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A Candidate for a New Serotype of Human Rotavirus

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We investigated genetic and serological characteristics of a human rotavirus isolate from Indonesia which had a "super short" RNA electrophoretic pattern (A. Hasegawa, S. Inouye, S. Matsuno, K. Yamaoka, R. Eko, and W. Suharyono, Microbiol. Immunol. 28:719–722, 1984). This virus, strain 69M, was found by RNA-RNA hybridization to have a low degree of homology with the representative strains of all four human serotypes. Furthermore, it could not be classified by neutralization analysis into any of these serotypes. Therefore, this virus might belong to a new serotype.

We have recently isolated 16 strains of rotavirus in tissue cultures from stool specimens of gastroenteritis patients in Indonesia; two of these strains showed a "super short" pattern when their RNA segments were analyzed by gel electrophoresis. These two strains were classified into subgroup I (1). This study describes our further work on the serotypic characterization of one of these super short isolates, strain 69M.

In this study, the following cultivable human rotaviruses were used as references: strain Wa of serotype 1 (supplied by R. G. Wyatt, National Institutes of Health, Bethesda, Md.) strains S2 and YO of serotypes 2 and 3, respectively (supplied by S. Urasawa, Department of Hygiene and Epidemiology, Sapporo Medical College, Sapporo, Japan); and strain Hochi of serotype 4 (supplied by Y. Inaba, National Institute of Animal Health, Ibaraki, Japan). These viruses, together with strain 69M, were propagated in MA-104 cells by using acetylated trypsin (2 μg/ml) (Sigma Chemical Co., St. Louis, Mo.) in the maintenance medium. Figure 1 shows the RNA electrophoretic patterns of these five viruses. As shown, the segment 10 of strain 69M migrated more slowly than that of strain S2.

To establish the genetic relationships between strain 69M and the reference strains, strain 69M RNA and the RNAs from the reference strains were compared by RNA-RNA cross-hybridization. The single-stranded RNAs were prepared in vitro by a previously described method (4) with slight modifications. The final composition of the reaction mixture was: 1.25 mM ATP, CTP, GTP, and UTP (including 20 μCi of [^{3}H]UTP), 10 mM Tris-HCl (pH 8.1), 7 mM $MgCl_2$, 8 mM phosphoenolpyruvate, 40 μg of pyruvate kinase, 20 μg of bentonite, and 200 μg of inner-shell particles in a total volume of 200 μl. The reaction was carried out at 40°C for 2 h, and then the reaction mixture was phenol extracted. The RNAs were precipitated overnight with 2.5 volumes of ethanol at −20°C and suspended in 10 mM Tris-HCl (pH 8.0). The ^{3}H-labeled single-stranded RNAs were reprecipitated overnight with 2 M LiCl at 4°C and suspended in 10 mM Tris-HCl (pH 8.0)–1 mM EDTA. The conditions of cross-hybridization tests were as previously described (4). Table 1 shows that strain 69M had a low degree of homology (less than 50%) with the other reference human rotavirus double-stranded RNAs. This indicates that the RNAs of strain 69M are significantly different from the RNAs of the reference strains used.

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To study the antigenic relationships between strain 69M and the reference strains, we performed cross-neutralization tests by the 50% plaque reduction method. All hyperimmune antisera used in the plaque reduction neutralization tests were prepared in guinea pigs as described previously (3). The guinea pigs used for immunization had neither complement fixation nor immune adherence hemagglutination antibody activities against strains Wa and S2 before immuniza-

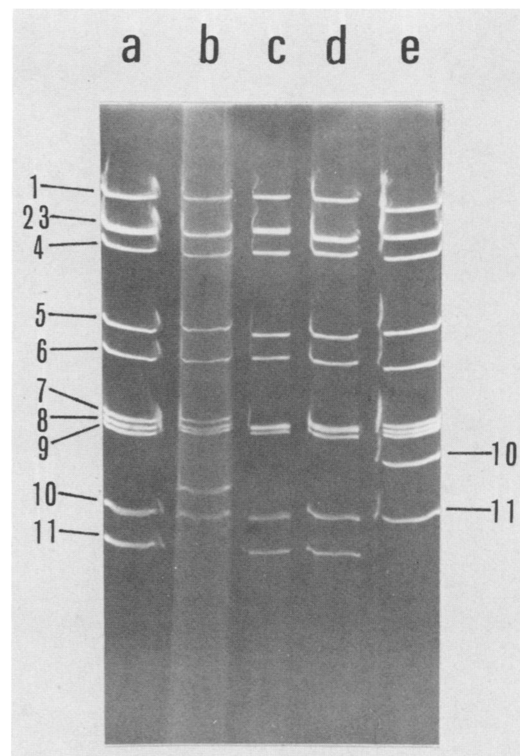

FIG. 1. RNA electrophoretic patterns of strain 69M and reference strains. Double-stranded RNAs were extracted from concentrated virions with phenol and precipitated with ethanol. Electrophoresis of double-stranded RNA was performed on 10% polyacrylamide gel with 3% stacking gels in a discontinuous Tris-glycine buffer system as described by Laemmli (2). Gel was stained with ethidium bromide before photography under UV light. Lanes: a, Wa; b, S2; c, YO; d, Hochi; e, 69M.

TABLE 1. Genetic relationship between human rotavirus strains by RNA-RNA cross-hybridization

^{3}H-labeled single-stranded RNAs	Double-stranded RNAs (%)				
	Wa	S2	YO	Hochi	69M
Wa	100.0	21.4	51.4	81.6	12.8
S2	24.6	100.0	19.5	20.9	45.6
YO	75.6	18.8	100.0	69.4	12.9
Hochi	88.1	28.1	71.4	100.0	18.2
69M	25.7	43.2	18.6	19.1	100.0

tion. The results are shown in Table 2. Strain 69M showed one-way cross-reactivity to the antisera against strains YO and Hochi (serotypes 3 and 4, respectively). Also, strain S2 reacted to the strain 69M antiserum in a unidirectional way.

TABLE 2. Antigenic relationship between human rotavirus strains as determined by plaque neutralization

Antisera to:	50% plaque neutralization titer to:				
	Wa	S2	YO	Hochi	69M
Wa	15,500	NT[a]	NT	NT	<100
S2	NT	90,000	NT	NT	<100
YO	NT	NT	6,200	NT	270
Hochi	NT	NT	NT	6,550	240
69M	<100	240	<100	<100	5,050

[a] NT, Not tested.

However, homologous anti-69M titers were at least 20-fold higher than the heterologous titers.

Because of the low degree of genetic homology and the 20-fold difference in neutralization titers, which are criteria for a distinct serotype (5, 6), we suggest that strain 69M belongs to a serotype different from the four so far recognized.

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