JOURNAL OF VIROLOGY, Jan. 1992, p. 548–553
0022-538X/92/010548-06$02.00/0

Vol. 66, No. 1

Identification of VP7 Epitopes Associated with Protection against Human Rotavirus Illness or Shedding in Volunteers

KIM Y. GREEN* AND ALBERT Z. KAPIKIAN

Epidemiology Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

Received 25 June 1991/Accepted 3 October 1991

Sera from 17 of 18 adult volunteers challenged with a virulent serotype 1 rotavirus strain (D) were examined for prechallenge antibody levels against several well-defined rotavirus VP7 and VP4 neutralization epitopes by a competitive epitope-blocking immunoassay (EBA) in order to determine whether correlates of resistance to diarrheal illness could be identified. The presence of prechallenge serum antibody at a titer of ≥1:20 that blocked the binding of a serotype 1 VP7-specific monoclonal antibody (designated 2C9) that maps to amino acid residue 94 in antigenic site A on the serotype 1 VP7 was significantly associated with resistance to illness or shedding ($P < 0.001$) or illness and shedding ($P < 0.01$) following challenge with the serotype 1 virus. In addition, an EBA antibody titer of ≥1:20 in prechallenge serum against a serotype 3 VP7-specific epitope (defined by monoclonal antibody 954/159) that maps to amino acid 94 on the serotype 3 VP7 was also significantly associated with resistance to illness or shedding ($P = 0.02$), with a trend for protection against illness and shedding. A trend was also noted between the presence of EBA antibody against a cross-reactive VP4 epitope common to many human rotavirus strains, including the challenge virus, or a rhesus monkey rotavirus strain-specific VP4 antigenic site, and resistance to illness or shedding. These data confirm that the presence of serum antibody correlates with resistance to rotavirus illness or shedding but, in addition, demonstrate the association of antibody to a specific epitope with resistance to illness or shedding. These data also suggest that antigenic site A on the rotavirus VP7, composed of amino acids 87 to 96, may be involved in the formation of a major protective epitope. Further study of the role of this epitope in the development of homotypic and heterotypic immunity to rotaviruses following natural or vaccine-induced infection may be important in the development of strategies for control of rotavirus diarrheal disease.

Rotaviruses are characteristically the major known etiologic agents of severe diarrhea in infants and young children under 2 years of age in developed and developing countries and are estimated to cause 873,000 deaths annually in developing countries (6). A top public health priority worldwide is the development and implementation of an effective vaccine against severe rotavirus diarrhea during the first 2 years of life. Several rotavirus vaccine candidates have already been evaluated for efficacy in clinical trials with variable success (20). The most extensively examined candidates in humans are animal rotavirus strains of bovine or simian origin (19). Studies in animals suggested the feasibility of this Jennerian approach; calves that were immunized in utero with a VP7 serotype 6 bovine rotavirus strain failed to develop illness following challenge with a human rotavirus VP7 serotype 1 strain, whereas most unimmunized control animals were not protected (44, 45). When administered orally to infants, bovine (serotype 6) or simian (serotype 3) vaccine candidate strains were sufficiently attenuated and antigenic (4, 5, 15, 28, 39). However, their efficacy was inconsistent in placebo-controlled trials (1, 3, 5, 8, 10, 12, 16, 23, 28–30, 32, 33, 38, 40). It has been suggested that the variable efficacy is a result of the inability of the animal strains to induce adequate heterotypic immunity against the four epidemiologically important human rotavirus serotypes in young infants not primed by a previous rotavirus infection (14).

The mechanisms responsible for the induction of immunity to rotavirus illness in infants and young children have not been completely defined. In animal studies, antibodies directed against the two outer capsid proteins VP4 and VP7, which are independent neutralization antigens, have been associated with protection against challenge in both active and passive immunization models (17, 27). However, in vaccine trials, the role of homotypic and heterotypic immunity in the development of resistance to rotavirus illness in humans has been particularly difficult to evaluate. In part, this is because of the high but variable rate of asymptomatic, and therefore frequently unidentified, rotavirus infection prior to vaccination of infants and young children (31, 38). In addition, although local antibody levels in the intestine are of major importance in the development of immunity to rotaviruses (35), most studies have not addressed the role of these antibodies; they have focused predominantly on the role of serum antibodies, and indeed, in several studies, such antibodies correlated with resistance to rotavirus illness. For example, Chiba et al. found that a critical level of serotype-specific neutralizing antibody (≥1:128) in the serum was associated with homotypic resistance to illness (2).

In previous studies in adult volunteers who were challenged with a human rotavirus serotype 1 strain, the presence of a critical level of serum neutralizing antibody against serotype 1, 2, or 6 correlated with resistance to illness (21, 22). The characterization of monoclonal antibodies that recognize epitopes on VP7 and VP4 has made it possible to dissect responses to antigenic sites on these proteins by using an adaptation of a competitive binding immunoassay, designated epitope-blocking assay (EBA) (14, 34). In the present study, the prechallenge serum antibody levels of these volunteers were reexamined by EBA in an attempt to identify epitopes associated with protection against rotavirus

* Corresponding author.

disease. In addition, by EBA we examined (i) serotype-specific responses to the VP7 of each of four rotavirus serotypes, (ii) differential responses to two epitopes of serotype 1 or 3 that map to different sites on the VP7, (iii) responses to a human rotavirus cross-reactive antigenic site on the VP4, and (iv) responses to a rhesus monkey rotavirus (RRV) strain-specific VP4 antigenic site. In these studies, antibodies to certain epitopes were found to correlate with protection against rotavirus challenge.

MATERIALS AND METHODS

Administration of rotavirus D strain to adult volunteers. Adult volunteers were studied at the National Institutes of Health and the University of Maryland in six separate phases as previously described (21, 22). Eighteen individuals between 18 and 35 years of age were administered by the oral route 1 ml of a 0.2% human stool filtrate derived from patient D, who had a diarrheal illness. The 2% stool filtrate was considered at the time of the study as rotavirus rich by electron microscopy, and the rotavirus was later found to belong to VP7 serotype 1 (22, 43). Serum samples were collected before and about 1 month after challenge (with a single exception when serum was obtained approximately 2 months after challenge). Two volunteers were challenged with the same inoculum about 19 months later. Clinical data have been described previously (21, 22).

PRN. Plaque reduction neutralization (PRN) assays were performed as previously described (43). Titers are expressed as the reciprocal of the serum dilution that caused 60% reduction in the number of plaques per well and were reported previously (21).

EBA. EBA was performed as previously described (14). Characteristics of the monoclonal antibodies (MAbs) used in this study were summarized previously (14). Briefly, the protein specificity and amino acid residue to which each MAb mapped by sequence analysis of neutralization-resistant mutants were as follows: MAb 2C9, serotype 1 VP7, amino acid 94; MAb KU-4, serotype 1 VP7, amino acid 213; MAb S2-2G10, serotype 2 VP7, amino acid 190; MAb YO-1E2, serotype 3 VP7, amino acid 221; MAb 954/159, serotype 3 VP7, amino acid 94; MAb ST-2G7, serotype 4 VP7, amino acid 145 (16a); MAb 954/23, RRV VP4, amino acid 100; and MAb YO-2C2, YO VP4, amino acid 305. MAbs KU-4, S2-2G10, YO-1E2, ST-2G7, and YO-2C2 were kindly supplied by Koki Taniguchi and Shozo Urasawa (Sapporo Medical College, Sapporo, Japan), and MAb 2C9 was kindly supplied by Harry Greenberg (Stanford University, Palo Alto, Calif.). The EBA titer represents the reciprocal of the serum dilution that gave an optical density at 410 nm that was ≤50% of that of the unblocked control. The starting dilution of sera in the EBA was either 1:10 or 1:20, depending on the quantity of serum available. With the exception of YO-2C2, which exhibited cross-reactivity with the VP4 of human rotavirus VP7 serotypes 1, 3, and 4 (37), each of the MAbs has homotypic neutralization activity.

RESULTS

Analysis of rotavirus-specific neutralizing serum antibody by PRN. Eighteen adult volunteers were given serotype 1 human rotavirus D strain by the oral route; two of them (A and B) were challenged a second time 19 months later. The relationship between prechallenge serum neutralizing antibody to homotypic serotype 1 (Wa), heterotypic serotype 2 (DS-1), or serotype 6 (UK) strains and the development of illness or virus shedding following rotavirus challenge has been described previously (21, 22). In brief, the presence of a critical level of preinoculation neutralizing antibody to each of these three strains individually correlated with resistance to diarrheal illness or virus shedding. For example, only 1 of 5 volunteers who developed illness or shed rotavirus possessed a prechallenge neutralizing antibody titer of ≥1:100 to the homotypic Wa virus, whereas 12 of 13 individuals who did not develop illness or shed virus had such antibody ($P < 0.008$). The D virus has been reported as similar to the Wa strain in hybridization (11) and antigenic (18, 43) studies. A similar pattern was observed in neutralization assays with heterotypic DS-1 or UK strains, indicating that either homotypic or heterotypic serum neutralizing antibody was associated with protection.

Further examination of available selected sera by PRN employing the DS-1 strain as antigen in place of the reassortant DS-1×UK strain (VP7:2; VP4:UK) that was used in the original study was essentially confirmatory (data not shown).

Analysis of rotavirus-specific serum antibody by EBA. In an attempt to identify rotavirus epitopes associated with protection against challenge, we reexamined the sera from the volunteers by EBA in an attempt to measure specific antibody responses to epitopes on the outer capsid protein VP7 or VP4 of various rotavirus strains. MAbs that reacted with either of two epitopes on VP7 of serotype 1 or 3, a single VP7 epitope of serotype 2 or 4, a single VP4 epitope representing a cross-reactive VP4 epitope shared with most human rotavirus strains, and a single VP4 epitope of RRV were available.

Table 1 shows the prechallenge serum antibody titer of each of the volunteers by neutralization or by EBA for VP7 serotypes 1 and 2 and by EBA only for VP7 serotypes 3 and 4. The EBA antibody responses to a cross-reactive human rotavirus VP4 epitope defined by MAb YO-2C2 and a RRV VP4 epitope are also shown. With regard to a comparison of the sensitivity of the EBA and neutralization assay, antibody titers were consistently higher by the latter assay (Table 1). However, the efficiency of detection of a ≥fourfold antibody response by these assays was comparable when the total number of EBA responses was compared with the number of neutralization responses. Nine of 16 volunteers developed a response to the homotypic VP7 epitope defined by MAb 2C9. It was of interest that only 2 of 16 volunteers developed a response to the other homotypic VP7 serotype 1 epitope defined by MAb KU-4.

Antibody levels in prechallenge sera to VP7 epitopes of serotype 1, 2, 3, or 4 and the cross-reactive human rotavirus or RRV VP4 epitopes were examined in an attempt to determine whether one or more correlates of resistance to challenge could be identified (Table 2). It was striking that the presence of serum antibody to the homotypic 2C9 epitope of rotavirus serotype 1 correlated to a high degree with resistance to illness or shedding ($P = 0.0007$) or illness and shedding ($P = 0.009$) with the homotypic strain, whereas antibody to the other serotype 1 epitope KU-4 that mapped to a different site failed to correlate with such resistance (Table 2). Antibody to serotype 2 or 4 VP7 epitopes also failed to correlate with resistance. However, antibody to the serotype 3 VP7 epitope defined by MAb 954/159 was associated with resistance to illness or shedding ($P = 0.02$) with a trend for protection against illness and shedding ($P = 0.07$), whereas antibody to the other serotype 3 VP7 epitope, YO-1E2, which mapped to a different site, was not significantly associated with resistance. In addition, serum antibodies to the cross-reactive human rotavirus VP4 epitope

defined by MAb YO-2C2 (located in the VP5 cleavage fragment of VP4) or to the VP4 of RRV (located in the VP8 cleavage fragment of VP4), although not significantly associated with protection, showed a trend in that direction (Table 2). Human rotavirus-specific MAbs directed against the VP8 cleavage fragment of VP4 that has been reported to contain serotype-specific antigens (24) were not available for this study. As noted in Table 2, the coefficient of association was highest with antibody to the MAb 2C9 epitope. Thus, it appears that EBA antibody to a serotype 1 VP7 epitope defined by MAb 2C9 was a major correlate of resistance to rotavirus challenge.

Volunteers A and B, both of whom developed a diarrheal disease after initial challenge, were rechallenged about 19 months later with the same inoculum and failed to develop a diarrheal illness on rechallenge. Prerechallenge serum antibody titers for volunteer A were as follows: by neutralization versus serotype 1 (Wa), 1:227, and versus serotype 2 (DS-1xUK), 1:38; by EBA versus serotype 1 VP7 (defined by MAb 2C9), 1:80; versus serotype 1 VP7 (defined by MAb KU-4), <1:10; versus serotype 2 VP7 (defined by MAb S2-2G10), 1:20; versus serotype 3 VP7 (defined by MAb 954/159), 1:80; versus serotype 3 VP7 (defined by MAb YO-1E2), 1:80; versus serotype 4 VP7 (defined by MAb ST-2G7), <1:10; versus a human rotavirus cross-reactive VP4 epitope (defined by MAb YO-2C2), 1:160; and versus an RRV-specific VP4 epitope (defined by MAb 954/23), 1:40. Prechallenge serum from volunteer B was not available for testing by EBA. The presence of antibody to the homotypic VP7 epitope defined by 2C9 and the absence of antibody to the homotypic KU-4 epitope with resistance of volunteer A to diarrheal illness upon rechallenge is consistent with the correlation with resistance described above for these epitopes in the initial challenge.

DISCUSSION

Correlates of resistance to rotavirus illness in adult volunteers challenged with a serotype 1 rotavirus strain were evaluated in this study. Previously, certain levels of serum-neutralizing antibodies to human rotavirus VP7 serotype 1 or 2 or bovine serotype 6 were shown to be associated with resistance to illness or shedding following challenge (21, 22). A significantly greater number of adult volunteers who were resistant to illness than those who were not protected following challenge with the VP7 serotype 1 D strain possessed a serum-neutralizing antibody titer of (i) ≥1:100 against the homotypic Wa strain or (ii) ≥1:60 against the heterotypic serotype 2 reassortant DS-1×UK strain (22). In addition, the presence of serum-neutralizing antibody to the homotypic Wa strain (≥1:100) or the heterotypic DS-1×UK reassortant (≥1:60) or the bovine UK strain (≥1:10) was associated with resistance to shedding of rotavirus. Thus, it was suggested that heterotypic as well as homotypic antibody may be important in protection. Because individuals older than 5 years of age through adulthood only infrequently become ill with rotavirus diarrhea, it has been suggested that rotavirus reinfections induce heterotypic immunity via cross-reactive epitopes among rotavirus strains (14).

In the present study, the sera from the same volunteers were examined by EBA in an attempt to dissect the specificity of the antibodies associated with protection previously. The presence of serum antibodies that blocked the

TABLE 1. Pre- and postchallenge serum antibody titers of adult volunteers as determined by neutralization and EBA[a]

Volunteer	Antibody titers (prechallenge/postchallenge serum [reciprocal]) against various antigens by indicated assay									
	Serotype 1 VP7			Serotype 2 VP7		Serotype 3 VP7		Serotype 4 VP7	YO VP4	RRV VP4
	PRN Wa	EBA 2C9 aa 94[b]	EBA KU-4 aa 213[b]	PRN DS-1×UK	EBA S2-2G10 aa 190[b]	EBA 954/159 aa 94[b]	EBA YO-1E2 aa 221[b]	EBA ST-2G7 aa 145[b,c]	EBA YO-2C2 aa 305[b]	EBA 954/23 aa 100[b]
A[d]	<20/**1,259**	<10/**320**	<10/<10	**20/86**	<10/**80**	<10/**160**	<10/**320**	<10/<20	<10/≥**640**	<10/**160**
C[d]	77/**1,324**	<10/**80**	<20/<20	53/**549**	**20/160**	<10/10	<10/**20**	<10/<10	<10/**40**	<20/**80**
D[d]	83/>**5,120**	<20/**320**	<20/>**40**	<20/**506**	**10/40**	<20/**40**	NT	<10/<10	<10/**160**	<20/≥**1,280**
E[e]	<20/**125**	<10/<10	<20/<20	<20/<20	<10/<20	<10[f]	<10/**20**	<10/<20	<10/**20**	<20/<20
F[g]	1,881[f]	320/≥**1,280**	**20/320**	**95/610**	80/≥**640**	**10/40**	**20/160**	<10/<10	≥640[f]	<20/**40**
G[g]	**69/996**	<60/**320**	<60/40	118/445	NT	NT	NT	NT	NT	NT
H[g]	288/**2,845**	<100/**800**	<280[f]	109/**10,863**	NT	NT	NT	NT	<100/<400	200[f]
I[g]	221/452	160/**640**	<20/<20	23/<20	<10/10	80/80	160/160	<10/<10	40/**160**	20/40
J[g]	357/334	20/40	<20/<20	82/94	80/160	80/80	160/160	<10/<10	80/160	20/20
K[g]	227/**1,558**	20/20	<20/<20	528/1,098	40/40	80/80	<10/<10	<10/<10	10/<10	20/20
L[g]	>5,120/2,399	1,600/400	<100/<100	364/900	NT	NT	NT	NT	NT	100/100
M[g]	388/417	**40/320**	<20/<20	399/265	40/40	80/40	10/20	<10/<10	160/160	20/20
N[g]	2,219/896	320/160	<20/<20	89/40	<10/<10	20/20	20/10	<10/<10	40/40	20/<20
O[g]	363/288	80/40	<20/<20	<20/<20	<10/<20	<10/<10	<10/<10	<10/<10	<10/<10	<20/<20
P[g]	540/784	160/160	<20/<60	45/47	NT	80[f]	NT	NT	NT	NT
Q[g]	>5,120[h]/2,485	≥1,280[f]	160/160	269/267	80/40	160/160	400/400	40/40	≥640/160	<20/<20
R[g]	1,202/1,354	200/**640**	200/80	271/438	NT	NT	NT	NT	NT	<100[f]

[a] Paired sera were not available for volunteer B for testing by EBA; numbers in boldface indicate serologic response as indicated by a ≥fourfold rise in titer in paired sera obtained before and after challenge. PRN data from Kapikian et al. (21). NT, not tested; aa, amino acid.
[b] MAb reviewed by Green et al. (14).
[c] Data from reference 16a.
[d] Developed diarrheal illness and shed rotavirus following challenge.
[e] Did not develop diarrheal illness but shed rotavirus following challenge.
[f] Titer reported is from analysis of prechallenge serum only.
[g] Did not develop diarrheal illness or shed rotavirus after challenge.
[h] Data modified from Kapikian et al. (21).

TABLE 2. Relationship of prechallenge serum rotavirus antibody by EBA and the development of diarrheal illness or virus shedding following oral challenge with human rotavirus serotype 1 strain D

Prechallenge antibody titer by EBA with indicated MAb (specificity)	No. of volunteers (%) who developed diarrheal illness or shed rotavirus after challenge		P^a (Kendall coefficient of association)
	Yes	No	
2C9 (VP7 serotype 1)			
<1:20	4 (100)	0	0.0007 (1.00)
≥1:20	0	11	
KU-4 (VP7 serotype 1)			
<1:20	4 (36)	7	0.5[b] (0.33)
≥1:20	0	3	
S2-2G10 (VP7 serotype 2)			
<1:20	3 (50)	3	0.55[b] (0.35)
≥1:20	1	5	
YO-1E2 (VP7 serotype 3)			
<1:20	3 (50)	3	0.18[b] (0.56)
≥1:20	0	5	
954/159 (VP7 serotype 3)			
<1:20	4 (67)	2	0.02 (0.72)
≥1:20	0	7	
ST-2G7 (VP7 serotype 4)			
<1:20	4 (36)	7	1.0[b] (0.21)
≥1:20	0	1	
YO-2C2 (VP4 human cross-reactive)			
<1:20	4 (67)	2	0.06[b] (0.70)
≥1:20	0	6	
954/23 (VP4 RRV)			
<1:20	4 (57)	3	0.07[b] (0.63)
≥1:20	0	7	

[a] Fisher exact test, two-tailed.
[b] Not significant.

binding of MAb 2C9, a VP7 serotype 1-specific MAb mapping to amino acid 94 of VP7, correlated with resistance to illness or shedding in the adult volunteers. EBA antibody against another VP7 epitope (954/159), known to be serotype 3 specific and mapping to the same antigenic site (amino acid 94), was also found to correlate with resistance to illness or shedding. Amino acid 94 is located in antigenic site A, as described previously (9). Amino acids involved in the formation of both homotypic and heterotypic VP7 epitopes have been mapped to antigenic site A by using several different MAbs (25, 36). Thus, antibodies (whether homotypic or heterotypic) directed against or near this region of the VP7 may be important in the development of resistance to rotavirus illness. Of interest is that the majority of VP7s from serotype 1 or 3 rotavirus strains examined thus far share the amino acid asparagine at residue 94 (13). It has been suggested that serotypes 1 and 3 share an immunodominant neutralization site (7), and it is possible that amino acid residue 94 is involved in the formation of this shared site.

A prechallenge EBA antibody titer of ≥1:20 against other serotype-specific 1, 2, 3, or 4 VP7 epitopes examined in this study that mapped to sites other than A did not correlate with resistance to illness or shedding. This may be because of the role of antigenic site A as an immunodominant protective epitope. Alternatively, it is possible that the failure to correlate the presence of antibodies to antigenic sites other than A with resistance to illness or shedding in this study was due to experimental limits related to the sensitivity of the EBA. In addition, the use of different MAbs and antigens in the EBA may yield additional information regarding protective epitopes.

Because rotavirus vaccines have induced variable protection against illness, it has been difficult to define an absolute correlate of resistance by studying serum antibody responses. Moreover, in young infants lacking prior experience with rotavirus, the serum antibody response to a vaccine is predominantly serotype specific (14, 42). Although in the present study it appeared that homotypic serum antibodies (as defined by blocking of the 2C9 serotype 1 VP7 epitope) had a high degree of correlation with resistance to illness in adults, it is clear from data generated from at least two recently described rotavirus vaccine studies in which protection was independent, at least in part, of the development of serotype-specific serum antibodies (26, 41) that other antigens or host factors may be important in the development of immunity. In addition, antigenic variation in circulating strains must also be considered in the analysis of vaccine efficacy trials. It is possible that critical amino acid substitutions in key epitopes among circulating strains may allow escape from the limited antibody repertoire of young infant vaccinees. Studies are in progress to determine whether the presence of antibodies specific for the epitopes associated with resistance to illness in adults correlates with resistance to illness in younger individuals who receive rotavirus vaccines. Perhaps examination of antibody responses to VP7 antigenic site A, which is associated with homotypic and heterotypic antibodies, will enable us to better understand the natural history of rotavirus infection.

REFERENCES

1. **Bernstein, D. I., V. E. Smith, D. S. Sander, K. A. Pax, G. M. Schiff, and R. L. Ward.** 1990. Evaluation of WC3 rotavirus vaccine and correlates of protection in healthy infants. J. Infect. Dis. **162**:1055–1062.
2. **Chiba, S., T. Yokoyama, S. Nakata, Y. Morita, T. Urasawa, K. Taniguchi, S. Urasawa, and T. Nakao.** 1986. Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies. Lancet **ii**:417–421.
3. **Christy, C., H. P. Madore, M. E. Pichichero, C. Gala, P. Pincus, D. Vosefski, Y. Hoshino, A. Kapikian, and R. Dolin.** 1988. Field trial of rhesus rotavirus vaccine in infants. Pediatr. Infect. Dis. J. **7**:645–650.
4. **Christy, C., H. P. Madore, J. J. Treanor, K. Pray, A. Z. Kapikian, R. M. Chanock, and R. Dolin.** 1986. Safety and immunogenicity of live attenuated rhesus monkey rotavirus vaccine (letter). J. Infect. Dis. **154**:1045–1047.
5. **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.
6. **Committee on Issues and Priorities for New Vaccine Development, Institute of Medicine.** 1986. The prospects for immunizing against rotavirus, p. 308–318. *In* New vaccine development: establishing priorities, vol. 2. Diseases of importance in developing countries. National Academy Press, Washington, D.C.
7. **Coulson, B. S., J. M. Tursi, W. J. McAdam, and R. F. Bishop.** 1986. Derivation of neutralizing monoclonal antibodies to human rotavirus and evidence that an immunodominant site is shared between serotypes 1 and 3. Virology **154**:302–312.
8. **De Mol, P., G. Zissis, J.-P. Butzler, A. Mutwewingabo, and F. E. André.** 1986. Failure of live, attenuated oral rotavirus vaccine. Lancet **ii**:108.

9. **Dyall-Smith, M. L., I. Lazdins, G. W. Tregear, and I. H. Holmes.** 1986. Location of the major antigenic sites involved in rotavirus serotype-specific neutralization. Proc. Natl. Acad. Sci. USA **83**:3465–3468.
10. **Flores, J., I. Pérez-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.
11. **Flores, J., M. Sereno, C.-J. Lai, E. Boeggeman, I. Perez, R. Purcell, A. Kalica, H. Greenberg, R. Wyatt, J. Hansen, A. Kapikian, and R. Chanock.** 1983. Use of single-stranded rotavirus RNA transcripts for the diagnosis of rotavirus infection, the study of genetic diversity among rotaviruses, and the molecular cloning of rotavirus genes, p. 115–127. *In* R. W. Compans and D. H. L. Bishop (ed.), Double-stranded RNA viruses. Elsevier Science Publishing Co., Inc., New York.
12. **Gothefors, L., G. Wadell, P. Juto, K. Taniguchi, A. Z. Kapikian, and R. I. Glass.** 1989. Prolonged efficacy of rhesus rotavirus vaccine in Swedish children. J. Infect. Dis. **159**:753–757.
13. **Green, K. Y., J. F. Sears, K. Taniguchi, K. Midthun, Y. Hoshino, M. Gorziglia, K. Nishikawa, S. Urasawa, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1988. Prediction of human rotavirus serotype by nucleotide sequence analysis of the VP7 protein gene. J. Virol. **62**:1819–1823.
14. **Green, K. Y., K. Taniguchi, E. R. Mackow, and A. Z. Kapikian.** 1990. Homotypic and heterotypic epitope-specific antibody responses in adult and infant rotavirus vaccinees: implications for vaccine development. J. Infect. Dis. **161**:667–669.
15. **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 vaccines: safety and immunogenicity in adults, infants and children. J. Infect. Dis. **158**:1261–1267.
16. **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 **i**:1342–1345.

16a. **Hoshino, Y.** Personal communication.

17. **Hoshino, Y., L. J. Saif, M. M. Sereno, R. M. Chanock, and A. Z. Kapikian.** 1988. Infection immunity of piglets to either VP3 or VP7 outer capsid protein confers resistance to challenge with a virulent rotavirus bearing the corresponding antigen. J. Virol. **62**:744–748.
18. **Kapikian, A. Z., W. L. Cline, H. B. Greenberg, R. G. Wyatt, A. R. Kalica, C. E. Banks, H. B. James, Jr., J. Flores, and R. M. Chanock.** 1981. Antigenic characterization of human and animal rotaviruses by immune adherence hemagglutination assay (IAHA): evidence for distinctness of IAHA and neutralization antigens. Infect. Immun. **33**:415–425.
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. Nishikawa, R. M. Chanock, L. Potash, and I. Pérez-Schael.** 1989. Strategies for the development of a rotavirus vaccine against infantile diarrhea with an update on clinical trials of rotavirus vaccines. Adv. Exp. Med. Biol. **257**:67–89.
21. **Kapikian, A. Z., R. G. Wyatt, M. M. Levine, R. E. Black, H. B. Greenberg, J. Flores, A. R. Kalica, Y. Hoshino, and R. M. Chanock.** 1983. Studies in volunteers with human rotaviruses. Dev. Biol. Standard. **53**:209–218.
22. **Kapikian, A. Z., R. G. Wyatt, M. M. Levine, R. H. Yolken, D. H. VanKirk, R. Dolin, H. B. Greenberg, and R. M. Chanock.** 1983. Oral administration of human rotavirus to volunteers: induction of illness and correlates of resistance. J. Infect. Dis. **147**:95–106.
23. **Lanata, C., R. E. Black, R. del Agula, A. Gil, H. Verastegui, G. Gerna, J. Flores, A. Z. Kapikian, and F. E. Andre.** 1989. Protection of Peruvian children against rotavirus diarrhea of specific serotypes by one, two or three doses of the RIT 4257 attenuated bovine rotavirus vaccine. J. Infect. Dis. **159**:452–458.
24. **Larralde, G. L., B. Li, A. Z. Kapikian, and M. Gorziglia.** 1991. Serotype-specific epitope(s) present on the VP8 subunit of rotavirus VP4 protein. J. Virol. **65**:3213–3218.
25. **Mackow, E. R., R. D. Shaw, S. M. Matsui, P. T. Vo, D. A. Benfield, and H. B. Greenberg.** 1988. Characterization of homotypic and heterotypic VP7 neutralization sites of rhesus rotavirus. Virology **165**:511–517.
26. **Madore, H. P., C. Christy, M. Pichichero, C. Long, P. Pincus, D. Vosefsky, the Panorama Pediatric Group and the Westfall Pediatric Group, A. Z. Kapikian, and R. Dolin.** Submitted for publication.
27. **Offit, P. A., H. F. Clark, G. Blavat, and H. B. Greenberg.** 1986. Reassortant rotaviruses containing structural proteins VP3 and VP7 from different parents induce antibodies protective against each parental serotype. J. Virol. **60**:491–496.
28. **Pérez-Schael, I., D. Garcia, M. Gonzalez, R. Gonzalez, N. Daoud, M. Perez, W. Cunto, A. Z. Kapikian, and J. Flores.** 1990. Prospective study of diarrheal diseases in Venezuelan children to evaluate the efficacy of rhesus rotavirus vaccine. J. Med. Virol. **30**:219–229.
29. **Rennels, M. B., G. A. Losonsky, M. M. Levine, A. Z. Kapikian, and the Clinical Study Group.** 1986. Preliminary evaluation of the efficacy of rhesus rotavirus vaccine strain MMU18006 in young children. Pediatr. Infect. Dis. J. **5**:587–588.
30. **Rennels, M. B., G. A. Losonsky, A. E. Young, C. L. Shindledecker, A. Z. Kapikian, M. M. Levine, and the Clinical Study Group.** 1990. An efficacy trial of the rhesus rotavirus vaccine in Maryland. Am. J. Dis. Child. **144**:601–604.
31. **Ruuska, T., T. Vesikari, A. Delem, F. E. Andre, G. M. Beards, and T. H. Flewett.** 1990. Evaluation of RIT 4237 bovine rotavirus vaccine in newborn infants: correlation of vaccine efficacy to season of birth in relation to rotavirus epidemic period. Scand. J. Infect. Dis. **22**:269–278.
32. **Santosham, M., G. W. Letson, M. Wolff, R. Reid, S. Gahagan, R. Adams, C. Callahan, R. B. Sack, and A. Z. Kapikian.** 1991. A field study of the safety and efficacy of two candidate rotavirus vaccines in a Native American population. J. Infect. Dis. **163**:483–487.
33. **Senturia, Y. D., C. S. Peckman, M. Cordery, I. A. Chrystie, J. E. Banatvala, and F. E. André.** 1987. Live attenuated oral rotavirus vaccine. Lancet **ii**:1091–1092.
34. **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.
35. **Snodgrass, D. R., and P. W. Wells.** 1978. Passive immunity in rotaviral infections. J. Am. Vet. Med. Assoc. **173**:565–568.
36. **Taniguchi, K., Y. Hoshino, K. Nishikawa, K. Y. Green, W. L. Maloy, Y. Morita, S. Urasawa, A. Z. Kapikian, R. M. Chanock, and M. Gorziglia.** 1988. Cross-reactive and serotype-specific neutralization epitopes on VP7 of human rotavirus: nucleotide sequence analysis of antigenic mutants selected with monoclonal antibodies. J. Virol. **62**:1870–1874.
37. **Taniguchi, K., W. L. Maloy, K. Nishikawa, K. Y. Green, Y. Hoshino, S. Urasawa, A. Z. Kapikian, R. M. Chanock, and M. Gorziglia.** 1988. Identification of cross-reactive and serotype 2-specific neutralization epitopes on VP3 of human rotavirus. J. Virol. **62**:2421–2426.
38. **Vesikari, T., E. Isolauri, A. Delem, E. D'Hondt, F. Andre, 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.
39. **Vesikari, T., E. Isolauri, E. D'Hondt, A. Delem, F. E. Andre, and G. Zissis.** 1984. Protection of infants against rotavirus diarrhoea by RIT 4237 attenuated bovine rotavirus strain vaccine. Lancet **i**:977–980.
40. **Vesikari, T., T. Rautenen, T. Varis, G. M. Beards, and A. Z. Kapikian.** 1990. Rhesus rotavirus vaccine candidate. Clinical trial in children vaccinated between 2 and 5 months of age. Am. J. Dis. Child. **144**:285–289.

41. **Vesikari, T., T. Ruuska, K. Green, J. Flores, and A. Z. Kapikian.** Submitted for publication.
42. **Ward, R. L., D. S. Sander, G. M. Schiff, and D. I. Berstein.** 1990. Effect of vaccination on serotype-specific antibody responses in infants administered WC3 bovine rotavirus before or after a natural rotavirus infection. J. Infect. Dis. **162**:1298–1303.
43. **Wyatt, R. G., H. B. Greenberg, W. D. James, A. L. Pittman, A. R. Kalica, J. Flores, R. M. Chanock, and A. Z. Kapikian.** 1982. Definition of human rotavirus serotypes by plaque reduction assay. Infect. Immun. **37**:110–115.
44. **Wyatt, R. G., A. Z. Kapikian, and C. A. Mebus.** 1983. Induction of cross-reactive serum neutralizing antibody to human rotavirus in calves after in utero administration of bovine rotavirus. J. Clin. Microbiol. **18**:505–508.
45. **Wyatt, R. G., C. A. Mebus, R. H. Yolken, A. R. Kalica, H. D. James, Jr., A. Z. Kapikian, and R. M. Chanock.** 1979. Rotaviral immunity in gnotobiotic calves: heterologous resistance to human virus induced by bovine virus. Science **203**:548–550.