Identification of Cognate Genes among Heterologous Strains of Group B Rotavirus

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The genetic relatedness of group B rotavirus (GBR) strains has previously been documented by hybridization with probes derived from whole genomic sequences, but the relationship of individual genes of heterologous GBR strains has not been evaluated. Definition of cognate GBR genes would facilitate investigation of the determinants of group specificity, serotype identity, and neutralization epitopes. Therefore, we investigated the genetic relatedness of three GBR strains by means of Northern (RNA) blot hybridization with isotopically labeled probes prepared from each of the 11 genes of the IDIR strain of GBR. Under low-stringency conditions, hybridization between each of the IDIR gene probes and genomic RNA from the ADRV strain of GBR was observed. Genomic RNA obtained from a bovine strain of GBR hybridized with 9 of the 11 IDIR gene probes. In most cases, cognate genes of each of the GBR strains appeared to migrate to similar positions following polyacrylamide gel electrophoresis. However, the electropherotype positions of GBR genes 5, 6, and 7 were different for each of the three GBR strains. Identification of these genomic segments among GBR strains should prove helpful in future evaluations of GBR structure and function.

Group B rotaviruses (GBR) are etiologic agents of gastroenteritis in humans and a variety of other mammals (2, 3, 6, 8, 13, 15, 20, 23, 24). While morphologically similar to other rotaviruses, GBR are antigenically and genetically distinct from the group A rotaviruses (GAR), which are usually associated with wintertime outbreaks of infantile gastroenteritis (6, 15). GBR are also distinguished from GAR by differences in the electropherotype patterns which are generated by polyacrylamide gel electrophoresis (PAGE) of the segmented double-stranded RNA genome (2, 15). When rotavirus genes are arranged in groups according to their relative PAGE migrations, the electropherotype of GAR may be characterized as a 4-2-3-2 pattern (11). Divergence from this electropherotype pattern may be noted, but GAR generally maintain the 4-2-3-2 pattern. In contrast, GBR strains exhibit a number of different electropherotypes, including patterns of 4-2-2-1-1-1, 4-3-1-1-1-1, and 4-2-1-1-1-1 (2, 6, 10, 15, 24). Among these heterologous GBR electropherotypes, little is known concerning the relative migrations of cognate genes which encode similar proteins. Nucleic acid sequence analysis has established that genes 9 and 11 of the IDIR (infectious diarrhea of infant rats) virus are similar to genes 9 and 11 of the GBR strain ADRV (adult diarrhea rotavirus) (4, 5, 16). Gene 3 of the IDIR virus also appears to correspond to gene 3 of a bovine strain of GBR, as determined by Northern (RNA) blot hybridization (7). The genetic relatedness and PAGE migrations of other gene segments of these diverse GBR strains have not been reported. Evaluation of the relationship of these GBR genes is needed because genomic segments which encode similar proteins may not necessarily migrate at equivalent positions following PAGE. For example, the genome segment which encodes the major outer capsid glycoprotein (VP7) of GAR can migrate at electropherotype position 7, 8, or 9, depending upon the virus strain (11, 14). If such variations are present among GBR genomic segments, establishment of

their identities will be helpful for comparison of the various GBR strains.

We have recently synthesized cDNA clones from all 11 genomic segments of the IDIR agent (16, 18). We now report Northern blot hybridization of these cDNA clones in order

FIG. 1. PAGE of GBR genomic RNA. Genomic RNA was purified and separated on a 7.5% polyacrylamide gel as described in the text. The gel was stained with ethidium bromide, and RNA bands were visualized by means of UV light exposure. Lanes: a, IDIR agent; b, bovine GBR; c, ADRV GBR; d, TRV. Gene numbers are indicated at the left.

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FIG. 2. Autoradiographs following hybridization of genomic RNA with ^{32}P -labeled probes from individual genes of the IDIR strain of GBR. Genomic RNA used for Fig. 1 was transferred to DBM-paper as described in the text and then hybridized with a probe from IDIR gene 5 (A). The DBM-paper was then hybridized with a probe from IDIR gene 1 (B). The lower portion of the DBM-paper was later hybridized with a cDNA probe of IDIR gene 11, while the upper portion of the paper was hybridized with a cDNA clone of IDIR gene 3 (C). Subsequently, the upper portion of the paper was rehybridized with a cDNA clone of IDIR gene 2, and the lower portion of the paper was rehybridized with IDIR gene 7 (D). The virus strains in lanes a through d are as noted for Fig. 1. Gene numbers are indicated at the right.

to identify nucleic acid homology with individual genomic segments of diverse strains of GBR.

The IDIR strain of GBR was propagated in infant CD rats (Charles River Laboratories) as previously described (25). Calf fecal specimens containing GBR were obtained from Alfonso Torres (New York State College of Veterinary Medicine, Cornell University, Ithaca) (24). Human fecal specimens containing the ADRV strain of GBR were provided by Fang Zhao Yin (Institute of Virology, Chinese Academy of Preventive Medicine, Beijing, China) (12). The TRV strain of fish rotavirus-like virus was provided by Siba K. Samal of the University of Maryland School of Veterinary Medicine (17).

CsCl purification of ADRV and the extraction of genomic RNA have been described elsewhere by Fang and coworkers (12). Genomic RNAs from fecal specimens of IDIR and bovine strains of GBR were obtained by means of CF-11 chromatography (22).

IDIR genomic double-stranded RNA was denatured, polyadenylated, and cloned as noted in prior reports (7, 16). These cDNA clones permitted determination of the genomic terminal sequences, and oligonucleotide primers were subsequently synthesized according to these sequences. Terminal primers were then employed in a reverse transcriptase-polymerase chain reaction in order to construct full-length cDNA clones of individual IDIR genomic segments (9). By this means, full-length cDNA clones were generated from genes 3 and 5 to 11 of the IDIR virus. A nearly full-length ($\geq 95\%$) cDNA clone of IDIR gene 2 was identified from the library originally constructed from IDIR RNA. Clones of IDIR genes 1 and 4 represented approximately 65 and 75% of the genomic sequence, respectively. ^{32}P -labeled probes with a specific activity of approximately 10^8 cpm/ μg were synthesized from the cloned cDNA inserts with *Escherichia coli*

FIG. 3. Autoradiographs following hybridization of genomic RNA with ^{32}P -labeled probes from individual genes of the IDIR strain of GBR. Genomic RNA used for Fig. 1 was transferred to DBM-paper as described in the text and then hybridized with a probe from IDIR gene 10 (A). Following autoradiography, the DBM-paper was hybridized with probes from IDIR genes 5 (B) and 4 (C). The virus strains in lanes a through d are as noted for Fig. 1. Gene numbers are indicated at the right.

DNA polymerase I and DNase I (Bethesda Research Laboratories, Inc., Gaithersburg, Md.)

Templates for Northern blot hybridization were prepared by PAGE of viral double-stranded RNA segments and transfer to diazobenzylxymethyl-paper (DBM-paper) (1, 21). Preliminary experiments indicated that hybridization between cDNA probes and homologous RNA would result in markedly darker autoradiograph signals than those generated by annealing with RNA from heterologous GBR strains. The intensity of the signal between the probe and homologous RNA often obscured weaker signals produced by the hybridization of the IDIR probe with heterologous RNA. In

FIG. 4. Autoradiographs following hybridization of genomic RNA with ^{32}P -labeled probes from individual genes of the IDIR strain of GBR. Genomic RNA used for Fig. 1 was transferred to DBM-paper as described in the text and then with a probe from IDIR gene 8 (A). Following autoradiography, the DBM-paper was successively hybridized with probes from IDIR genes 9 (B), 3 (C), and 6 (D). The virus strains in lanes a through d are as noted for Fig. 1. Gene numbers are indicated at the right.

order to observe hybridization with heterologous GBR strains, smaller quantities of IDIR RNA were employed as templates in subsequent Northern blot reactions. Genomic RNAs of the other virus strains were applied in equal amounts (Fig. 1). Prehybridization and hybridization buffers, as well as washing conditions for the hybridizations, have been described previously (1, 6). All hybridizations were performed in 10% formamide for approximately 15 h at 45°C, permitting annealing of nucleic acids with $\leq 35\%$ base mismatch. These particular conditions were employed because higher-stringency conditions prevented annealing between many of the genome segments of heterologous GBR strains (6). In addition, previous studies with diverse animal and human strains of GAR had demonstrated that few genome segments would exhibit annealing under high-stringency conditions (19). Therefore, in the present study, lower-stringency conditions were employed in order to determine the relationship of as many cognate GBR genes as possible. Under these conditions, probes from individual IDIR genes hybridized to no more than one genomic segment of each of the GBR strains. No hybridization by Northern blot with the control RNA (TRV) was noted. Hybridization between IDIR cDNA and SA11 RNA was also not detected by dot blot hybridization (data not shown). Thus, the annealing conditions were judged to be of sufficient stringency to assure specific hybridization only with related genome segments of GBR.

In this Northern blot format, IDIR gene 1 hybridized to gene 1 of both ADRV and bovine GBR strains (Fig. 2). IDIR gene 2 hybridized with ADRV gene 2, but no hybridization with bovine GBR RNA was detected (Fig. 2D).

Genes 3 and 4 of IDIR each hybridized with ADRV and bovine GBR genes of similar sizes (Fig. 2 to 4). However, genes 3 and 4 of the ADRV and bovine GBR were poorly resolved by means of PAGE with a variety of gel concentrations, and it was not possible to distinguish hybridizations which had occurred with one or the other of these probes.

Although genes 5, 6, and 7 of the IDIR agent migrated as a triplet in PAGE, these genes were well separated for ADRV and bovine GBR strains (Fig. 1). Northern blot hybridization revealed that the cognate genes of these three GBR strains occupied different positions within their respective electropherotypes. IDIR gene 5 annealed to gene 7 of the ADRV and bovine GBR genomes (Fig. 3B and C and D). Conversely, hybridization between IDIR gene 7 and gene 5 of the bovine GBR was noted. IDIR gene 7 also annealed to gene 6 of ADRV (Fig. 2D). IDIR gene 6 annealed to bovine GBR gene 6 and to ADRV gene 5 (Fig. 4D). Preliminary sequence data have confirmed the relationship of IDIR gene 6 and ADRV gene 5, with approximately 71.5% nucleic acid sequence identity in these two genes (5a).

Probes prepared from the remaining IDIR genes (7, 9–11) annealed to heterologous RNA genome segments which occupied similar positions within their respective electropherotypes. IDIR gene 8 probe and gene 9 (Fig. 4) hybridized to both ADRV and bovine RNA. Whereas IDIR gene 10 hybridized with ADRV RNA, no signal was detected with the bovine GBR RNA (Fig. 3). Annealing between IDIR gene 11 probe and the bovine GBR RNA was observed, but the signal produced by this hybridization was notably weaker than that seen with ADRV gene 11 (Fig. 2).

For several of the IDIR genes (genes 2, 5, 6, 7, 10, and 11), hybridization with the bovine GBR RNA was not detectable or was more difficult to detect than hybridization with the ADRV RNA (Fig. 5). These results may indicate that IDIR is more closely related to ADRV than to the bovine GBR

FIG. 5. Hybridization of IDIR gene probes with RNA of ADRV and bovine strains of GBR. Arrows indicate results of hybridization with single-gene probes as described in the text. Dashed arrows indicate hybridizations observed only after more prolonged exposure (≥ 2 days). Numbers indicate genes.

isolate. Comparison of the nucleic acid sequences of individual genes in future investigations should be valuable in confirming the relationship of these virus strains. Such comparisons may provide insight into the evolution of the GBR and their relationship to other rotavirus groups.

The identification of homologous genomic segments among GBR strains in this report should also prove helpful in future evaluations of the antigenic relatedness of this virus group. As antigenic epitopes and gene coding assignments are determined for individual strains of GBR, cDNA clones of additional GBR strains may be selected for comparison. Such evaluations are likely to aid our understanding of the mechanisms of rotavirus attachment, replication, and pathogenesis.

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