Isolation from Diarrheal and Asymptomatic Kittens of Three Rotavirus Strains That Belong to the AU-1 Genogroup of Human Rotaviruses

MASAMI MOCHIZUKI,1 TOYOKO NAKAGOMI,2 AND OSAMU NAKAGOMI2*

Laboratory of Clinical Microbiology, Kyoritsu Shoji Co., Tokyo 102,1 and Department of Microbiology, Akita University School of Medicine, Akita 010,2 Japan

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A survey of 143 stool specimens collected during a 17-month period in Japan from diarrheal and asymptomatic kittens identified three rotavirus strains that were similar in their genomic RNA constellation to human rotavirus AU-1 (G3P3[9]), lending strong support to the view that rotaviruses belonging to the same genogroup are circulating in both the human and the feline population.

Group A rotaviruses, members of the genus Rotavirus in the family Reoviridae, have been established as the single most important etiologic agents of severe diarrhea of infants and young children worldwide (11). While rotaviruses are recovered from humans and a number of other animal species, there appears to be host-range restriction as indicated by poor growth of rotaviruses from one animal species in the intestines of another animal species (11). This natural attenuation in the heterologous host forms the basis for the Jennerian approach of rotavirus vaccine (10, 12). It was initially thought that host-range restriction was reflected by the distinctness of overall genomic RNA constellation (now termed genogroup) as assessed by RNA-RNA hybridization assays under high-stringency conditions when Flores et al. (3) demonstrated that homology is greater among strains derived from the same animal species than among strains derived from different animal species.

Extensive analysis by genogrouping has revealed, however, that a genogroup can be shared by rotavirus strains derived from two different host species, and this has been taken as evidence that rotaviruses can cross the host-species barriers (16). The first such evidence was exemplified by the observation that unusual human rotavirus isolates AU-1 and AU228 shared a genogroup with feline rotavirus strain FRV-1 (19). Evidence suggesting that AU-1 and FRV-1 have the same animal origin was fortified by further molecular analysis including VP4 sequence comparison (8). Furthermore, approximately 1% of human rotavirus isolates collected over a 10-year period from diarrheal children were shown to share the genogroup with AU-1/FRV-1 (7) and to have the same combination of G (defined by VP7) and P (defined by VP4) types, i.e., G3P3[9] (9) according to the recently proposed nomenclature system (2). However, isolation of increasing numbers of human rotaviruses belonging to the AU-1 genogroup has raised a question regarding the direction of virus transmission between humans and cats because molecular evidence shows only the similarity of the genome and does not indicate the direction of transmission. Here, we report isolation from cats of three strains that belong to the AU-1 genogroup and show serological evidence suggesting that cats were exposed to AU-1/FRV-1-like rotaviruses.

A total of 143 feline fecal specimens were collected in Kagoshima, Japan, during the period from January 1993 through May 1994, and they were examined for rotavirus antigen by commercially available reversed passive hemagglutination assay (Denka-Seiken, Tokyo, Japan). Five rotavirus antigen-positive specimens were identified, and strains designated FRV303, FRV317, FRV348, FRV381, and FRV384 were isolated in MA104 cells.

Serotyping was performed by plaque reduction neutralization assays as previously described (14). G and P serotypes were predicted by the typing method based on reverse transcription-PCR as previously described (5, 6).

Human and animal rotavirus strains used in this study were as follows: human strains AU-1, AU125, AU242, AU387, AU785, and AU938, all of which are G3P3[9] (15, 18); human strain PA151 (G6P3[9]) (4); feline strain FRV-1 (G3P3[9]) (19); and bovine strain 0510 (G6P7[5]) (13).

Genogrouping by RNA-RNA hybridization was performed as previously described (17). Briefly, the 32P-labeled single-stranded RNA probes from strains AU-1, FRV-1, and FRV317 were hybridized to the denatured genomic RNAs from a panel of human and feline rotaviruses. Hybridization was allowed to occur at 65°C for 16 h in a buffer containing 25 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, and 0.1% sodium dodecyl sulfate (pH 8.0). The resulting hybrids were then precipitated with ethanol and separated on a 10% polyacrylamide gel. Reannealed genomic RNAs were visualized by staining with ethidium bromide under UV illumination. Dried gels were exposed to X-Omat AR films (Eastman Kodak Co., Rochester, N.Y.) to make autoradiographs.

Neutralizing abilities of cat sera were determined by plaque reduction neutralization assays using as test strains rotaviruses possessing different combinations of G and P serotypes (14). Briefly, approximately 100 PFU of a test strain was incubated with serially diluted serum specimens for 1 h at 37°C. Each mixture was inoculated into the monolayer of MA104 cells in a 60-mm plastic dish and overlaid with agarose containing 1.0 mg of trypsin per ml. When plaques were developed, the number of plaques was counted with neutral red stain. Antibody titer was expressed as the highest dilution of serum neutralizing 75% or more of the input virus.

Of 143 fecal specimens collected from cats during the period from January 1993 through May 1994, 5 (3.5%) were positive
for rotavirus antigen by reversed passive hemagglutination assay. A single rotavirus strain was successfully isolated in MA104 cells from each of these five fecal specimens, and the strains were designated FRV303, FRV317, FRV348, FRV381, and FRV384. Only the kitten from which FRV317 was isolated had severe diarrhea, while the others were asymptomatic. These animals were epizootiologically unrelated to each other.

The electropherotypes of these new isolates were determined by polyacrylamide gel electrophoresis to be long patterns and were distinct from each other except for FRV381 and FRV384, which had identical gel migration profiles (data not shown). The serotype of these new isolates was determined by plaque reduction neutralization assays to be G3 (data not shown), as are those of all feline rotaviruses described in the literature to date (1, 14). Among feline rotaviruses, however, there are strains carrying two distinct VP4 alleles, correspond-
ing to either the absence or the presence of hemagglutinating ability of the virus as well as to two distinct P serotypes, P3 and P5 (14). According to the proposed new nomenclature system (2), P serotypes 3 and 5 correspond to P genotypes [9] and [3], respectively. Thus, nonhemagglutinating feline rotaviruses (e.g., FRV-1) have VP4 of P3[9] and hemagglutinating feline rotaviruses (e.g., FRV64) have VP4 of P5[3]. Since P serotypes are hard to determine serologically, a reverse transcription-PCR assay was used to determine the P genotype, and this was used to predict the corresponding P serotype. FRV317, FRV381, and FRV384 were determined to possess P3[9], while FRV303 and FRV348 were predicted to possess P5[3]. Thus, three isolates were possible new examples of long-sought-after FRV-1-type feline rotaviruses.

To determine to what extent these new G3P3[9] isolates were similar in their genomic RNA constellation to prototype G3P3[9] strains, i.e., AU-1 and FRV-1, RNA-RNA hybridization was performed with probes made from AU-1, FRV-1, and FRV317. Both AU-1 and FRV-1 probes formed 9 to 10 hybrid bands with the genomic RNA of FRV317, FRV381, and FRV384, similar in number and intensity to those formed between the probe and the genomic RNA of the homologous strain as well as other human strains belonging to the AU-1 genogroup (Fig. 1). Conversely, the FRV317 probe formed 9 to 10 hybrid bands with the genomic RNA of AU-1, FRV-1, FRV381, and FRV384, similar in number and intensity to those formed between the probe and the homologous virus (Fig. 2). These results showed that strains FRV317, FRV381, and FRV384 are new members of the AU-1 genogroup, indicating that the AU-1/FRV-1-like rotaviruses have been circulating among cats. By contrast, FRV303 and FRV348 were shown not to belong to the AU-1/FRV-1 genogroup because the number of hybrids formed between these strains and the FRV-1 probe was limited to a few (data not shown).

To serologically support these molecular observations, we examined whether cats are exposed in nature to infection with rotaviruses carrying P3[9] VP4. We randomly chose 30 serum specimens from a panel of cat sera collected during the period between 1989 and 1994 and tested them in neutralization assays against three rotavirus strains possessing different combinations of G and P serotypes to determine whether each serum specimen had neutralization activity directed at P3[9] antigenic determinants. Specifically, the serum specimens were tested first against FRV-1(G3P3[9]), and the sera that neutralized FRV-1 were further tested against PA151(G6P3[9]) and 0510(G6P7[5]). Of 15 serum specimens that neutralized FRV-1, 7 neutralized PA151, which shares only P serotype with FRV-1, but they did not neutralize 0510, which shares G se-

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<tr>
<th>Cat serum</th>
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FIG. 2. Hybridization patterns between genomic RNAs from the indicated virus strains and the 32P-labeled plus-strand transcription probes prepared from FRV317. Shown is an ethidium bromide-stained gel under UV light illumination (a), revealing genomic RNAs from the indicated strain. Faint bands represent aberrantly migrating hybrid bands formed between the probe and genomic RNAs and correspond to the hybrid bands appearing on the autoradiograph (b). Approximate positions of the RNA segments of the FRV317 strain are indicated to the left.
rototype with PA151 but is serologically unrelated to FRV-1 (Table 1). Thus, at least 23% (7 of 30) of the serum specimens contained neutralization activity directed at the P3[9] antigenic determinants. We therefore speculate that approximately a quarter of cats were exposed to infection with AU-1/FRV-1-like rotaviruses.

Interspecies transmission of rotaviruses from one animal species to another has been addressed from the perspective of genogroup, which is determined by RNA-RNA hybridization under high-stringency conditions. Genogroup is defined by the number and the intensity of hybrid bands formed between the probe and the genomic RNA of a test strain. It has been previously established that rotaviruses of a given host species belong usually to one genogroup or, at most, to a few genogroups but that rotaviruses deriving from different animal origins rarely belong to the same genogroup (16). This rare exception of the sharing of a genogroup by different animal rotaviruses has been interpreted as molecular evidence for interspecies transmission of rotaviruses as exemplified by the human strain AU-1 and feline strain FRV-1. However, the sharing of a genogroup, i.e., possession of a closely related genomic RNA constellation, does not indicate by itself the direction of transmission. Thus, the presence of many AU-1-like strains in humans in contrast to the absence of FRV-1-like strains in cats raised the possibility that the cat from which FRV-1 was isolated might have been infected with a human rotavirus similar to the AU-1 strain. In this context, by presenting the data that three of five feline rotaviruses isolated during a 17-month period belonged to the AU-1 genogroup and that approximately a quarter of cats had been exposed to the AU-1/FRV-1-like rotaviruses, this study has provided strong support for the view that the AU-1/FRV-1-like rotaviruses, i.e., rotaviruses belonging to the AU-1 genogroup, circulate among cats. When it is taken into consideration that a number of AU-1-like viruses were isolated from humans (7, 20), members of the AU-1 genogroup are likely to circulate both in the human and in the feline population. The question remains, however, what percentage of the strains belonging to the AU-1/FRV-1 genogroup directly resulted from recent interspecies transmission.

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REFERENCES