERATURE CITED

1. **Clark, H. F., F. E. Borian, L. M. Bell, K. Modesto, V. Gouvea, and S. A. Plotkin.** 1988. Protective effect of WC3 vaccine against rotavirus diarrhea in infants during a predominantly serotype 1 rotavirus season. J. Infect. Dis. **158**:570–587.
2. **Clark, H. F., T. Furukawa, L. M. Bell, P. A. Offit, P. A. Perrella, and S. A. Plotkin.** 1986. Immune response of infants and children to low-passage bovine rotavirus (strain WC3). Am. J. Dis. Child. **140**:350–356.
3. **Davidson, G. P., R. J. Hogg, and C. P. Kirubakaran.** 1983. Serum and intestinal immune response to rotavirus enteritis in children. Infect. Immun. **40**:447–452.
4. **DeMol, P., G. Zissis, J. P. Butzler, A. Mutwewingabo, and F. E. André.** 1986. Failure of live, attenuated oral rotavirus vaccine. Lancet **ii**:108.
5. **Flores, J., G. Daoud, N. Daoud, M. Puig, M. Martinez, I. Perez-Schael, R. Shaw, H. B. Greenberg, K. Midthun, and A. Z. Kapikian.** 1988. Reactogenicity and antigenicity of rhesus rotavirus vaccine (MMU-18006) in newborn infants in Venezuela. Pediatr. Infect. Dis. J. **7**:776–780.
6. **Flores, J., I. Perez-Schael, M. Blanco, M. Vilar, D. Garcia, M. Perez, N. Daoud, K. Midthun, and A. Z. Kapikian.** 1989. Reactions to and antigenicity of two human-rhesus rotavirus reassortant vaccine candidates of serotypes 1 and 2 in Venezuelan infants. J. Clin. Microbiol. **27**:512–518.
7. **Flores, J., I. Perez-Schael, M. Gonzalez, D. Garcia, M. Perez, N. Daoud, W. Cunto, R. M. Chanock, and A. Z. Kapikian.** 1987. Protection against severe rotavirus diarrhoea by rhesus rotavirus vaccine in Venezuelan infants. Lancet **i**:882–884.
8. **Grauballe, P. C., A. Hornsleth, K. Hjelt, and P. A. Krasilnikoff.** 1986. Detection by ELISA of immunoglobulin G subclass-specific antibody responses in rotavirus infections in children. J. Med. Virol. **18**:277–281.
9. **Grimwood, K., J. C. S. Lund, B. S. Coulson, I. L. Hudson, R. F. Bishop, and G. L. Barnes.** 1988. Comparison of serum and mucosal antibody responses following severe acute rotavirus gastroenteritis in young children. J. Clin. Microbiol. **26**:732–738.
10. **Gust, I. D., R. C. Pringle, G. L. Barnes, G. P. Davidson, and R. F. Bishop.** 1977. Complement-fixing antibody response to rotavirus infection. J. Clin. Microbiol. **5**:125–130.
11. **Halsey, N. A., E. L. Anderson, S. D. Sears, M. Steinhoff, M. Wilson, R. B. Belshe, K. Midthun, A. Z. Kapikian, R. M. Chanock, R. Samorodin, B. Burns, and M. L. Clements.** 1988. Human-rhesus reassortant rotavirus vaccines: safety and immunogenicity in adults, infants, and children. J. Infect. Dis. **158**: 1261–1267.
12. **Hanlon, P., L. Hanlon, V. Marsh, P. Byass, F. Shenton, M. Hassan-King, O. Jobe, H. Sillah, R. Hayes, B. H. M'Boge, H. C. Whittle, and B. M. Greenwood.** 1987. Trial of an attenuated bovine rotavirus vaccine (RIT 4237) in Gambian infants. Lancet **ii**:1342–1345.
13. **Hjelt, K., P. C. Grauballe, L. Andersen, P. O. Schiotz, P. Howitz, and P. A. Krasilnikoff.** 1986. Antibody response in serum and intestine in children up to six months after a naturally acquired rotavirus gastroenteritis. J. Pediatr. Gastroenterol. Nutr. **5**:74–80.
14. **Hjelt, K., P. C. Grauballe, A. Paerregaard, O. H. Nielsen, and P. A. Krasilnikoff.** 1987. Protective effect of preexisting rotavirus-specific immunoglobulin A against naturally acquired rotavirus infection in children. J. Med. Virol. **21**:39–47.
15. **Hjelt, K., P. C. Grauballe, P. O. Schiotz, L. Andersen, and P. A. Krasilnikoff.** 1985. Intestinal and serum immune response to a naturally acquired rotavirus gastroenteritis in children. J. Pediatr. Gastroenterol. Nutr. **4**:60–66.
16. **Jayashree, S., M. K. Bhan, R. Kumar, P. Raj, R. Glass, and N. Bhandari.** 1988. Serum and salivary antibodies as indicators of rotavirus infection in neonates. J. Infect. Dis. **158**:1117–1120.
17. **Kapikian, A. Z., W. L. Cline, H. W. Kim, A. R. Kalica, R. G. Wyatt, D. H. VanKirk, R. M. Chanock, H. D. James, Jr., and A. L. Vaughn.** 1976. Antigenic relationships among five reovirus-like (RVL) agents by complement fixation (CF) and development of new substitute CF antigens for the human RVL agent of infantile gastroenteritis (39434). Proc. Soc. Exp. Biol. Med. **152**:535–539.
18. **Kapikian, A. Z., W. L. Cline, C. A. Mebus, R. G. Wyatt, A. R. Kalica, H. D. James, Jr., D. VanKirk, R. M. Chanock, and H. W. Kim.** 1975. New complement-fixation test for the human reovirus-like agent of infantile gastroenteritis. Lancet **i**:1056–1061.
19. **Kapikian, A. Z., J. Flores, Y. Hoshino, R. I. Glass, K. Midthun, M. Gorziglia, and R. M. Chanock.** 1986. Rotavirus: the major etiologic agent of severe infantile diarrhea may be controllable by a "Jennerian" approach to vaccination. J. Infect. Dis. **153**:815–822.
20. **Kapikian, A. Z., J. Flores, K. Midthun, Y. Hoshino, K. Y. Green, M. Gorziglia, K. Taniguchi, K. Nishikawa, R. M. Chanock, L. Potash, I. Perez-Schael, R. Dolin, C. Christy, M. Santosham, N. A. Halsey, M. L. Clements, S. D. Sears, R. E. Black, M. M. Levine, G. A. Losonsky, M. B. Rennels, L. Gothefors, G. Wadell, R. I. Glass, T. Vesikari, E. L. Anderson, R. B. Belshe, P. F. Wright, and S. Urasawa.** 1988. Development of a rotavirus vaccine by a "Jennerian" and a modified "Jennerian" approach, p. 151–159. *In* R. M. Chanock, R. A. Lerner, F. Brown, and H. Ginsberg (ed.), Modern approaches to new vaccines including prevention of AIDS. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Kapikian, A. Z., K. Midthun, Y. Hoshino, J. Flores, R. G. Wyatt, R. I. Glass, J. Askaa, O. Nakagomi, T. Nakagomi, R. M. Chanock, M. M. Levine, M. L. Clements, R. Dolin, P. F. Wright, R. B. Belshe, E. L. Anderson, and L. Potash.** 1985. Rhesus rotavirus: a candidate vaccine for prevention of human rotavirus disease, p. 357–367. *In* R. A. Lerner, F. Brown, and R. M. Chanock (ed.), Vaccines 85: molecular and chemical basis of resistance to parasitic, bacterial, and viral diseases. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Losonsky, G. A., M. B. Rennels, A. Z. Kapikian, K. Midthun, P. J. Ferra, D. N. Fortier, K. M. Hoffman, A. Baig, and M. M. Levine.** 1986. Safety, infectivity, transmissibility and immunogenicity of rhesus rotavirus vaccine (MMU 18006) in infants. Pediatr. Infect. Dis. **5**:25–29.
23. **Losonsky, G. A., M. B. Rennels, Y. Lim, G. Krall, A. Z. Kapikian, and M. M. Levine.** 1988. Systemic and mucosal immune responses to rhesus rotavirus vaccine MMU 18006. Pediatr. Infect. Dis. J. **7**:388–393.
24. **McLean, B., S. Sonza, and I. H. Holmes.** 1980. Measurement of immunoglobulin A, G, and M class rotavirus antibodies in serum and mucosal secretions. J. Clin. Microbiol. **12**:314–319.
25. **Midthun, K., H. B. Greenberg, Y. Hoskino, A. Z. Kapikian, R. G. Wyatt, and R. M. Chanock.** 1985. Reassortant rotaviruses as potential live rotavirus vaccine candidates. J. Virol. **53**: 949–954.
26. **Midthun, K., Y. Hoshino, A. Z. Kapikian, and R. M. Chanock.** 1986. Single gene substitution rotavirus reassortants containing the major neutralization protein (VP7) of human rotavirus serotype 4. J. Clin. Microbiol. **24**:822–826.
27. **Perez-Schael, I., M. Gonzalez, N. Daoud, M. Perez, I. Soto, D. Garcia, G. Daoud, A. Z. Kapikian, and J. Flores.** 1987. Reactogenicity and antigenicity of the rhesus rotavirus vaccine in Venezuelan children. J. Infect. Dis. **155**:334–338.
28. **Riepenhoff-Talty, M., S. Bogger-Goren, P. Li, P. J. Carmody, H. J. Barrett, and P. L. Ogra.** 1981. Development of serum and intestinal antibody response to rotavirus after naturally acquired rotavirus infection in man. J. Med. Virol. **8**:215–222.
29. **Sarkkinen, H. K., O. H. Meurman, and P. E. Halonen.** 1979. Solid-phase radioimmunoassay of IgA, IgG, and IgM antibodies to human rotavirus. J. Med. Virol. **3**:281–289.
30. **Shaw, R. D., K. J. Fong, G. A. Losonsky, M. M. Levine, Y. Maldonado, R. Yolken, J. Flores, A. Z. Kapikian, P. T. Vo, and H. B. Greenberg.** 1987. Epitope-specific immune responses to rotavirus vaccination. Gastroenterology **93**:941–950.
31. **Vesikari, T., E. Isolauri, A. Delem, E. D'Hondt, F. E. André, G. M. Beards, and T. H. Flewett.** 1985. Clinical efficacy of the RIT 4237 live attenuated bovine rotavirus vaccine in infants vaccinated before a rotavirus epidemic. J. Pediatr. **107**:189–194.
32. **Vesikari, T., E. Isolauri, A. Delem, E. D'Hondt, F. E. André, and G. Zissis.** 1983. Immunogenicity and safety of live oral

attenuated bovine rotavirus vaccine strain RIT 4237 in adults and young children. Lancet ii:807–811.

33. **Vesikari, T., E. Isolauri, E. D'Hondt, A. Delem, F. E. André, and G. Zissis.** 1984. Protection of infants against rotavirus diarrhoea by RIT 4237 attenuated bovine rotavirus strain vaccine. Lancet i:977–981.
34. **Wright, P. F., T. Tajima, J. Thompson, K. Kokubun, A. Z. Kapikian, and D. T. Karzon.** 1987. Candidate rotavirus vaccine (rhesus rotavirus strain) in children: an evaluation. Pediatrics 80:473–480.
35. **Wyatt, R. G., H. D. James, Jr., A. L. Pittman, Y. Hoshino, H. B. Greenberg, A. R. Kalica, J. Flores, and A. Z. Kapikian.** 1983. Direct isolation in cell culture of human rotaviruses and their characterization into four serotypes. J. Clin. Microbiol. 18: 310–317.
36. **Yolken, R. H., R. G. Wyatt, H. W. Kim, A. Z. Kapikian, and R. M. Chanock.** 1978. Immunological response to infection with human reovirus-like agent: measurement of anti-human reovirus-like immunoglobulin G and M levels by the method of enzyme-linked immunosorbent assay. Infect. Immun. 19:540–546.
37. **Zheng, B., S. Han, Y. Yan, X. Liang, G. Ma, Y. Yang, and M. H. Ng.** 1988. Development of neutralizing antibodies and group A common antibodies against natural infections with human rotavirus. J. Clin. Microbiol. 26:1506–1512.