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Complete Nucleotide Sequence of the Gene Encoding VP4 of a Human Rotavirus (Strain K8) Which Has Unique VP4 Neutralization Epitopes

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In our previous study (K. Taniguchi, Y. Morita, T. Urasawa, and S. Urasawa, J. Virol. 62:2421–2426, 1987) in which the cross-reactive neutralization epitopes on VP4 of human rotaviruses were analyzed, one strain, K8, was found to bear unique VP4 neutralization epitopes. This strain, which belongs to subgroup II and serotype 1, was not neutralized by any of six anti-VP4 neutralizing monoclonal antibodies which reacted with human rotavirus strains of serotypes 1, 3, and 4 or serotypes 1 through 4. We determined the complete nucleotide sequence of the gene encoding VP4 of strain K8 by primer extension. The VP4 gene is 2,359 base pairs in length, with 5′ and 3′ noncoding regions of 9 and 25 nucleotides, respectively. The gene contains a long open reading frame of 2,325 bases capable of coding for a protein of 775 amino acids. When compared with those of other human rotaviruses, VP4 of strain K8 had an insertion of one amino acid after residue 135, as found in simian rotavirus strains, and in addition, it had a deletion of one amino acid (residue 575). The amino acid homology of VP4 of strain K8 and those of other virulent human rotaviruses was only 60 to 70%. This was unusual, since over 90% VP4 homology has been found among the other virulent human rotavirus strains. In contrast, the VP7 amino acid sequence of the K8 strain was quite similar (over 98% homology) to those of other serotype 1 human rotaviruses. Thus, the K8 strain appears to have a unique VP4 gene previously not described.

The rotavirus virion contains 11 double-stranded RNA segments enclosed within the inner shell of its double-shelled capsid (14). Two capsid proteins, VP4 and VP7, are constituents of the outer capsid, and they are independent neutralization antigens (9, 14, 22). VP7 encoded by RNA segment 8 or 9 defines serotype specificity (8, 12). The product of RNA segment 4, VP4, is responsible for growth restriction of fastidious rotavirus strains in cell culture and for protease-enhanced plaque formation (2, 3, 11). VP4 is also associated with rotavirus virulence. In a murine model, the gene encoding VP4 determined the dose of SA-11 and NCDV rotaviruses required to induce experimental gastroenteritis (23). Also, in humans, a dimorphism of VP4 sequence is found between virulent and asymptomatic strains (5–7). Moreover, VP4 appears to be responsible for some cross-neutralization or cross-protection among serotypes (15, 27, 28, 36–38).

We previously prepared six anti-VP4 neutralizing monoclonal antibodies (N-MAbs) reactive with human rotavirus (HRV) strains of serotypes 1 through 4 or serotypes 1, 3, and 4 (28, 30). By sequencing the VP4 gene of antigenic mutants resistant to each of the six N-MAbs, we identified the amino acid residues involved in cross-neutralization (27). However, one HRV strain (K8) did not react with any of the six anti-VP4 N-MAbs, although it was neutralized by each of five anti-VP7 serotype 1-specific N-MAbs directed against different epitopes (21, 26). These results implied that strain K8 has a unique VP4, prompting us to characterize its VP4 gene by sequence analysis. In this report, we present the complete nucleotide sequence of the gene encoding VP4 of strain K8 and compared it with those of other human and simian rotavirus strains.

Strain K8 was derived from a stool sample from a schoolboy of 14 years of age, who became ill during an outbreak of gastroenteritis which occurred in Kitami, Hokkaido, Japan, in 1977. The outbreak affected over 53 people, of whom 39 (73.6%) were over 6 years of age. The virus was successfully cultivated in cell culture (34). Subsequently, the virus was grown in MA-104 cells in the presence of trypsin (1 μg/ml) and purified by fluorocarbon treatment and CsCl gradient centrifugation. Viral mRNA was synthesized in vitro by the endogenous transcriptase present in viral cores obtained by treatment with EDTA. Synthetic oligonucleotide primers were used to sequence mRNA by reverse transcriptase in the presence of dideoxynucleotide as described previously (7). The 3′-terminal 200 nucleotides of the viral RNA were determined by using denatured double-stranded virion RNA.

In our previous studies (28, 33–35), strain K8 was serologically characterized as belonging to subgroup II by the use of anti-VP6 subgroup-specific MAbs and to serotype 1 by neutralization and an enzyme-linked immunosorbent assay (ELISA), using polyclonal antibodies or N-MAbs, respectively. However, it did not react by neutralization or ELISA with six anti-VP4 cross-reactive N-MAbs that recognize VP4s of serotype 1 strains (28).

Figure 1 shows the complete nucleotide and the deduced amino acid sequence of the K8 VP4 gene. The sequence has a single long open reading frame of 2,325 bases, which begins with ATG at position 10 to 12 and terminates with TAG at position 2335 to 2337. The first ATG conforms to the consensus sequence for strong translation initiation (AN NATGG) (16). By direct amino acid sequence analysis of

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          12          24          36          48          60          72          84          96         108         120
GGC TAT AAA ATG GCT TCT TTA ATT TAT AGA CAG TTA TTA TCA AAC TCA TAT GTT ACA AAC ATC TCT GAC GAA GTT AAT GAA ATT GGA ACT AAA AAA ACA ACT AAC GTT ACT GTT AAT CCA
          MET Ala Ser Leu Ile Tyr Arg Gln Leu Leu Ser Asn Ser Tyr Val Thr Asn Ile Ser Asp Glu Val Asn Glu Ile Gly Thr Lys Lys Thr Thr Asn Val Thr Val Asn Pro

         132         144         156         168         180         192         204         216         228         240
GGG CCA TTC GCA CAA ACG GGA TAT GCG CCT GTT GAC TGG GGA CAT GGT GAA TTG CCT GAT TCT ACA TTG GTG CAG CCA ACT CTT GAT GGT CCA TAC CAA CCC ACC TCA CTC AAC TTA CCA
Gly Pro Phe Ala Gln Thr Gly Tyr Ala Pro Val Asp Trp Gly His Gly Glu Leu Pro Asp Ser Thr Leu Val Gln Pro Thr Leu Asp Gly Pro Tyr Gln Pro Thr Ser Leu Asn Leu Pro

         252         264         276         288         300         312         324         336         348         360
GTT GAT TAT TGG ATG TTA ATT GCG CCT ACT AGA GAA GGA AAA GTT GCT GAA GGT ACA AAT ACG ACT GAC AGA TGG TTC GCT TGT GTA CTA GTT GAA CCA AAT GTG CAA AAT ACA CAA AGA
Val Asp Tyr Trp MET Leu Ile Ala Pro Thr Arg Glu Gly Lys Val Ala Glu Gly Thr Asn Thr Thr Asp Arg Trp Phe Ala Cys Val Leu Val Glu Pro Asn Val Gln Asn Thr Gln Arg

         372         384         396         408         420         432         444         456         468         480
CAA TAC GTA TTA GAT GGG CAA AAT GTC CAA TTG CAT GTC TCA AAC GAT TCA AGT ACT TCG TGG AAA TTT ATA TTA TTC ATT AAA TTG ACG CCT TAT GGA ACG TAC ACT CAA TAT TCA ACA
Gln Tyr Val Leu Asp Gly Gln Asn Val Gln Leu His Val Ser Asn Asp Ser Ser Thr Ser Trp Lys Phe Ile Leu Phe Ile Lys Leu Thr Pro Tyr Gly Thr Tyr Thr Gln Tyr Ser Thr

         492         504         516         528         540         552         564         576         588         600
TTA TCA ACG CCG CAT AAG TTG TGC GCG TGG ATG AAA AGA GAT AAC AGA GTG TAC TGG TAT CAA GGA GCG ACA CCG AAC GCA TCA GAG AGC TAT TAC TTG ACC ATA AAC AAT GAT AAC AGC
Leu Ser Thr Pro His Lys Leu Cys Ala Trp MET Lys Arg Asp Asn Arg Val Tyr Trp Tyr Gln Gly Ala Thr Pro Asn Ala Ser Glu Ser Tyr Tyr Leu Thr Ile Asn Asn Asp Asn Ser

         612         624         636         648         660         672         684         696         708         720
AAC GTT TCA AGT GAC GCT GAA TTT TAT TTA ATA CCG CAA TCG CAG ACC GCT ATG TGT ACA CAA TAT ATA AAC AAT GGT TTA CCA CCA ATT CAG AAT ACC AGG AAT ATT GTA CCA GTA AAT
Asn Val Ser Ser Asp Ala Glu Phe Tyr Leu Ile Pro Gln Ser Gln Thr Ala MET Cys Thr Gln Tyr Ile Asn Asn Gly Leu Pro Pro Ile Gln Asn Thr Arg Asn Ile Val Pro Val Asn

         732         744         756         768         780         792         804         816         828         840
ATT ACA TCT AGA CAG ATT AAA GAC GTA AGA GCT CAG ATG AAT GAA GAC ATA GTA ATA TCA AAA ACC TCG TTA TGG AAA GAA ATG CAA TAT AAT AGA GAT ATA ATC ATT AGA TTT AAA TTT
Ile Thr Ser Arg Gln Ile Lys Asp Val Arg Ala Gln MET Asn Glu Asp Ile Val Ile Ser Lys Thr Ser Leu Trp Lys Glu MET Gln Tyr Asn Arg Asp Ile Ile Ile Arg Phe Lys Phe

         852         864         876         888         900         912         924         936         948         960
GCT AAT TCA ATA ATC AAA TCA GGT GGA CTA GGT TAT AAG TGG TCG GAA ATA TCA TTT AAG CCC ATG AAC TAT CAA TAT ACG TAT ACG AGA GAT GAA GAA GAA GTA ACA GCA CAT ACA ACA
Ala Asn Ser Ile Ile Lys Ser Gly Gly Leu Gly Tyr Lys Trp Ser Glu Ile Ser Phe Lys Pro MET Asn Tyr Gln Tyr Thr Tyr Thr Arg Asp Glu Glu Glu Val Thr Ala His Thr Thr

         972         984         996        1008        1020        1032        1044        1056        1068        1080
TGT TCA GTT GGT GGT GTC AAT GAT TTT AAT TAT AAT GGA GGT ACG TTG CCT ACT GAT TTT GCA ATA TCG AGA TTT GAG GTC ATA AAG GAA AAT TCT TAT GTA TAT GTA GAT TAT TGG GAT
Cys Ser Val Gly Gly Val Asn Asp Phe Asn Tyr Asn Gly Gly Thr Leu Pro Thr Asp Phe Ala Ile Ser Arg Phe Glu Val Ile Lys Glu Asn Ser Tyr Val Tyr Val Asp Tyr Trp Asp

        1092        1104        1116        1128        1140        1152        1164        1176        1188        1200
GAT TCA CAA GCA TTT AGA AAT ATG GTA TAT GAA GGT ACG TTA GCT GCA AAT TTG AAT GAT GTA GTA TGC AGT GGA GGT TCT TAC AGT TTT GCG TTA CCT GTA GGC AAT CAT CCG GTG ATA
Asp Ser Gln Ala Phe Arg Asn MET Val Tyr Glu Gly Thr Leu Ala Ala Asn Leu Asn Asp Val Val Cys Ser Gly Gly Ser Tyr Ser Phe Ala Leu Pro Val Gly Asn His Pro Val Ile

        1212        1224        1236        1248        1260        1272        1284        1296        1308        1320
GAT GGT GGT GCA GTG ACC TTA ACA TCT GCT GGC GTA ACA CTA TCA ACC CAG TAT ACA GAT TAT GTA TCA TTA AAT TCA TTG CAA TTC AGA TTC AGA TTG GCG GTG AGC GAA CCA TCA TTT
Asp Gly Gly Ala Val Thr Leu Thr Ser Ala Gly Val Thr Leu Ser Thr Gln Tyr Thr Asp Tyr Val Ser Leu Asn Ser Leu Gln Phe Arg Phe Arg Leu Ala Val Ser Glu Pro Ser Phe

        1332        1344        1356        1368        1380        1392        1404        1416        1428        1440
TCC ATC TCG CGA ACT AGA ATG AGT GGC ATA TAT GGA TTA CCA GCT GTA AAT CCA AAT AAT AGC GCA GAA TAT TAT GAG ATA GCT GGT AGA TTC TCA CTC ATA TCA CTA GTA CCA ACA AAT
Ser Ile Ser Arg Thr Arg MET Ser Gly Ile Tyr Gly Leu Pro Ala Val Asn Pro Asn Asn Ser Ala Glu Tyr Tyr Glu Ile Ala Gly Arg Phe Ser Leu Ile Ser Leu Val Pro Thr Asn

        1452        1464        1476        1488        1500        1512        1524        1536        1548        1560
GAT GAC TAT CAA ACG CCA ATC GCT AAT TCA GTT ACC GTA AGA CAA GAT TTA GAG AGA CAA TTA GGC GAG TTA AGA GAA GAA TTT AAT TCG TTG TCA CAA GAA ATA GCT GTT TCC CAA CTT
Asp Asp Tyr Gln Thr Pro Ile Ala Asn Ser Val Thr Val Arg Gln Asp Leu Glu Arg Gln Leu Gly Glu Leu Arg Glu Glu Phe Asn Ser Leu Ser Gln Glu Ile Ala Val Ser Gln Leu

        1572        1584        1596        1608        1620        1632        1644        1656        1668        1680
ATA GAT CTA GCA ACA TTA CCG CTT GAT ATG TTC TCA ATG TTC TCT GGA ATA AAA TCA ACG GTA GAG GCA GTA AAA TCT ATG ACT ACG AAT GTG ATG AAA AGA TTT AAA ACA TCA AGT TTA
Ile Asp Leu Ala Thr Leu Pro Leu Asp MET Phe Ser MET Phe Ser Gly Ile Lys Ser Thr Val Glu Ala Val Lys Ser MET Thr Thr Asn Val MET Lys Arg Phe Lys Thr Ser Ser Leu

        1692        1704        1716        1728        1740        1752        1764        1776        1788        1800
GCA AAC GCC ATA TCT GAT TTA ACA AGT AAT ATG TCG GAA GCG GCA TCA TCT GTA AGA TTG ACG TCA GTG AGA TCG GTG GGC ACT ATT ACA TTG CCA AGA GCT AGG GTT TCA TTG CAA GTG
Ala Asn Ala Ile Ser Asp Leu Thr Ser Asn MET Ser Glu Ala Ala Ser Ser Val Arg Leu Thr Ser Val Arg Ser Val Gly Thr Ile Thr Leu Pro Arg Ala Arg Val Ser Leu Gln Val

        1812        1824        1836        1848        1860        1872        1884        1896        1908        1920
GGC GAT GAC TTG AGG TCC ATG CAA GAC GTA TCA ACA CAA GTG TCA AAT GTG AGT AGA AAT TTA AGA TTG AAA GAG TTC ACG ACG CAA ACT GAT ACT TTA AGC TTT GAT GAC ATC TCT GCA
Gly Asp Asp Leu Arg Ser MET Gln Asp Val Ser Thr Gln Val Ser Asn Val Ser Arg Asn Leu Arg Leu Lys Glu Phe Thr Thr Gln Thr Asp Thr Leu Ser Phe Asp Asp Ile Ser Ala

        1932        1944        1956        1968        1980        1992        2004        2016        2028        2040
GCT GTA TTG AAG ACG AAA CTA GAT AAA TCG ACG CAA ATT TCA CAA CAA ACA ATG CCA GAT ATT ATA GCT GAG TCA TCT GAG AAG TTT ATA CCG AAG AGA TCG TAT AGA ATA GTT GAT GAG
Ala Val Leu Lys Thr Lys Leu Asp Lys Ser Thr Gln Ile Ser Gln Gln Thr MET Pro Asp Ile Ile Ala Glu Ser Ser Glu Lys Phe Ile Pro Lys Arg Ser Tyr Arg Ile Val Asp Glu

        2052        2064        2076        2088        2100        2112        2124        2136        2148        2160
GAT ATT CGA TTC GAA ACT GGA ATT GAC GGA ACG TTT TAT GCT TAC AAA GTC GAT ACA TTT AAT GAA ATT CCG TTT GAT ATG GAA CGA TTT AAT AAA TTA ATA ACA GAT TCA CCA GTT TTA
Asp Ile Arg Phe Glu Thr Gly Ile Asp Gly Thr Phe Tyr Ala Tyr Lys Val Asp Thr Phe Asn Glu Ile Pro Phe Asp MET Glu Arg Phe Asn Lys Leu Ile Thr Asp Ser Pro Val Leu

        2172        2184        2196        2208        2220        2232        2244        2256        2268        2280
TCA GCA ATA ATA GAC TTT AAG ACG TTA AAG AAC TTA AAC GAC AAT TAT GGA ATA ACA AAG AAA CAA GCC ATG GAA CTA TTA CAT TCA AAT CCA AAG ACA TTA AAA GAG TTT ATA AAT AAT
Ser Ala Ile Ile Asp Phe Lys Thr Leu Lys Asn Leu Asn Asp Asn Tyr Gly Ile Thr Lys Lys Gln Ala MET Glu Leu Leu His Ser Asn Pro Lys Thr Leu Lys Glu Phe Ile Asn Asn

        2292        2304        2316        2328        2340        2352
AAT AAT CCA ATA ATT AGA AAT AGA ATC GAA AAC TTA ATA TCG CAG TGT AGG TTG TAG CTG TCT ATT TTA AGA TGT GAC C
Asn Asn Pro Ile Ile Arg Asn Arg Ile Glu Asn Leu Ile Ser Gln Cys Arg Leu
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TABLE 1. Nucleotide and amino acid sequence homology of VP4 of strain K8 and of other human or simian rotavirus strains

Strain (serotype)	% Homology with VP4 of strain K8	
	Nucleotide sequence	Amino acid sequence
Virulent human rotavirus		
KU (1)	65.4	64.3
Wa (1)	65.6	64.3
DS-1 (2)	66.3	65.7
RV-5 (2)	65.9	65.2
P (3)	66.0	65.0
VA70 (4)	65.2	63.7
Asymptomatic human rotavirus		
M37 (1)	64.4	65.8
1076 (2)	64.0	65.0
McN13 (3)	64.2	65.7
ST-3 (4)	64.3	65.4
Simian rotavirus		
SA-11 (3)	67.6	70.5
RRV (3)	66.8	69.7

SA-11 VP5, two trypsin cleavage sites (arginine residues 241 and 247) have been identified previously (18). These two arginine residues are also conserved in strain K8. However, the six amino acids of the connecting peptide of K8, i.e., amino acids 242 to 247, were quite different from those found in other human rotavirus strains (Fig. 2). Furthermore, when compared with other HRV strains, VP4 of strain K8 was found to have an insertion of one amino acid after residue 135 (as described in two simian rotavirus strains), and in addition, it lacked one amino acid at residue 575.

The K8 nucleotide and amino acid sequences were compared with those of several virulent and asymptomatic HRV strains as well as two simian rotavirus strains. The overall nucleotide sequence homology of VP4 of strain K8 and of other virulent HRV strains was only 65 to 67% (Table 1). This was in sharp contrast to the high degree of homology (90% or more) found among other virulent HRV strains (6, 7). In recent studies (6, 7), asymptomatic neonatal HRV strains representing each of four serotypes were found to have a high degree of nucleotide homology (more than 90%) among themselves but a relatively low degree of nucleotide sequence homology (68 to 72%) with virulent strains of corresponding serotype. The K8 strain also had a low degree of sequence homology (64 to 65%) with the asymptomatic strains. In addition, a low degree of homology (66 to 68%) between strain K8 and two simian rotavirus strains was also observed. These differences were also reflected at the amino acid level (Table 1).

Several neutralization epitopes on the VP8 fragment of VP4 have been identified in rhesus rotavirus (RRV) (19), although these sites have not yet been defined on human strains. These epitopes seem to be responsible for strain-specific neutralization, since amino acid divergence in the VP8 region was high among rotavirus strains (6) and the N-MAbs directed against VP8 showed limited neutralization of heterotypic rotavirus strains (19). The amino acid sequence homology in VP8 of strain K8 and of the other rotavirus strains was quite low (Fig. 2). Compared with the low homology of amino acid sequence in VP8 of rotavirus strains, the N-terminal half of VP5, the larger cleaved product of VP4, was relatively conserved among rotavirus strains including strain K8 (Fig. 2). In our previous study (27), we identified an amino acid critical to individual cross-reactive neutralization epitopes on the VP5 fragment of VP4 by using KU (serotype 1 human strain) antigenic mutants resistant to each of the six anti-VP4 cross-reactive N-MAbs. At the positions where amino acid substitutions occurred in the antigenic mutants, there was a correlation between conservation of sequence among different HRVs and the reactivity of these viruses with antibodies used for selection of mutants. Amino acid residue 305 (Leu) which is found to be critical to epitope I was different in strain K8 (Thr for residue 306) from the amino acid in other strains (Fig. 3). In addition, the amino acid critical to epitope II was shown to be position 392 (Ala) or 439 (Leu), but the amino acid corresponding to these positions (393 or 440 in strain K8) was different in strain K8 (Asn or Ser). The amino acid critical to epitope III was conserved at position 434 of the strain K8, but an amino acid sequence difference at the preceding position (433 for strain K8 and 432 for other human strains) was found. Our previous study (27) suggested that this preceding residue is also critical to epitope III. These differences in amino acid residues may explain the lack of reactivity of strain K8 with the anti-VP4 cross-reactive N-MAbs.

The gene encoding VP7 of strain K8 was also sequenced by primer extension. The deduced VP7 amino acid sequence of strain K8 exhibited an extremely high homology to those of other serotype 1 human strains; only two or five amino acid residues were different between K8 and KU or Wa, respectively (data not shown).

Thus, strain K8, which shares a VP7 similar to those of the serotype 1 viruses, carries a unique VP4 gene which has not been previously detected. These data strongly support the concept that VP4 and VP7 have independent neutralization specificities (9). With respect to VP7 among HRV strains, six different serotype specificities have been reported previously (1, 10, 20). There have been only a few studies examining the number of neutralization specificities of VP4 (6, 7, 19, 27). By serological characterization and amino acid sequence analysis, it has been shown that among virulent strains the VP4s of HRV strains of serotypes 1, 3, and 4 are highly conserved and that there is an apparent diversity between VP4 of serotype 2 strains and those of serotype 1, 3, and 4 strains (6, 7, 27, 29). Furthermore, a considerably lower degree of homology has been found between the VP4s of virulent and asymptomatic HRV strains. In this study, the VP4 of K8 was found to be unique. Thus, there appear to exist at least four varieties of VP4 among HRV strains: (i) virulent strains of serotypes 1, 3, and 4; (ii) virulent strains of serotype 2; (iii) asymptomatic strains of serotypes 1, 2, 3, and 4; and (iv) the virulent K8 strain of serotype 1 and other strains having K8-like VP4.

Strain K8 has several other characteristics that follow. (i) In comparison with other HRV strains, it grows efficiently in cell culture and produces relatively large plaques (34). (ii) In

FIG. 1. Complete nucleotide sequence and deduced amino acid sequence of the VP4 gene of strain K8. The numbers above the sequence refer to the nucleotide positions. Cleavage sites are indicated by arrows, and the corresponding connecting peptide is underlined. The positions of nucleotide insertion and deletion found in K8 VP4 gene are shown boxed and indicated by the arrowhead, respectively.

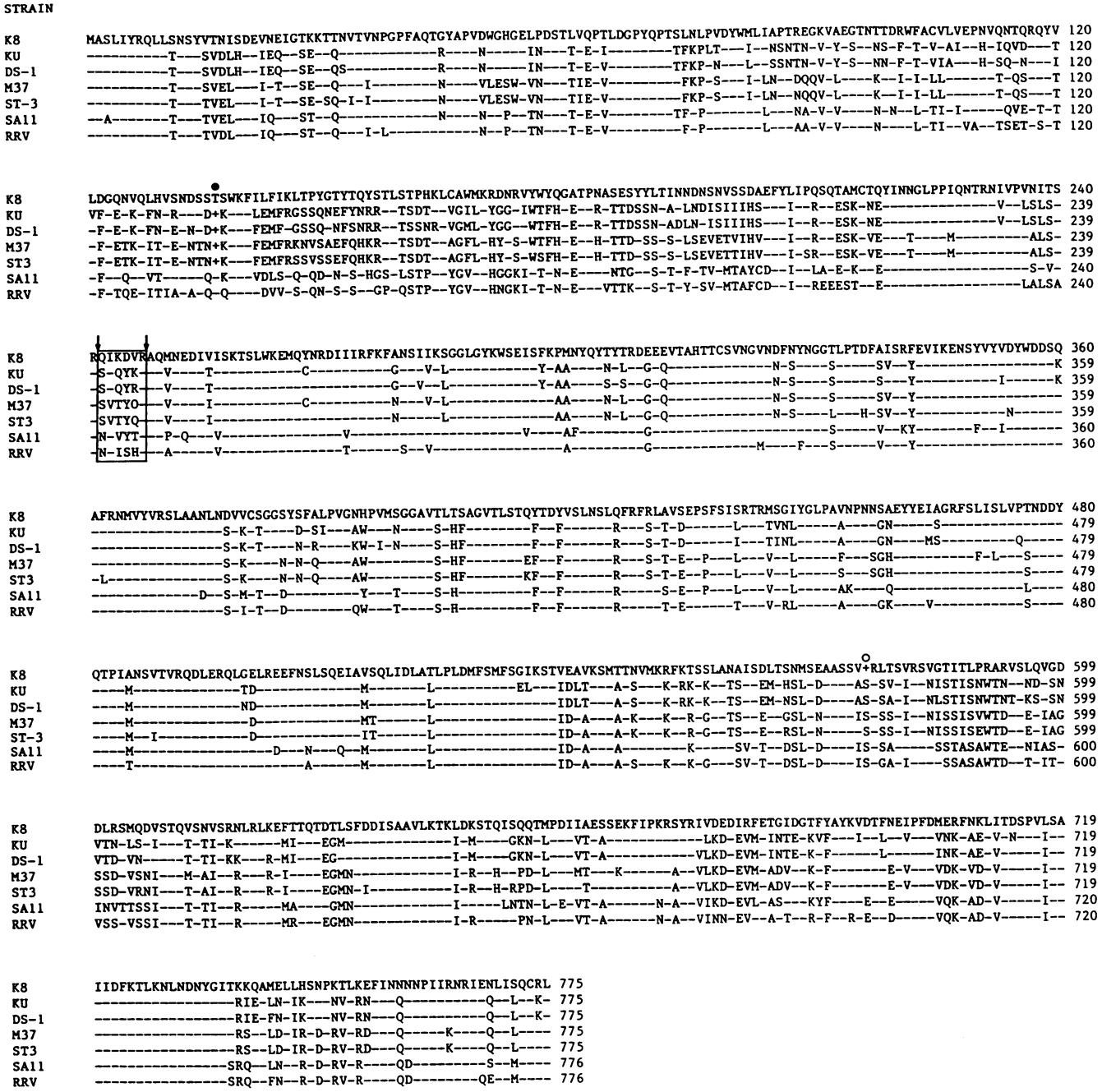

FIG. 2. Comparison of VP4 amino acid sequence of strain K8 with those of other human and simian rotavirus strains. The entire VP4 amino acid sequences of KU, DS-1, M37, ST-3, SA-11, and RRV have been previously described (6, 7, 17–19, 27). The positions of the predicted amino acid insertion (●) and deletion (○) in K8 VP4 are indicated. The cleavage sites are shown by arrows, and the site of connecting peptide is boxed.

its RNA pattern, the differences in electrophoretic mobility between segments 2 and 3 and between 10 and 11 are wider than those for other serotype 1 and subgroup II strains examined thus far (29). (iii) The strain is not recognized by anti-VP2 MAb (YO-60 antibody), which is reactive exclusively with subgroup II strains (32). The relationship of the K8 VP4 gene with a variety of animal rotavirus strains needs further study. In an RNA-RNA hybridization analysis using ^{32}P-labeled single-stranded K8 probe and virion RNA from bovine (NCDV and UK), simian (SA-11 and MMU18006), porcine (Gottfried), equine (H2 and FI-14), and feline (Cat 97) strains, we could not detect any evidence of genetic relatedness between the VP4 gene of K8 and the VP4 genes of these animal strains (Fig. 4). Similar studies with a variety of naturally occurring human rotavirus strains and K8 virus need to be carried out. In addition, the preparation of N-MAbs specific to K8 VP4 would be useful in examining the prevalence of rotavirus strains which have a K8-like VP4 by using ELISA as the detection method in both humans and animals (31).

Strain (Serotype)	Amino acid critical to epitope I		Amino acid critical to epitope II		Amino acid critical to epitope III	Amino acid critical to epitope II	
KU (1)	296	KAANYQYNYLRDGEQVTA	313	380 TGGSYDFSIPVGAWPVMNGGA 400	428	SLTVDEPSFSILRTRTVNLY	447
Wa (1)	296	----------------	313	380 -----N---------- 400	428	-------P----------	447
K8 (1)	297	-PM----T-T--E-E---	314	381 S----S-AL---NH---S--- 401	429	R-A-S-----S---MSGI-	448
DS-1 (2)	296	-------S-S--------	313	380 -----N-RL---K--I----- 400	428	----------I----I---	447
RV-5 (2)	296	-------S-S--------	313	380 -------RL---G--I----- 400	428	----------I----I---	447
P (3)	296	----------------	313	380 ---------------- 400	428	----------------	447
VA-70 (4)	296	----------------	313	380 ---------------- 400	428	----------M---I----	447

FIG. 3. Comparison of amino acid residues critical to cross-reactive neutralization epitopes on VP4 of human rotavirus strains. The entire VP4 amino acid sequences of the strains other than strain K8 have been previously described (6, 7, 13, 19, 27).

It has been shown that antibody to VP4 can mediate protection (24). The probability of antibody acquisition in infants against VP4 of K8-like strains may be less, since K8 VP4 lacks the cross-reactive neutralization epitopes found in serotype 1, 3, and 4 strains. The survey of prevalence of neutralizing antibodies to HRV in different age groups has shown that antibody distribution patterns were quite different between the two serotype 1 strains (KU and K8); the appearance of antibodies against the K8 strain after birth was delayed significantly (35). This is most likely reflected by divergent VP4s, since a difference of only two amino acids was observed on VP7 between the two strains. Thus, the unique gene 4 of the K8 strain may have a role in allowing the K8 strain to cause epidemic illness in older children. We have recently studied the antibody response to each of several neutralization epitopes on VP7 and VP4 in human sera by competition binding assay using N-MAbs (25; K. Taniguchi et al., manuscript in preparation). It will also be of interest to examine the antibody prevalence to the unique VP4 of strain K8.

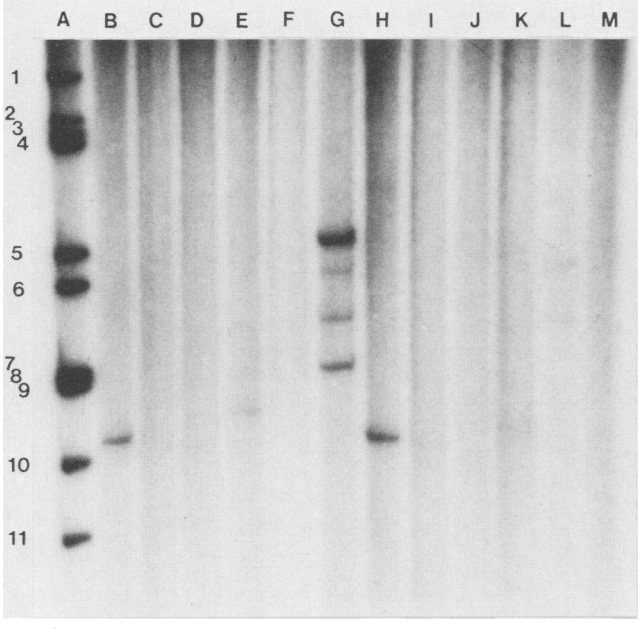

FIG. 4. Hybridization of a labeled single-stranded RNA probe from strain K8 to genomic RNAs from human, feline, bovine, simian, equine, and porcine rotavirus strains. Before the RNA-RNA hybridization experiments, the amount of the double-stranded RNA from different strains was checked by staining the double-stranded RNA in the gel with ethidium bromide; we employed almost equal amounts of each double-stranded RNA for the hybridization tests. The RNA-RNA hybridization was performed as described previously (4). Lanes: A, K8 (human); B, P (human); C, DS-1 (human); D, Taka (feline); E, NCDV (bovine); F, SA-11 (simian); G, KU (human); H, ST-3 (human); I, RRV (simian); J, H2 (equine); K, UK (bovine); L, Gottfried (porcine); M, FI-14 (equine). Numbers to the left of the gel show the RNA segment order.

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