Molecular Identification by RNA-RNA Hybridization of a Human Rotavirus That Is Closely Related to Rotaviruses of Feline and Canine Origin

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With a few exceptions subgroup I group A human rotavirus strains have short RNA patterns, whereas most animal rotavirus strains belong to subgroup I and have long RNA patterns. Thus, new isolates of subgroup I human rotaviruses with long RNA patterns are considered to have a high likelihood of being animal rotaviruses. A group of human rotaviruses represented by the AU-1 strain has recently been shown to be genetically related to a feline rotavirus (FRV-1) isolated in Japan. A human rotavirus, strain Rol845, which is similar to the AU-1 strain in its subgroup (I), serotype (3), and electropherotype (long), was compared with various human and animal strains by RNA-RNA hybridization to determine its genogroup, a term proposed to classify rotaviruses based on their gene homology. The Rol845 strain did not show a significant level of homology with AU-1, FRV-1, or other human strains, indicating that the Rol845 strain is different in its genogroup not only from the AU-1 strain but also from other human strains. However, the Rol845 strain showed a high degree of homology with another feline rotavirus (Cat97) isolated previously in Australia, suggesting that the Rol845 strain might originate from a feline rotavirus that is genetically distinct from the Japanese FRV-1 strain. Furthermore, the Rol845 strain as well as the Cat97 strain were related genetically to the canine rotavirus RS15 strain. Taken together, these results indicate that at least two genogroups are present in feline rotaviruses, one resembling the AU-1 strain and the other resembling the Rol845 strain as well as canine rotaviruses.

Rotavirus is the major etiological agent of acute diarrhea of infants and young children as well as young animals of many species (20). The genus Rotavirus has been classified into five groups, A to E, based on group-specific antigens detected by immune electron microscopy (38, 39). Group A rotaviruses constitute the majority of rotaviruses and have been the focus of extensive studies. Group A rotaviruses can be assigned to at least nine serotypes by neutralization assays (4, 16, 20, 26, 47). Distinct from the serotype, another antigenic specificity called subgroup specificity can be identified by enzyme-linked immunosorbent assays with monoclonal antibodies specific for subgroup antigens located on the inner capsid (13).

The rotavirus genome comprises 11 segments of double-stranded RNA (dsRNA) which are encased within a double-layered capsid (20). Upon analysis by polyacrylamide gel electrophoresis, extensive heterogeneity is noted in the migration patterns of these dsRNA genome segments, allowing a given rotavirus strain to be assigned to a distinct electropherotype (for a review, see reference 5). Despite this diversity in electropherotypes, however, two distinct RNA patterns are apparent. Short patterns possess slower-moving gene segments 10 and 11, and long patterns possess faster-moving gene segments 10 and 11 (19, 22). Short and long RNA electropherotypes are, in most instances, associated with subgroups I and II, respectively (1, 3, 19, 22, 34, 40–45). In addition, serotype 2 strains have short RNA electrophero-
types and serotype 1, 3, and 4 strains have long RNA electropherotypes (33, 43). However, such apparent correlation between RNA electropherotype and subgroup or serotype has no genetic basis because the subgroup antigen, which is located on VP6, is encoded by gene segment 6 (12) and serotype-specific antigens, which are located on VP4 and VP7, are determined, respectively, by gene segment 4 and gene segments 8 or 9 (15, 37).

On the other hand, RNA-RNA hybridization studies on human rotavirus strains obtained from field studies lent strong support to the observation that the association of two major RNA electropherotypes and subgroups appeared to be more coincidental. Flores et al. (7–9) described the genetic dimorphism of human rotaviruses at the molecular level by an RNA-RNA hybridization assay and showed that this molecular dimorphism is associated with two major RNA electropherotypes, i.e., short and long. Thus, the genetic differences of two major rotavirus groups are not confined to gene segment 6, but the differences extend to most, if not all, of the other 10 genes. These observations formed the basis of the genetic classification of rotaviruses by RNA-RNA hybridization for which we have proposed the term genogroup (36). It was recently shown by RNA-RNA hybridization that human rotaviruses basically fall into any one of three distinct genogroups (30), i.e., the first genogroup that includes strains related to the Wa strain (serotype 1, subgroup II, long RNA electropherotype), the second genogroup that includes strains related to the DS-1 strain (serotype 2, subgroup I, short RNA electropherotype), or

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the third genogroup that includes strains related to the AU-1 strain (serotype 3, subgroup I, long RNA electropherotype).

As to animal rotaviruses, a previous study by Flores et al. (6) showed that rotaviruses recovered from the same species share a high degree of gene homology, whereas this degree of homology was not observed between the viruses derived from different animal species. We have recently shown that bovine rotaviruses constitute a single genogroup that is distinct from any of the three human genogroups, even though the bovine rotavirus strains examined belonged to two different bovine rotavirus serotypes (25). Thus, interspecies transmission is suspected if a given rotavirus isolate shares a genogroup with rotaviruses derived from a different animal species.

The AU-1 genogroup is of particular interest in terms of possible interspecies transmission of animal rotavirus to humans. We have recently shown by RNA-RNA hybridization that human rotaviruses belonging to the AU-1 genogroup are genetically related to a feline rotavirus (36). Since increasing numbers of subgroup I human rotaviruses with long RNA patterns are being discovered (1, 3, 10, 31, 34, 40, 41) and some of these strains were adapted to grow in cell cultures, we have attempted to compare one such strain (the Ro1845 strain isolated in Israel [1]) with the AU-1 strain, a prototype strain that possesses these novel characteristics (31). Furthermore, the Ro1845 strain was compared genetically with feline and other animal rotavirus strains. Here we report that the Ro1845 strain is genetically distinct from either the AU-1 strain or the feline FRV-1 strain but that the Ro1845 strain shares a high degree of homology with the Cat97 strain isolated in Australia as well as the canine RS15 strain isolated in Japan.

MATERIALS AND METHODS

Viruses. The following tissue culture-adapted human rotavirus strains were used in this study: Wa, serotype 1, subgroup II (46); KUN, serotype 2, subgroup I (23); P, serotype 3, subgroup II (47); MO, serotype 3, subgroup II (23); McN, serotype 3, subgroup II (11); AU-1, serotype 3, subgroup I (21, 30); AU228, serotype 3, subgroup I (34, 36); F16, serotype 3, subgroup I (30); S106, serotype 3, subgroup I (30); Ro1845, serotype 3, subgroup I (1); ST3, serotype 4, subgroup II (47); 69M, serotype 8, subgroup I (26); and WI61, serotype 9, subgroup II (4). In addition, the following animal strains were used: feline FRV-1, serotype 3, subgroup I (29, 18); feline Cat2, serotype 3 (2); feline Cat97, serotype 3 (2); canine RS15, serotype 3, subgroup I (28, 35); simian SA11, serotype 3, subgroup I (24); equine H123, serotype 3 (18); bovine NCDV, serotype 6, subgroup I (27).

Preparation of dsRNA. Genomic dsRNA was extracted with phenol-chloroform from the partially purified virions which were prepared from infected MA104 cells by pelleting them at 40,000 rpm for 1.5 h in a rotor (RP42; Hitachi) and then by sedimentation through 30% (wt/vol) sucrose at 38,000 rpm for 2 h in a rotor (RPS40T; Hitachi).

Preparation of ssRNA transcripts. Single-stranded RNA (ssRNA) probes (mRNA) were prepared by in vitro transcription of rotavirus single-shelled particles in 250 μl of 70 mM Tris acetate buffer (pH 8.0) that contained 20 mM magnesium acetate, 100 mM sodium acetate, 8 mM ATP, 0.5 mM GTP, 2.5 mM CTP, 2.5 mM UTP, 0.5 mM β-adenosylmethionine, 0.1% bentonite, and [α-32P]GTP (25 μCi per reaction). After 6 h of incubation at 42°C, ssRNAs were purified by phenol-chloroform extraction and lithium chloride precipitation.

RNA-RNA hybridization. The 32P-labeled ssRNA probes from strains AU-1, FRV-1, Ro1845, RS15, Cat2, and Cat97 were hybridized to the denatured genomic RNAs from a panel of human and animal rotaviruses as described previously (30, 33). Denaturation of dsRNA (approximately 1 μg) was accomplished by 2 min of incubation at 100°C followed by quenching on ice for 2 min. 32P-labeled probes (10,000 cpm for each denatured dsRNA) were added; and hybridization was allowed to occur at 65°C for 16 h in a buffer containing 5 mM Tris acetate, 150 mM NaCl, 1 mM EDTA, and 0.1% sodium dodecyl sulfate (pH 7.5). After hybridization, the RNAs were precipitated with ethanol and dissolved in a sample buffer containing 62.5 mM Tris hydrochloride (pH 6.8), 5% (vol/vol) 2-mercaptoethanol, 10% (vol/vol) glycerol, 2% (wt/vol) sodium dodecyl sulfate, and 0.001% (wt/vol) bromophenol blue. The resulting hybrids, consisting of negative-strand genomic RNA and the positive-strand probe, were separated on a 10% polyacrylamide gel with a 4% stacking gel and then were stained with ethidium bromide. Autoradiographs were prepared by exposing dried gels to X-Omat AR films (Eastman Kodak Co., Rochester, N.Y.) at −80°C.

RESULTS

When the AU-1 probe was hybridized to the genomic RNA prepared from the Ro1845 strain and three feline strains (FRV-1, Cat2, and Cat97), only the genomic RNA from the FRV-1 strain produced hybrids similar to those formed between the probe and the homologous dsRNA (Fig. 1). This degree of homology was not observed between the AU-1 probe and the genomic RNA from the other two feline strains (Cat2 and Cat97) isolated in Australia as well as those from the Ro1845 strain (Fig. 1). Of two Australian feline strains, the Cat2 strain appeared to be more closely related to the AU-1 strain since the AU-1 probe formed six hybrids with the genomic RNA from the Cat2 strain, while this probe formed only three hybrids with the genomic RNA from the Cat97 strain (Fig. 1). Correspondingly, the Ro1845 probe did not show a high level of homology with the genomic RNA from the AU-1 strain (Fig. 1). We therefore compared the genetic relatedness of the Ro1845 strain and the other rotavirus strains that possess long RNA patterns yet that belong to subgroup I. We selected the AU228, S106, and F16 strains because these three human strains were recently shown by RNA-RNA hybridization to belong to the AU-1 genogroup (30). As expected, the Ro1845 probe did not show a high level of homology with the genomic RNA from the AU228, S106, and F16 strains (Fig. 2). These results indicate that the Ro1845 strain does not share a sufficient degree of genetic homology to be assigned to the AU-1 genogroup, even though it shares common characteristics, i.e., electropherotype (long pattern), subgroup (subgroup I), and serotype (serotype 3), with the AU-1 strain.

Since the Ro1845 strain was genetically distinct from the AU-1 strain, we next examined the genetic relatedness of the Ro1845 strain and other human rotavirus strains representing serotypes 1 to 4, 8, and 9 and some animal strains, including three feline strains. While the Ro1845 probe did not significantly hybridize with the genomic RNA from the Wa (serotype 1), KUN (serotype 2), P (serotype 3), ST3 (serotype 4), 69M (serotype 8), WI61 (serotype 9), simian SA11 (serotype 3), equine H123 (serotype 3), feline FRV-1 (serotype 3), feline Cat2 (serotype 3), and bovine NCDV (serotype 6) strains, the probe formed 7 (or 8) and 10 hybrids with canine RS15 and Cat97 strains, respectively (Fig. 2 and
Correspondingly, the RS15 probe formed 8 hybrids with the genomic RNA from the Ro1845 strain, and the Cat97 probe formed 10 (or 11) hybrids that were almost indistinguishable from the homologous bands (Fig. 4). These results indicate that the Ro1845 strain is closely related to the Cat97 strain and is to a lesser degree, but still significantly, related to the canine RS15 strain. More than 11 hybrid bands seen in the homologous reaction of the Cat97 strain need to be mentioned. A plausible explanation is that the seed stock of the Cat97 strain contains more than one population of rotavirus because polyacrylamide gel electrophoresis showed two closely migrating gene segments 5 (data not shown). Although we did not plaque purify the Cat97 strain in our laboratory, the RNA pattern of this strain did not change during several passages in our laboratory. The origin of two hybrid bands seen above gene segment 1 could not be identified.

The RS15 strain showed a high degree of homology with the Cat97 strain (Fig. 4). Strain FRV-1 showed a higher degree of homology with the Cat2 strain than it did with the RS15 and Cat97 strains (Fig. 4). Conversely, the Cat2 strain showed a higher degree of homology with the FRV-1 strain than it did with the RS15 and Cat97 strains (Fig. 5).

We have previously reported that the AU-1 probe does not hybridize with genomic RNA from other human rotaviruses belonging to either the Wa or the DS-1 genogroup (31). However, gene segment 9, which encodes that outer capsid glycoprotein VP7, shows a high degree of homology with that of serotype 3 human rotavirus strains, but not with that of serotype 3 animal rotavirus strains (31). We examined whether similar results were obtained when the Ro1845 probe was hybridized with genomic RNA from serotype 3 human rotavirus strains (the P, MO, and McN strains). The absence of a hybrid band comigrating with genomic RNA segments 8 or 9 suggested that the VP7 gene of the Ro1845 strain is different from those of human serotype 3 strains (Fig. 5).

**DISCUSSION**

The genetic relatedness between the AU-1 and Ro1845 strains and their relationship to other prototype human and animal rotavirus strains representing serotypes 1 to 4, 6, 8,
and 9 were examined by RNA-RNA hybridization under stringent conditions which were calculated to allow up to an 18% mismatch of the nucleotide sequences. In this assay, homologous reactions were identified by bands on the autoradiogram which comigrated with genomic dsRNA segments visualized by ethidium bromide staining of the gel. Although hybrids were not treated with S1 nuclease before gel electrophoresis throughout this study, previous studies have shown that these hybrids are resistant to digestion with S1 nuclease (7, 36). On the other hand, the hybrids formed between the genes that were related but not completely homologous could be identified by bands with a lesser intensity that did not necessarily comigrate with their parental genomic RNAs (Fig. 1 to 5). Thus, such aberrantly migrating hybrid bands can be interpreted as an indication of partial homology between the probe and genomic RNAs, but exact identification of the origin of the gene segments involved in such hybrids is not always possible.

Molecular characterization by RNA-RNA hybridization under stringent conditions provides a unique opportunity to study the genetic relationships among rotaviruses from humans as well as animals. The term genogroup has been proposed to describe rotavirus strains that can be placed together on the basis of their gene homology (36). Sharing of a genogroup by two rotavirus strains derived from different animal species can be interpreted as a consequence of interspecies transmission of rotaviruses from one animal species to another or as a result of the sharing of a common ancestor by two viruses. As an example, we have shown previously (36) that the AU228 strain, a human rotavirus that was isolated from a symptomatic child with a history of contact with a cat, is closely related genetically to the feline rotavirus FRV-1 strain.

Of interest in this study with regard to the genogroup classification of rotaviruses is that the Ro1845 strain did not share genogroup specificity with AU-1 and similar strains or with a Japanese feline rotavirus FRV-1 strain, even though
very similar to the canine genogroup, and they may be placed together in the category of the canine-feline genogroup. Further genetic analysis on a larger number of feline and canine isolates is required to address this question further. Unfortunately, there are only a few feline (2, 14, 36) and canine strains available for study. Birch et al. (2) previously speculated that cats might act as a source of rotavirus infection in humans. Results of this study as well as those of our previous study (36) support the hypothesis of Birch et al. (2) and may justify the need to explore rotaviruses of canine and feline origin, because such viruses are considered to be of medical significance in terms of the zoonotic origin of novel human rotavirus strains.

In summary, we observed that (i) the Ro1845 strain does not belong to the AU-1 genogroup, although the Ro1845 strain is similar to the AU-1 strain in its serotype, subgroup, and electropherotype; (ii) the Ro1845 strain is closely related genetically to a feline rotavirus strain; and (iii) at least two genogroups are present in feline rotavirus strains, one resembling the AU-1 strain and the other resembling the Ro1845 strain as well as a canine rotavirus. Understanding of the genetic diversity and similarity of human rotavirus genomes in relation to animal rotavirus genomes will expand our knowledge of the evolution of rotavirus genes in nature.

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LITERATURE CITED


