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Isolation and Characterization of a Novel Reassortant between Avian Ty-1 and Simian RRV Rotaviruses

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A reassortant, TyRh, was isolated after coinfection of MA104 cells with avian Ty-1 and simian RRV rotaviruses. Hybridization and serological studies showed that the reassortant's 4th gene, which encodes Vp4, was derived from the simian RRV rotavirus parent, whereas the remaining 10 genes were derived from the avian Ty-1 rotavirus parent.

Rotavirus is the major viral pathogen responsible for acute neonatal gastroenteritis in humans and animals (6). The rotavirus genome consists of 11 segments of double-stranded RNA, so that reassortment of gene segments occurs readily during coinfection of cells in tissue culture. There have been many examples of reassortants, either naturally occurring or those made in vitro, between rotaviruses of different mammalian species as well as reassortants between strains of human and animal rotaviruses, but no reassortants between avian and mammalian rotaviruses have previously been described (10, 12, 14, 22, 23, 25).

Reassortants have been useful in gene-coding and gene function studies. For example, the main inner capsid protein, Vp6, is encoded by the sixth gene, which contains domains specifying common and subgroup antigens (9, 18). The fourth gene codes for Vp4, an outer capsid protein that is the second neutralizing antigen (11, 30) which is also responsible for hemagglutination (17), and virulence (30) and has the site for trypsin cleavage which activates infectivity (17, 19). The major outer-shell protein Vp7 is encoded by the seventh, eighth, or ninth gene segment, depending on the strain, and is the major neutralization antigen (5, 9, 18, 23, 24, 29). Analyses of particular reassortants have also demonstrated the independent segregation of Vp7 and Vp4 neutralization specificities (11, 15, 22). Both Vp4 and Vp7 have been found to be protective as immunogens (13, 31). In this study, we report on the isolation and characterization of a novel reassortant, TyRh, resulting from the coinfection of MA104 cells with an avian rotavirus strain, Ty-1, and a simian rotavirus strain, RRV.

The growth and neutralization tests of the rotavirus strains were carried out as previously described (26). The reassortant TyRh and the Ty-1 strains were individually plaque purified three times in the presence of trypsin by published methods (17). Rabbit hyperimmune sera to RRV, Ty-1, and the reassortant TyRh were raised in seronegative rabbits according to an immunization schedule described previously (1). Genomic double-stranded RNAs were extracted from cell lysates and electrophoresed on a 10% polyacrylamide gel and stained with silver nitrate as previously described (26). The single-stranded RNA probes (mRNA) were made by in vitro transcription as described by Cohen et al. (4). Northern (RNA) blots were prepared by alkaline transfer of double-stranded RNA separated by polyacrylamide gel electrophoresis (PAGE) to Zeta Probe membranes as previously described (27). High-stringency prehybridization and hybridization conditions used were similar to those described by Flores et al. (7). The membranes were incubated for 2 h at 55°C in a solution containing 1.5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 10 mM EDTA [pH 7]), 1% sodium dodecyl sulfate, 50% formamide, 1% skim milk powder, and 0.2 mg of sonicated salmon sperm DNA per ml. To this solution, the ^{32}P-labelled mRNA probe was added, and the mixture was incubated for 16 h at 55°C. After hybridization, washes were carried out as outlined by Nakagomi et al. (27). Under such hybridization conditions, and with an assumed GC content of 43%, the effective hybridization temperature for RNA was T_m − 24°C (2), thus allowing RNA with a homology of 75.6% or greater to bind. The RRV, Ty-1, and reassortant TyRh viruses were tested in plaque reduction neutralization tests as previously described (33).

In the course of plaque purifying the Ty-1 strain from a stock that was copassaged three times with RRV, a virus with an RNA pattern different from that of either Ty-1 or RRV was isolated. This isolate was plaque purified another three times. From the RNA pattern, it appeared that the isolate contained the Vp4 gene segment from RRV, whereas the remaining 10 segments were all of Ty-1 origin (Fig. 1). Under high-stringency hybridization conditions, a ^{32}P-labelled probe of TyRh mRNA was hybridized to TyRh, RRV, and Ty-1 double-stranded RNA (Fig. 2). At this level of stringency, the probe bound to all 11 TyRh gene segments but only to the Vp4 of RRV and to 10 segments of Ty-1, since the 4th segment (Vp4) of Ty-1 was not bound. This hybridization procedure clearly demonstrated TyRh to be a reassortant of Ty-1 and RRV, consisting of 10 segments of Ty-1 with a single gene substitution (segment 4) encoding Vp4 from RRV. This was also confirmed by serological characterization of the reassortant. Cross-neutralization tests with rabbit hyperimmune sera to RRV, TyRh, and Ty-1 were carried out with each of the three rotavirus strains (Table 1). Sera raised against RRV and Ty-1 neutralized the respective homologous viruses RRV and Ty-1 to high titers (titer, 204,800). RRV antisera neutralized Ty-1 to low levels (titer, 200), just as antisera to Ty-1 neutralized RRV to a titer of only 200. These heterologous titers differ by >20-fold compared with the homologous titers, supporting earlier findings that RRV and Ty-1 belong to different Vp7 (G) serotypes,

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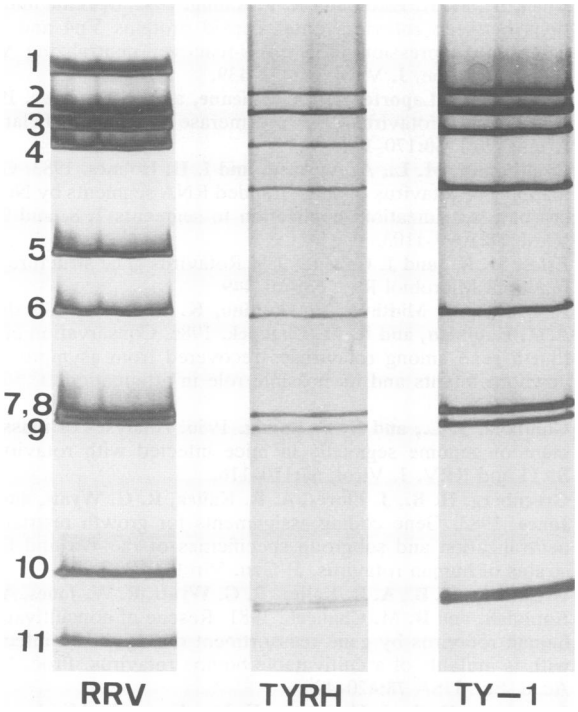

FIG. 1. PAGE of simian, reassortant, and avian rotavirus double-stranded RNA segments. Lanes from left to right: RRV, TyRh, and Ty-1.

designated serotype 3 and serotype 7, respectively (16). The antisera raised to Ty-1 neutralized TyRh to a high titer of 129,015 via the Ty-1 Vp7 antigen. Neutralization of the reassortant TyRh to a titer of 2,015 by the RRV antiserum must be via the Vp4, since the Vp7 antigens of RRV and Ty-1 have been shown to be distinct. The same antiserum caused only low-level neutralization (titer, 200) against Ty-1, which has both Vp4 and Vp7 of Ty-1 origin. Antisera to the reassortant TyRh neutralized TyRh and Ty-1 to high titers (titer, 81,274) and neutralized RRV to a titer of 3,200 via the Vp4 antigen. The results indicate that the reassortant TyRh contains Vp7 from Ty-1 and Vp4 from RRV. These cross-neutralization tests also suggest that the Vp4 genes of RRV and Ty-1 are antigenically different. In addition, partial RNA sequencing of the Vp4 gene of the reassortant TyRh at nucleotide position 864 to 1009 showed 100% homology to the same region of the Vp4 sequence of RRV (data not shown). Preliminary sequencing of the Ty-1 Vp4 shows the nucleotide sequence to be different from that of the TyRh and RRV Vp4 sequence. The degree of difference and whether the Vp4 antigens of RRV and Ty-1 are distinct Vp4 (P) serotypes may be further understood only after complete sequencing of the Ty-1 Vp4 gene and its expression in the absence of Vp7.

A series of anti-Vp4 monoclonal antibodies (MAbs), M11, M14 (20), 7A12, 1A9, 5C4, and 2G4 (32), were used in plaque reduction neutralization tests against RRV, TyRh, and Ty-1 (Table 2). With the exception of the MAb 2G4, which shows heterotypic neutralization specificity, these MAbs react exclusively with RRV (20). The MAbs 1A9, 2G4, M11, and 5C4 all demonstrated reduced reactivities against TyRh, which contains RRV Vp4 in the presence of Ty-1 Vp7, in comparison with RRV, which has both Vp4 and Vp7 from RRV. The

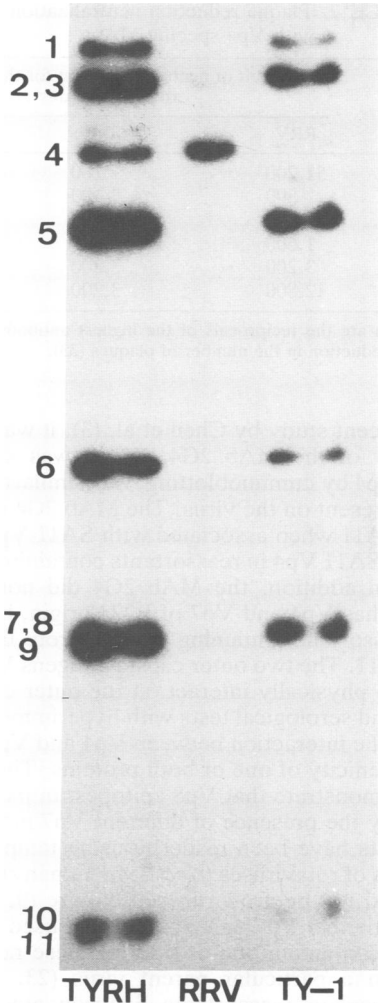

FIG. 2. Autoradiograph of Northern blot hybridization of a ^{32}P-labelled TyRh mRNA probe to genomic double-stranded RNA from the following rotavirus strains (lanes from left to right) immobilized on a Zeta Probe membrane after PAGE: reassortant TyRh, simian RRV, and avian Ty-1.

reactivity of the MAb 1A9 against RRV Vp4 in the reassortant TyRh was abolished, whereas the reactivities of MAbs 2G4, M11, and 5C4 were reduced to 25, 12.5, and <12.5%, respectively. These results suggest that the Ty-1 Vp7 inhibits the abilities of MAbs 1A9, 5C4, and M11 (all directed at the Vp8 portion of Vp4) and MAb 2G4 (which is directed at the Vp5 region of Vp4) to bind and neutralize the reassortant

TABLE 1. Serological relationship of reassortant TyRh to RRV and Ty-1 by fluorescent-focus neutralization test

Rotavirus strain	Reciprocal titer of antisera to[a]:		
	Ty-1	TyRh	RRV
Ty-1	**204,800**	81,274	200
TyRh	129,015	**81,274**	2,015
RRV	200	3,200	**204,800**

[a] The numbers are the reciprocals of the highest serum dilutions giving a 50% reduction in fluorescent foci. Titers are the geometric means of three determinations. Homologous values are in boldface type.

TABLE 2. Plaque reduction neutralization test with Vp4-specific MAbs

MAb	Result of neutralization test for the following rotavirus strains[a]:		
	RRV	TyRh	Ty-1
1A9	51,200	<100	<200
M11	800	100	<200
5C4	800	<100	200
M14	1,600	800	200
7A12	3,200	3,200	<200
2G4	12,800	3,200	<200

[a] The numbers are the reciprocals of the highest antibody dilutions that result in a 60% reduction in the number of plaques (33).

virus. In a recent study by Chen et al. (3), it was found that the reactivity of the MAb 2G4, which was shown to be specific for Vp4 by immunoblotting, was similarly dependent on the Vp7 present on the virus. The MAb 2G4 reacted with the Vp4 of SA11 when associated with SA11 Vp7 but failed to react with SA11 Vp4 in reassortants containing the bovine B223 Vp7. In addition, the MAb 2G4 did not react with B223, which has Vp4 and Vp7 of B223 origin, but did react against a reassortant containing the Vp4 from B223 and the Vp7 from SA11. The two outer capsid antigens Vp4 and Vp7 are known to physically interact on the outer capsid of the virion (34), and serological tests with hyperimmune sera (14) suggest that the interaction between Vp4 and Vp7 can affect the immunogenicity of one or both proteins. The findings of this study demonstrate that Vp8 epitopes, in particular, can be affected by the presence of different Vp7 molecules.

Reassortants have been made by using temperature-sensitive mutants of rotaviruses to retrieve human strains that in the past could not be grown in cell culture (9, 10, 12, 17). MAbs against either Vp7 or Vp4 were also used to select for reassortants containing one or both of these neutralization antigens from a particular parent virus (23, 24). Earlier attempts to isolate reassortants between avian rotavirus strains other than Ty-1 and mammalian rotaviruses with or without the use of MAbs as a method of selection failed to yield any reassortants (21, 28). Since there was no need for any antibody pressure on the coinfection mix to produce the reassortant TyRh in vitro, it can no longer be assumed that such reassortment with avian rotaviruses cannot occur in vivo. The human neonatal isolate M37 has been shown to share Vp4 neutralization specificity with the serotype 4 strain ST3, while the Vp7 neutralization specificity is shared with the serotype 1 strain Wa. This naturally occurring isolate and the porcine SB-1A strain appear to be examples of in vivo reassortment (14). This, along with evidence of high-frequency reassortment of two simian rotaviruses in vivo in mice (8), suggests that the possibility of avian rotaviruses reassorting with human or animal rotaviruses within the different hosts cannot be discounted.

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