JOURNAL OF CLINICAL MICROBIOLOGY, July 1993, p. 1783–1787
0095-1137/93/071783-05$02.00/0

Vol. 31, No. 7

Identification of Feline- and Canine-Like Rotaviruses Isolated from Humans by Restriction Fragment Length Polymorphism Assay

AMI VONSOVER,[1]* ILAN SHIF,[1] ILANA SILBERSTEIN,[1] HAGIT RUDICH,[1] YAIR ABOUDY,[1] ELLA MENDELSON,[1] LESTER SHULMAN,[2] TOYOKO NAKAGOMI,[3] AND OSAMU NAKAGOMI[3]

Central Virology Laboratory[1] and Institute of Nephrology,[2] Chaim Sheba Medical Center, Tel-Hashomer 52621, Israel, and Department of Microbiology, Akita University School of Medicine, Akita, Japan[3]

Received 13 January 1993/Accepted 5 April 1993

Restriction fragment length polymorphism assay of reverse-transcribed and polymerase chain reaction-amplified rotavirus gene segment 9 was developed to differentiate human serotype 3 rotaviruses from animal serotype 3 rotaviruses. On the basis of similarities or differences in *Hin*fI and *Dde*I restriction profiles, unusual group A serotype 3 human rotaviruses that belonged to subgroup I were shown to be of feline and canine origin. By this approach, the new human rotavirus isolates 5193, AU-387, AU-720, AU-785, and AU-1115 were shown to resemble certain feline-like human rotaviruses. Similar results were previously obtained by Nakagomi et al. (O. Nakagomi, A. Hoshima, Y. Aboudy, I. Shif, M. Mochizuki, T. Nakagomi, and T. Gotlieb-Stematsky. J. Clin. Microbiol. 28:1198–1203, 1990) by using RNA-RNA cross hybridization with established feline rotaviruses. The restriction fragment length polymorphism assay can provide fast and valuable information on the interspecies transmission of rotaviruses in nature.

Rotaviruses are considered the most prevalent viral agent associated with acute gastroenteritis in children under 3 years of age. Although detection of rotavirus in clinical specimens has become a routine diagnostic procedure, characterization of rotavirus isolates for specific gene products has remained difficult, despite the need for such information both for analyzing natural rotavirus variants and their circulation in human populations and for mapping out a strategy for developing a vaccine against rotaviruses.

The genus *Rotavirus* has been classified into five serogroups, A to E, on the basis of group-specific antigens detected by immune electron microscopy (25, 26). Group A is divided into two antigenic subgroups: I and II (11). Group A rotaviruses are further classified into at least 13 serotypes on the basis of their VP-7 antigenicities (4, 29). VP-7, which is the major component of the outer shell of rotavirus particles, is involved in virus neutralization and also defines the serotype specificity. It is encoded by gene 9, which is 1,062 nucleotides in length. Of the 13 VP-7 serotypes or G (glycoprotein) types, seven serotypes (G-1 to G-4, G-8, G-9, and G-12) are known to occur in human rotaviruses (HRVs), with serotypes 1 through 4 being the most prevalent. Among all rotaviruses, serotype 3 is the most abundant in many species.

It is known that rotaviruses cross species barriers. Experimentally, HRVs may infect animals and induce diarrheal illness (13). Conversely, animal rotaviruses can infect humans, as observed in field studies performed to evaluate the safety and efficacy of rotavirus vaccines (31).

Animal group A rotaviruses are differentiated from the human strains by virtue of the serotype. Certain serotypes such as G-5, G-6, G-7, G-10, G-11, and G-13 are recovered only from animals. Serotype 3 isolates are found in both humans and animals. However, the strains from humans belong to subgroup II, while the strains from animals belong to subgroup I. Only certain animal strains, but not human strains, possess active hemagglutinin capable of agglutinating the erythrocytes of several species (6).

Animal rotaviruses and HRVs were proven to belong to different genogroups by RNA-RNA cross hybridization. In recent years unusual serotype 3 viruses belonging to subgroup I rather than subgroup II were recovered from infants with diarrhea in several countries (1, 9). Several of these unusual human rotavirus isolates were studied in detail by RNA-RNA cross hybridization and were shown to be genetically related to rotaviruses of cats and dogs (20). So far, only one HRV isolate among these (Ro-1845) was found to possess active hemagglutinin (19).

Many attempts have been made to assess whether field isolates of HRVs originated from animal rotavirus strains. Development of a simplified, rapid method of differentiating human from animal rotaviruses was needed.

DNA fingerprinting of hypervariable minisatellite genes, developed recently for genealogic studies of human populations, is based on the similarities of the restriction fragment length polymorphism (RFLP) patterns of genes of related individuals. By analogy with DNA fingerprinting, we reasoned that it would be possible to identify particular rotaviruses by examining the restriction patterns of genome segments from different strains. By combination of the polymerase chain reaction (PCR) with reverse transcription, it is possible to produce a large number of DNA copies from RNA and to compare restriction patterns in different strains of double-stranded RNA (dsRNA) viral genomes.

In this report we present evidence that the polymorphism within gene 9 of rotavirus can be used to characterize rotavirus isolates and enables differentiation between HRVs and animal rotaviruses. Through this approach we were able to show identity between several unusual serotype 3 HRVs isolated in Japan and Israel and rotaviruses of feline and canine origin.

* Corresponding author.

MATERIALS AND METHODS

Viruses. Local HRV isolates of serotypes 1 through 4 were obtained from hospitalized Israeli children from different parts of the country and during different seasons, since the rates of rotavirus infections vary throughout the year. They were serotyped by using a set of serotype-specific monoclonal antibodies (Silenus Co., Hawthorn, Victoria, Australia) and a specific enzyme-linked immunosorbent assay (28).

HRVs also included serotype 3, subgroup I tissue culture-adapted isolates from Japan (AU-1 [21], AU-228 [20], and AU-125 and AU-379 [20]) and Israel (Ro-1845 [1]); all of these isolates were shown to be of feline and canine origin by RNA-RNA cross hybridization. Additional HRV strains used included recent isolates from Japan (AU-382, AU-720, AU-785, and AU-1115) and Israel (5193); the genetic relatedness of these isolates to animal rotaviruses has not yet been determined.

Established animal rotaviruses, all of which were serotype 3, subgroup I, included SA-11, a simian rotavirus isolated in South Africa by Malherbe and Strickland-Cholmley (12); feline rotaviruses FRV-1 (16), FRV-64, FRV-70, FRV-72, and FRV-73 (15), all isolated in Japan; Cat-2, Cat-3, and Cat-97 feline rotaviruses isolated in Australia (3); and canine rotaviruses K-9, a canine rotavirus isolated in the United States (8), and RS-15, a canine rotavirus isolated in Japan (14).

Virus isolation from fecal specimens was carried out in MA-104 cell cultures in the presence of trypsin by a previously reported procedure (2).

Viral RNA preparation and purification. Rotavirus dsRNA was extracted from stool suspensions or infected tissue cultures in the manner commonly used for RNA electropherotyping in polyacrylamide gels (1). Following overnight ethanol precipitation at −20°C, the dsRNA was dried and then resuspended in 100 μl of buffer containing 10 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, and 0.1% sodium dodecyl sulfate and was layered onto a 1.0-ml column of G-50 Sephadex (Fine; Pharmacia, Uppsala, Sweden) equilibrated in the same buffer (the column was made in a 1.0-ml tuberculin syringe). Following centrifugation at 2,400 rpm for 25 min at room temperature, the eluted dsRNA was purified by one phenol-chloroform extraction and one chloroform extraction and was then precipitated with 2.5 volumes of ethanol at −20°C.

Primers. Oligonucleotide primers specific for gene segment 9 of rotaviruses were synthesized complementary to the 3′ ends of both viral RNA strands. These primers, Beg^9 and End^9, were selected to produce full-length copies of gene 9 from any group A rotaviruses (10).

Reverse transcription and enzymatic amplification by PCR. Synthesis of cDNA was performed in a reaction mixture containing viral dsRNA, 50 pmol of End^9 primer, reverse transcriptase buffer (50 mM Tris-HCl [pH 8.3], 6 mM $MgCl_2$, 40 mM KCl, 10 mM dithiothreitol), each deoxynucleoside triphosphate at 1 mM, 40 U of RNA guard (Pharmacia), and 9 U of avian myeloblastosis reverse transcriptase (Promega Biotec) in a total volume of 20 μl.

Following denaturation of viral RNA at 97°C for 3 min and cooling to 42°C for 5 min (annealing of the End^9 primer to the RNA template), reverse transcriptase buffer, two deoxynucleoside triphosphates, and reverse transcriptase were added and the mixture was held at 42°C for an additional 30 min, after which time the cDNA was denatured by boiling at 100°C for 5 min and was then immediately chilled in iced water. PCR was performed on the whole volume of the cDNA-containing reverse transcriptase mixture.

The total volume (20 μl) was adjusted to 100 μl by the addition of 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 50 pmol each of End^9 and Beg^9 primers, and 7% dimethyl sulfoxide. The mixture was subjected to one thermal cycle (Programmable Thermal Controller; MJ Research, Inc.) of 5 min of denaturation at 94°C and then 5 min of annealing at 42°C. After a short centrifugation, 2.5 U of *Taq* polymerase (Boehringer, Mannheim, Germany) and 100 μl of light mineral oil (Sigma) were added and extension was carried out for 2 min at 72°C. Reaction mixtures were then subjected to 30 automated cycles of 1 min of denaturation at 94°C, 2 min of annealing at 42°C, and 1 min of extension at 72°C.

Analysis of PCR amplification products. A 10% aliquot of the amplified product was electrophoresed through a 1.2% agarose (Seakem LE) gel and visualized by staining with ethidium bromide. A unique electrophoresed band of about 1,062 bp was considered to be the product of a specific amplification of the targeted rotavirus genome segment.

Restriction enzyme mapping. Generally, 10 μl of the amplified cDNA product was digested with either 10 U of *Hin*fI or 10 U of *Dde*I and was then adjusted to a final volume of 20 μl with the corresponding buffer. Cleavage reaction mixtures were incubated at 37°C for 2 h, and the DNA fragments were electrophoresed through 2% agarose (LE) and 1% NuSieve GTG agarose (FMC) gels containing ethidium bromide and were visualized under UV light.

RESULTS

Polymorphism of segment 9 of rotavirus genome. To determine whether gene segment 9 of the rotavirus genome had the properties necessary to be effective as a polymorphic gene, we determined the RFLP patterns of the amplified cDNAs from different HRV isolates with known serotypes. The restriction enzymes *Hin*fI and *Dde*I were selected by computer-assisted analysis of the four HRV serotypes (serotypes 1 to 4) with known nucleotide sequences to generate serotype-specific restriction patterns.

Twenty-eight amplified cDNAs from different HRV isolates serotyped by enzyme immunoassay with monoclonal antibodies (12 were serotype 1, 5 were serotype 2, 6 were serotype 3, and 5 were serotype 4) were compared by their RFLP profiles following cleavage with the *Hin*fI and *Dde*I restriction enzymes.

The RFLP patterns of two representative strains from each serotype are shown in Fig. 1. From the results of that test (Fig. 1), it can be seen that isolates of each serotype of the four serotypes analyzed had a unique restriction pattern when the results from separate digestions with *Hin*fI and *Dde*I were combined. While isolates of serotypes 3 and 4 could not be distinguished on the basis of *Hin*fI alone (both lack the *Hin*fI restriction site), they had different *Dde*I restriction patterns. Within each serotype, all isolates had the same pattern; thus, with all 28 cDNAs tested, there was 100% agreement between the RFLP assay and serotyping with specific monoclonal antibodies.

RFLP analysis of gene 9 of serotype 3 HRVs and animal rotaviruses. An attempt was made to differentiate serotype 3 isolates from different species according to the restriction profiles generated by the *Hin*fI and *Dde*I restriction enzymes. We tried to determine whether strain-specific RFLP profiles could be generated for the various serotype 3 strains analyzed: human (5335), simian (SA-11), and two different

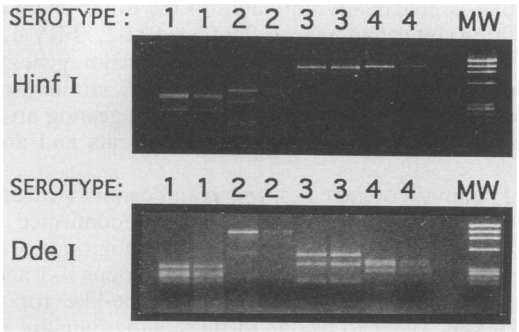

FIG. 1. RFLP patterns of HRVs belonging to serotypes 1 to 4. Genomic segment 9 of different HRV isolates (two different isolates for each serotype) was reverse transcribed, and the resulting cDNAs were amplified as described in the text. Each of the resulting DNA fragments (1,062 bp) was separately digested with *Hin*fI or *Dde*I. The digested products were run on 3% gels (2% Seakem LE agarose plus 1% Nusieve GTG agarose) in parallel with a molecular weight (MW) marker, φX174 digested with *Hae*III (sizes of markers: 1,353, 1,078, 872, 603, 310, 281, 271, 234, and 194 bp).

genogroups of feline (FRV-64 and Cat-2) and canine (RS 15) rotaviruses.

The results depicted in Fig. 2 demonstrated unique RFLP patterns for the various cDNAs analyzed. While similar *Hin*fI patterns were obtained for FRV-64 and RS-15 strains, *Dde*I patterns could differentiate between them. Different *Hin*fI and *Dde*I profiles were demonstrated for the two groups of feline rotaviruses represented by FRV-64 and Cat-2, confirming previous results which indicated that they belong to two different genogroups. Additional feline rotavirus isolates (FRV-70, FRV-72, and FRV-73) exhibited an RFLP profile identical to that of FRV-64, while other feline strains (Cat-3, FRV-1) yielded RFLP patterns similar to that

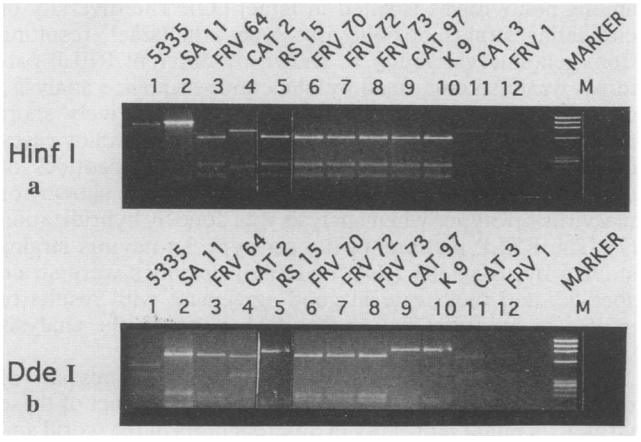

FIG. 2. RFLP patterns of serotype 3 rotaviruses of human and animal origin. Genomic segment 9 of serotype 3 HRVs and rotaviruses from various animals was reverse transcribed and amplified. The resulting DNA fragments were digested separately with *Hin*fI or *Dde*I restriction enzymes and were processed as described in the legend to Fig. 1. Lane 1, HRV strain 5335; lane 2, simian rotavirus SA-11; lanes 3 to 12, feline and canine strains FRV-64, Cat-2, RS-15, FRV-70, FRV-72, FRV-73, Cat-97, K-9, Cat-3, and FRV-1, respectively. The molecular weight marker (M) is the φX174 restriction fragment DNA digested with *Hae*III as described in the legend to Fig. 1.

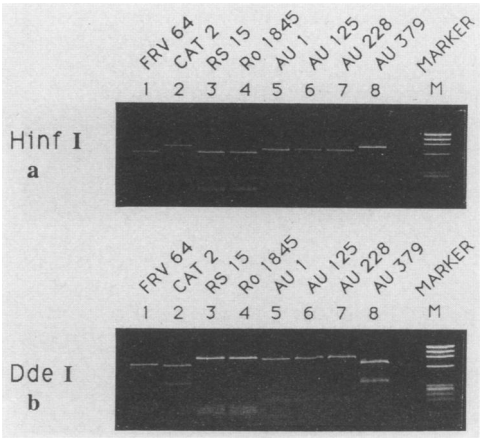

FIG. 3. RFLP profiles of feline (FRV-64, Cat-2), canine (RS-15), and feline- and canine-like serotype 3 rotaviruses isolated from humans (Ro-1845, AU-1, AU-15, AU-228, and AU-397). The genomic dsRNAs were extracted, and segment 9 was reverse transcribed and amplified as described in the legend to Fig. 1. (a) *Hin*fI pattern. (b) *Dde*I pattern. The molecular weight marker (M) is the φX174 restriction fragment DNA digested with *Hae*III as described in the legend to Fig. 1.

shown for Cat-2. Cat-97 and the canine rotavirus K-9 demonstrated a pattern common with that of canine RS-15.

RFLP analysis of gene 9 of "feline- and canine-like" and "unusual" human viruses of unknown origin. Five feline- and canine-like rotaviruses isolated from humans and identified as such by RNA-RNA cross hybridization (18, 20) were analyzed by the RFLP assay. Complementary cDNAs were prepared from strains Ro-1845, AU-1, AU-125, AU-228, and AU-379, and their RFLP patterns were compared with those of established feline and canine isolates (FRV-64, Cat-2, and RS-15). The results presented in Fig. 3 indicate that the Ro-1845 and RS-15 strains were identical in regard to their *Hin*fI and *Dde*I profiles. Similarly, strain AU-379 shared an homologous pattern with strain Cat-2. Strains AU-1, AU-125, and AU-228 revealed a common pattern with both enzymes but were different from the three groups presented by RS-15, FRV-64, and Cat-2.

An additional five unusual serotype 3 rotaviruses of unknown origin isolated recently from humans were characterized by the RFLP assay. These included strain 5193 isolated in Israel and strains AU-387, AU-720, AU-785, and AU-1115 isolated recently in Japan. The results presented in Fig. 4 show a complete homology in the RFLP patterns between strains 5193, AU-387, and AU-720 and the Cat-2 strain, while strains AU-785 and AU-115 shared profiles common with that of AU-1.

On the basis of the *Hin*fI and *Dde*I restriction profiles, serotype 3 HRVs could be distinguished from serotype 3 rotavirus strains of feline and canine origin isolated from children suffering from acute diarrhea. HRVs of feline or canine origin have been assigned to three groups on the basis of the similarities or differences in their RFLP profiles (Table 1).

DISCUSSION

The data presented in this report demonstrate the usefulness of analyzing RFLP profiles of reverse-transcribed, PCR-amplified segment 9 of serotype 3 rotaviruses and support the use of RFLP analysis in differentiating HRVs

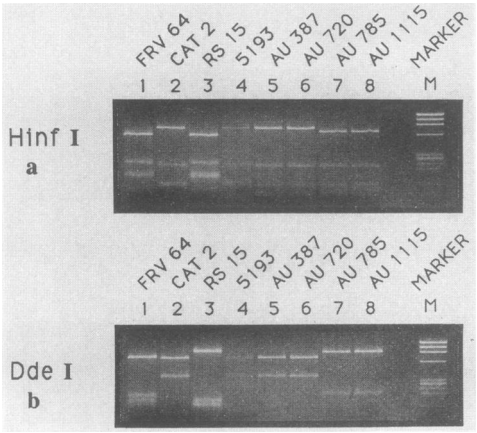

FIG. 4. RFLP profiles of gene 9 of unusual human viruses of unknown origin. Five unusual serotype 3 rotaviruses of unknown origin (characterization by RNA-RNA cross hybridization was not available) were characterized by their RFLP profiles. (a) *Hin*fI profile. (b) *Dde*I profile. Lane 1, strain 5193 isolated in Israel; lanes 2 to 5, strains AU-387, AU-720, AU-785, and AU-1115, respectively, all recently isolated in Japan. The molecular weight marker (M) is the ϕX174 restriction fragment DNA-digested with *Hae*III as described in the legend to Fig. 1.

from animal rotaviruses and in determining the origins of unusual serotype 3 strains from humans. The restriction patterns obtained with these enzymes were species specific and thus could distinguish the feline and canine rotavirus strains from serotype 3 human and simian rotavirus strains.

Group A rotaviruses belonging to serotype 3 can be isolated from at least eight species including humans, monkeys, horses, pigs, rabbits, cats, dogs, and mice. Among serotype 3 strains, Nishikawa et al. (24) defined nine regions in the deduced amino acid sequences of gene 9. The nine regions were species specific and were almost identical in their amino acid sequences among the serotype 3 strains isolated from the same species, but differed from those belonging to other host species. An exception was the finding of two distinct feline rotavirus genogroups represented by strains Cat-2 and Cat-97. Cat-97 is assigned to the same genogroup that includes canine rotaviruses RS-15 and K-9. RNA-RNA hybridization confirmed the relatedness of Cat-97 to feline rotaviruses FRV-64, FRV-70, FRV-72, and FRV-73 and canine rotaviruses K-9 and RS-15, all of which agglutinate erythrocytes (15, 23). Thus, feline and canine hemagglutinating rotaviruses can be included in one genogroup that is genetically unrelated to the other feline group represented by nonhemagglutinating Cat-2, FRV-1, and other isolates. Interestingly, these two major genogroups were present in certain unusual serotype 3 strains isolated from humans in Japan and Israel, thus suggesting an interspecies transmission of rotaviruses from cats and dogs to humans.

TABLE 1. Classification of feline, canine and feline- and canine-like HRV groups according to RFLP analysis of amplified gene segment 9

RFLP group	Established feline and canine viruses	Feline- and canine-like viruses from humans
Group A-1 (hemagglutinin +)	RS-15, Cat-97, K-9	Ro-1845
Group A-2 (hemagglutinin +)	FRV-64, FRV-70, FRV-72, FRV-73	
Group B (hemagglutinin −)		AU-1, AU-125, AU-228, AU-785, AU-1115
Group C (hemagglutinin −)	Cat-2, Cat-3, FRV-1	AU-379, AU-387, AU-720, 5193

RFLP analysis of gene 9 of feline and canine viruses with the restriction enzymes *Hin*fI and *Dde*I confirmed these results and could further classify the two subgroups of feline hemagglutinating rotaviruses into RFLP groups A-1 and A-2 (Table 1). One human feline- and canine-like rotavirus, Ro-1845, was assigned to one of these subgroups by RFLP analysis, confirming previous results obtained by molecular hybridization (18). No representative of the second subgroup from humans has been identified so far. The second and third RFLP groups, B and C, consisted of nonhemagglutinating feline and feline-like human rotavirus isolates, all of which were included in the AU-1 genogroup of Mochizuki et al. (15). These two groups (B and C) were specified by their different restriction profiles obtained with both enzymes (*Hin*fI and *Dde*I). While group B had no feline or canine counterparts, three of its members, namely, AU-1, AU-125, and AU-228, were proven by RNA-RNA hybridization to originate in cats. Group C contained both feline (Cat-2, Cat-3, and FRV-1) and feline-like (AU-379, 5193, AU-387 and AU-720) strains from humans. RNA-RNA cross hybridization between established feline and canine rotaviruses and feline- and canine-like isolates from humans, which allowed up to an 18% mismatch of the nucleotide sequences, included RFLP groups A and A-1 on the one hand and groups B and C on the other in only two distinct genogroups. Thus, the RFLP method allowed a further extension of that classification. By the RFLP approach, five additional unusual serotype 3 human isolates for which no genetic information was available (5193, AU-387, AU-750, AU-785, and AU-1115) could be assigned to two of the three major RFLP groups (Table 1).

We previously used RFLP analysis to study relationships among polioviruses isolated in Israel (32). The diversity of circulating strains of poliovirus type 1 in Israel, resulting from genomic variability as shown by different RFLP patterns, was also confirmed by nucleotide sequence analysis, although the sequencing was limited to a relatively short fragment. Most genetic relationships among polioviruses could be determined from only 75 to 100 nucleotides of sequence information (27). The same was also shown for rotavirus serotypes when analysis was done by hybridization (7). The RFLP patterns of the serotype 3 rotavirus strains studied by using the *Hin*fI and *Dde*I enzymes were strain specific, and they were in good agreement with results of molecular hybridization studies (18), although the analysis was made with just gene 9.

Evidence for the natural interspecies transmission of rotaviruses is growing (5, 30). However, the impact of these viruses on infant morbidity in different parts of the world and in different environments has not yet been explored. We do not even know what population is at risk of being infected with animal rotaviruses and what proportion of infected infants develop asymptomatic infections. Depending on their impact on infant morbidity or mortality in certain geographical regions, vaccines against animal rotaviruses will have to be included in future vaccine formulations. The RFLP assay for the rapid characterization of field isolates of rotaviruses and determination of their origin may be useful in investigating these issues.

ACKNOWLEDGMENTS

We thank C. J. Birch for Cat-2, Cat-3, and Cat-97 viruses; M. Mochizuki for isolates FRV-1, FRV-64, FRV-70, FRV-72, and FRV-79; and Etti Tilles for typing the manuscript.

L.S. is an ICRF CDA fellow.

REFERENCES

1. **Aboudy, Y., I. Shif, I. Silberstein, and T. Gotlieb-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.
2. **Aboudy, Y., I. Shif, I. Silberstein, and T. Gotlieb-Stematsky.** 1989. Efficiency of isolation of human rotavirus in primary African green monkey kidney cells. J. Virol. Methods **25**:250–258.
3. **Birch, C. J., R. L. Heath, J. A. Marshall, S. Liu, and I. D. Gust.** 1985. Isolation of feline rotaviruses and their relationship to human and simian isolates by electropherotype and serotype. J. Gen. Virol. **66**:2731–2735.
4. **Browning, G. F., R. M. Chalmers, T. A. Fitzgerald, and D. R. Snodgrass.** 1991. Serological and genomic characterization of L338, a novel equine group A rotavirus G serotype. J. Gen. Virol. **72**:1059–1064.
5. **Brussow, H., O. Nakagomi, G. Gerna, and W. Eichhorn.** 1992. Isolation of avianlike group A rotavirus from a calf with diarrhea. J. Clin. Microbiol. **39**:67–73.
6. **Fauvel, M., L. Spence, A. Babiuk, R. Petro, and S. Bloch.** 1978. Hemagglutinin and hemagglunination-inhibition studies with a strain of Nebraska calf diarrhea virus (bovine rotavirus). Intervirology **9**:95–105.
7. **Flores, J., J. Sears, I. Perez Schael, L. White, D. Garcia, C. Lanata, and A. Z. Kapikian.** 1990. Identification of human rotavirus serotype by hybridization to polymerase chain reaction-generated probes derived from a hyperdivergent region of the gene encoding outer capsid protein VP-7. J. Virol. **64**:4021–4024.
8. **Fulton, R. W., C. A. Johnson, N. J. Pearson, and G. N. Woode.** 1981. Isolation of a rotavirus from a newborn dog with diarrhea. Am. J. Vet. Res. **42**:841–843.
9. **Gerna, G., A. Sarasini, A. Di Matteo, L. Zentilin, P. Miranda, M. Parea, F. Baldanti, S. Arista, G. Milanesi, and M. Battaglia.** 1990. Serotype 3 human rotavirus strains with subgroup I specificity. J. Clin. Microbiol. **28**:1342–1347.
10. **Gouvea, V., R. I. Glass, P. Woods, K. Taniguchi, H. F. Clark, B. Forrester, and Z. Y. Fang.** 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J. Clin. Microbiol. **28**:276–282.
11. **Greenberg, H. B., V. McAuliffe, J. Voldesuso, R. C. Wyatt, J. Flores, A. R. Kalica, Y. Hoshino, and N. H. Singh.** 1983. Serological analysis of the subgroup protein of rotavirus using monoclonal antibodies. Infect. Immun. **39**:91–99.
12. **Malherbe, H. H., and M. Strickland-Cholmley.** 1967. Simian virus SA11 and the related "O" agent. Gestamte Virusforsch. **22**:235–245.
13. **Mebus, C. A., R. G. Wyatt, R. L. Sharpee, M. M. Sereno, A. R. Kalica, A. Z. Kapikian, and M. J. Twienhaus.** 1976. Diarrhea in gnotobiotic calves caused by the reovirus-like agent of infantile gastroenteritis. Infect. Immun. **14**:471–474.
14. **Mochizuki, M., and S. W. Hsuan.** 1984. Isolation of a rotavirus from a canine diarrhea feces. Jpn. J. Vet. Sci. **46**:905–908.
15. **Mochizuki, M., O. Nakagomi, and S. Shibata.** 1992. Hemagglutinin activity with two distinct genogroups of feline and canine rotavirus strains. Arch. Virol. **122**:373–383.
16. **Mochizuki, M., and M. Yamakawa.** 1987. Detection of rotavirus in cat feces. Jpn. J. Vet. Sci. **49**:159–160.
17. **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. **Nakagomi, O., A. Hoshima, Y. Aboudy, I. Shif, M. Mochizuki, T. Nakagomi, and T. Gotlieb-Stematsky.** 1990. Molecular identification by RNA-RNA hybridization of a human rotavirus that is closely related to rotaviruses of feline and canine origin. J. Clin. Microbiol. **28**:1198–1203.
19. **Nakagomi, O., M. Mochizuki, Y. Aboudy, I. Shif, I. Silberstein, and T. Nagagomi.** 1992. Hemagglutination by a human rotavirus isolate as evidence for transmission of animal rotaviruses to humans. J. Clin. Microbiol. **30**:1011–1013.
20. **Nakagomi, O., T. Nakagomi, K. Akatani, and N. Ikegami.** 1989. Identification of rotavirus genogroups by RNA-RNA hybridization. Mol. Cell. Probes **3**:251–261.
21. **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.
22. **Nakagomi, O., H. Oyamada, and T. Nakagomi.** 1991. Experience with serotyping rotavirus strains by reverse transcription and two-step polymerase chain reaction with generic and type-specific primers. Mol. Cell. Probes **5**:285–289.
23. **Nakagomi, T., Y. Matsuda, A. Ohshima, M. Mochizuki, and O. Nakagomi.** 1989. Characterization of a canine rotavirus strain by neutralization and molecular hybridization assays. Arch. Virol. **106**:145–150.
24. **Nishikawa, K., Y. Hoshino, K. Taniguchi, K. Y. Green, H. B. Greenberg, A. Z. Kapikian, R. M. Chanock, and M. Garziglia.** 1989. Rotavirus VP-7 neutralization epitopes of serotype 3 strains. Virology **171**:503–515.
25. **Pedley, S., J. C. Bridger, J. G. Brown, and A. McCrae.** 1983. Molecular characterization of rotaviruses with distinct group antigens. J. Gen. Virol **64**:2093–2101.
26. **Pedley, S., J. C. Bridger, D. Chasey, and A. McCrae.** 1986. Definition of two new groups of atypical rotaviruses. Arch. Virol. **67**:131–137.
27. **Rico-Hesse, R. M., A. Pallansch, B. K. Nottay, and O. M. Kew.** 1987. Geographic distribution of wild poliovirus type 1 genotypes. Virology **160**:311–322.
28. **Shif, I., I. Silberstein, Y. Aboudy, E. Mendelson, A. Mates, and T. Gotlieb-Stematsky.** 1992. Uniformity of serotype and electropherotype in local human rotavirus isolates during each of three successive cold seasons. Int. J. Epidemiol. **21**:399–405.
29. **Taniguchi, K., T. Urasawa, N. Kobayashi, M. Gorziglia, and S. Urasawa.** 1990. Nucleotide sequence of VP-4 and VP-7 genes of human rotaviruses with subgroup I specificity and long RNA pattern. Implication for new G serotype specificity. J. Virol. **64**:5640–5644.
30. **Urasawa, S., A. Hasegawa, T. Urasawa, K. Taniguchi, F. Wakasugi, H. Suzuki, S. Inouye, B. Pongprot, J. Supawadee, S. Suprasert, O. Rangsiyanond, S. Tonusin, and Y. Yamazi.** 1992. Anigenic and genetic analysis of human rotaviruses in Chiang Mai, Thailand. Evidence for a close relationship between human and animal rotaviruses. J. Infect. Dis. **166**:227–234.
31. **Vesikari, T. E., E. Isolauri, A. d'Hondt, F. Delem, E. Andre, and G. Zissis.** 1984. Protection of infants against human rotavirus diarrhea by RIT 4237 live attenuated bovine rotavirus vaccine. Lancet **i**:977–981.
32. **Vonsover, A., R. Handsher, M. Neuman, S. Guillot, J. Balanant, H. Rudich, E. Mendelson, T. Swartz, and R. Crainic.** 1993. Molecular epidemiology of polioviruses type 1 isolated in Israel and defined by restriction fragment length polymorphism assay. J. Infect. Dis. **167**:199–203.