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Isolation and Characterization of Two Distinct Human Rotavirus Strains with G6 Specificity

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Two new human rotavirus (HRV) strains, PA151 and PA169, with subgroup I specificity and a long RNA pattern, yet with a serotype G (VP7) specificity different from those of any of the six well-established HRV serotypes (G1 to G4, G8, and G9), were isolated 3 months apart from two children with acute gastroenteritis in Sicily, southern Italy, in the winter season of 1987 and 1988. The HRV isolates were adapted to growth in cell cultures and were then characterized by neutralization and RNA-RNA (Northern blot) hybridization. Cross-neutralization studies with type-specific immune sera to RV serotypes 1 to 10 showed the antigenic relatedness of the two strains with serotype 6 bovine strains UK and NCDV. Monoclonal antibodies to VP7 of UK were able to recognize UK and NCDV strains as well as both HRV isolates. Cross-hybridization studies showed a genetic relatedness of PA151 and PA169 to bovine strains for all genes except gene 4. Gene 4 of PA151 appeared to be genetically related to that of AU228 (a human strain of subgroup I and with serotype G3 specificity that belongs to a feline genogroup), whereas gene 4 of PA169 appeared to be unique, yet it was related to gene 4 of two recently reported subgroup I HRV strains, one (PA710) with serotype G3 specificity and the other (HAL1271) with serotype G8 specificity. The new HRV strains must be taken into consideration when deciding strategies for the development of an effective RV vaccine.

Group A human rotavirus (HRV) strains, which represent the major cause of acute gastroenteritis in infants and young children, possess common group, subgroup (I and II), and VP7 serotype (G1 to G4, G8, and G9) antigenic specificities. With a few exceptions (19, 34–36), serotype 2 and 8 HRV strains belong to subgroup I; and serotype 1, 3, 4, and 9 strains belong to subgroup II. While serotypes 1 to 4 have been found to circulate at a variable rate in different geographic areas around the world (2, 15), serotype 8 strains, which were first isolated by Matsuno et al. (29) in Indonesia, and serotype 9 strains, which were initially described by Clark et al. (7) in the United States, seem to circulate poorly. Although in the last few years a limited number of HRV strains sharing genetic homology with animal strains of the same serotype have been reported (19, 22, 34–36), no HRV strain sharing serotype G specificity with bovine serotype 6 has so far been described.

During an epidemiological study on the prevalence of HRV serotypes in Italy, we were initially unable to type a strain by an enzyme-linked immunosorbent assay (ELISA) system using VP7-specific monoclonal antibodies (MAbs) to HRV serotypes 1 to 4, 8, and 9. This strain, PA151, was isolated in Palermo, Sicily, in November 1987 from a 19-month-old child with acute gastroenteritis. The serotype of a second strain, PA169, isolated in the same urban area 3 months later from a 5-month-old infant with gastroenteritis could not be identified either. Following propagation in cell cultures, the two strains were shown to be antigenically related to NCDV and UK bovine strains of serotype 6. In this study, we report results showing the antigenic relatedness of the two new HRV strains with bovine strains of serotype 6 at the VP7 level, while by RNA-RNA hybridization studies, we show that the gene 4 sequences of the two HRV strains differ from each other as well as from the sequences of the corresponding genes of both UK and NCDV.

MATERIALS AND METHODS

HRV specimens. During an epidemiological survey carried out from 1985 to 1989 in Palermo, Sicily, southern Italy (1), 19 of 238 HRV strains could not be serotyped by ELISA by using type-specific MAbs (14). Some of these strains were successfully adapted to growth first in African green monkey kidney and then in MA104 cell cultures in the presence of trypsin, according to previously reported procedures (13, 42). Two of these strains, PA151 and PA169, were isolated 3 months apart from the stools of a 19-month-old child and of a 5-month-old infant, both of which had acute gastroenteritis, and these strains were further studied and characterized.

Virus strains. The following cell culture-adapted reference RV strains with the indicated serotype G specificity were used in this study: human Wa, G1 (24); human DS-1, G2 (24); human YO and simian SA11, G3 (24); human PCP5, MZ58, and PA710, G3, with subgroup I specificity and long RNA pattern (19); human AU228 (generously provided by O. Nakagomi [36]) and canine CU1 (24), both with subgroup I and G3 specificities; human ST3 (24) and VA70 (13), G4A and G4B, respectively (16); porcine OSU, G5 (24); bovine UK and NCDV, G6 (24); avian Ch-2, G7 (24); human 69M and bovine 678, G8 (29, 37, 39); human HAL1166,

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HAL1271, and HAL 8590, G8, with subgroup I specificity and long RNA pattern (22); human WI61, G9 (7); and bovine B223 (39, 44) and V1005 (6), both with G10 specificity.

Preparation of immune sera and neutralizing MAbs to UK, PA151, and PA169. Immune sera were prepared by immunizing guinea pigs and rabbits, free of RV antibodies, with partially purified virus (13). Each animal received a first dose of virus plus complete Freund adjuvant intramuscularly at day 0, a second intramuscular dose of virus plus incomplete Freund adjuvant at day 7, and a third dose of virus in phosphate-buffered saline intraperitoneally at day 21. Animals were bled 8 to 10 days later. Neutralizing MAbs to RV strains UK, PA151, and PA169 were developed by using BALB/c mice immunized with partially purified virus, as reported previously (18). Hybridomas showing neutralizing activity were subcloned twice by limiting dilution and then grown as ascites tumors in pristane-primed syngeneic mice. The specific reactivities of different MAbs with viral proteins were determined by Western blot (immunoblot) analysis and radioimmunoprecipitation, as reported previously (16, 18). The immunological reactivities with reference RV serotypes G1 to G10 were then tested by neutralization and ELISA. The immunoglobulin isotypes of different MAbs were determined by ELISA (Mouse-Typer Sub-Isotyping Kit; Bio-Rad Laboratories, Richmond, Calif.).

RV detection, subgrouping, serotyping, and electropherotyping. RV detection (17) and subgrouping (20) were performed by ELISA by using polyclonal antibodies as capture antibodies and MAbs labeled with peroxidase as detector antibodies. RV serotyping (14) and subtyping of serotype 4 strains (16) was done by ELISA by using type- or subtype-specific immune sera and type- or subtype-specific neutralizing MAbs as capture and detector antibodies, respectively. Electropherotyping was performed by genomic RNA extraction from samples, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (25), as reported previously (12).

Neutralization assay. The neutralization assay was performed in MA104 cell cultures grown in microdilution plates as described previously (13). Briefly, equal volumes of a trypsin-treated virus suspension (approximately 200 focus-forming units per 0.1 ml) and serial dilutions of immune sera or MAbs were incubated for 30 min at 37°C and were 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 ^{32}P were prepared by in vitro transcription of purified PA151, PA169, UK, and NCDV strains, as described previously (10). The integrity of all 11 RNA segments in each probe was assessed by electrophoresis in 3% denaturing polyacrylamide gels. Genomic double-stranded RNAs of the different RV strains tested were obtained from partially purified virus preparations by phenol-chloroform extraction followed by ethanol precipitation (34). 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) with 25 mM phosphate buffer (pH 6.5). The ^{32}P-labeled single-stranded RNA probes (0.5×10^6 cpm) were hybridized to the blotted RNAs under conditions of high stringency for 20 h at 52°C in a solution containing 2.5× SSC (0.375 M Na^+ plus 0.0375 M sodium citrate), 50 mM phosphate 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 (9).

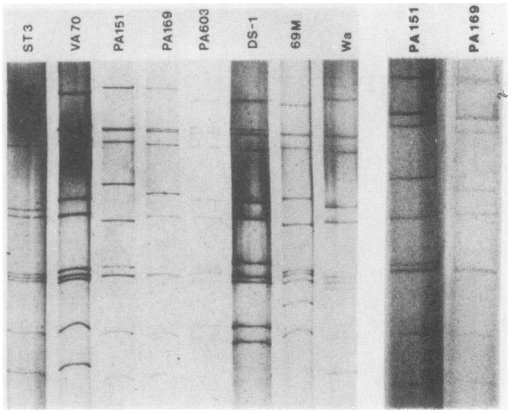

FIG. 1. Electrophoretic analysis of genomic RNAs of cell culture-adapted HRV strains. Lanes: ST3 and VA70, known strains of serotypes 4A and 4B, respectively; PA151 and PA169, serotype 6 HRV strains; PA603 and DS-1, known strains with short RNA patterns; 69M, reference serotype 8 HRV strain with a super short RNA pattern; Wa, reference strain with a long RNA pattern. For comparison, the electrophoretic patterns of PA151 and PA169 genomic RNAs extracted from stool samples are shown on the right.

RESULTS

Propagation and characterization of PA151 and PA169 HRV strains. Strains PA151 and PA169 were untypeable by serotyping ELISA on fecal material by using VP7-specific MAbs directed to HRV serotypes 1 to 4, 8, and 9. They were both subgroup I, but they showed long electrophoretic patterns of RNA migration that were distinct from each other (Fig. 1). The two strains were adapted to growth first in primary African green monkey kidney and then in MA104 cell cultures. Following 10 to 15 passages, they started growing at a high titer and were shown to infect 30 to 50% of the cell monolayers. The RNA patterns of cell culture-adapted strains were identical to those obtained with stool samples. Similarly, subgroup I and the undeterminable serotype were confirmed on cell culture-adapted virus preparations. Virus strains were then purified and used for the preparation of animal immune sera as well as MAb development. By using PA151 and PA169 guinea pig immune sera in cross-neutralization assays, it was found that, among the 10 RV G (VP7) serotypes so far established, both strains appeared to be antigenically related to bovine prototype strains of serotype 6, UK and NCDV (Table 1). However, NCDV antiserum neutralized PA151 and PA169 at titers that were 32- and 16-fold lower than the homologous titer, respectively. In addition, the two strains appeared to be antigenically related to each other in only one way, since PA169 was neutralized at a comparable titer by both immune sera, whereas PA151 was neutralized only by the homolo-

TABLE 1. Cross-neutralization assays of PA151 and PA169 with reference RV strains of different serotypes

RV strain (serotype)	Neutralizing antibody titer of guinea pig immune serum to RV strain (serotype)[a]:															
	Wa (1)	DS-1 (2)	YO (3)	ST3 (4A)	VA70 (4B)	OSU (5)	NCDV (6)	UK (6)	PA151 (6)	PA169 (6)	Ch-2 (7)	69M (8)	678 (8)	WI61 (9)	B223 (10)	V1005 (10)
Wa (1)	**10,240**								<40	<40						
DS-1 (2)		**10,240**							<40	<40						
YO (3)			**10,240**						<40	<40						
ST3 (4A)				**40,960**					<40	80						
VA70 (4B)					**5,120**				<40	<40						
OSU (5)						**81,920**			<40	<40						
NCDV (6)							**2,560**	640	640	640						
UK (6)							2,560	**2,560**	640	640						
PA151 (6)	<40	160	<40	<40	160	80	80	160	**5,120**	80	<40	160	80	<40	<80	<40
PA169 (6)	<40	80	320	<40	320	80	160	320	2,560	**5,120**	<40	320	<80	40	<80	<40
Ch-2 (7)									160	160	**20,480**					
69M (8)									80	<40		**5,120**				
678 (8)									40	<40			**2,560**			
WI61 (9)									40	40				**163,480**		
B223 (10)									<80	<80					**5,120**	
V1005 (10)									<80	<80						**5,120**

[a] Titers are expressed as reciprocals. Homologous titers are given in boldface type. Titers not reported are at least 16-fold lower than homologous titers.

gous antiserum. No antigenic relationship was detected with bovine strains of serotypes other than serotype 6, i.e., with strains 678 (serotype 8) or B223 or V1005 (both belonging to serotype 10).

A type-specific neutralizing MAb directed to VP7 of bovine RV UK (3B4) was found to detect both bovine UK and NCDV strains as well as both PA151 and PA169 HRV strains (although at different titers). Among the additional MAbs that were developed, it was found that two other MAbs directed to VP7 of UK (3B5 and 1C1) behaved as 3B4 in recognizing both bovine and human strains (Table 2). When the three VP7-specific UK MAbs were added to the MAb panel currently used for serotyping of HRV strains by ELISA, the human serotype 6 isolates were correctly identified (Table 3). Neutralizing MAbs directed to PA151 and PA169 were also developed. However, they appeared to be directed to VP4, and their reactivities were not found to be serotype specific by both neutralization and ELISA.

Neutralizing antibody response to PA151 and PA169 in convalescent-phase sera from infants and young children with acute RV gastroenteritis. Results of the study of the type-specific neutralizing antibody response in convalescent-phase sera from infants and young children with gastroenteritis caused by a primary or secondary HRV infection are reported in Table 4. The first six cases reported are primary HRV infections caused by serotype 1 (two patients), serotype 2 (two patients), serotype 4A (one patient), and serotype 4B (one patient). In no case was a heterotypic neutralizing antibody response to PA151 or PA169 or to RV prototypes of serotype 6, 8, or 9 detected. In two additional cases (patients 65 and 80), the actual infecting serotype (in the absence of a sufficient amount of virus in the stool samples to allow typing) resembled those observed in children infected with serotypes G1 and G4B. Finally, in the last two cases (patients 27 and 63) of gastroenteritis caused by a secondary HRV infection, different levels of neutralizing antibody titers to both PA151 and PA169 were detected. In addition, in patient 27 a fairly high neutralizing titer to UK

TABLE 2. Cross-neutralization assays of UK VP7-specific neutralizing MAbs with RV strains of different serotypes

RV strain (serotype)	Neutralization antibody titer of VP7-specific MAbs to RV strain (serotype)[a]:										
	Wa (1)	DS-1 (2)	YO (3)	ST3 (4A)	VA70 (4B)	UK (6)			B37 (8)	WI61 (9)	B223 (10)
						3B4	3B5	1C1			
Wa (1)	**10,000**					<1,000	<1,000	<1,000			
DS-1 (2)		**30,000**				<1,000	<1,000	<1,000			
YO (3)			100,000			<1,000	<1,000	<1,000			
ST3 (4A)				**300,000**		<1,000	<1,000	<1,000			
VA70 (4B)					**30,000**	<1,000	<1,000	<1,000			
OSU (5)						<1,000	<1,000	<1,000			
NCDV (6)						64,000	32,000	16,000			
UK (6)						**256,000**	**64,000**	**64,000**			
PA151 (6)	<1,000	<1,000	<1,000	<1,000	<1,000	16,000	8,000	4,000	<1,000	<1,000	<1,000
PA169 (6)	<1,000	<1,000	<1,000	<1,000	<1,000	128,000	128,000	32,000	<1,000	<1,000	<1,000
Ch-2 (7)						<1,000	<1,000	<1,000			
69M (8)						<1,000	<1,000	<1,000	**60,000**		
678 (8)						<1,000	<1,000	<1,000			
WI61 (9)						<1,000	<1,000	<1,000		**30,000**	
B223 (10)						<1,000	<1,000	<1,000			**30,000**

[a] Titers are expressed as reciprocals. Homologous titers are given in boldface type. Titers not reported are <1:1,000.

TABLE 3. Identification of serotype 6 HRV isolates by ELISA by using type-specific MAbs

RV strain (serotype)	Absorbance (10^2) with type-specific MAbs to RV strain (serotype)[a]:									
	Wa (1)	DS-1 (2)	YO (3)	ST3 (4A)	VA70 (4B)	UK (6)			69M (8)	WI61 (9)
						3B4	3B5	1C1		
Wa (1)	**135**					5	3	2		
DS-1 (2)		**125**				2	0	1		
YO (3)			**132**			0	2	4		
ST3 (4A)				**148**		4	5	2		
VA70 (4B)					**115**	0	4	5		
OSU (5)						1	1	0		
NCDV (6)	0	3	4	1	5	>200	>200	>200	0	0
UK (6)	0	2	1	0	3	**>200**	**>200**	**>200**	0	0
PA151 (6)	4	2	3	2	0	>200	>200	>200	0	0
PA169 (6)	2	6	0	7	4	>200	>200	>200	0	0
69M (8)						4	3	2	**131**	
WI61 (9)						0	2	4		**159**
B223 (10)						0	0	1		

[a] Homologous reactivity values are given in boldface type. Absorbance values not reported are <10.

was found. These data (confirmed in other patients [data not shown]) suggest that neutralizing antibody to HRV strains of serotype 6 are not due to a heterotypic response to infection by strains of serotypes 1 to 4.

Northern blot hybridization. Cross-hybridization studies were performed to verify the level of genetic relatedness between human and bovine serotype 6 strains by using ^{32}P-labeled RNA probes prepared from PA151, PA169, NCDV, and UK strains. Under high-stringency conditions, the labeled PA151 probe hybridized strongly to PA169 at the level of all the RNA segments except segment 4. On the other hand, gene 4 of PA151 appeared to be related to the corresponding genes of PCP5 and MZ58, but not to gene 4 of strain PA710 (Fig. 2). All three strains, as recently reported by our laboratory (19), possess subgroup I and G3 specificities and display long electrophoretic patterns of RNA migration. This prompted us to study the genetic relationships between PA151 and AU228, an HRV strain with subgroup I and G3 specificities and with a long RNA pattern (34). As shown in Fig. 3, the labeled AU228 probe hybridized strongly, at the level of segment 4, to PA151; similarly, the labeled PA151 probe hybridized strongly to segment 4 of AU228. By using the labeled PA169 probe, the two serotype 6 HRV strains again appeared to be highly related to each other at the level of all segments except segment 4 (Fig. 4). However, the PA169 probe hybridized strongly to segments 2 to 4 of HAL1271, a G8 HRV strain of subgroup I with a long RNA electropherotype isolated in Finland (22). In addition, PA169 appeared to be related to bovine UK and NCDV strains at the level of all segments except gene 4 and to segments 1 to 4 of HRV strain PA710. With the labeled NCDV probe, cross-hybridization was detected for segments 1 to 3 and 6 to 11 among the bovine and the human serotype 6 strains (Fig. 5). In addition, the NCDV probe reacted with most of the HAL1271 segments. Finally, when the ^{32}P-labeled UK RNA probe was used, the genetic relatedness among the bovine and the human serotype 6 strains was confirmed (Fig. 6). As a general observation, it must be noted that in all cross-hybridization experiments that were performed, all probes also reacted with some or most segments of both serotype 3 (19) and serotype 8 (22) anomalous subgroup I strains in a fairly strong fashion.

DISCUSSION

To date, at least 10, and maybe 12, RV serotypes have been identified, including RV strains from humans and animals of different species. Recently, HRV strains of subgroup I and long RNA electropherotype serologically distinct from known serotypes 1 to 6, 8, and 9 have been described as members of a presumptive seventh HRV serotype (41). Although in recent years a few HRV strains with genetic relatedness to animal strains have been reported (19, 22, 34–36), all of these strains possess G specificities already described in human serotypes. During an extended epidemiological survey performed on Peruvian children vaccinated with the attenuated bovine RV strain RIT4237, we never have been able to identify serotype 6 bovine RV strains in stool samples collected during episodes of diarrhea in vaccinees, even when highly sensitive serotyping techniques such as solid-phase immune electron microscopy are used (26). On this basis, the finding reported here of the first isolation, identification, and characterization of two HRV strains that share G6 specificity with the well-known bovine RV prototypes UK and NCDV appears to be particularly interesting.

It must first be noted that no peculiar epidemiological findings, such as close contacts with animals or residence in rural areas, were identified in the anamnestic analysis of the two children with acute gastroenteritis and from whom the two virus strains were isolated in the same urban area of Palermo 3 months apart. Unfortunately, only acute-phase serum samples were available from both children. However, the lack of neutralizing antibodies to any of the 10 RV serotypes tested in the serum obtained from the patient infected with strain PA151 suggested that a true primary RV infection occurred. This might explain the severity of symptoms, which requires hospital admission. On the other hand, in the acute-phase serum sample from the patient infected with strain PA169, a neutralizing antibody titer of 1:80 to the homologous virus versus a titer of <1:40 to all the other RV serotypes tested suggested that a specific neutralizing antibody response was being mounted. The extension of the previously reported (21) analysis of the neutralizing antibody response in convalescent-phase sera from Italian children with primary rotavirus infections caused by HRV strains of

TABLE 4. Neutralizing antibody response to human and animal RV strains of different serotypes in convalescent-phase sera from infants and young children with acute gastroenteritis caused by an HRV strain of known or unknown serotype

Patient no. (age)	Infecting RV serotype	Neutralizing antibody titer to rotavirus strain (serotype)[a]:										
		Wa (1)	DS-1 (2)	YO (3)	SA11 (3)	ST3 (4A)	VA70 (4B)	UK (6)	PA151 (6)	PA169 (6)	69M (8)	WI61 (9)
26 (21 mo)	1	**640**	<40	<40	<40	160	80	<40	<40	<40	<40	<40
60 (7 mo)	1	**640**	<40	<40	<40	160	80	<40	<40	<40	<40	<40
25 (13 mo)	2	80	**320**	<40	<40	80	40	<40	<40	<40	<40	<40
29 (14 mo)	2	80	**320**	<40	<40	80	40	<40	<40	<40	<40	<40
181 (2 yr)	4A	640	40	80	80	**1,280**	320	<40	<40	<40	<40	<40
75 (7 mo)	4B	640	<40	<40	<40	640	**640**	<40	<40	<40	<40	<40
65 (14 mo)	NT,[b] 4B[c]	320	<40	<40	<40	320	320	<40	<40	<40	<40	<40
80 (7 mo)	NT, 1[c]	1,280	40	40	<40	320	160	<40	<40	<40	<40	<40
27 (4 yr)[d]	NT	2,560	>2,560	2,560	>2,560	1,280	2,560	640	>2,560	1,280	320	1,280
63 (23 mo)[d]	NT	320	160	160	80	160	320	<40	80	160	<40	320

[a] Homotypic titers are given in boldface type.
[b] NT, not typed.
[c] Serotype inferred from serologic response.
[d] Secondary infection.

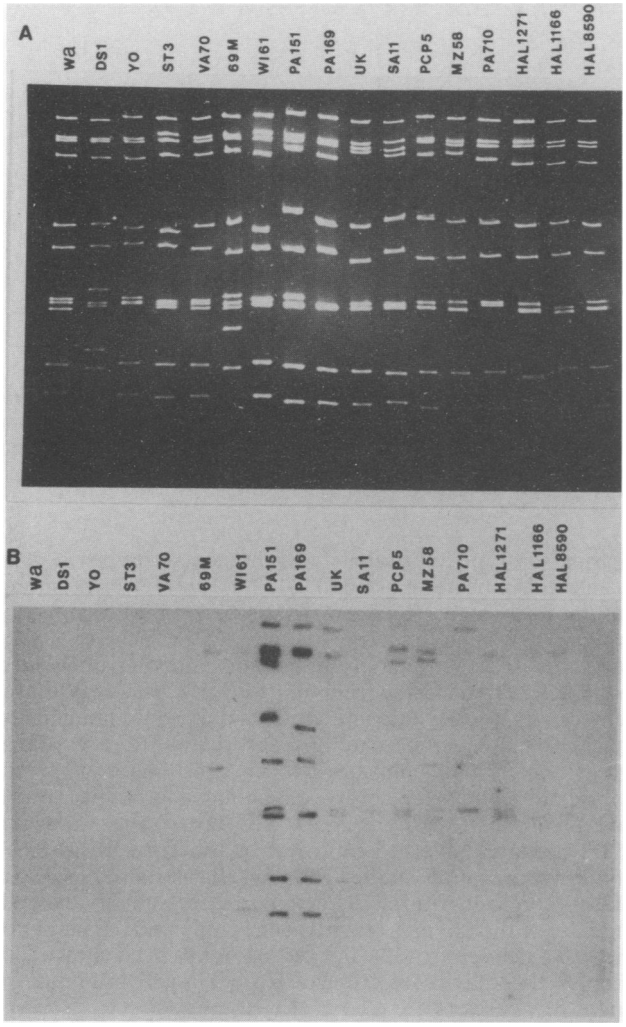

FIG. 2. Northern blot hybridization of ^{32}P-labeled RNA probe prepared from PA151 HRV to denatured genomic RNAs of the indicated strains. (A) UV light photograph of the gel after ethidium bromide staining; (B) the corresponding autoradiogram.

serotypes 1, 2, 4A, or 4B to the new HRV strains showed that no cross-reactive antibodies to PA151 and PA169 were elicited, as a rule, during primary infections by the four established HRV serotypes. On the other hand, study of the type-specific neutralizing antibody response in children with secondary infections often showed the presence of neutralizing antibody to most serotypes, with the highest titers directed to PA151 and/or PA169 (11). Neutralizing antibody to serotype 6 bovine strains were also found in these cases as well as in children from other countries (5). However, these serological data do not allow us to draw any conclusion on the epidemiological significance of serotype 6 HRV strains. We are reexamining all previously untyped HRV strains from different geographic areas by using type-specific neutralizing MAbs directed to UK in our ELISA system for serotyping. Thus far, according to preliminary data, an additional serotype 6 HRV strain has been found in northern Italy.

On the basis of the results of cross-hybridization studies, the two serotype 6 HRV strains seem to belong to the bovine genogroup (28), since all their genome segments except gene 4 hybridized to the corresponding segments of serotype 6 bovine strains. If this is true, PA151 and PA169 represent two new HRV strains that show genetic homology with

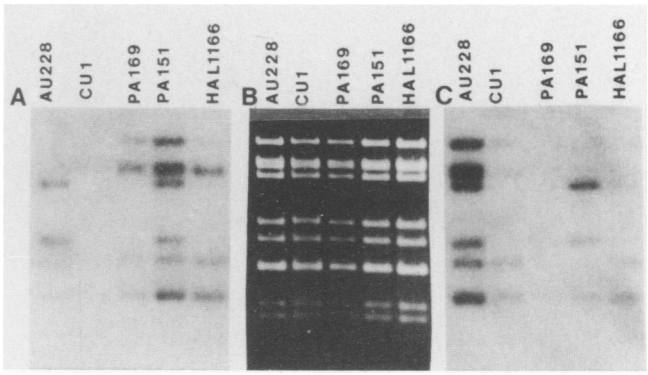

FIG. 3. Northern blot hybridization of the ^{32}P-labeled RNA probes from PA151 (A) and AU228 (C) HRV to denatured genomic RNAs of the indicated strains. (B) Ethidium bromide-stained gel; (A and C) the corresponding autoradiograms.

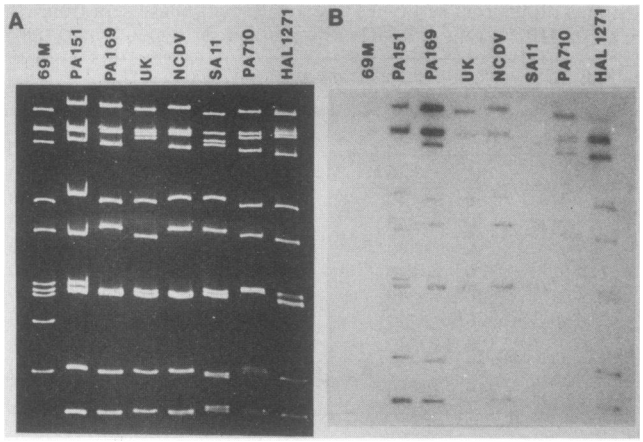

FIG. 4. Northern blot hybridization of ^{32}P-labeled PA169 RNA probe to denatured genomic RNAs of the indicated RV strains. (A) Ethidium bromide-stained gel; (B) the corresponding autoradiogram.

strains from animals. This has been already reported for a few subgroup I, serotype 3, HRV strains belonging to a feline RV genogroup (36) or to a genogroup including feline and canine strains (35). In addition, porcine RV strains

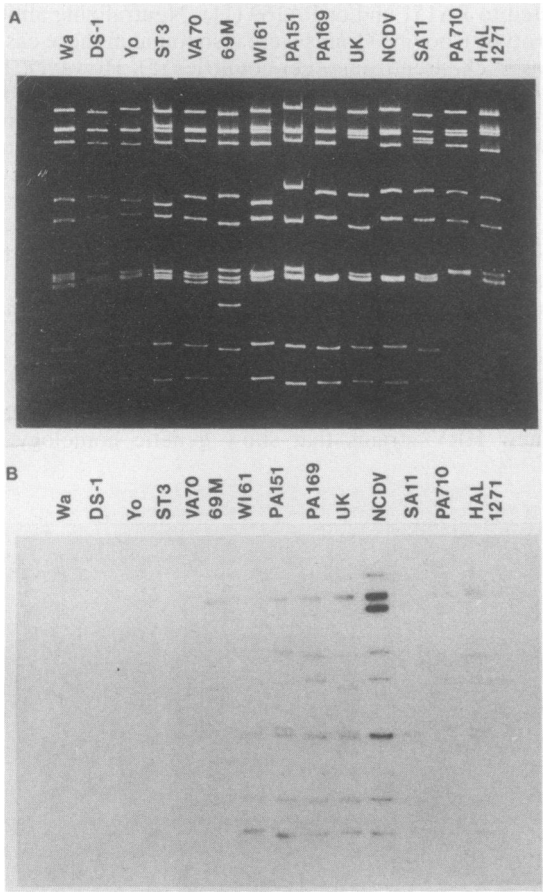

FIG. 5. Northern blot hybridization of the ^{32}P-labeled RNA probe prepared from bovine NCDV to denatured genomic RNAs of the indicated RV strains. (A) Ethidium bromide-stained gel; (B) the corresponding autoradiogram.

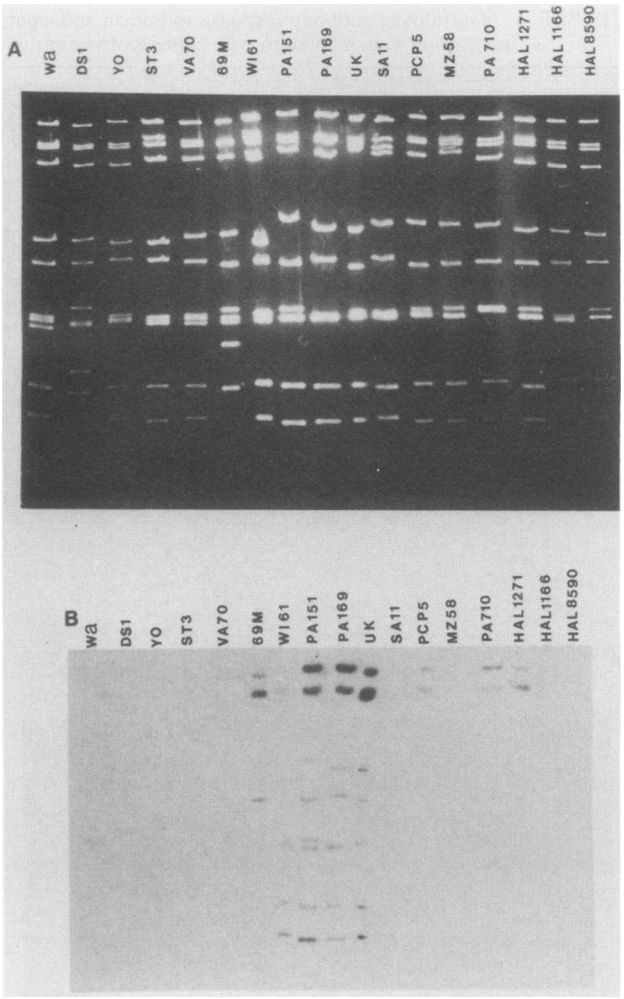

FIG. 6. Northern blot hybridization of ^{32}P-labeled RNA prepared from bovine UK to denatured genomic RNAs of the indicated strains. (A) Ethidium bromide-stained gel; (B) the corresponding autoradiogram.

sharing serotype antigenicity with human serotypes 1, 2, 3, 4, and 8 have been reported (3, 24, 30, 31, 38). Furthermore, natural reassortants between HRV strains of different genogroups have been described recently (43). These data suggest that some strains from humans may have evolved ancestrally from strains from animals, that they may represent strains directly transmitted from animals to humans, or that they may even be natural reassortants between strains from humans and animals. The multiple bands of cross-hybridization between serotype 6 human and bovine strains and subgroup I, serotype 3 and 8, HRV strains with long RNA patterns have recently been clarified by RNA-RNA liquid hybridization studies showing that strains PCP5 and MZ58 represent naturally occurring reassortants between a member of the bovine rotavirus genogroup and a member of the AU-1 genogroup (32). In addition, it has been shown that Finnish G8 rotaviruses are genetically similar to bovine G6 and G10 viruses (4).

It is noteworthy that gene 4 of strain PA151 is genetically related to the corresponding gene of AU228, an HRV strain of serotype 3 belonging to a feline genogroup (34): this finding documents for the first time its association with G6

specificity. On the other hand, gene 4 of PA169 appears to be unique; i.e., it is different from the gene 4 alleles that have thus far been identified among HRVs (27). Preliminary sequence data confirm the genetic relationships among gene 9 of human and bovine serotype 6 strains, as well as the relatedness between PA151 and AU228 at the level of gene 4 (8). These preliminary results also confirm that gene 4 of PA169 appears to be unique, since it has not been possible to derive its sequence from any of over 20 gene 4 primers tested so far. However, it is noteworthy that gene 4 of PA169 appears to be genetically related, on the basis of Northern blot hybridization, to the corresponding genes of a subgroup I, serotype 3, HRV strain, PA710 (19), and of a subgroup I, serotype 8, HRV strain (HAL1271) with a long RNA pattern (22); both of these strains were previously shown to be genetically related to strains from animals.

Thus, five different alleles of gene 4 have been identified among the following HRV strains: Wa and DS-1 (9), M37 (23), K8 (33, 40), and PA169. Molecular and immunological characterization of gene 4 of PA169 and its product could have important implications in the correct development of RV vaccine strategies.

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