JOURNAL OF VIROLOGY, Nov. 1988, p. 4022–4026
0022-538X/88/114022-05$02.00/0

Vol. 62, No. 11

Comparative Analysis of the VP3 Gene of Divergent Strains of the Rotaviruses Simian SA11 and Bovine Nebraska Calf Diarrhea Virus

K. NISHIKAWA,[1]* K. TANIGUCHI,[1] A. TORRES,[2] Y. HOSHINO,[1] K. GREEN,[1] A. Z. KAPIKIAN,[1] R. M. CHANOCK,[1] AND M. GORZIGLIA[1]

Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892,[1] and Norden Laboratories, Inc., Lincoln, Nebraska 68521[2]

Received 9 May 1988/Accepted 2 August 1988

The gene encoding outer capsid protein VP3 of subpopulations of two animal rotaviruses, simian SA11 and Nebraska calf diarrhea virus (NCDV), was analyzed. Two laboratory strains of simian SA11 rotavirus (SA11-SEM and SA11-FEM) differed with respect to VP3. This dimorphism was indicated by a difference in electrophoretic mobility and a difference in reactivity with anti-VP3 monoclonal antibodies. The overall VP3 amino acid homology between the two SA11 VP3 proteins was 82.7%, whereas the VP3 protein of SA11-FEM was 98.5% homologous in amino acid sequence to NCDV VP3, suggesting that SA11-FEM VP3 was derived by gene reassortment in the laboratory during contamination with a bovine rotavirus. A comparison of the deduced amino acid sequence of the VP3 of two virulent NCDV strains and an attenuated NCDV strain (RIT 4237), revealed only five amino acid differences which were scattered throughout the protein but did not involve the trypsin cleavage sites. Of interest, the VP3 of the standard strain of NCDV which is virulent for cows differed in only one amino acid (position 23, Gln to Lys) from the VP3 of an NCDV mutant which was attenuated both for cows and for children.

Rotaviruses possess two outer capsid proteins, designated VP7 and VP3, each of which can induce neutralizing antibodies (5, 18). VP3, which is the product of double-stranded RNA segment 4, is also responsible for hemagglutination and trypsin enhancement of infectivity (7) and appears to play a major role in virulence in vivo (4, 19).

Simian rotavirus SA11 (serotype 3), first isolated in Africa in 1957 (15), has been used as a reference strain of the rotavirus genus in numerous studies. The complete nucleotide sequence of segment 4 of this virus has been determined (11, 12). However, it has been reported that SA11 consists of at least two virus subpopulations which differ in electrophoretic migration of RNA segment 4 (20). In addition, Lopez et al. (12) showed that the cDNA of the SA11 VP3 gene which was used for nucleotide sequence analysis did not hybridize with the corresponding gene segment of another laboratory strain of SA11.

Nebraska calf diarrhea virus (NCDV), first isolated in 1971 (16), has been used as a reference rotavirus strain of serotype 6. A live vaccine (RIT 4237) containing this virus was recently evaluated in several clinical trials in Finland and was found to be effective in protecting children against clinically significant rotavirus diarrhea (27). However, the mechanism for induction of resistance to human rotaviruses of serotypes 1 through 4 by a bovine strain of serotype 6 is not well understood. Recent studies suggest that this heterotypic protection may be due to the presence of cross-reactive neutralization antigenic sites on VP3 (14, 24).

MATERIALS AND METHODS

Virus. Two strains of SA11 and three strains of NCDV were studied. Strains of SA11 were supplied by R. L. Ward (10) and by H. H. Malherbe (15). The standard Cody strain of bovine rotavirus NCDV was received from C. A. Mebus in 1977, and the Adamson field isolate of the Cody strain of NCDV was received from A. Torres in 1987. The NCDV rotavirus vaccine strain, designated RIT 4237 (NCDV-Lincoln) (batch 82 D28/L1109; Smith Kline-RIT, Rixensart, Belgium) and studied in a rotavirus vaccine field trial in Whiteriver, Arizona, in 1986, was also analyzed. These viruses, except the Adamson isolate of Cody strain, were propagated in MA104 cells. Trypsin was used at a final concentration of 10 μg/ml for 60 min at 37°C to activate virus infectivity. After adsorption of virus, cells were fed with Eagle minimal essential medium supplemented with 1 μg of trypsin per ml. Rotavirus vaccine strain RIT 4237 was used after two passages in the MA104 cells. The virus of the Adamson isolate of the Cody strain was purified directly from the stool of infected calves.

PRN assay and ELISA. Hyperimmune antiserum against each of the viruses was prepared in guinea pigs as described previously (6). The plaque reduction neutralization (PRN) assay was performed in plastic six-well plates of MA104 cell monolayers to measure neutralizing antibody, as previously described (6). An enzyme-linked immunosorbent assay (ELISA) using monoclonal antibody as a capture antibody was performed as described previously (24). Infected culture fluid (10^7 to 10^8 PFU/ml) was used as an antigen. A_{405} was measured with a V_{max} kinetic microplate reader (Molecular Devices, Palo Alto, Calif.).

Polyacrylamide gel electrophoresis of RNA. Viral RNA was extracted from viral cores with phenol-chloroform and subjected to electrophoresis in acrylamide-bisacrylamide (10%: 0.33%) by the method of Laemmli (17). After electrophoresis, the gel was stained with ethidium bromide and photographed under UV light.

In vitro transcription and dideoxynucleotide sequencing. Viral mRNA was produced by viral cores as described previously (3). The total mRNA was hybridized to a series of 18- to 21-mer synthetic primers, and sequence was determined by the dideoxy-chain termination method by using avian myeloblastosis virus reverse transcriptase as described previously (4). To determine the 3′ end of each gene segment, double-stranded RNA extracted with phenol-chlo-

* Corresponding author.

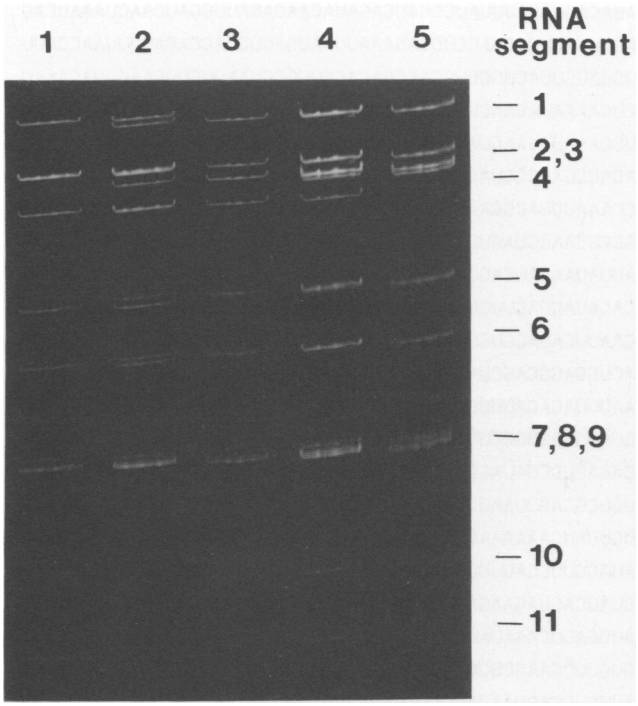

FIG. 1. Comparison of two SA11 subpopulations and a bovine rotavirus strain. Lanes: 1, NCDV; 3, SA11-FEM from R. L. Ward; 5, SA11-SEM from H. H. Malherbe; 2, mixture of NCDV and SA11-FEM; 4, mixture of SA11-FEM and SA11-SEM.

roform from the viral core was sequenced by the methods just described, except that primers were annealed to viral RNA which had been heated for 2 min at 100°C in the presence at 90% dimethyl sulfoxide in 10 mM Tris (pH 6.8), and the mixture was chilled quickly on ice and diluted fivefold in cold water. This initial set of treatments was performed to denature double-stranded viral RNA.

RESULTS

SA11 VP3 dimorphism: relationship of one form of SA11 VP3 to bovine rotavirus VP3. The two strains of SA11 which exhibit VP3 dimorphism have been designated SA11-FEM and SA11-SEM. The VP3 gene of the SA11-FEM strain has a faster electrophoretic migration pattern than the corresponding gene of strain SA11-SEM (Fig. 1). The former was supplied by R. L. Ward (10), and the latter was supplied by H. H. Malherbe (15). SA11-SEM was received from Malherbe in 1976, passaged once, and stored at −70°C for 11 years. The VP3 of the standard strain of bovine rotavirus NCDV, which is known to be virulent in calves, was also analyzed and compared with the two SA11 variants. The VP3 genes of SA11-FEM and standard NCDV showed identical RNA migration patterns (Fig. 1). Table 1 summarizes the antigenic relationships among the dimorphic forms of SA11 and standard NCDV. Although an antigenic relationship was not observed between SA11-SEM and NCDV, hyperimmune antiserum prepared against SA11-FEM neutralized not only homologous SA11-FEM but also SA11-SEM and NCDV at high titer, indicating that SA11-FEM shares neutralization epitopes with both SA11-SEM and the standard NCDV.

The reactivity of standard NCDV and the dimorphic forms of SA11 was also examined by ELISA using three cross-

TABLE 1. Antigenic relationships among two SA11 subpopulations and NCDV as determined by PRN

Virus strain	Reciprocal of 60% PRN titer of hyperimmune antisera to indicated rotavirus strain:		
	SA11-SEM	SA11-FEM	NCDV
SA11-SEM	10,240	10,240	160
SA11-FEM	20,480	10,240	10,240
NCDV	<80	2,560	10,240

reactive VP3 neutralizing monoclonal antibodies induced by a human serotype 3 rotavirus, YO, and a single serotype 3-specific anti-VP7 monoclonal antibody (24, 25) (Table 2). Two of the VP3 monoclonal antibodies, YO-1S3 and YO-1E6, showed high reactivity with SA11-SEM but little or no reactivity with SA11-FEM or NCDV. Moreover, monoclonal antibody YO-2C2 reacted strongly with SA11-FEM and NCDV and to a lesser degree with SA11-SEM. In addition, YO-1E2, the monoclonal antibody directed to serotype 3 VP7, reacted with both SA11 subpopulations but showed little reactivity with NCDV.

The sequence of the VP3 gene of each these strains was also analyzed. The complete nucleotide sequence of SA11-SEM is presented in Fig. 2. The VP3 gene of each of the three strains contained 2,362 nucleotides which included a long open reading frame of 2,328 nucleotides capable of encoding a protein of 776 amino acids. The open reading frame starts at nucleotide 10 and ends at nucleotide 2337. The 5′ conserved sequence of rotavirus, GGCUUUAAAA, is present in NCDV, whereas in the SA11 subpopulations, the U at position 5 is replaced by A. The 3′ conserved sequence AUGUGACC is present in each strain (data not shown). An extra nucleotide triplet (CAA) not found in human rotavirus VP3 is present in each strain at nucleotide positions 382 to 384 (4, 8).

An alignment of the predicted VP3 amino acid sequence of the two forms of SA11 and the standard NCDV is shown in Fig. 3. The deduced amino acid sequence of SA11-FEM agreed with that of Lopez et al. (11, 12), except at amino acid position 300, where phenylalanine was replaced by asparagine. Also, between amino acid positions 230 to 260, our SA11-FEM and SA11-SEM were identical with SA114fM and SA11, respectively, described by Lopez et al. (13), with the exception of two differences at positions 230 and 236 between SA11-SEM and SA11. The major trypsin cleavage sites of VP3 were present at amino acid positions 241 and 247 in each strain. A comparison of the predicted amino acid sequences of this region (amino acid positions 236 to 251) of SA11-FEM and NCDV revealed no amino acid differences. However, the cleavage region of SA11-SEM was quite different from that of SA11-FEM. The two SA11 variants exhibited only 37.5% homology in this region.

TABLE 2. ELISA reactivity pattern of VP3 and VP7 neutralizing monoclonal antibodies with two SA11 subpopulations and NCDV

Virus strain	Result of ELISA with monoclonal antibody[a]:			
	YO-1S3 (VP3)	YO-1E6 (VP3)	YO-2C2 (VP3)	YO-1E2 (VP7)[b]
SA11-SEM	**2,897**	**3,290**	**484**	**4,590**
SA11-FEM	69	69	**1,643**	**3,886**
NCDV	64	77	**1,504**	186

[a] The results are expressed as the sum of the A_{405} × 1,000 for two wells. Values over 300 are boldfaced and are considered positive.

[b] Serotype 3-specific monoclonal antibody YO-1E2 reactive with VP7 (25).

FIG. 2. Complete nucleotide sequence of the VP3 gene of SA11-SEM. Underlined bases indicate the positions of the initiation and termination codons.

The overall VP3 amino acid homology between SA11-FEM and SA11-SEM was 82.7% (Table 3). This value was similar to the relatedness values among rhesus rotavirus (14), SA11-SEM, and NCDV, but was somewhat higher than that described by Kantharidis et al. (8) for a comparison of SA11 and human rotavirus RV-5 (serotype 2), i.e., 71% homology. In contrast, SA11-FEM VP3 and NCDV VP3 were 98.5% homologous. A similarly high degree of VP3 homology between SA11-FEM and another bovine rotavirus (strain C486) was described previously (21).

Bovine NCDV rotavirus VP3: analysis of virulent strains and an attenuated vaccine mutant. In the experimental mouse model of rotavirus disease, VP3 appears to be associated with virulence of simian rotavirus SA11 and bovine rotavirus NCDV (19). In an attempt to better understand the basis for attenuation of the RIT NCDV vaccine strain which is attenuated both in cows and in children, we compared it to two additional bovine rotavirus strains designated NCDV which were both virulent for cows. The virulent strain, designated NCDV-V, is the Adamson field isolate of the Cody strain of NCDV. This virus retained its virulence for calves during 26 passages; eight times in gnotobiotic calves, twice in embryonic bovine testicular cells, once in gnotobiotic calves, three times in primary bovine lung cells, and 12 times in gnotobiotic calves. The standard Cody strain was passaged 99 times in cell culture and distributed to many laboratories. This strain is considered to be virulent because after an additional 81 passages in tissue culture it still caused diarrhea in calves (C. A. Mebus, personal communication). The attenuated NCDV strain, designated RIT 4237 (NCDV-Lincoln), which is a candidate live virus vaccine strain for use in infants and children, was passaged 147 times in primary bovine kidney cells and 5 times in primary CMK cells (2). This strain was cold adapted during the bovine kidney cell passage and was shown to be attenuated in calves (C. A. Mebus, personal communication). Analysis of the virulent NCDV-V and cold-adapted, attenuated NCDV RIT 4237 strains revealed 6 nucleotide and 5 amino acid differences (including a serine-to-proline substitution) (Table 4). Three amino acid differences were observed in the VP8 cleavage product of VP3, and two were observed in the other VP3 cleavage product, VP5; however, these changes did not involve the trypsin cleavage region. Significantly, the RIT vaccine mutant differed from the standard NCDV (Cody) strain in only one amino acid position; a Gln-to-Lys difference was detected at position 23 (Table 4).

DISCUSSION

Among myxoviruses and paramyxoviruses, the trypsin cleavage site of surface glycoproteins is of considerable significance in pathogenesis because one or two amino acid substitutions in this region of the hemagglutinin of influenza A virus or the fusion glycoprotein of Newcastle disease virus can bring about a marked change in virulence (9, 26). For example, substitution of one or two nonbasic amino acids for basic amino acid residues in the connecting peptide just upstream of the cleavage site of the hemagglutinin of a highly virulent strain of influenza A virus decreases the cleavability of this surface glycoprotein. Also, the cleavage activation site of the fusion glycoprotein of virulent Newcastle disease virus strains contains two sets of dibasic amino acid residues separated by an intervening nonbasic amino acid, whereas avirulent Newcastle disease virus strains contain two single basic amino acid residues separated by two uncharged residues in the corresponding region (26). Although the

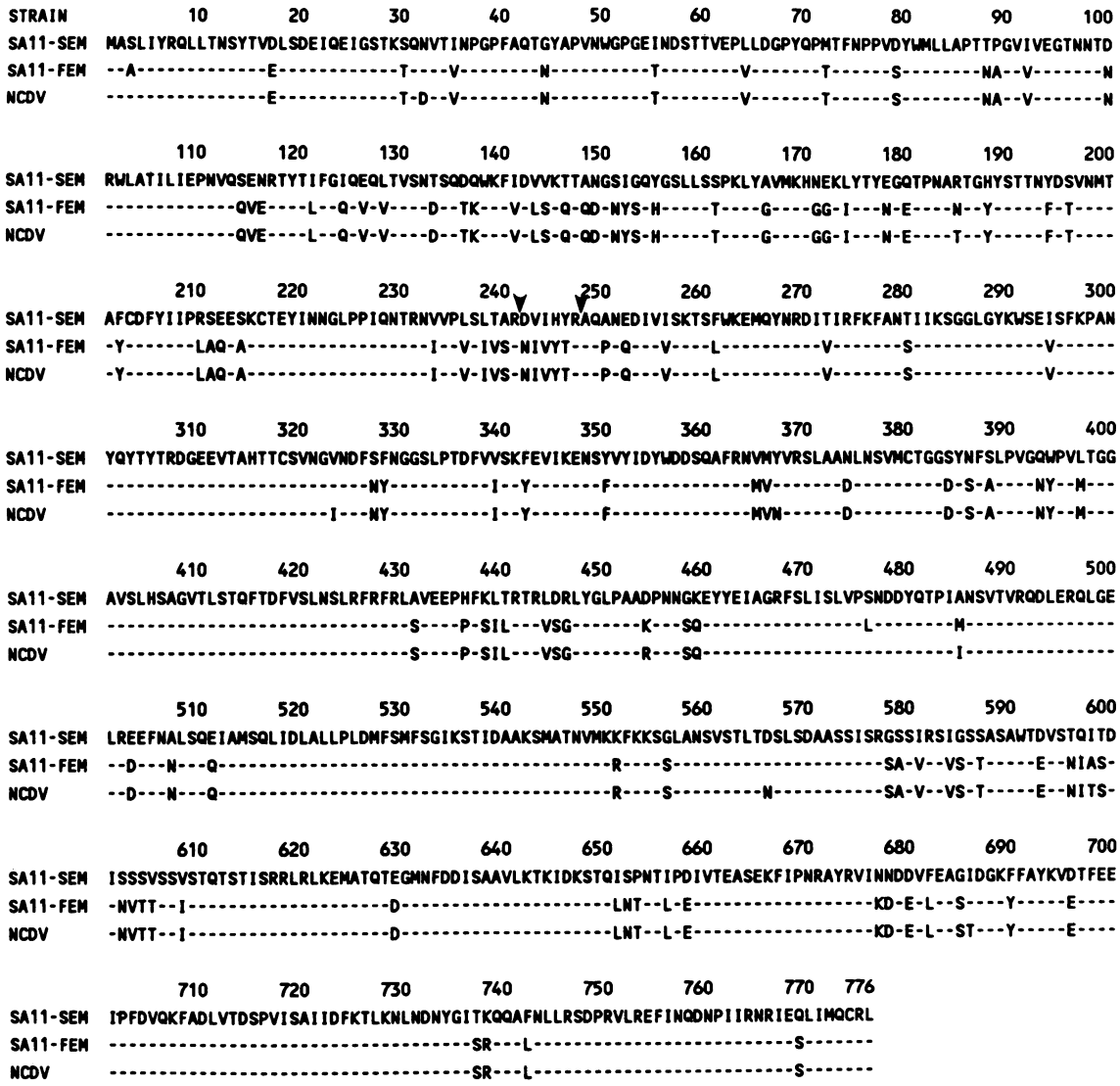

FIG. 3. Comparison of the deduced amino acid sequences of VP3 of two subpopulations of SA11 and standard NCDV. ▼, Cleavage site.

relationship between the cleavability of VP3 and virulence still remains to be established for rotavirus, a pair of basic amino acids is present just upstream of the VP3 cleavage site of virulent but not avirulent human rotavirus strains (4). In this study, a pair of basic amino acids was not found near the cleavage site of any of the NCDV strains, which included two viruses that were virulent. Also the small number of amino acid differences in VP3 between virulent and cold-adapted, avirulent bovine rotavirus strains did not involve the cleavage area. Thus, it is unlikely that attenuation of the RIT strain of NCDV can be ascribed to mutations in VP3. It is more likely that a gene (or genes) other than the VP3 gene

TABLE 3. Percent amino acid and nucleotide homology in VP3 among three animal rotaviruses and one human rotavirus

Virus strain	% Amino acid homology (% nucleotide homology) with:				
	SA11-SEM	SA11-FEM	NCDV	RRV[a]	RV-5[a]
RV-5	71.7 (70.6)	71.4 (69.6)	71.6 (69.9)	72.0 (68.5)	
RRV	87.8 (78.1)	84.5 (75.5)	84.8 (76.2)		
NCDV	82.6 (76.1)	98.5 (97.0)			
SA11-FEM	82.7 (76.2)				
SA11-SEM					

[a] Nucleotide sequences of RV-5 and RRV were quoted from Kantharidis et al. (8) and Mackow et al. (14), respectively.

TABLE 4. Comparison of nucleotides and deduced amino acid sequences of VP3 gene among NCDV-V (virulent), standard NCDV (virulent), and RIT 4237 (avirulent) strains

Changed nucleotide (amino acid) position	Substitution in strain:		
	NCDV-V (Cody)	Standard NCDV (Cody)	RIT 4237 (Lincoln)
5 (—)[a]	A	T	T
76 (23)	CAA (Gln)	CAA (Gln)	AAA (Lys)
103 (32)	AAC (Asn)	GAC (Asp)	GAC (Asp)
478 (157)	CCT (Pro)	TCT (Ser)	TCT (Ser)
1108 (367)	TAT (Tyr)	AAT (Asn)	AAT (Asn)
1174 (389)	ATT (Ile)	CTT (Leu)	CTT (Leu)

[a] —, Noncoding region.

sustained mutations which were responsible for attenuation of the RIT strain of NCDV. It is still possible, however, that VP3 amino acid substitutions outside the cleavage region could affect cleavability of this outer capsid protein.

Genomic heterogeneity of simian rotavirus SA11 has been reported for gene 5 (22) as well as for gene 4 (20). Our data suggest that heterogeneity of SA11 VP3 is not due to a divergence which developed during prolonged independent passage of the SA11-SEM and SA11-FEM, because the three NCDV bovine rotaviruses examined were found to have fewer than six nucleotide differences in spite of prolonged independent passage of these strains. A very low mutation rate of rotavirus was also described by Arias et al. (1). In a comparison of sequences determined from different cDNA clones derived from the same region of an SA11 strain which had been repeatedly propagated for 6 years without plaque purification, they found a frequency of 1 nucleotide substitution per 2,000 bases. On the other hand, the observed dimorphism of SA11 VP3 could result from a selection of different subpopulations which were present in different proportions in the original suspension of SA11 (22, 23). More likely, it resulted from gene reassortment which occurred during contamination of the SA11 strain with a bovine rotavirus while SA11 was being passaged in the laboratory. The SA11 VP3 gene present in the virus originally received from Malherbe and only passaged once in our laboratory probably represents the authentic SA11 gene. Additional evidence supporting this view comes from the previously described high level of amino acid sequence homology between the VP3 of SA11-FEM and that of another independent isolate of bovine rotavirus (21).

ACKNOWLEDGMENTS

We thank R. L. Ward for supplying virus and guinea pig SA11 antiserum and P. Collins and M. Hill for preparation of oligonucleotide primers. We also appreciate the editorial assistance of L. Jordan, S. Chang, and C. Fonseca.

LITERATURE CITED

1. **Arias, C. F., S. Lopez, and R. T. Espejo.** 1986. Heterogeneity in base sequence among different DNA clones containing equivalent sequences of rotavirus double-stranded RNA. J. Virol. **57:** 1207–1209.
2. **Delem, A., M. Lobmann, and N. Zygraich.** 1984. A bovine rotavirus developed as a candidate vaccine for use in humans. J. Biol. Stand. **12:**443–445.
3. **Flores, J., H. B. Greenberg, J. Myslinski, A. R. Kalica, R. G. Wyatt, A. Z. Kapikian, and R. M. Chanock.** 1982. Use of transcription probes for genotyping rotavirus reassortants. Virology **121:**288–295.
4. **Gorziglia, M., Y. Hoshino, A. Buckler-White, I. Blumentals, R. Glass, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1986. Conservation of amino acid sequence of VP8 and cleavage region of 84-kDa outer capsid protein among rotaviruses recovered from asymptomatic neonatal infection. Proc. Natl. Acad. Sci. USA **83:**7039–7043.
5. **Hoshino, Y., M. M. Sereno, K. Midthum, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1985. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82:** 8701–8704.
6. **Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Flores, and A. Z. Kapikian.** 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. J. Infect. Dis. **149:**694–702.
7. **Kalica, A. R., J. Flores, and H. B. Greenberg.** 1983. Identification of the rotaviral gene that codes for hemagglutination and protease-enhanced plaque formation. Virology **125:**194–205.
8. **Kantharidis, P., M. L. Dyall-Smith, and I. H. Holmes.** 1987. Marked sequence variation between segment 4 genes of human RV-5 and simian SA11 rotaviruses. Arch. Virol. **93:**111–121.
9. **Kawaoka, Y., and R. G. Webster.** 1988. Sequence requirements for cleavage activation of influenza virus hemagglutinin expressed in mammalian cells. Proc. Natl. Acad. Sci. USA **85:** 324–328.
10. **Knowlton, D. R., and R. L. Ward.** 1985. Effect of mutation in immunodominant neutralization epitopes on the antigenicity of rotavirus SA-11. J. Gen. Virol. **66:**2375–2381.
11. **Lopez, S., and C. F. Arias.** 1987. The nucleotide sequence of the 5′ and 3′ ends of rotavirus SA11 gene 4. Nucleic Acids Res. **15:** 4691.
12. **Lopez, S., C. F. Arias, J. R. Bell, J. H. Strauss, and R. T. Espejo.** 1985. Primary structure of the cleavage site associated with trypsin enhancement of rotavirus SA11 infectivity. Virology **144:**11–19.
13. **Lopez, S., C. F. Arias, E. Mendez, and R. T. Espejo.** 1986. Conservation in rotaviruses of the protein region containing the two sites associated with trypsin enhancement of infectivity. Virology **154:**224–227.
14. **Mackow, E. R., R. D. Shaw, S. M. Matsui, P. T. Vo, M.-N. Dang, and H. B. Greenberg.** 1988. The rhesus rotavirus gene encoding protein VP3: location of amino acids involved in homologous and heterologous rotavirus neutralization and identification of a putative fusion region. Proc. Natl. Acad. Sci. USA **85:**645–649.
15. **Malherbe, H. H., and M. Strickland-Cholmley.** 1967. Simian virus SA11 and the related O agent. Arch. Gesamte Virusforsch. **22:**235–245.
16. **Mebus, C. A., M. Kono, N. R. Underdahl, and M. J. Twiehaus.** 1971. Cell culture propagation of neonatal calf diarrhea (scours) virus. Can. Vet. J. **12:**69–72.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
18. **Offit, P. A., and G. Blavat.** 1986. Identification of the two rotavirus genes determining neutralization specificities. J. Virol. **57:**376–381.
19. **Offit, P. A., G. Blavat, H. B. Greenberg, and H. F. Clark.** 1986. Molecular basis of rotavirus virulence: role of gene segment 4. J. Virol. **57:**46–49.
20. **Pereira, H. G., R. S. Azeredo, A. M. Fialho, and M. N. P. Vidal.** 1984. Genomic heterogeneity of simian rotavirus SA11. J. Gen. Virol. **65:**815–818.
21. **Potter, A. A., G. Cox, M. Parker, and L. A. Babiuk.** 1987. The complete nucleotide sequence of bovine rotavirus C486 gene 4 cDNA. Nucleic Acids Res. **15:**4361.
22. **Sabara, M., D. Deregt, L. A. Babiuk, and V. Misra.** 1982. Genetic heterogeneity within individual bovine rotavirus isolates. J. Virol. **44:**813–822.
23. **Spencer, E. G., L. F. Avendano, and B. I. Garcia.** 1983. Analysis of human rotavirus mixed electopherotypes. Infect. Immun. **39:** 569–574.
24. **Taniguchi, K., Y. Morita, T. Urasawa, and S. Urasawa.** 1987. Cross-reactive neutralization epitopes on VP3 on human rotavirus: analysis with monoclonal antibodies and antigenic variants. J. Virol. **61:**1726–1730.
25. **Taniguchi, K., T. Urasawa, Y. Morita, H. B. Greenberg, and S. Urasawa.** 1987. Direct serotyping of human rotavirus in stools by an enzyme-linked immunosorbent assay using serotype 1-, 2-, 3-, and 4-specific monoclonal antibodies to VP7. J. Infect. Dis. **155:**1159–1166.
26. **Toyoda, T., T. Sakaguchi, K. Imai, N. M. Inocencio, B. Gotoh, M. Hamaguchi, and Y. Nagai.** 1987. Structural comparison of the cleavage-activation site of the fusion glycoprotein between virulent and avirulent strains of Newcastle disease virus. Virology **158:**242–247.
27. **Vesikari, T., E. Isolauri, E. d'Hondt, A. Delem, F. E. Andre, and G. Zissis.** 1984. Protection of infants against rotavirus diarrhea by RIT 4237 attenuated bovine rotavirus strain vaccine. Lancet **i:**977–981.