Genetic Variation in VP7 Gene of Human Rotavirus Serotype 2 (G2 Type) Isolated in Japan, China, and Pakistan

Leying Wen1,2, Hiroshi Ushijima*,1,3, Junko Kakizawa1,3, Zhao-Yin Fang2, Osamu Nishio1, Shigeru Morikawa4, and Takashi Motohiro5

1 Institute of Public Health, Minato-ku, Tokyo 108, Japan, 2 Institute of Virology, Chinese Academy of Preventive Medicine, Beijing 100052, China, 3 Department of Maternal and Child Health, Faculty of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan, 4 National Institute of Health, Musashimurayama, Tokyo 208, Japan, and 5 Department of Pediatrics, Kurume University School of Medicine, Kurume, Fukuoka 830, Japan

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Abstract: Sequence analysis of the gene encoding the major neutralization glycoprotein (VP7) was performed on sixteen human isolates of serotype 2 of rotavirus in Japan, China, and Pakistan and their genetic variations were examined. Comparative studies of their nucleotide and deduced amino acid sequences between the sixteen isolates and the HU5 strain revealed an overall homology of more than 94%. A higher degree of homology in nucleotides was observed among the sixteen isolates than between HU5 and the isolates. A total of thirteen amino acid residues frequently converted to another amino acid. Out of the thirteen, five amino acid residues belonging to the major neutralizing epitope regions (C, E, and F in this communication) converted frequently. From the amino acid sequences three subtypes, subtype 1, subtype 2, and intermediate, were suggested to be classified as previously reported for serotype 1 (Xia et al, Virology, 1993, 197: 813–816).

Key words: Rotavirus, Serotype 2, Gene, Amino acid

Sequence data from this article have been deposited in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases under Accession nos. D50112 (CHIN1 strain), D50113 (CHIN3 strain), D50114 (CHIN5 strain), D50115 (CHIN7 strain), D50116 (CHIN8 strain), D50117 (JAPAN0022 strain), D50118 (JAPAN038 strain), D50119 (JAPAN076 strain), D50120 (JAPAN085 strain), D50121 (JAPAN137 strain), D50122 (JAPAN21 strain), D50123 (JAPAN58 strain), D50124 (KUN strain), D50125 (PAK426 strain), D50126 (PAK458 strain), and D50127 (TMC-II strain).

Rotavirus is an important agent causing acute gastroenteritis worldwide, especially in developing countries. Since effective anti-rotavirus drugs have not been developed, a rotavirus vaccine is an essential goal. Vaccine trials have indicated that an effective vaccine should contain strains antigenically similar to those circulating in the community (5, 6, 14). Because there are four main human serotypes, it is also possible that immunity to each of the rotavirus serotypes may be required to induce complete protection. Rhesus rotavirus tetravalent reassortant vaccine is safer and reduces the incidence of rotavirus gastroenteritis to a greater degree than the monovalent vaccine (2).

Group A rotaviruses are most commonly recognized and they have been divided into fourteen serotypes (G types) based on reaction to VP7-specific neutralizing antibodies (3). Antibody resistance patterns and sequence analyses have revealed that six clustered sequence divergences on VP7, located at amino acids 39-50 (A region), 87-101 (B region), 120-130 (C region), 143-152 (D region), 208-221 (E region), and 233-242 (F region), are involved in the neutralization of human rotaviruses (12). When the same regions were compared among rotaviruses belonging to the same serotype, a high degree of homology, ranging from 91–99%, was detected (4, 10, 12).

We have continued epidemiological studies of rotavirus infections for more than ten years by enzyme immunoassay using serotype-specific monoclonal anti-

Abbreviations: cDNA, complementary DNA; EIA, enzyme immunoassay; G, glycoprotein; MAb, monoclonal antibody; P, proteolytic protein; PCR, polymerase chain reaction; RT, reverse transcription; VP, virus protein.
bodies. These studies showed that serotype 1 was the predominant type and that serotype 2 was the second or third most predominant type (17, 18).

Recent development of the reverse transcription and polymerase chain reaction (RT-PCR) has encouraged analysis of the nucleotide sequence of the VP7 gene (12, 19). In order to develop an effective rotavirus vaccine, it may be necessary to determine the heterogeneity, especially within the principal neutralizing regions, of the VP7 gene. Here we attempt to compare the nucleotide and the deduced amino acid sequences of G2 isolated in Japan in different years and also in China and Pakistan following our first report of human rotavirus G1 in Japan and China (19).

Sixteen isolates from children with gastroenteritis were used in this experiment: Three isolates (no. 21, no. 58, and no. 137) were collected from October 1992 to March 1993 in Kurume City, Fukuoka Prefecture, Japan; two (no. 0022 and no. 038) in 1983 in Aichi Prefecture, Japan; two (no. 076 and no. 085) in 1993 in Aichi Prefecture; five (no. 1, no. 3, no. 5, no. 7, and no. 8) from October 1989 to March 1990 in Henan Province, China; two (PAK426 and PAK458) in 1993 in Karachi, Pakistan; TMC-II in Tokyo, 1983, and cultured in MA104 cells; and KUN in Sendai, Japan, 1980, and cultured in MA104 cells (15, 16).

The sixteen isolates were determined to be serotype 2 by RNA polyacrylamide gel electrophoresis, enzyme immunoassay using mouse monoclonal antibodies (1), and RT-PCR with serotype-specific primer mixture following the modified method of Gouvea et al (11, 18).

The viral RNAs were extracted from the supernatant of 10% stool suspensions using Gentsch’s method (7). Full-length cDNA of the VP7 gene were reverse-transcribed with primer pairs of Beg 9 (5'-GGCTTTAAAA-GAGAGAATTCTCGTG-3', nucleotides 1-28) and End 9 (5'-GGTCACTCATACATCTTACCTAG-3', complementary sequence nucleotides 1062-1036), and avian myeloblastosis virus RT XL. Complementary DNAs were then amplified by PCR with the same primer pairs and EX Taq DNA polymerase. The first PCR product was amplified again with four sets of primer pairs as follows: Beg 9 and VP7-1' (5'-ACTGATCCTGTTGGC-GCCATTTTTC-3', complementary sequence nucleotides 395-373), VP7-6 (5'-TTATGATCTCCAGGACACCACTA-3', nucleotide 295-316) and RV1248 (5'-TGTTATTAGTTGACATATTTC-3'), complementary sequence nucleotide 650-628), VP7-8 (5'-TGGATATCGAGAAGACT-3', complementary sequence nucleotides 824-804), and VP7-4 (5'-ACTGATTTGTAAATG-3', complementary sequence nucleotides 824-804), and VP7-4 (5'-ACTGATTTGTAAATG-3', complementary sequence nucleotide 650-628). The nucleotide sequence of the second PCR product was determined by using a dideoxynucleotide sequencing kit.

The nucleotide and the deduced amino acid sequences of the sixteen isolates were compared with the sequences of other human strains of serotype 1 (Wa isolated in the United States in 1974), serotype 2 (HU5, S2, DS1, and HN126 isolated in Australia in 1985, in Japan in 1982, in the United States in 1976, and Venezuela in 1981, respectively), serotype 3 (P isolated in the United States in 1974), and serotype 4 (ST3 isolated in England in 1975 and VA70 isolated in Italy in 1981 (12)).

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Table 1. Percent homology in nucleotide sequences (upper-right side) and amino acid sequences (lower-left side) among 16 isolates, HU5 and S2.
Homology of 95–99% was noted between each isolate for both nucleotide and amino acid sequences. HU5 was less homologous to our serotype 2 isolates (94–96%) (Table 1). The results indicate that there were no remarkable differences in homology among the samples from Japan, China, and Pakistan.

The deduced amino acid sequences of the VP7 gene are shown in Fig. 1. HU5, DS1, and HN126 are quite similar sequences and the homology of amino acids among the three is 99% (12). Amino acids 51, 75, 113, 125, 129, 178, 213, 236, 242, 255, 287, 306, and 319 are frequently converted to other amino acids. Among them, amino acids 125, 129, 213, 236, and 242 belong to the major neutralizing epitope regions (C, E, and F). These changes are within serotype 2 and the amino acid sequences are quite different from those in other serotypes (Wa for serotype 1, P for serotype 3, and ST3 and VA70 for serotype 4) (12).

Serotype 4 rotavirus strains have been classified into two antigenic “subtypes” by solid-phase immune electron
microscopy techniques (8), VP7-specific neutralizing monoclonal antibodies (9), and the deduced amino acid sequences (13). Serotype 1 strains were suggested to be divided into three subtypes from the deduced amino acid sequence (19). We may differentiate serotype 2 rotavirus into subtype 1 ("HU5 like"): HU5, DS1, HN126, subtype 2 ("JPN085 like"): CHIN1, CHIN3, CHIN5, CHIN7, CHIN8, JPN137, JPN58, JPN21, JPN0022, JPN038, JPN076, JPN085, PAK426, PAK458, S2), and intermediate type (TMC-II, KUN) by our deduced amino acid sequences. However, within clustered sequence divergences including the C, E, and F region in this report, four other subtypes may be differentiated as follows: 1) subtype 1', amino acid residues 125, 128, 213, 236, and 242 are N:V:N:N:N in strains HU5, HN126; 2) subtype 2', T:M:D:K:S in S2, CHIN3, CHIN7, JPN21, JPN0022, JPN038, JPN076, JPN085, PAK458; 3) subtype 3', T:I:D:K:S in JPN137, JPN58; and finally 4) intermediates between N:V:N:N:N and T:M:D:K:S in strains DS1, CHIN1, CHIN5, CHIN8, PAK426, TMC-II, KUN in which more than one amino acid residue changed from subtype 1' to 2' and vice versa. Further studies with neutralizing monoclonal antibodies may be necessary in order to confirm the subtypes.

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References
