JOURNAL OF VIROLOGY, June 1988, p. 1870–1874
0022-538X/88/061870-05$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 62, No. 6

Cross-Reactive and Serotype-Specific Neutralization Epitopes on VP7 of Human Rotavirus: Nucleotide Sequence Analysis of Antigenic Mutants Selected with Monoclonal Antibodies

K. TANIGUCHI,[1†*] Y. HOSHINO,[1] K. NISHIKAWA,[1] K. Y. GREEN,[1] W. L. MALOY,[2] Y. MORITA,[3] S. URASAWA,[3] A. Z. KAPIKIAN,[1] R. M. CHANOCK,[1] AND M. GORZIGLIA[1]

Laboratory of Infectious Diseases[1] and Laboratory of Immunogenetics,[2] National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892, and Department of Hygiene, Sapporo Medical College, Sapporo 060, Japan[3]

Received 10 December 1987/Accepted 16 February 1988

The neutralization epitopes of human and simian rotavirus protein VP7 were studied by producing six neutralizing monoclonal antibodies (N-MAbs) and using these N-MAbs to select antigenic mutants that resisted neutralization by the N-MAbs used for their selection. Cross-neutralization tests between the N-MAbs and the antibody-selected antigenic mutants identified one cross-reactive and five distinct serotype-specific neutralization epitopes which operationally overlapped one another and constituted a single antigenic site. In addition, the amino acid substitutions in human rotavirus VP7 that are responsible for the antigenic alterations in the mutants selected with anti-VP7 cross-reactive or serotype-specific N-MAbs were identified. All the amino acid substitutions in the antigenic mutants occurred in one of two variable regions: amino acids 87 to 101 and 208 to 221.

Seven group A rotavirus (RV) serotypes have been established by cross-neutralization tests (13). In addition, several new candidate group A RV serotypes have been described recently (3, 16). Serotype specificity has been attributed mainly to an outer capsid protein, VP7, on the basis of analysis of reassortant viruses with various constellations of RNA segments (11, 14) and protein specificity of serotype-specific neutralizing monoclonal antibodies (N-MAbs) (4, 21). VP7 is coded for by gene 8 or 9 depending on the RV strain (11, 14). The nucleotide sequence of the VP7 gene of more than 20 RV strains of various serotypes has recently been determined. A comparative analysis of their deduced amino acid sequences revealed six discrete divergent regions (A to F) among different serotypes (9, 10a, 12, 19): amino acids 39 to 50 (A), 87 to 101 (B), 120 to 130 (C), 143 to 152 (D), 208 to 221 (E), and 233 to 242 (F). Since these six regions were highly conserved within a given serotype, it was likely that some or all of them were responsible for defining the serotype specificity of RV. By selecting antigenic mutants of simian RV SA-11 (serotype 3) with serotype 3-specific N-MAbs, Dyall-Smith et al. (7) identified three regions, corresponding to B, D, and E above (i.e., amino acids 87 to 96, 145 to 150, and 211 to 223), as the antigenic sites involved in serotype-specific neutralization. They also suggested that regions B and E appeared to be in close proximity in the folded protein but widely spaced in the linear molecule (7). In addition, the sequences of the B and E regions of a variety of reference and field strains have been shown to correlate absolutely with the serotype determined by standard immunologic methods such as neutralization (10b).

We previously prepared five serotype 1-specific N-MAbs, designated KU-2, KU-4, KU-3C7, KU-5H1, and KU-6A11, directed to human RV (HRV) VP7 and used these antibodies to select four antigenic escape mutants (V-KU-4, V-KU-3C7, V-KU-5H1, and V-KU-6A11) of strain KU (serotype 1); each of the N-MAb escape mutants was resistant to neutralization by the antibody used for its selection (17a). Analysis of the five N-MAbs and the four escape mutants by cross-neutralization indicates that VP7 has at least five overlapping serotype-specific neutralization epitopes which make up a single antigenic site (17a).

In this study, antigenic analysis of VP7 was extended by preparing several antigenic mutants resistant to anti-VP7 cross-reactive N-MAb YO-4C2 which neutralized HRV strains of serotypes 1, 3, and 4. Moreover, amino acid substitutions in the VP7 responsible for the antigenic alteration in the mutants selected with serotype-specific or cross-reactive N-MAbs against HRV were identified.

MATERIALS AND METHODS

Virus. The following 14 HRV strains were used for characterizing N-MAbs: KU, S12, and Wa (serotype 1); S2, P1, AK26, DS-1, and HN-126 (serotype 2); YO, S3, P2, and AK35 (serotype 3); and ST-3 and Hochi (serotype 4). Three representative animal RVs, simian SA-11 (serotype 3), simian RV strain MMU 18006 (RRV, serotype 3), and bovine neonatal calf diarrhea virus (NCDV, serotype 6), were also studied. The viruses were pretreated with 10 μg of trypsin per ml, propagated in MA-104 cells in the presence of trypsin (1 μg/ml), and harvested 1 to 3 days after infection.

Preparation of N-MAbs. Five anti-VP7 serotype 1-specific N-MAbs (KU-2, KU-4, KU-3C7, KU-5H1, and KU-6A11) were previously prepared by using strain KU as the immunizing antigen (17a). YO-4C2 antibody, which neutralizes strains of serotypes 1, 3, and 4, was obtained in a fusion experiment with strain YO (serotype 3) as the immunizing antigen. Immunoprecipitation with [^{35}S]methionine-labeled SA-11-infected viral lysate, which was carried out as described previously (20), showed that the YO-4C2 antibody was directed to VP7.

Antigenic mutants resistant to N-MAbs. HRV strains KU (serotype 1), P (serotype 3), and ST-3 (serotype 4) and RRV

* Corresponding author.
† Present address: Department of Hygiene, Sapporo Medical College, South-1, West-17, Chuo-ku, Sapporo 060, Japan.

TABLE 1. Heterotypic reactivity of anti-VP7 YO-4C2 N-MAb as determined by neutralization and ELISA

Virus strain		Reactivity pattern of YO-4C2 antibody	
Serotype and designation	Host of origin	Neutralization titer[a]	ELISA[b]
1			
KU	Human	3,200	1,322
S12	Human	3,200	1,096
Wa	Human	6,400	1,324
2			
S2	Human	400	140
AK-26	Human	100	190
P1	Human	400	68
DS-1	Human	<100	125
HN-126	Human	<100	127
3			
YO	Human	≧25,600	1,338
S3	Human	≧25,600	922
P2	Human	≧25,600	1,000
AK-35	Human	400	1,364
SA-11	Simian	≧25,600	1,357
4			
Hochi	Human	≧25,600	1,334
ST-3	Human	6,400	845
6			
NCDV	Bovine	<100	71

[a] The neutralization titer is expressed as the reciprocal of the highest dilution of ascitic fluid that reduced the fluorescent-focus count by more than 60%.

[b] The ELISA was carried out by using YO-4C2 ascitic fluid as a capture antibody (22). ELISA results are shown as the sum of $A_{410} \times 1,000$ in two wells. Values over 300 in each ELISA test were considered positive.

(serotype 3) were used as parental viruses. A KU antigenic mutant was selected with the KU-4, KU-3C7, KU-5H1, or KU-6A11 anti-VP7 serotype 1-specific N-MAb. Also, KU, P, ST-3, and RRV antigenic mutants were selected with YO-4C2 N-MAb. We used several tubes for each selection as described previously (17a, 20) and isolated two or three independently selected clones for each mutant. Each mutant is designated by a V followed by the name of the N-MAb used for its selection, and the parental virus of each mutant is given in parentheses.

ELISA and neutralization test. An enzyme-linked immunosorbent assay (ELISA) for examining the reactivity of N-MAbs with virus strains was carried out as described previously (22). The neutralization test was performed by a fluorescent-focus reduction or plaque reduction method (13, 23).

Nucleotide sequence determination. The nucleotide sequence of the VP7 gene of the parent strain and the antigenic mutants was determined by primer extension analysis as described previously (10).

Preparation of synthetic peptides. Synthetic peptides were prepared by the solid-phase method with a Beckman 990 peptide synthesizer. An ELISA for examining the reactivity of N-MAbs with synthetic peptides was carried out as follows. Polyvinyl microdilution plates were coated with peptide (5 μg per well) overnight at 37°C. After being washed with phosphate-buffered saline containing 0.05% Tween 20, the wells were saturated with 10% fetal calf serum. Aliquots (50 μl) of dilutions of ascitic fluid were placed in the wells and incubated for 2 h at 37°C. After being washed with phosphate-buffered saline containing 0.05% Tween 20, the samples were incubated for a further 2 h with alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G. Finally, 100 μl of a *p*-nitrophenylphosphoric acid disodium salt solution was added. Values over 300 when expressed as the sum of the $A_{405} \times 1,000$ for two wells were considered positive.

RESULTS

Relationship among serotype-specific and cross-reactive neutralization epitopes on VP7. In our previous study (17a), we characterized five anti-VP7 serotype 1-specific N-MAbs (KU-2, KU-4, KU-3C7, KU-5H1, and KU-6A11) prepared by using strain KU as the immunizing antigen, and we prepared four antigenic mutants (V-KU-4, V-KU-3C7, V-KU-5H1 and V-KU-6A11) selected with the respective N-MAbs. In this study we obtained a cross-reactive anti-VP7 N-MAb, YO-4C2, which neutralized HRV strains of serotypes 1, 3, and 4 (Table 1). The YO-4C2 antibody immunoprecipitated VP7 of [^{35}S]methionine-labeled SA-11-infected viral lysate (data not shown). By propagating KU (serotype 1), P (serotype 3), RRV (serotype 3), or ST-3 (serotype 4) in the presence of the YO-4C2 antibody, we prepared antigenic mutants of each of these viruses.

The KU antigenic mutants and their selecting N-MAbs were analyzed by cross-neutralization (Table 2). Although the epitopes recognized by each of the six N-MAbs differed from one another, they overlapped functionally. For example, V-KU-6A11 (KU) was not neutralized or was weakly neutralized by serotype 1-specific KU-3C7, KU-5H1, KU-6A11, and KU-4 antibodies as well as cross-reactive YO-4C2 antibody. V-YO-4C2$_{I}$ (KU) (an antigenic mutant selected with YO-4C2 antibody) was not neutralized by cross-reac-

TABLE 2. Epitope analysis of KU (serotype 1) antigenic mutants selected with anti-VP7 N-MAbs as determined by neutralization

N-MAb	Neutralizing antibody titer for[a]						
	V-KU-3C7	V-KU-5H1	V-KU-6A11	V-KU-4	V-YO-4C2$_{I}$ (KU)	V-YO-4C2$_{II}$ (KU)	Parental virus (strain KU)
KU-3C7[b]	<200	<200	200	6,400	<200	6,400	12,800
KU-5H1[b]	<200	<200	200	6,400	<200	3,200	6,400
KU-6A11[b]	<200	200	<200	800	200	51,200	51,200
KU-4[b]	204,800	51,200	200	<200	204,800	204,800	204,800
KU-2[b]	<200	12,800	3,200	6,400	12,800	12,800	12,800
YO-4C2[c]	<200	6,400	200	12,800	<200	<200	25,600

[a] The neutralizing antibody titer is expressed as the reciprocal of the highest dilution of ascitic fluid that reduced the plaque count by more than 60%.

[b] Serotype 1-specific N-MAbs.

[c] Cross-reactive N-MAb which neutralizes serotype 1, 3, and 4 strains.

```
1                                                              64
MYGIEYTTILIFLISIILLNYILKSVTRIMDYIIYRFLLITVALFALTRAQNYGLNLPITGSMD
65                                                             128
                                ▼ ▽▼  ▽
TVYTNSTQEEVFLTSTLCLYYPTEASTQINDGDWKDSLSQMFLTKGWPTGSVYFKEYSSIVDFS
129                                                            192
VDPQLYCDYNLVLMKYDQSLELDMSELADLILNEWLCNPMDITLYYYQQSGESNKWISMGSSCT
193                                                            256
                      ▼ ▼
VKVCPLNTQTLGIGCQTTNVDSFEMVAENEKLAIVDVVDGINHKINLTTTTCTIRNCKKLGPRE
257                                                            320
NVAVIQVGGSNVLDITADPTTNPQTERMMRVNWKKWWQVFYTIVDYINQIVQVMSKRSRSLNSA
321  326
AFYYRV
```

FIG. 1. Complete deduced amino acid sequence of the VP7 gene of strain KU. The numbers above the sequence refer to the amino acid positions. The positions where amino acid substitutions were found in the KU antigenic mutants are indicated by ▼ (for mutants selected with serotype-specific N-MAbs) or ▽ (for mutants selected with cross-reactive YO-4C2 antibody). The amino acid sequence corresponding to the region from which peptides were synthesized is underlined.

tive YO-4C2 antibody or by serotype 1-specific KU-3C7, KU-5H1, or KU-6A11 antibody.

Identification of serotype-specific and cross-reactive neutralization epitopes on VP7 of HRV. To identify the amino acid substitutions responsible for antigenic variation of the N-MAb escape mutants, we sequenced the VP7 gene of the parental KU strain and its antigenic mutants which were resistant to the N-MAb used for their selection. The entire VP7 gene of strain KU consisted of 1,062 nucleotides, with two potential open reading frames beginning at an AUG at positions 49 to 51 or 136 to 138 and ending at an UAG at positions 1,027 to 1,029. The open reading frames had the coding capacity for a VP7 of 297 or 326 amino acids. The deduced amino acid sequence of VP7 of strain KU is shown in Fig. 1.

Only a single nucleotide change was found on the entire VP7 gene in each mutant. Table 3 shows the nucleotide and deduced amino acid sequence changes in the mutants. Two or three clones independently selected with the same N-MAbs were sequenced, and the same amino acid substitution at the same position was obtained for all antigenic mutants except for V-YO-4C2 (KU). V-KU-3C7 (KU) and V-KU-5H1 (KU) sustained a single amino acid substitution at positions 94 (Asn to Ser) and 97 (Asp to Gly), respectively. In V-KU-6A11 (KU) and V-KU-4 (KU) the amino acid substitution occurred at residues 211 (Asn to Asp) and 213 (Asp to Gly), respectively. In contrast, two independently selected KU antigenic mutants, V-YO-4C2$_{I}$ (KU) and V-YO-4C2$_{II}$ (KU), which showed different reactivity patterns with the five serotype 1-specific N-MAbs, differed in the position at which an amino acid substitution occurred; in the first instance it was at position 96 (Gly to Asp), and in the second instance it was at position 99 (Lys to Glu). The P, ST-3, and RRV antigenic mutants selected with the cross-reactive YO-4C2 antibody sustained amino acid substitutions at positions 96 (Asn to Asp), 94 (Ser to Asn), and 98 (Trp to Leu), respectively. Thus, all the antigenic mutants selected with N-MAbs had an amino acid substitution in either variable region B (amino acids 87 to 101) or E (amino acids 208 to 221) (9, 10a, 12).

Reactivity of N-MAbs with synthetic peptides. To examine whether the amino acid substitutions found in the N-MAb-selected RV mutants are located inside the binding site of N-MAbs, we examined the reactivity of N-MAb with two synthetic peptides (amino acids 87 to 104 and 204 to 221) corresponding to the amino acid sequence of parental strain KU in the region of the amino acid substitutions found in the mutants (Fig. 1). However, neither of the synthetic peptides could be recognized by any of the five serotype 1-specific N-MAbs in ELISA (data not shown).

DISCUSSION

Cross-neutralization tests involving five serotype 1-specific N-MAbs and mutants selected with four of the N-MAbs showed that the five serotype 1-specific neutralization epitopes recognized by the N-MAbs were distinct from each other, but overlapped operationally as constituents of a single antigenic site (17a). Sequence analysis of the antigenic mutants revealed that amino acid substitutions were limited to two variable regions on VP7, B (amino acids 87 to 101) and E (amino acids 208 to 221) (9, 10a, 12). These results suggest that these two regions of VP7, B and E, are in close proximity in the three-dimensional structure of VP7, although they are widely separated in the linear sequence. Our observations with an HRV serotype 1 strain are similar to those previously reported by Dyall-Smith et al. for a simian RV serotype 3 strain (SA-11) (7). Accordingly, it appears that immunodominant neutralization epitopes of VP7 are located on the same site in viruses of different serotypes as well as different host species of origin. It is of interest that the amino acid substitutions found in the antigenic mutants resistant to serotype-specific N-MAbs (serotype 1 [KU] and serotype 3 [SA-11]) yielded aspartic acid and asparagine in most instances (7).

It is of interest that the antigenic mutants developed amino acid substitutions which corresponded to amino acids

TABLE 3. Nucleotide and amino acid sequence changes found in the antigenic mutants selected with anti-VP7 N-MAbs

Parental virus	Selecting antibody	Neutralizing activity of selecting antibody with different HRV serotypes[a]	Antigenic mutant	Codon change (position)	Amino acid change (position)
KU	KU-3C7	Serotype 1	V-KU-3C7 (KU)	AAT to AGT (329)	Asn to Ser (94)
KU	KU-5H1	Serotype 1	V-KU-5H1 (KU)	GAC to GGC ([illegible])	Asp to Gly (97)
KU	KU-6A11	Serotype 1	V-KU-6A11 (KU)	AAC to GAC ([illegible])	Asn to Asp (211)
KU	KU-4	Serotype 1	V-KU-4 (KU)	GAC to GGC ([illegible]36)	Asp to Gly (213)
KU	YO-4C2	Serotypes 1, 3, and 4	V-YO-4C2$_{I}$ (KU)	GGT to GAT (335)	Gly to Asp (96)
KU	YO-4C2	Serotypes 1, 3, and 4	V-YO-4C2$_{II}$ (KU)	AAA to GAA (343)	Lys to Glu (99)
P	YO-4C2	Serotypes 1, 3, and 4	V-YO-4C2 (P)	AAT to GAT (334)	Asn to Asp (96)
ST-3	YO-4C2	Serotypes 1, 3, and 4	V-YO-4C2 (ST-3)	AGT to AAT (329)	Ser to Asn (94)
RRV	YO-4C2	Serotypes 1, 3, and 4	V-YO-4C2 (RRV)	TGG to TTG (341)	Trp to Leu (98)

[a] Neutralizing activity of N-MAbs was determined by a fluorescent-focus reduction assay.

present at the same position in other rotavirus serotypes. For example, with regard to V-KU-3C7 (KU), the amino acid (Asn) at residue 94 of the parent KU strain was replaced by Ser, which is the residue at the corresponding position of RV serotypes 2 and 4 (10a), and V-KU-6A11 (KU) underwent an amino acid substitution at position 211 from Asn to Asp, the latter being the amino acid in this position in serotypes 2, 3, and 5 (10a). These observations suggest that there are constraints governing which amino acids can be tolerated in specific positions in the functional VP7.

Heterotypic protective immunity has been observed following RV infection, but this has not been a consistent finding (2, 8, 15, 18, 24, 25). An understanding of the viral determinants responsible for eliciting such heterotypic protective immunity is fundamental to the construction of an effective RV vaccine. In previous studies (20), we demonstrated that VP3 has at least three cross-reactive neutralization epitopes. Furthermore, recently, the location of the cross-reactive neutralization epitopes was identified, at the molecular level, by sequencing the VP3 genes of the antigenic mutants resistant to cross-reactive N-MAbs (K. Taniguchi, Y. Hoshino, K. Nishikawa, K. Y. Green, W. L. Maloy, Y. Morita, S. Urasawa, A. Z. Kapikian, R. M. Chanock, and M. Gorziglia, submitted for publication). Offit et al. (18) showed that in a murine model, passively acquired antibodies to VP3 are capable of protecting mice from heterotypic RV challenge. Also, Hoshino et al. demonstrated similar protection in piglets following active immunization with VP3 (12a). Thus, VP3 may be more important for heterotypic immunity than VP7, which is the major protein determining a serotype-specific antibody response. However, the role of VP7 in heterotypic immunity is still not clear, since we obtained one N-MAb, YO-4C2, which was capable of neutralizing serotype 1, 3, and 4 strains. Coulson et al. (5) also reported the derivation of an N-MAb which neutralized serotypes 1 and 3. This indicates that VP7 also possesses cross-reactive neutralization epitopes. Unexpectedly, cross-neutralization tests between six N-MAbs including the cross-reactive YO-4C2 antibody and antibody-selected antigenic mutants showed that the cross-reactive neutralization epitope recognized by YO-4C2 antibody was located within variable region B, which also contains serotype-specific neutralization epitopes. The KU antigenic mutants resistant to YO-4C2 antibody had an amino acid substitution at position 96 or 99, depending on the mutant analyzed. Similarly, the P, ST-3, and RRV antigenic mutants resistant to YO-4C2 antibody sustained an amino acid substitution in the same region: 96 (Asn to Asp), 94 (Ser to Asn), and 98 (Trp to Leu), respectively.

Sequence analysis of the VP7 gene of antigenic mutants selected with serotype-specific or cross-reactive N-MAbs allowed us to identify the amino acid substitution responsible for the antigenic alteration of each mutant. However, the position of an amino acid substitution does not necessarily correspond to the actual binding site of the N-MAb. For example, most amino acid substitutions found in the N-MAb-selected poliovirus type 1 mutants were located outside the binding site of N-MAb, whereas all mutations of poliovirus type 3 were found to cluster between amino acids 93 and 100 of VP1, which is the N-MAb-binding site (1, 6, 17). In this study, the failure of reactivity of N-MAbs with synthetic peptides corresponding to the amino acid sequence in the region of amino acid substitutions found in the RV mutants may suggest that the regions of amino acid substitutions found in the RV mutants are outside the N-MAb-binding site. Alternatively, the regions form the conformational antigenic site which resulted in the failure of recognition by N-MAbs, even if the positions of amino acid substitutions are located inside the N-MAb-binding site.

ACKNOWLEDGMENTS

We thank P. Collins and M. Hill for preparation of oligonucleotide primers, R. Jones for technical assistance, and S. Chang for editorial assistance.

LITERATURE CITED

1. **Blondel, B., R. Crainic, O. Fichot, G. Dufraisse, A. Candrea, D. Diamond, M. Girard, and F. Horaud.** 1986. Mutations conferring resistance to neutralization with monoclonal antibodies in type 1 poliovirus can be located outside or inside the antibody-binding site. J. Virol. **57**:81–90.
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. **Clark, H. F., Y. Hoshino, L. M. Bell, J. Groff, G. Hess, P. Bachman, and P. A. Offit.** 1987. Rotavirus isolate WI61 representing a presumptive new human serotype. J. Clin. Microbiol. **25**:1757–1762.
4. **Coulson, B. S., K. J. Fowler, R. F. Bishop, and R. G. H. Cotton.** 1985. Neutralizing monoclonal antibodies to human rotavirus and indication of antigenic drift among strains from neonates. J. Virol. **54**:14–20.
5. **Coulson, B. S., J. M. Tursi, W. J. McAdam, and R. F. Bishop.** 1986. Derivation of neutralizing monoclonal antibodies to human rotaviruses and evidence that an immunodominant neutralization site is shared between serotypes 1 and 3. Virology **154**:302–312.
6. **Diamond, D. C., B. A. Jameson, J. Bonin, M. Kohara, S. Abe, H. Ito, T. Komatsu, M. Arita, S. Kuge, A. D. M. E. Osterhaus, R. Crainic, A. Nomoto, and E. Wimmer.** 1985. Antigenic variation and resistance to neutralization in poliovirus type 1. Science **229**:1090–1093.
7. **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.
8. **Gaul, S. K., T. F. Simpson, G. N. Woode, and R. W. Fulton.** 1982. Antigenic relationships among some animal rotaviruses: virus neutralization in vitro and cross-protection in piglets. J. Clin. Microbiol. **16**:495–503.
9. **Glass, R. I., J. Keith, O. Nakagomi, T. Nakagomi, J. Askaa, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1985. Nucleotide sequence of the structural glycoprotein VP7 gene of Nebraska calf diarrhea virus rotavirus: comparison with homologous genes from four strains of human and animal rotaviruses. Virology **141**:292–298.
10. **Gorziglia, M., Y. Hoshino, A. Buckler-White, I. Blumenthals, 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.

10a. **Green, K. Y., K. Midthun, M. Gorziglia, Y. Hoshino, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1987. Comparison of the amino acid sequences of the major neutralization protein of four human rotavirus serotypes. Virology **161**:153–159.

10b. **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.

11. **Greenberg, H. B., J. Flores, A. R. Kalica, R. G. Wyatt, and R. Jones.** 1983. Gene coding assignments for growth restriction, neutralization and subgroup specificities of the W and DS-1 strains of human rotavirus. J. Gen. Virol. **64**:313–320.
12. **Gunn, P. R., F. Sato, K. F. H. Powell, A. R. Bellamy, J. R. Napier, D. R. K. Harding, W. S. Nanock, L. J. Liegman, and**

G. W. Both. 1985. Rotavirus neutralizing protein VP7: antigenic determinants investigated by sequence analysis and peptide synthesis. J. Virol. **54**:791–797.

12a. **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.

13. **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.

14. **Kalica, A. R., H. B. Greenberg, R. G. Wyatt, J. Flores, M. M. Sereno, A. Z. Kapikian, and R. M. Chanock.** 1981. Genes of human (strain Wa) and bovine (strain UK) rotaviruses that code for neutralization and subgroup antigens. Virology **112**:385–390.

15. **Kapikian, A. Z., and R. M. Chanock.** 1985. Rotaviruses, p. 863–906. *In* B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman, and R. E. Shope (ed.), Virology. Raven Press, New York.

16. **Matsuno, S., A. Hasegawa, A. Mukoyama, and S. Inouye.** 1985. A candidate for a new serotype of human rotavirus. J. Virol. **54**:623–624.

17. **Minor, P. D., G. C. Schild, J. Bootman, D. M. A. Evans, M. Ferguson, P. Reeve, M. Spitz, G. Stanway, A. J. Cann, R. Hauptman, L. D. Clarke, R. C. Mountford, and J. W. Almond.** 1983. Location and primary structure of a major antigenic site for poliovirus neutralization. Nature (London) **301**:674–679.

17a. **Morita, Y., K. Taniguchi, T. Urasawa, and S. Urasawa.** 1988. Analysis of serotype-specific neutralization epitopes on VP7 of human rotavirus by the use of neutralizing monoclonal antibodies and antigenic variants. J. Gen. Virol. **69**:451–458.

18. **Offit, P. A., R. D. Shaw, and H. B. Greenberg.** 1986. Passive protection against rotavirus-induced diarrhea by monoclonal antibodies to surface proteins VP3 and VP7. J. Virol. **58**:700–703.

19. **Richardson, M. A., A. Iwamoto, N. Ikegami, A. Nomoto, and Y. Furuichi.** 1984. Nucleotide sequence of the gene encoding the serotype-specific antigen of human (Wa) rotavirus: comparison with the homologous genes from simian SA-11 and UK bovine rotaviruses. J. Virol. **51**:860–862.

20. **Taniguchi, K., Y. Morita, T. Urasawa, and S. Urasawa.** 1987. Cross-reactive neutralization epiotpes on VP3 on human rotavirus: analysis with monoclonal antibodies and antigenic variants. J. Virol. **61**:1726–1730.

21. **Taniguchi, K., S. Urasawa, and T. Urasawa.** 1985. Preparation and characterization of neutralizing monoclonal antibodies with different reactivity patterns to human rotaviruses. J. Gen. Virol. **66**:1045–1053.

22. **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.

23. **Urasawa, S., T. Urasawa, and K. Taniguchi.** 1982. Three human rotavirus serotypes demonstrated by plaque neutralization of isolated strains. Infect. Immun. **38**:781–784.

24. **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.

25. **Woode, G. N., N. E. Kelso, T. F. Simpson, S. K. Gaul, L. E. Evans, and K. Babiuk.** 1983. Antigenic relationships and cross-protection among some bovine rotavirus: serum neutralization and cross-protection in gnotobiotic calves. J. Clin. Microbiol. **18**:505–508.