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Distinctive Ribonucleic Acid Patterns of Human Rotavirus Subgroups 1 and 2

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The ribonucleic acid migration patterns of 7 subgroup 1 and 16 subgroup 2 human rotaviruses recovered from four geographic areas were compared. The subgroup 1 ribonucleic acid patterns had strikingly slower-moving segments 10 and 11, suggesting a correlation between the ribonucleic acid pattern and the subgroup specificity.

In 1978, we reported that electrophoresis of viral ribonucleic acids (RNAs) revealed three distinct patterns among eight human rotaviral strains which were amplified by passage in gnotobiotic calves after their recovery from patients at Children's Hospital National Medical Center, Washington, D.C. (5). Two of these strains were from a patient who developed sequential gastroenteric illnesses 11 months apart. Rotavirus was detected during each episode, and the strains were designated DS-1 and DS-2. Subsequently, seven of the eight strains, including DS-2, were typed by the enzyme-linked immunosorbent assay (ELISA) as serotype 2, and one strain, DS-1, was typed as serotype 1 (6; Yolken et al., unpublished data).

Serological differences, first observed by specific complement fixation, immune electron microscopy, and ELISA (7, 8), were used to designate serotypes, and it was assumed that they would be identical to serotypes that would eventually be identified by the conventional neutralization assay. However, recently, antigens detected by ELISA or the immune adherence hemagglutination assay (IAHA) were shown to be distinct from those detected by cell culture neutralization (4, 4a, 6). Thus, a new scheme was proposed for designating antigenic specificities among the rotaviruses (6). In this scheme, the term "serotype" is reserved for designation of neutralization specificity, and the term "subgroup" is used, in place of serotype, to indicate differences detected by ELISA, immune electron microscopy, complement fixation, or IAHA. This new scheme is employed in the present report.

In our original report, a comparison of the DS-1 and DS-2 strains revealed only one minor difference in their RNA migration patterns; that is, segment 5 of DS-1 moved slightly slower than that of DS-2. Furthermore, the DS-1 strain had an RNA migration pattern identical to the RNA pattern of strains L and G, which were subsequently shown to belong to rotavirus subgroup 2. Thus, the pattern of RNA migration in gels could not be correlated with ELISA subgroup reactivity.

Espejo et al. (1-3) used electrophoresis of viral RNAs for analysis of rotaviral strains recovered from patients with gastroenteritis in Mexico City from 1976 to 1978. Based on the migration of the second RNA segment, they distinguished two major RNA patterns, designated 2s and 2l, respectively, for the small and large second segment. Another obvious difference between these two patterns was in the migration of segments 10 and 11, which moved more slowly in the 2s RNA pattern than in the 2l pattern. Viruses with the 2s pattern were recovered from only 6 of 52 (11%) patients studied in 1977, but in 1978, the 2s pattern predominated and was found in rotaviruses from 8 of 9 gastroenteritis patients (3).

Recently, we discovered that our previously reported pattern for DS-1 was incorrect due to mislabeling in the laboratory. The purpose of this communication is to correct this error and to describe a correlation between the RNA pattern and ELISA or IAHA subgroup reactivity, or both, among the human rotaviruses.

The strains listed in Table 1 were compared for their RNA patterns and subgroup specificities as determined by ELISA or IAHA or both. ELISA was recently modified to provide a more specific method for subgrouping rotaviruses, and IAHA was similarly adapted to distinguish rotavirus subgroups (6). By ELISA, an approximate 50% or greater difference in optical density with one subgroup serum compared to the other

TABLE 1. *Correlation of ELISA or IAHA subgroup or both with viral RNA polyacrylamide gel electrophoresis pattern for human rotaviruses*

Strain	Location	RNA pattern[a]	ELISA[b]		IAHA[c]	
			Subgroup 1	Subgroup 2	Subgroup 1	Subgroup 2
DS-1	Washington, D.C.	1	94	25	≥128	<2
Bang 268	Bangladesh	1	T-1[d]	0	ND[e]	ND
HRV 1383	Mexico City	1[f]	25	12	8	<2
HRV 23/86	Mexico City	1[f]	ND	ND	32	<2
HRV 24/75	Mexico City	1[f]	ND	ND	32	<2
Ven 8a	Venezuela	1	86	51	256	<32
Ven HNC4	Venezuela	1	62	28	64	<16
D	Washington, D.C.	2	23	118	<2	32
Fh	Washington, D.C.	2	29	84	<2	≥128
G	Washington, D.C.	2	42	116	<2	64
W	Washington, D.C.	2	41	129	ND	ND
L	Washington, D.C.	2	21	46	<2	≥256
M	Washington, D.C.	2	41	128	<2	32
DS-2	Washington, D.C.	2	39	117	<2	16
Wa	Washington, D.C.	2	29	89	<2	32
HRV 22/344	Mexico City	2[f]	ND	ND	<8	32
HRV 20/292	Mexico City	2[f]	22	68	<5	320
Ven HNC1	Venezuela	2	15	29	<2	32
Ven HNC10	Venezuela	2	58	82	4	16
Ven HNC11a	Venezuela	2	26	69	4	128
Ven HNC19	Venezuela	2	28	69	<2	≥256
Ven HNC21	Venezuela	2	71	149	2	128
Ven HNC22	Venezuela	2	34	60	<2	128

[a] RNA pattern 1 has slower-moving segments 10 and 11; RNA pattern 2 has faster-moving segments 10 and 11.

[b] Optical density at 400 nm of undiluted antigen with calf postinfection human rotavirus antiserum or absorbed guinea pig hyperimmune human rotavirus antiserum (6).

[c] Reciprocal of antigen titer with calf postinfection human rotavirus antiserum.

[d] Type 1 according to Yolken et al. (Science 201:259–262, 1978; specimen not available for further testing).

[e] ND, Not determined.

[f] RNA patterns determined by Espejo et al. (1–3); all others determined in the Laboratory of Infectious Diseases.

was considered significant and served as the basis for subgroup designation. A fourfold or greater difference in antigen titer served as the basis for designation of subgroup specificity of the test antigen by IAHA.

When newly prepared RNAs of the DS-1 virus from the original gnotobiotic calf fecal material were tested, segments 10 and 11 exhibited a strikingly slower mobility than noted previously (5). This pattern was similar, with respect to the migration of segments 10 and 11, to the 2s pattern of Espejo et al. (1, 2). In addition, RNAs from another subgroup 1 rotaviral strain (Bang 268) recovered from a patient with gastroenteritis in Bangladesh were found to have slower-moving segments 10 and 11 (A. R. Kalica and R. H. Yolken, unpublished data). Figure 1 shows the easily recognizable differences in migration of segments 10 and 11 between two subgroup 1 and two subgroup 2 rotaviruses. The patterns with the slower-migrating, smallest RNA segments are designated pattern 1, and those patterns with the faster-moving segments are designated pattern 2 in Table 1. We also studied the RNA pattern of two additional rotaviruses (HRV 2017 and HRV 4185) previously studied in Mexico City; one had been shown to have the 2s pattern and the other had the 2l pattern. These strains exhibited the expected patterns 1 and 2 under our electrophoresis conditions (5). Due to the limited quantity, these specimens could not be subgrouped.

Five rotaviral strains from Mexico City were subgrouped (Table 1). Three strains (HRV 1383, HRV 23/86, and HRV 24/75) were found to belong to subgroup 1; each had previously been shown by Espejo et al. (1–3) to have a 2s RNA pattern. The remaining strains (HRV 22/344 and HRV 20/292) were found to belong to subgroup 2 and were previously shown by Espejo et al. (1–3) to have a 2l RNA pattern.

Subgroup testing and RNA analysis were also carried out on eight rotaviral strains recovered from children in Venezuela (Table 1). These

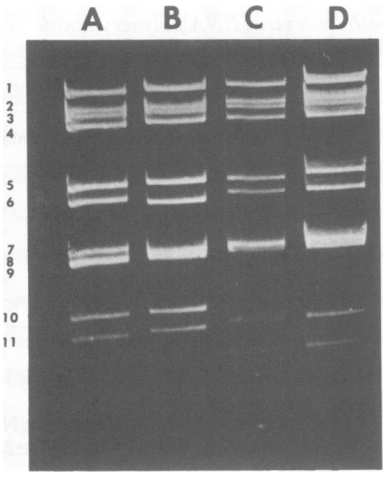

FIG. 1. *Polyacrylamide-agarose gel electrophoresis of human rotaviral RNAs. (A) DS-1 strain, subgroup 1; (B) Bang 268 strain, subgroup 1; (C) D strain, subgroup 2; (D) Wa strain, subgroup 2.*

strains were kindly supplied by Irene Perez-Schael (Instituto Nacional de Dermatologia, Caracas). Two of the Venezuelan strains (Ven 8a and Ven HNC4) were subgroup 1, and their segments 10 and 11 migrated similarly to those of the other subgroup 1 viruses. The remaining strains from Venezuela were subgroup 2 and had pattern 2 RNA migration. Strain Ven HNC10 from Venezuela presented some difficulty in subgrouping reactions. By ELISA, it reacted more strongly with subgroup 2 serum than with subgroup 1 serum, but it did not give a twofold or greater difference. However, there was a fourfold difference by IAHA despite the presence of some anticomplementary activity.

From these preliminary results with rotaviruses derived from four geographic areas and representing both subgroups, it appears that the slower-moving segments 10 and 11 may serve to identify subgroup 1 human rotaviruses. Many smaller, less obvious differences in migration in other segments exist among human rotaviral strains, but no discernable pattern which could be correlated with subgroup reactivity was evident. Recently, two human rotavirus neutralization serotypes were demonstrated using a cultivatable human rotavirus (Wa strain) and previously non-cultivatable human rotaviruses that were rescued by coinfection with a cultivatable bovine rotavirus (4). The neutralization and subgroup specificities of human strain Wa, subgroup 2, and bovine strain UK, subgroup 1, rotaviruses could be dissociated by genetic reassortment and were shown to be functions coded by two separate genes. The neutralization specificity of the Wa strain was coded by gene 9, and IAHA specificity was coded by gene 6. In addition, gene 6 of the UK strain was associated with its subgroup 1 specificity and gene 8 or 9 was associated with its neutralization specificity (4a). The genes responsible for neutralization and subgroup specificities of the human strain DS-1, subgroup 1 rotavirus are currently under investigation. Preliminary data from this investigation indicate that the slower-moving segments (genes) 10 and 11 of DS-1 are not associated with its subgroup or neutralization specificities (Greenberg et al., manuscript in preparation). It should be noted that the epidemiological significance, if any, of the subgroup antigen(s) of the human rotaviruses must still be determined.

The practical significance of the correlation between the RNA pattern and the antigenic subgroup is the convenience offered by RNA analysis, because the large differences in migration of the smallest RNA segments between subgroup 1 and 2 viral RNAs are easily resolved. Furthermore, this method offers an alternative which does not depend on a supply of serological reagents that are presently not readily available. A relatively simple procedure for characterization of viral RNAs, similar to that of Espejo et al. (1), should facilitate the subgrouping of rotaviruses. Before this approach is adopted for routine use, however, more human strains must be examined, particularly subgroup 1 strains, to confirm the correlation between the electrophoretic pattern of viral RNAs and the antigenic subgroups.

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