

Serotype 3 Human Rotavirus Strains with Subgroup I Specificity

GIUSEPPE GERNA,^{1*} ANTONELLA SARASINI,¹ ANGELA DI MATTEO,¹ LORENA ZENTILIN,²
PAOLA MIRANDA,² MAURIZIO PAREA,¹ FAUSTO BALDANTI,¹ SERENELLA ARISTA,³
GABRIELE MILANESI,² AND MASSIMO BATTAGLIA⁴

Virus Laboratory, Institute of Infectious Diseases, University of Pavia,¹ and Consiglio Nazionale delle Ricerche Istituto di Genetica, Biochimica ed Evoluzionistica,² 27100 Pavia, Institute of Microbiology, University of Palermo, 90127 Palermo,³ and Consiglio Nazionale delle Ricerche Institute of Experimental Medicine, 00137 Rome,⁴ Italy

Received 31 October 1989/Accepted 6 March 1990

During an epidemiological study on human rotavirus (HRV) infections in Italy, three subgroup I strains not associated with serotype 2 reactivity were detected. All three strains were serotype 3, each with a distinct RNA pattern showing fast-moving tenth and eleventh segments (long electropherotype). Following successful adaptation to growth in cell cultures, the serotype 3 strains (MZ58, PCP5, and PA710) were further characterized by neutralization and by RNA-RNA (Northern blot) hybridization. Antiserum to reference HRV strain YO (subgroup II, serotype 3), as well as a monoclonal antibody to VP7 of YO neutralized, at comparable titers, the homologous virus, the three unusual HRV strains, and two reference simian strains (SA11 and RRV-2, both subgroup I, serotype 3), whereas SA11 antiserum and a monoclonal antibody to VP7 of SA11 neutralized simian strains more efficiently. However, antiserum to PCP5 neutralized the three unusual isolates and the simian strains at significantly higher titers than it did with reference strain YO. With ³²P-labeled RNA from MZ58 as a probe, a high degree of homology was detected by Northern blot hybridization with strains PCP5, PA710, SA11, and UK (bovine rotavirus) at the level of several segments and with strain YO only at the level of genes 7 to 9. Conversely, labeled RNA of strain YO hybridized extensively with Wa (subgroup II, serotype 1 HRV strain) but only at the level of genes 7 to 9 with MZ58, PCP5, PA710, SA11, and UK. Finally, the labeled SA11 probe hybridized at the level of RNA segments 1 to 3 and 6 to 11 to the three unusual strains. These findings suggest that the unusual subgroup I, serotype 3, strains isolated from humans are more likely to be animal rotaviruses rather than natural reassortants between different HRV strains.

Group A human rotavirus (HRV), the single most important etiologic agent of acute nonbacterial gastroenteritis in infants and young children, possesses two major antigenic specificities, i.e., subgroup and serotype, in addition to common group antigen. Human and animal strains may share subgroup or serotype antigens. Subgroup is specified by the major inner capsid protein, VP6 (17), and to a lesser extent by VP2 (28). The outer capsid proteins VP7 and, to a lesser extent, VP4 are responsible for serotype specificity (26). Both specificities are now defined by enzyme-linked immunosorbent assay (ELISA) with specific monoclonal antibodies (5, 17, 27, 29). For several years, subgroup specificity of HRV strains has been found by polyacrylamide gel electrophoresis analysis of viral genomic RNA to be associated with distinctive patterns (19); i.e., subgroup I strains have more slowly moving gene segments 10 and 11 (short pattern), whereas subgroup II strains have more quickly moving gene segments 10 and 11 (long pattern). However, most animal rotavirus (RV) strains belong to subgroup I and possess long RNA electropherotypes (18). Thus, subgroup I RV strains with a long electropherotype are likely to be animal RVs. In addition, despite a few exceptions (20, 25), subgroup I HRV strains with a short (or supershort) RNA pattern have been found to be associated with serotype 2 (18) and 8 (3, 22) specificities, whereas subgroup II strains with a long RNA pattern were associated with serotype 1, 3, 4, and 9 specificities (18). Furthermore, RNA-RNA hybridization studies have shown that HRV strains belong to two major families (9, 10) or genogroups (25), referred to as the DS-1 and Wa families, and that strains

not falling into either family are likely to be of animal origin (17). Recently, two subgroup I HRV strains with a long RNA pattern and serotype 3 specificity isolated in Japan were shown to resemble a feline RV strain (20, 25).

In the last few years, during an epidemiological survey on HRV strains circulating in Italy, we have identified three subgroup I strains which belonged to serotype 3. Serotype 3 strains all had long RNA electropherotypes. Neutralization and RNA-RNA hybridization studies showed that all three serotype 3 strains resemble simian strains rather than natural HRV reassortants.

MATERIALS AND METHODS

Rotavirus specimens. During an epidemiological study in the period from 1981 to 1989, more than 1,000 HRV strains were detected in stools from patients living in different regions of Italy. Among these strains, three (MZ58, PCP5, and PA710) were unusual in that they had serotype 3 VP7 specificity and a long RNA pattern, but belonged, unlike common human strains, to subgroup I. These strains were obtained from infants and young children suffering from acute gastroenteritis. Strain MZ58 was collected from a 6-month-old infant in Lombardia, a region of northern Italy, in 1987, whereas the other two strains, PCP5 and PA710, were collected in Sicily, a region of southern Italy, from a 36-month-old child and a 3-month-old infant in 1984 and 1988, respectively. All three strains were successfully adapted to growth in MA104 cell cultures in the presence of trypsin by a previously reported procedure (12).

RV reference strains. The following cell culture-adapted reference strains were used in our study: (i) HRV strain DS-1 (subgroup I, serotype 2) and Wa (subgroup II, serotype 1) kindly provided by R. G. Wyatt (National Institute of

* Corresponding author.

Allergy and Infectious Diseases, Bethesda, Md.); (ii) human strain YO (subgroup II, serotype 3) provided by S. Urasawa (Sapporo Medical College, Sapporo, Japan); (iii) human strain 69M (subgroup I, proposed serotype 8) provided by S. Matsuno (National Institute of Public Health, Tokyo, Japan); (iv) simian strains SA11 (subgroup I, serotype 3), provided by S. Kalter (Southwest Foundation for Animal Research, San Antonio, Tex.), and RRV-2 provided by R. F. Bishop (Melbourne, Australia); and (v) bovine strain UK (subgroup I, serotype 6) provided by T. H. Flewett (Birmingham, United Kingdom). In addition, HRV strains VA70 (subgroup II, serotype 4B) (11) and HAL1271 (subgroup I, proposed serotype 8 with long RNA pattern), recently isolated in our laboratory (G. Gerna, A. Sarasini, L. Zentilin, A. Di Matteo, P. Miranda, M. Parea, M. Battaglia, and G. Milanesi, Arch. Virol., in press), were also used.

RV detection. Virus was detected in stools and in cell culture fluids by ELISA with a polyclonal antibody as capture antibody and an RV group A-specific monoclonal antibody labeled with peroxidase as detector antibody, as recently reported (16).

RV subgrouping. RV strains were subgrouped by ELISA with subgroup I and subgroup II polyclonal antibodies as capture antibodies and subgroup I- and subgroup II-specific monoclonal antibodies labeled with peroxidase as detector antibodies (G. Gerna, A. Sarasini, M. Torsellini, A. Di Matteo, F. Baldanti, M. Parea, and M. Battaglia, Arch. Virol., in press).

RV serotyping and serotype 4 subtyping. Typing was done by ELISA, as previously described (14) with serotype-specific animal antisera and serotype-specific neutralizing mouse monoclonal antibodies as capture and detector antibodies, respectively. Reactivity was revealed by a biotinylated anti-mouse immunoglobulin, which was then detected by avidin-biotinylated peroxidase complexes. Subtyping of serotype 4 strains was done by ELISA by the same methodology and subtype 4A- and 4B-specific monoclonal antibodies, as recently reported (13, 15).

Electrophoretotyping. RV genomic RNA was extracted from samples and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (21), as previously reported (11).

Neutralization assay. Serotype 3 strains were also examined by neutralization. Antisera against RV strains PCP5, YO, and SA11 were prepared by immunizing guinea pigs, free of RV antibodies, with partially purified virus (12). Each animal received two sequential doses of virus plus Freund complete adjuvant intramuscularly at days 0 and 7 and a third dose of virus in phosphate-buffered saline intraperitoneally at day 21. Animals were bled 8 to 10 days after the last inoculation. Neutralizing monoclonal antibodies against strains YO and SA11 were produced by using BALB/c mice immunized with partially purified virus, as previously described (15). The neutralization assay was performed in MA104 cell cultures grown in microdilution plates, as previously described (12). Briefly, equal volumes of a trypsin-treated virus suspension (approximately 200 focus-forming units per 0.1 ml) and serial dilutions of sera were incubated for 30 min at 37°C and then inoculated onto MA104 cell monolayers. After incubation at 37°C for 18 to 24 h, cells were fixed and stained by the immunoperoxidase technique for the detection of nonneutralized virus. The neutralizing antibody titer was expressed as the reciprocal of the highest dilution giving at least 50% reduction in the number of infected cells compared with that in the virus control wells.

Northern blot hybridization. Single-stranded RNA probes labeled with ³²P were prepared by in vitro transcription of

TABLE 1. Subgrouping and serotyping of the three unusual HRV strains by ELISA

HRV strain and source	Absorbance (10 ²) in test system						
	Subgroup		Serotype				
	I	II	1	2	3	4A	4B
PCP5							
Stools	145	9	6	7	>200	6	4
MK/4-MA/15	200	3	5	5	124	7	7
MZ58							
Stools	103	11	4	7	>200	5	2
MK/8-MA/11	160	0	0	5	187	5	0
PA710							
Stools	26	3	0	2	26	4	3
MK/6-MA/7	95	0	3	6	104	4	5

purified MZ58 and YO HRV strains, as previously described (8). The integrity of all 11 RNA segments in each probe was assessed by electrophoresis in 3% denaturing polyacrylamide gels. Genomic double-stranded RNAs of HRV strains DS-1, Wa, YO, PCP5, MZ58, simian strain SA11, and bovine strain UK were obtained from partially purified virus preparations by phenol-chloroform extraction followed by ethanol precipitation (23). Ten micrograms of RNA from each strain was analyzed by electrophoresis in 7.5% discontinuous polyacrylamide gels. The gels were stained with ethidium bromide, photographed, and then immersed in 0.2 M NaOH for 10 min. For Northern blot hybridization, the NaOH was neutralized and genomic RNAs were blotted onto nylon membranes (Genescreen; Dupont, NEN Research Products, Boston, Mass.) for 18 h at 250 mA in a Transblot unit (Bio-Rad Laboratories, Richmond, Calif.) with 25 mM phosphate buffer (pH 6.5). The ³²P-labeled single-stranded RNA probes (0.5 × 10⁶ cpm) were hybridized for 20 h to the blotted RNAs under conditions of high stringency at 52°C, in a solution containing 2.5 × SSC (0.375 M Na⁺ + 0.0375 M sodium citrate), 50 mM NaPO₄ buffer (pH 6.5), 0.1% sodium dodecyl sulfate, 0.02% bovine serum albumin, 0.02% Ficoll (Pharmacia, Inc., Piscataway, N.J.), 50% formamide, 100 μg of sheared and denatured salmon sperm per ml, and 5% dextran sulfate (7).

RESULTS

Detection of unusual subgroup I HRV strains. During an epidemiological survey, the three unusual strains drew our attention because of their serotype 3 specificity associated with subgroup I reactivity. ELISA subgrouping and typing results for all three strains, both on stool extracts and infected cell culture fluids, are reported in Table 1. In addition, typing of the three strains was performed by neutralization with animal immune sera to RV serotypes 1 through 4, 8, and 9 (Table 2). The RNA patterns of these unusual serotype 3 HRV strains were different from those of the two reference serotype 3 HRV strains YO and P (subgroup II), as well as from those of the two reference serotype 3 simian strains SA11 and RRV-2 (subgroup I) (Fig. 1). These were the only serotype 3 HRV strains found in Italy in the period from 1981 to 1989 among a large collection of about 1,000 isolates. In the same period, subgroup II, serotype 3, strains were never detected.

Neutralization assays. The unusual serotype 3 isolates were further characterized by cross-neutralization assays. As shown in Table 3, YO antiserum neutralized all of the

TABLE 2. Typing of the three unusual HRV strains by neutralization

Antiserum to HRV strain (serotype)	Neutralizing antibody titer to HRV strain ^a :		
	PCP5	MZ58	PA710
Wa (1)	80 (128)	160 (64)	320 (32)
DS-1 (2)	40 (256)	<40 (>256)	<40 (>256)
YO (3)	10,240 (1)	5,120 (2)	5,120 (2)
PCP5 (3)	20,480 (1)	20,480 (1)	20,480 (1)
ST3 (4A)	<40 (>1,024)	<40 (>1,024)	<40 (>1,024)
VA70 (4B)	<40 (>128)	<40 (>128)	160 (32)
69M (8)	<40 (>128)	<40 (>128)	80 (64)
WI61 (9)	<40 (>4,096)	<40 (>4,096)	<40 (>4,096)

^a Numbers in parentheses indicate homologous over heterologous antibody titer ratios; for numbers <16, the relevant antiserum identifies the strain serotype.

serotype 3 strains at high titers, whereas PCP5 antiserum neutralized strain YO at a titer significantly (16-fold) lower and SA11 antiserum neutralized all of the human strains, except PA710, at lower titers (Table 3). PA710 was neutralized by SA11 immune serum at a titer eightfold higher than were the other serotype 3 HRV strains. When monoclonal antibodies directed to VP7 of YO³ and SA11 were tested against the same human and animal RV strains, results were comparable to those of the corresponding immune sera, i.e., the monoclonal antibody to YO neutralized all strains at high titers, whereas the monoclonal antibody to SA11 only neutralized simian strains and strain PA710 at a high titer. Neither the animal antisera nor the monoclonal antibodies appreciably neutralized (titers of <1:40 and <1:100, respectively) the reference strains of serotypes 1, 2, and 4 (data not shown).

Northern blot hybridization. In the three unusual HRV strains, serotype 3 specificity was associated with subgroup I reactivity, as was the case with simian strains. In addition, PCP5 antiserum neutralized the unusual serotype 3 HRV strains, as well as the simian strains, more efficiently than it did with the reference strain YO. Thus, cross-hybridization studies were performed to verify the level of genetic relatedness between these strains and simian RVs. Under high-stringency conditions, the labeled MZ58 probe hybridized strongly at the level of RNA segments 6 to 11 (or 5 to 11) to PCP5, PA710 (data not shown), SA11, and UK strains, whereas less intense reactions were observed with 69M and a 69M-like strain (HAL1271), and very weak reactions, at the level of segments 7 to 9, were observed with YO and VA70 (Fig. 2). No significant reaction was found with either

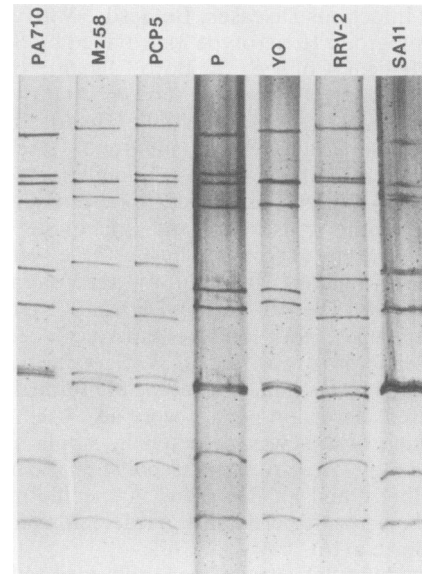


FIG. 1. Electrophoretic analysis of genomic RNAs of serotype 3 RV strains. Lanes: PA710, MZ58, and PCP5, unusual HRV isolates of subgroup I; P and YO, subgroup II reference HRV strains; RRV-2 and SA11, subgroup I simian strains.

DS-1 or Wa. On the other hand, the labeled YO probe hybridized strongly to Wa, whereas there was a low degree of hybridization to segments 7 to 9 of strains MZ58, PCP5, PA710, SA11, and UK. No reaction was observed with DS-1 (Fig. 3). Finally, with the labeled SA11 probe, cross-hybridization was detected for segments 1 to 3 and 6 to 11 among the three unusual strains and the simian strain (Fig. 4).

DISCUSSION

Two HRV strains, AU1 and AU228, belonging to subgroup I and serotype 3 and showing a long RNA pattern, were recently isolated in Japan and shown to be closely related in genogroup to a feline RV (20, 25). Another HRV isolate from Japan, AU125, has been shown to be genetically and antigenically related to AU1 (24). In addition, a subgroup I, serotype 3, strain with fast-moving tenth and eleventh segments has been recently isolated in Israel (1). Similarly, the three serotype 3 HRV strains from Italy reported in this study possessed a subgroup I reactivity and a long RNA pattern. Unfortunately, the strains previously

TABLE 3. Cross-neutralization tests on serotype 3 RV strains by using polyclonal and monoclonal antibodies

Antibody to RV strain	Neutralizing antibody titer to RV strain ^a :					
	YO	PCP5	MZ58	PA710	SA11	RRV-2
Animal antisera^b						
YO	10,240	10,240	5,120	5,120	40,960	10,240
PCP5	1,280	20,480	20,480	20,480	20,480	20,480
SA11	640	640	640	5,120	81,920	20,480
Monoclonal antibodies^c						
YO	80,000	80,000	80,000	640,000	640,000	640,000
SA11	2,000	4,000	2,000	160,000	160,000	160,000

^a Titers are expressed as reciprocals. Homologous titers are given in boldface.

^b Hyperimmune sera prepared in guinea pigs.

^c Ascitic fluid preparations of mouse monoclonal antibodies.

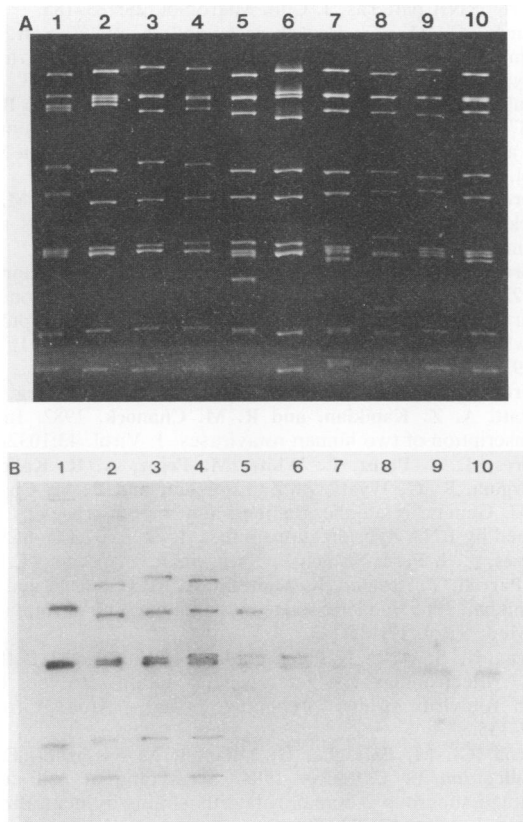


FIG. 2. Northern blot hybridization of ^{32}P -labeled RNA probe prepared from MZ58 HRV (subgroup I, serotype 3, long RNA pattern) to denatured genomic RNAs of the indicated strains. (A) UV light picture of the gel after ethidium bromide staining; (B) corresponding autoradiogram. Lanes: 1, simian SA11 (subgroup I, serotype 3); 2, bovine UK (subgroup I, serotype 6); 3, human PCP5 (subgroup I, serotype 3); 4, human MZ58 (subgroup I, serotype 3); 5, human 69M (subgroup I, proposed serotype 8); 6, human HAL1271 (subgroup I, serotype 8); 7, human Wa (subgroup II, serotype 1); 8, human DS-1 (subgroup I, serotype 2); 9, human YO (subgroup II, serotype 3); 10, human VA70 (subgroup II, serotype 4, subtype 4B).

isolated from Japan were not available in the laboratory to be included in our study and compared with our strains. At the moment, it cannot be excluded that they are closely related to each other. The finding that these strains represent the only serotype 3 HRVs found in Italy during the period from 1981 to 1989 out of more than 1,000 strains appears particularly interesting from an epidemiological standpoint. The circulation of such strains might not be occasional. In this respect, it is of interest to note that these three strains, each with an electropherotype distinct from the others, but all with the same unusual combination of subgroup I, serotype 3, and fast-moving tenth and eleventh RNA segments, were circulating during different years in different geographic areas of Italy.

Compared with the usual HRV strains of serotype 3, these strains possessed a major immunological difference, i.e., subgroup I instead of subgroup II reactivity. It is well known that several animal RV strains, such as simian or canine strains, are subgroup I and serotype 3 in association with a long electropherotype. Thus, in order to investigate the possible origin of these strains, two approaches were used. The first was to perform cross-neutralization studies in order

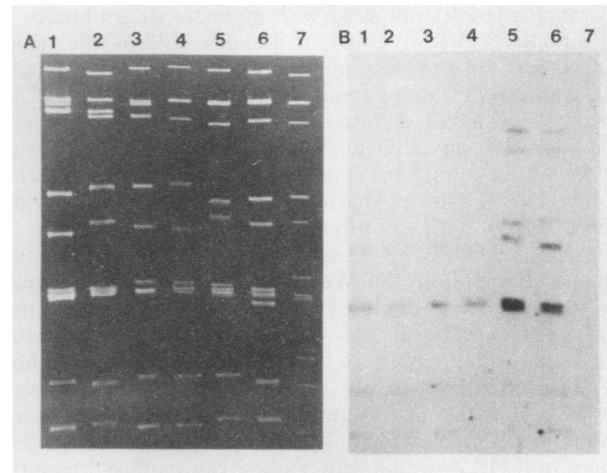


FIG. 3. Northern blot hybridization of ^{32}P -labeled YO RNA probe to denatured genomic RNAs from bovine UK (lane 1), simian SA11 (lane 2), human MZ58 (lane 3), human PCP5 (lane 4), human YO (lane 5), human Wa (lane 6), and human DS-1 (lane 7). (A) Ethidium bromide-stained gel; (B) corresponding autoradiogram.

to investigate the antigenic relationship among HRV strains, as well as between human and animal strains, of the same serotype. The second was to perform cross-hybridization studies in order to investigate whether these strains belonged to a genogroup closer to human or animal strains.

Among animal RV strains available in the laboratory, simian strains SA11 and RRV-2 were selected on the basis of identity of their subgroup and serotype specificities with unusual human strains, while results of cross-neutralization tests also appeared to suggest a closer antigenic relationship of human to simian strains. Bovine strain UK was used as a control. Although two-way cross-neutralization assays were done only for PCP5 and only one-way cross-neutralization assays were done for MZ58 and PA710, the rather low neutralizing activity of PCP5 antiserum versus YO and the relatively high neutralizing activity of SA11 antiserum versus PA710 virus suggested a possible closer antigenic relationship of the new strains to SA11 than to YO. In addition,

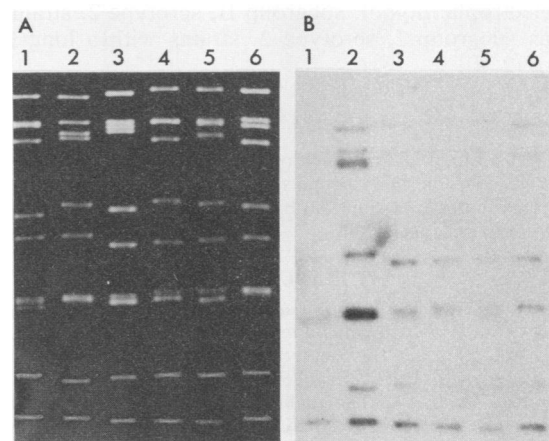


FIG. 4. Northern blot hybridization of ^{32}P -labeled SA11 RNA probe to denatured genomic RNAs from human VA70 (lane 1), simian SA11 (lane 2), bovine UK (lane 3), human PCP5 (lane 4), human MZ58 (lane 5), and human PA710 (lane 6). (A) Ethidium bromide-stained gel; (B) corresponding autoradiogram.

neutralizing results obtained with monoclonal antibodies to YO and SA11 VP7 seemed to identify VP7 as the major neutralizing antigen responsible for different neutralizing titers. However, monoclonal antibodies to VP7 of PCP5 were not available and the possible role of the gene 4 product, VP4, in determining differences in neutralizing antibody titers must be considered. In fact, hybridization studies (Fig. 3) showed sharing of gene 4 between PCP5 and MZ58, but not with any other strain studied.

For some years, it has been established that there are two human RV families, the Wa family and the DS-1 family, which have been identified by RNA-RNA hybridization studies and have been shown to include subgroup II strains with a long RNA pattern and subgroup I strains with a short RNA pattern, respectively (9, 10). Recently, it has been proposed that the term family be replaced with the term genogroup (25). However, it has been shown that a few subgroup II HRV strains with a long RNA pattern do not fall in either of the two human genogroups, whereas a few others seem to belong to both genogroups (10). In addition, Flores et al. (6) have shown that genomic homology, as studied by RNA-RNA hybridization, among strains recovered from the same animal species is generally higher than that among strains from different species.

The high level of cross-hybridization found in segments 6 to 11 among MZ58, PCP5, PA710, SA11, and UK suggests the following. (i) PCP5, MZ58, and PA710 are genetically different from reference serotype 3 HRV strain YO, except for genes 7 to 9. (ii) PCP5, MZ58, and PA710 are genetically related to simian RV SA11 and bovine RV UK. These three unusual RV strains belong to a genogroup different from that of YO, which belongs to the Wa family. They show a high degree of homology with UK and SA11 strains, known to display some cross-hybridization (6). Thus, while the usual serotype 3 HRV strains belong to the Wa genogroup, the unusual serotype 3 HRV strains might belong to the same genogroup as SA11 and UK. These conclusions suggest that the unusual serotype 3 strains are more likely to be animal strains infecting humans rather than natural reassortants between human strains. Whether these anomalous strains were directly transmitted from animals to humans or had evolved ancestrally from animal strains remains to be determined. So far, unusual RV strains isolated from humans include, in addition to subgroup I, serotype 3, strains (with a long electropherotype), subgroup II, serotype 2, strains, as well as subgroup I, serotype 2, strains with a long RNA pattern (2, 4).

ACKNOWLEDGMENTS

We thank Linda D'Arrigo for revision of the English.

This work was partially supported by contract HQ85056 from the World Health Organization and by Ministero della Pubblica Istruzione, Ricerca Scientifica 60%.

LITERATURE CITED

- Aboudy, Y., I. Shif, I. Zilberstein, and T. Gottlieb-Stematsky. 1988. Use of polyclonal and monoclonal antibodies and analysis of viral RNA in the detection of unusual group A human rotaviruses. *J. Med. Virol.* **25**:351-359.
- Ahmed, M. U., K. Taniguchi, N. Kobayashi, T. Urasawa, F. Wakasugi, M. Islam, H. Shaikh, and S. Urasawa. 1989. Characterization by enzyme-linked immunosorbent assay using subgroup- and serotype-specific monoclonal antibodies of human rotavirus obtained from diarrheic patients in Bangladesh. *J. Clin. Microbiol.* **27**:1678-1681.
- Albert, M. J., L. E. Unicom, and R. F. Bishop. 1987. Cultivation and characterization of human rotaviruses with "super short" RNA patterns. *J. Clin. Microbiol.* **25**:183-185.
- Beards, G. M., U. Desselberger, and T. H. Flewett. 1989. Temporal and geographic distributions of human rotavirus serotypes, 1983 to 1988. *J. Clin. Microbiol.* **27**:2827-2833.
- Coulson, B. S., L. E. Unicom, G. A. Pitson, and R. F. Bishop. 1987. Simple and specific enzyme immunoassay using monoclonal antibodies for serotyping human rotaviruses. *J. Clin. Microbiol.* **25**:509-515.
- Flores, J., Y. Hoshino, E. Boeggeman, R. Purcell, R. M. Chanock, and A. Z. Kapikian. 1986. Genetic relatedness among animal rotaviruses. *Arch. Virol.* **87**:273-285.
- Flores, J., K. Midthun, Y. Hoshino, K. Green, M. Gorziglia, A. Z. Kapikian, and R. M. Chanock. 1986. Conservation of the fourth gene among rotaviruses recovered from asymptomatic newborn infants and its possible role in attenuation. *J. Virol.* **60**:972-979.
- Flores, J., J. Myslinski, A. R. Kalica, H. B. Greenberg, R. G. Wyatt, A. Z. Kapikian, and R. M. Chanock. 1982. In vitro transcription of two human rotaviruses. *J. Virol.* **43**:1032-1037.
- Flores, J., I. Perez, L. White, M. Perez, A. R. Kalica, R. Margina, R. G. Wyatt, A. Z. Kapikian, and R. M. Chanock. 1982. Genetic relatedness among human rotaviruses as determined by RNA hybridization. *Infect. Immun.* **37**:648-655.
- Flores, J., I. Perez-Schael, E. Boeggeman, L. White, M. Perez, R. Purcell, Y. Hoshino, K. Midthun, R. M. Chanock, and A. Z. Kapikian. 1985. Genetic relatedness among human rotaviruses. *J. Med. Virol.* **17**:135-143.
- Gerna, G., S. Arista, N. Passarani, A. Sarasini, and M. Battaglia. 1987. Electropherotype heterogeneity within serotypes of human rotavirus strains circulating in Italy. *Arch. Virol.* **95**:129-135.
- Gerna, G., M. Battaglia, G. Milanesi, N. Passarani, E. Percivalle, and E. Cattaneo. 1984. Serotyping of cell culture-adapted subgroup 2 human rotavirus strains by neutralization. *Infect. Immun.* **43**:722-729.
- Gerna, G., N. Passarani, L. E. Unicom, M. Parea, A. Sarasini, M. Battaglia, and R. F. Bishop. 1989. Solid-phase immune electron microscopy and enzyme-linked immunosorbent assay for typing of human rotavirus strains by using monoclonal and polyclonal antibodies: a comparative study. *J. Infect. Dis.* **159**:335-339.
- Gerna, G., A. Sarasini, B. S. Coulson, M. Parea, M. Torsellini, E. Arbustini, and M. Battaglia. 1988. Comparative sensitivities of solid-phase immune electron microscopy and enzyme-linked immunosorbent assay for serotyping of human rotavirus strains with neutralizing monoclonal antibodies. *J. Clin. Microbiol.* **26**:1383-1387.
- Gerna, G., A. Sarasini, A. Di Matteo, M. Parea, P. Orsolini, and M. Battaglia. 1988. Identification of two subtypes of serotype 4 human rotavirus by using VP7-specific neutralizing monoclonal antibodies. *J. Clin. Microbiol.* **26**:1388-1392.
- Gerna, G., A. Sarasini, A. Di Matteo, M. Parea, M. Torsellini, and M. Battaglia. 1989. Rapid detection of human rotavirus strains in stools by single-sandwich enzyme-linked immunosorbent assay systems using monoclonal antibodies. *J. Virol. Methods* **24**:43-56.
- Greenberg, H., V. McAuliffe, J. Valdesuso, R. Wyatt, J. Flores, A. Kalica, Y. Hoshino, and N. Singh. 1983. Serological analysis of the subgroup protein of rotavirus, using monoclonal antibodies. *Infect. Immun.* **39**:91-99.
- 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.
- Kalica, A. R., H. B. Greenberg, R. T. Espejo, J. Flores, R. G. Wyatt, A. Z. Kapikian, and R. M. Chanock. 1981. Distinctive ribonucleic acid patterns of human rotavirus subgroups 1 and 2. *Infect. Immun.* **33**:958-961.
- Kitaoka, S., T. Nakagomi, N. Fukuhara, Y. Hoshino, H. Suzuki, O. Nakagomi, A. Z. Kapikian, T. Ebina, T. Konno, and N. Ishida. 1987. Serologic characteristics of a human rotavirus isolate, AU-1, which has a "long" RNA pattern and subgroup I specificity. *J. Med. Virol.* **23**:351-357.

21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680-685.
22. **Matsuno, S., A. Hasegawa, A. Mukoyama, and S. Inouye.** 1985. A candidate for a new serotype of human rotavirus. *J. Virol.* **54**:623-624.
23. **Nakagomi, O., T. Nakagomi, Y. Hoshino, J. Flores, and A. Z. Kapikian.** 1987. Genetic analysis of a human rotavirus that belongs to subgroup I but has an RNA pattern typical of subgroup II human rotaviruses. *J. Clin. Microbiol.* **25**:1159-1164.
24. **Nakagomi, O., H. Oyamada, S. Kuroki, Y. Kobayashi, A. Ohshima, and T. Nakagomi.** 1989. Molecular identification of a novel human rotavirus in relation to subgroup and electropherotype of genomic RNA. *J. Med. Virol.* **28**:163-168.
25. **Nakagomi, T., and O. Nakagomi.** 1989. RNA-RNA hybridization identifies a human rotavirus that is genetically related to feline rotavirus. *J. Virol.* **63**:1431-1434.
26. **Offit, P. A., and G. Blavat.** 1986. Identification of the two rotavirus genes determining neutralization specificities. *J. Virol.* **57**:376-378.
27. **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.
28. **Taniguchi, K., T. Urasawa, and S. Urasawa.** 1986. Reactivity patterns to human rotavirus strains of a monoclonal antibody against VP2, a component of the inner capsid of rotavirus. *Arch. Virol.* **87**:135-141.
29. **Taniguchi, K., T. Urasawa, S. Urasawa, and T. Yasuhara.** 1984. Production of monoclonal antibodies against human rotaviruses and their application to an enzyme-linked immunosorbent assay for subgroup determination. *J. Med. Virol.* **14**:115-125.