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Short communication

Selection of rotavirus VP7 gene in the genetic background of simian rotavirus SA11: implications for rotavirus reassortant vaccine development

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Abstract

We previously reported that the VP7 gene from simian rotavirus SA11 with G-serotype 3(G3-VP7 gene) was preferentially selected in the genetic background of SA11 compared with the G1- or G2-VP7 gene. In the present study, selection of the G4-VP7 gene in competition with G1-, G2- or G3-VP7 gene in the SA11 background was analyzed through mixed infection experiments using SA11 and SA11-human rotavirus single-VP7 gene-substitution reassortants with G-serotypes 1, 2, and 4 (G1-, G2- and G4-reassortant). In virus clones from coinfection of SA11 and G4-reassortant, the frequency of G4 virus decreased to 7% at the 3rd passage and the G4 virus disappeared at the 10th passage, whereas the majority of the clones possessed G3 specificity. However, the predominance of either of the viruses coinfected was not observed in the mixed infection with G4-reassortant and G1- or G2-reassortant. Although growth kinetics of SA11 and G4-reassortant was similar, G4-reassortant showed significantly smaller plaque size than SA11, G1- and G2-reassortant did. These results indicated that the G3-VP7 gene from SA11 might be preferentially selected in the SA11 genetic background compared with the G4-VP7 gene, and suggested that the introduction of a single G4-VP7 gene may affect growth characteristics of recipient virus SA11. These results together with our previous findings suggested the significance of genetic compatibility between recipient viral genes and foreign VP7 gene in the development of multivalent reassortant rotavirus vaccines.

Keywords: Rotavirus; VP7; Selection; Reassortant; G-serotype

Group A rotavirus is the most commonly recognized cause of severe diarrhea among infants in developed and developing countries, and the impact of rotavirus disease has been promoting research on vaccine development (Flores and Kapikian, 1992).

Rotavirus consists of outer capsid and inner capsid containing 11 segmented double-stranded (ds) RNAs. Rotavirus neutralization epitopes exist on two outer capsid proteins VP4 and VP7

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which define different serotype specificities, i.e. P-serotype and G-serotype, respectively (Estes and Cohen, 1989; Taniguchi et al., 1994b). In human rotavirus (HRV), four major G-serotypes (G1–G4) and two frequently identified P serotypes (P1A and P1B) have been described in epidemiological studies (Flores and Kapikian, 1992; Steele et al., 1993; Wu et al., 1994).

Neutralizing antibodies directed to both VP7 and VP4 can be elicited after primary infection with HRV in infants and are effective for protection against rotavirus infection (Green et al., 1990; Rojas et al., 1995). Hence, it is essential for effective rotavirus vaccine to elicit antibodies to these proteins. Live attenuated monovalent vaccines from animal rotavirus (bovine and simian rotavirus) were first developed for oral administration to children, and the protective efficacy of these monovalent vaccine candidates has been demonstrated by several field trials (Conner et al., 1994). However, infant vaccinees below 6 months of age exhibited mostly homotypic response against the immunizing virus (Green et al., 1990) and the vaccine sometimes failed to protect against diarrheal disease due to heterogenous HRV strains. Thus, subsequently developed multivalent vaccine containing strains with different G-serotype specificities has been expected to show a broader protective spectrum against HRV infection.

The most evaluated multivalent vaccine is rhesus rotavirus (RRV)-based tetravalent vaccine (RRV-TV) which is composed of RRV(G3) and RRV-reassortant with a single-VP7 gene expressing G1-, G2- or G4-specific antigens (Flores and Kapikian, 1992; Perez-Schael et al., 1990). The safety and immunogenicity of RRV-TV have been well established in several field trials. However, an unexpected result was that antibody response in vaccinees to HRV with each G-serotype and RRV was considerably different, even though identical doses of the four vaccine components were administered. A relatively higher neutralizing antibody response to RRV than to G1 through G4 HRVs was commonly observed in field trials with RRV-TV (Flores et al., 1990, 1993; Perez-Schael et al., 1990, 1994), and was considered to be due to the dominant production of anti-VP4 antibody to RRV, as indicated by epitope-blocking assay using VP4- and VP7-specific monoclonal antibodies (Green et al., 1990). However, the factor(s) causing uneven antibody response to HRV strains with G1 through G4 serotype remain(s) unknown. Interference among vaccine components has been suggested to be one of the possible reasons. Indeed, G-serotypes of the viruses shed from vaccinees did not distribute evenly (Kobayashi M. et al., 1994; Perez-Schael et al., 1990). However, interference or different growth properties among multivalent vaccine components has not been much analyzed.

In order to clarify this subject in vitro, we previously studied competitive growth among SA11 and single-VP7 gene reassortants based on SA11 with G1 or G2 specificity (Kobayashi et al., 1995). SA11 and the two single-VP7 gene reassortants were employed as a model of RRV-TV components, since both SA11 and RRV are of simian origin and possess G3 specificity. Although VP4 of SA11 belongs to a different genetic group (P-type or VP4-genotype) than RRV (Estes and Cohen, 1989), SA11 is one of the best characterized rotaviruses and has been used for experimental infection studies in animals (Conner et al., 1988; Gombold and Ramig, 1989). Our previous study indicated that the VP7 gene from SA11 (G3-VP7 gene) was preferentially selected in the genetic background of SA11 compared with G1- or G2-VP7 gene. In the present study, selection of G4-VP7 gene in the SA11 background was analyzed to extend our previous study on the VP7 gene selection depending on the G-serotype.

The mixed infection experiment was performed on MA-104 cells as described previously (Kobayashi et al., 1995) at a multiplicity of infection (m.o.i.) of 5 plaque forming units (PFU)/cell for each strain by employing SA11-L2 strain and single-VP7 gene reassortants with G1, G2 and G4 specificity. The G4-reassortant, SA11-L2/Hochi-R1, which had been prepared previously, possessed VP7 gene derived from G4 HRV strain Hochi in the genetic background of SA11 (Kobayashi N. et al., 1994). Three kinds of coinfections were performed: (A) G1-reassortant (SA11-L2/KU-R1) and G4-reassortant; (B) G2-reassortant (SA11-L2/DS1-R1) and G4-reassor-

tant; (C) SA11-L2 and G4-reassortant. Multiple passage was done until the 10th passage in MA-104 cells, employing coinfected culture fluid as inoculum for the following passage at an approximate m.o.i. of 5 PFU/cell. Virus clones were randomly isolated from plaques on CV-1 cells. In virus isolation, irrespective of the plaque size, all the single plaques were picked up from each well of 6-well plates (FALCON). Isolated viruses were propagated in MA-104 cells, and G-serotypes of each virus was determined by enzyme-linked immunosorbent assay (ELISA) using G-serotype 1–4-specific monoclonal antibodies, KU-6BG, S2-2G10, McN-10, and ST-2G7, respectively (Kobayashi et al., 1991a,b; Taniguchi et al., 1987).

In order to roughly estimate the relative amount of VP7 gene in the virus population yielded after coinfection, dsRNAs extracted from mixed culture A (G1-reassortant and G4- reassortant) were electrophoresed on a polyacrylamide gel. G1-VP7 gene could be distinguished from G4-VP7 gene because of its faster migration. As shown in Fig. 1, both genes could be seen almost evenly at the 3rd and the 6th passages, and a more intense band representing G4-VP7 gene was seen at the 10th passage.

Table 1 shows G-serotype distribution of virus clones isolated from each coinfection at the 3rd and the 10th passages. The detection rate of G4-virus in both coinfections A and B was extremely low (13.2% and 13.5%, respectively) at the 3rd passage. However, among clones at the 10th passage, G4-virus was detected at higher rates, 67.6% (A) and 41.7% (B). In coinfection C, in contrast, most of the virus clones (93%) at the 3rd passage, and all the clones at the 10th passage, belonged to G3.

One-step growth curves of SA11 and G4-reassortants were examined as described previously (Taniguchi et al., 1994a). As shown in Fig. 2, SA11 and G4-reassortant showed similar growth kinetics, although after the maturation phase (24–48 h postinfection), titer of G4-reassortant was approximately 0.2–0.4 $\log_{10}$ lower than that of SA11.

In addition, plaque sizes of SA11 and three single-VP7 gene reassortants were compared. At day 4 after inoculation of viruses onto CV-1 cells, average plaque size of G4-reassortant was significantly smaller than those of SA11-L2, G1- and G2-reassortants (Table 2). On the other hand, no significant difference in plaque size was found among SA11, G1-, and G2-reassortants.

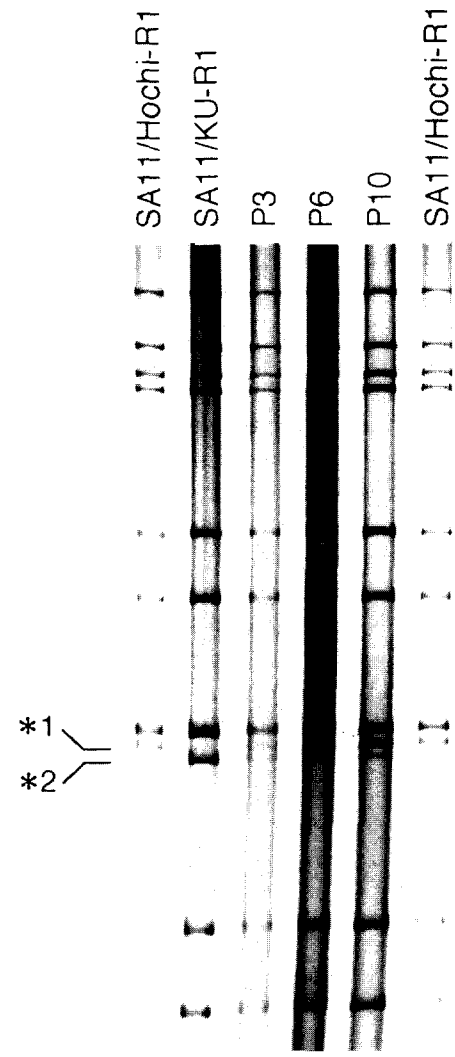

Fig. 1. Electrophoretic migration patterns of dsRNA extracted from G4-reassortant (SA11-L2/Hochi-R1), G1-reassortant (SA11-L2/KU-R1), and 3rd (P3), 6th (P6) and 10th (P10) passage of mixed infection A (G1 × G4-reassortant). *1 and *2 indicate G4- and G1-VP7 gene, respectively.

Table 1
G-serotype of rotavirus clones isolated from the 3rd and 10th passages of each mixed infection

Mixed infection of rotaviruses (G-serotype)	Passage no.	No. of clones examined	G-serotype			
			G1	G2	G3	G4
A:						
SA11-L2 KU-R1 (G1)	3	76	66 (86.8%)			10 (13.2%)
×SA11-L2 Hochi-R1 (G4)	10	37	12 (32.4%)			25 (67.6%)
B:						
SA11-L2 DS1-R1 (G2)	3	37		32 (86.5%)		5 (13.5%)
×SA11-L2 Hochi-R1 (G4)	10	36		21 (58.3%)		15 (41.7%)
C:						
SA11-L2(G3)	3	71			66 (93%)	5 (7%)
×SA11-L2 Hochi-R1 (G4)	10	35			35 (100%)	0 (0%)

The results obtained in the present study were consistent with our previous study (Kobayashi et al., 1995) showing that SA11 with G3 outgrew viruses with other G-serotypes in coinfection experiments. In our previous study, the SA11-VP7 gene was suggested to be more preferably assorted with other SA11 genes compared with G1- or G2-VP7 genes which were derived from HRV, since no significant difference in viral growth was detected among SA11 and G1- or G2-reassortants. Our present study indicated also that SA11-VP7 gene was preferentially selected in the genetic background of SA11 compared with G4-VP7 gene, since SA11 and G4-reassortant showed similar one-step growth curves.

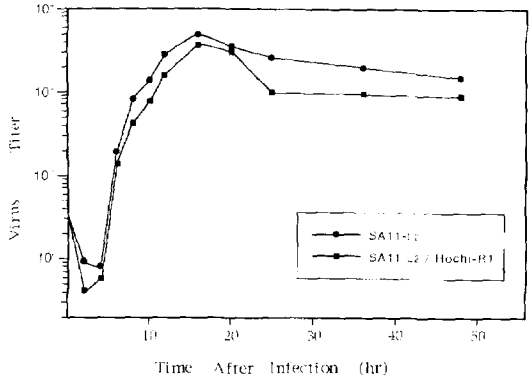

Fig. 2. One-step growth curves of SA11-L2 and SA11-L2 Hochi-R1. Virus titers are expressed as PFU/ml.

On the other hand, selection efficiency in the SA11 genetic background is not considerably different among VP7 genes encoding G1-, G2- and G4-specificity, as seen in our previous and present study. Although G1- and G2-reassortants were dominantly selected at the 3rd passage in coinfections A and B of the present study, respectively, preferential selection of either parental strains was not found in the 10th passage and RNA profiles of mixed infection A (G1- and G4-reassortants) indicated the presence of almost

Table 2
Plaque size of SA11-L2 and three SA11-HRV single-VP7 gene-substitution reassortants

Virus (G-serotype)	No. of plaques examined	Plaque size (mm)
		Mean ± S.D.
SA11-L2 KU-R1 (G1)	30	1.8 ± 0.7 *
SA11-L2 DS1-R1 (G2)	30	1.5 ± 0.7 *
SA11-L2 (G3)	30	1.8 ± 0.8 *
SA11-L2/Hochi-R1 (G4)	30	0.8 ± 0.2

For plaque size determination, plaques were formed in the presence of trypsin (2 μg ml), stained with neutral red and measured 4 days after infection.
* Significantly ($P<0.01$) large size compared with that of SA11-L2/Hochi-R1.

equal amounts of both VP7 genes at the 3rd passage. Therefore, this inconsistent result might be caused by poor plaque-forming capability of G4-reassortant compared with G1- or G2-reassortants.

These results may suggest that preferential SA11-VP7 gene selection, compared with G1-, G2- and G4-VP7 genes, is due to a difference in genetic compatibility between simian-simian and simian-human gene combinations: SA11-VP7 gene might be more compatible with other SA11 genes than VP7 gene derived from HRV. However, it is still unclear whether or not the G3-VP7 gene, irrespective of its origin, is preferentially selected in the SA11 genetic background.

Functional interaction between outer capsid proteins VP4 and VP7 has been suggested by the altered antigenicity depending on the combination of the two proteins (Chen et al., 1992; Lazdins et al., 1995; Xu and Woode, 1993). Moreover, (Xu and Woode, 1994) revealed that VP7 from certain rotavirus (bovine rotavirus B223) could confer replication advantage in the genetic background of another virus strain (HRV 69M). In their study, it was hypothesized that VP7 from B223 modified the efficiency of attachment of 69M-VP4 to cells. In our study, it was of note that plaque size of G4-reassortant was significantly smaller compared with SA11 and other reassortants. This finding may suggest that the introduction of G4-VP7 affected the infectivity of recipient virus SA11.

Our present and previous in vitro studies were performed to examine whether or not interference or relative growth difference exists among single-VP7 gene reassortants and their parental virus. While we evaluated the persistence of virus clones through multiple replication cycles in vitro, the results thus obtained may not accurately reflect the in vivo event in which vaccine strains appear to propagate in the gastrointestinal tract for relatively limited replication cycles. However, it should be noted that preferential selection of SA11 was detected in in vitro study even in early replication cycles; 88% and 93% of the virus clones were SA11 at the 3rd passage when SA11 was coinfected with G1- or G4-reassortant, respectively (Kobayashi et al., 1995 and the present data). It can be inferred from these data that SA11 was yielded about 1.9 or 2.4 times as much as the G1- or G4-reassortant virus during a single replication cycle, respectively. Such uneven viral growth, resulting in a 2-times or more difference in titer among viral components, if it occurs in vivo, should be taken into account in administering multivalent vaccines. Indeed, RRV was detected exclusively or preferably in viruses recovered from stools of vaccinees administered with RRV-TV (Kobayashi M. et al., 1994; Perez-Schael et al., 1990). However, it is not clear to what extent the relative amount of vaccine components propagated in the gastrointestinal tract is reflected in uneven antibody response to each G-serotype of HRV.

In conclusion, our in vitro observations may provide the following suggestions for development of reassortant vaccines: (i) genetic compatibility between recipient virus genomes and the VP7 gene may be different among reassortant vaccine components, and (ii) certain foreign VP7 genes may affect infectivity of recipient virus. Therefore, examination of growth characteristics for individual vaccine components seems to provide useful information to determine the effective combination of vaccine components to be administered.

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