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Preferential selection of heterologous G3-VP7 gene in the genetic background of simian rotavirus SA11 detected by using a homotypic single-VP7 gene-substitution reassortant

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Abstract

Introduction of segmented genomes into virion is an important process in viral replication of rotavirus. We previously studied the assortment of the VP7 gene segment (encoding outer capsid protein VP7) in the genetic background of simian rotavirus SA11 (G serotype 3, G3) and found the preferential selection of homologous G3 VP7 gene over VP7 gene of heterologous G serotype (G1, G2 or G4). In the present study, in order to clarify whether or not VP7 gene derived from different G3 rotavirus (heterologous G3-VP7 gene) is also preferentially selected in the SA11 background, a single-VP7 gene-substitution reassortant was prepared from SA11 through multiple steps of coinfection with rotaviruses in vitro. The isolated reassortant, SNR1, possessed VP7 gene derived from canine G3 rotavirus K9 and all other gene segments of SA11 origin, and showed an identical growth characteristic to that of SA11. Amino acid sequence of K9 VP7 gene showed a high degree of identity (93.6%) to SA11 VP7 gene. In analysis by mixed infection and multiple passages of SNR1 and a single VP7 gene (with G1, G2 or G4 specificity) reassortant in the SA11 background, the G3-VP7 gene became predominant at early passage numbers. However, in mixed infection with SA11 and SNR1, homologous G3-VP7 gene (SA11-VP7 gene) was preferentially selected into progenies over heterologous one (K9-VP7 gene). These results together with our previous findings suggested that G3-VP7 gene, irrespective of origin of species, was functionally adapted to the genetic background of SA11, although the homologous gene had a better fit with other SA11 genes than did heterologous one, providing suggestions for efficaciousness of multivalent reassortant rotavirus vaccine.

Keywords: Heterologous G3-VP7; Homotypic single-VP7; Simian rotavirus SA11

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1. Introduction

Rotavirus is the single most important viral agent of diarrheal disease in infants and young children throughout the world. Various candidates of rotavirus vaccine have been developed to date because of the high prevalence of rotavirus infection and thereby, high priority of vaccine development (Conner et al., 1994; Midthun and Kapikian, 1996). Outer capsid of rotavirus particle consists of two viral proteins VP7 and VP4 each having multiple neutralization epitopes, and antibodies directed to these epitopes play an important role in protection of rotavirus infection (Matsui et al., 1989; Hoshino and Kapikian, 1994). VP7 and VP4 define G (VP7) serotype and P (VP4) serotype, respectively. Four G serotypes (G1, G2, G3 and G4) and one P serotype (P1 containing subtypes P1-a and P1-b) are recognized as epidemiologically important in human rotaviruses (HRVs), although other serotypes have also been detected infrequently (Gentsch et al., 1996). One of the most critical problems of a Jennerian rotavirus vaccine which consisted of a single strain represented by rhesus rotavirus (RRV) with G3 specificity has been a lack of consistent heterotypic protection against the other major G serotypes. To overcome this problem, the tetravalent vaccine (RRV-TV) consisting of RRV (G3) and RRV-HRV reassortants was developed (Flores and Kapikian, 1992). Genes of the reassortant viruses in RRV-TV consisted of a single HRV gene encoding G1, G2 or G4 VP7 and remaining 10 RRV RNA segments. Since safety and efficacy of RRV-TV have been demonstrated through extensive field trials, RRV-TV is now considered the vaccine ready for general use (Kapikian et al., 1996). However, several reports on trials with RRV-TV described that the rate of antibody response against individual G serotype was considerably different, and that the amount of virus shed in the feces of vaccinees differed among component viruses (G1–G4), despite the administration of identical doses of the component viruses (Flores et al., 1990; Perez-Schael et al., 1990; Kobayashi M. et al., 1994; Bernstein et al., 1995; Rennels et al., 1996). To elucidate the mechanism of variable antibody response to different G serotypes seems important to increase the efficacy of rotavirus vaccine.

During mixed infection of different rotaviruses, exchange of viral genome segments occurs easily, yielding new progeny viruses known as genome reassortments. Therefore, we assumed that the efficiency of the VP7 gene assortment during virus multiplication might have been related to some extent with the uneven replication of the RRV-TV components (Kobayashi et al., 1995, 1996a). Although reassortment is recognized as one of the important processes of genomic evolution of rotavirus (Ramig and Ward, 1991), the mechanism by which each segment is selected into progeny virus during the mixed infection has not been well elucidated.

Previously, in order to analyze assortment of VP7 gene in vitro, we employed a simian rotavirus SA11 (G3) and three single VP7 gene reassortants with G1, G2 and G4 specificity, which were constructed on a genomic background of SA11, as a model of RRV-TV (Kobayashi et al., 1995, 1996a). When the same titers of SA11 and one of the single VP7 gene reassortants were coinfected to cell cultures and propagated viruses were passaged sequentially, virus with SA11 genotype became predominant at early passage numbers, although there is no difference in the growth rate between SA11 and the reassortants. Furthermore, in similar mixed infection and multiple passage experiments using G1-reassortant (SKR1) (VP7 gene from G1-HRV strain and the other gene segments of SA11 origin) and one of G3 rotaviruses (S3, YO, P, RRV or K9), VP7 gene and NSP1 gene were always selected more preferentially from G3 viruses than from G1-reassortant (Kobayashi et al., 1996b). These findings suggested that SA11 gene segments were more efficiently assorted with G3-VP7 gene, even if derived from a heterologous strain, than with G1-VP7 gene, although the mechanism of co-selection of G3-VP7 and NSP1 genes remained unclear.

The preferential selection of SA11-VP7 gene was presumed to be due to either of following reasons; SA11-VP7 gene was homologous to the genetic background of SA11, or it encodes VP7 homotypic to SA11 (G3). Subsequent coinfection experiment with SKR1 and various G3 viruses

suggested that G3-VP7 gene might be preferentially selected in the SA11 background compared with G1-VP7 gene (Kobayashi et al., 1996b), favoring the latter view mentioned above. However, ambiguity still remained because, in addition to G3-VP7 gene, some other RNA segments of G3 viruses were coselected in the SA11 background, suggesting the role of other gene segments on VP7 gene selection. Hence, in order to further clarify the mechanism of preferential selection of VP7 gene, we prepared a reassortant of SA11 (SNR1) whose VP7 gene alone was derived from heterologous G3 virus (K9 strain). Employing this reassortant, we studied selectivity of homologous vs. heterologous G3-VP7 genes and of homotypic vs. heterotypic VP7 genes.

2. Materials and methods

2.1. Isolation of a single VP7 gene substitution reassortant of SA11 (SNR1)

A single VP7 gene reassortant SA11/K9-R1 (SNR1) having VP7 gene from G3 canine rotavirus K9 in the genetic background of SA11 (SA11-L2 strain) was isolated from a culture fluid of mixed infection with a single gene- and a double gene-reassortants with the genetic background of SA11, SA11/KU-R1 (SKR1) and SNR2, respectively, reported previously (Kobayashi N. et al., 1994; Kobayashi et al., 1996b). SKR1 possesses VP7 gene derived from HRV strain KU (G1), while SNR2 has VP7 and NSP1 genes of K9. Coinfection of SKR1 and SNR2 generated four genotypes: genotype 1 (identical to SKR1); genotype 2 (identical to SNR2); genotype 3 with KU-VP7 gene and K9-NSP1 gene; and genotype 4 with K9-VP7 gene alone (SNR1). RNA profiles of SNR1 and the two parenral reassortant viruses are shown in Fig. 1. SNR1 was employed in the following study after plaque purification twice in CV-1 cell cultures. Single-step growth curve of SNR1 was examined as described previously (Kobayashi et al., 1995).

2.2. Viruses and mixed infection analysis

In addition to SKR1 mentioned above, two single VP7 gene reassortant viruses SA11/DS1-R1 (SDR1) and SA11/Hochi-R1 (SHR1) with G2 and G4 specificity, respectively (Kobayashi N. et al., 1994) were employed in this study. Parental strains of the reassortants are listed in Table 1. SNR1 and one of the single gene reassortants (SKR1, SDR1, SHR1) were simultaneously inoculated onto MA-104 cell monolayer in six well plate at a multiplicity of infection (m.o.i.) of five plaque forming unit (p.f.u.)/cell for each virus. Viruses pretreated with acetylated trypsin (20 μg/ml) at 37°C for 1 h were inoculated on MA-104 cells washed with Eagle's minimum essential medium. After 1 h adsorption, 1 ml of mainte-

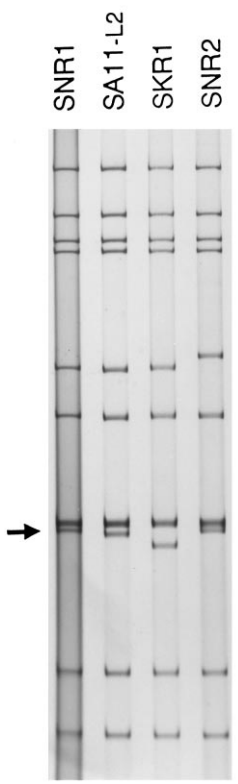

Fig. 1. RNA patterns of SA11-L2 and reassortants SNR1, SKR1 and SNR2. An arrow indicates VP7 gene segment derived from K9, which is present in SNR1 and SNR2.

Table 1
Single-VP7 gene-substitution reassortants employed in this study

Reassortant designation (abbreviation)	Derivation of VP7 gene (G serotype specificity)	Parent viruses used for coinfection to obtain reassortants
SA11/KU-R1 (SKR1)	KU (G1)	SA11, KU
SA11/DS1-R1 (SDR1)	DS1 (G2)	SA11, DS1
SA11/Hochi-R1 (SHR1)	Hochi (G4)	SA11, Hochi
SA11/K9-R1 (SNR1)	K9 (G3)	SKR1, SNR2[a]

[a] SNR2 is a double-gene reassortant having VP7 gene and NSP1 gene derived from K9 in the genetic background of SA11.

nance medium containing acetylated trypsin (2 μg/ml) was added and the cells were incubated until significant cytopathic effect appeared (1 or 2 days postinfection). The harvested culture fluid, after three cycles of freezing and thawing, was treated with acetylated trypsin and inoculated onto MA-104 cell monolayer at an approximate m.o.i. of 5 p.f.u./cell. Three or six sequential passages of the viruses were performed in a similar way. Mixed infection followed by multiple passages was also done for crosses SA11 and SKR1, and SA11 and SNR1.

2.3. Isolation and characterization of virus clones

From mixed culture at the first, third or sixth passage, virus clones forming plaques on CV-1 cells were randomly picked up and were propagated in MA-104 cells. Derivation of VP7 gene in the isolated viruses was determined by enzyme-linked immunosorbent assay with G serotype–specific monoclonal antibodies (Taniguchi et al., 1987) or polyacrylamide gel electrophoresis (PAGE) of dsRNA extracted from 300 μl of infected culture fluid as described previously (Kobayashi et al., 1989).

2.4. Nucleotide sequencing of K9-VP7 gene

Culture fluid of K9 was purified into single-shelled particles and mRNA was prepared according to the method described previously (Flores et al., 1982). Using the mRNA, nucleotide sequence of the VP7 gene was determined by primer extension method as described previously (Gorziglia et al., 1986).

3. Results

Electrophoretic profiles of dsRNA extracted from coinfection of homotypic (having G3 VP7 of

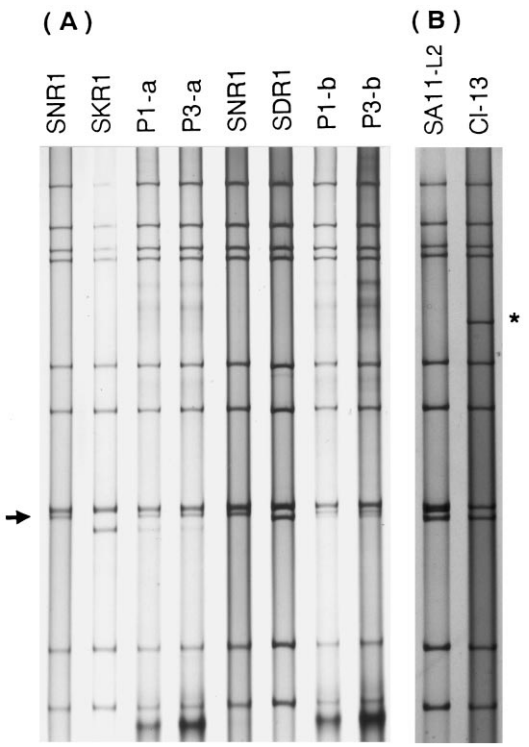

Fig. 2. (A) Electrophoretic migration patterns of dsRNA extracted from mixed culture of SNR1 and SKR1(P1-a, P3-a), or SNR1 and SDR1(P1-b, P3-b). P1 and P3 represent passage numbers 1 and 3, respectively. An arrow indicates VP7 gene segments derived from K9. (B) RNA patterns of SA11 and a virus clone (Cl-13) which was detected from the sixth passage of mixed infection of SA11 and SNR1. Putative rearranged RNA segment is shown by an asterisk.

Table 2
G serotype of rotavirus clones isolated from culture coinfected with homotypic (G3) and heterotypic (G1, G2 or G4) single-VP7 gene-reassortants

Mixed infection of rotavirus	Passage number	Number of clones examined	G serotype			
			G1	G2	G3	G4
SNR1 × SKR1	1	70	14 (20%)		56 (80%)	
	3	47	2 (4.3%)		45 (95.3%)	
SNR1 × SDR1	1	72		24 (33.3%)	48 (66.7%)	
	3	48		9 (18.8%)	39 (81.2%)	
SNR1 × SHR1	1	66			59 (89.4%)	7 (10.6%)
	3	44			41 (93.2%)	3 (6.8%)
SA11 × SKR1	1	66	13 (19.7%)		53 (80.3%)	
	3	45	0 (0%)		45 (100%)	

K9) and a heterotypic (having G1, G2 or G4 VP7) reassortant were examined in PAGE. As shown in Fig. 2(A) (lanes P1-a and P3-a), a segment representing K9-VP7 gene was found in greater intensity than G1-VP7 gene at the first and third passages of coinfection with SNR1 and SKR1. In coinfection with SNR1 and SDR1, the difference in intensity of the two VP7 gene segments was not distinct (Fig. 2(A), lanes P1-b and P3-b). Similar result was obtained in coinfection with SNR1 and SHR1 (data not shown).

More than 40 virus clones were isolated from each mixed infection and their G serotypes were determined (Table 2). In coinfections SNR1 × SKR1 and SNR1 × SHR1, G3 virus accounted for 80 and 89%, respectively, of the isolated clones at the first passage, and more than 90% of the clones possessed G3 specificity at the third passage. Also in the cross SNR1 × SDR1, G3 clones became predominant though the rate was a little slower than in the other crosses. Although these results seem not to be parallel with change in the density of VP7 genes in PAGE, it should be considered that the density of the genes in PAGE only roughly reflects the amount of dsRNA including that from non-infectious particles. Rapid increase of G3 clones were also observed in mixed infection of SA11 and SKR1.

In coinfection and multiple passage with SA11 and SNR1, clones with SA11-VP7 gene surpassed those with K9-VP7 gene after the first passage (Table 3), which became more distinct as the passage advances. A single virus clone (Cl-13) with SA11-VP7 isolated from the sixth passage of coinfected culture possessed an additional band between the fourth and the fifth RNA segments, while lacking the eighth RNA segment (NSP3 gene) at the normal position (Fig. 2(B)), suggesting that the unusual segment might be a rearranged genome of the eighth segment.

As shown in Fig. 3, single step growth curve of SNR1 was almost identical to that of SA11. Nucleotide sequence of the protein coding region of K9-VP7 gene (Gen Bank accession No. U97199) showed an identity of 82.6% to that of SA11. Between the deduced amino acid sequences of K9 and SA11, 21 amino acids were different (Table 3) and the identity was 93.6%. Most of these amino acids were located in variable regions that had been identified among rotaviruses with different G serotypes (Green et al., 1989).

4. Discussion

When a multivalent vaccine is put to practical use, interference or relative growth difference among viral components should be primarily considered for efficacy of the vaccine. A series of our study including present results have revealed various characteristics in assortment of VP7 gene of rotavirus strain in vitro, which is relevant to

Table 3
Frequency of homologous (SA11)- and heterologous (K9)-G3 VP7 gene in the clones obtained from co-infection with SA11 and SNR1

Passage number	Number of clones examined	Clones with	
		SA11-VP7 gene	K9-VP7 gene
1	67	45 (67.2%)	22 (32.8%)
3	44	36 (81.8%)	8 (18.2%)
6	42	38 (90.5%)	4 (9.5%)

interaction among components of rotavirus reassortant vaccine. The aim of our research is to obtain suggestions to improve the rotavirus vaccine from the viewpoint of interaction or VP7 gene selection among vaccine strains.

In the present study, a homotypic (G3) single VP7 gene reassortant (SNR1) surpassed heterotypic (G1, G2 or G4) single VP7 gene reassortants during multiple passages. Moreover, as observed for SKR1, SDR1 and SHR1, growth characteristic of SNR1 was identical to that of SA11 (Kobayashi et al., 1995, 1996a). These findings indicated that K9-VP7 gene, which was derived from homotypic but heterologous strain K9, might have been preferentially selected in the genetic background of SA11. Hence, it is suggested that irrespective of animal species from which the virus was derived, the gene segment encoding G3-VP7 may be functionally adapted to the genetic background of SA11. Furthermore, it was of note that SA11-VP7 gene was more preferentially selected than K9-VP7 gene. This result suggested that homologous VP7 gene might be better adapted to the other SA11 genes compared with heterologous VP7 gene, even though both genes encode the same G serotype.

Amino acid identity of VP7 among strains with different G serotypes has been reported to be 71–86%, whereas, among the strains with the same G serotype, the identity is higher (more than 90%) as found between SA11 and K9 (Green et al., 1987). Therefore, the preferential selection of G3-VP7 gene in the SA11 background might have been associated with the distinct diversity of G3-VP7 gene from the VP7 genes of the other G serotypes. On the other hand, genetic variation also exists in VP7 genes from viruses with the same G serotype, and larger sequence diversity was observed in G3 viruses than in viruses of the other G serotypes (Nishikawa et al., 1989). The sequence diversity among different G serotypes resides mostly in several variable regions (Green et al., 1989). In these regions, amino acid variations in G3 viruses were also located (Nishikawa et al., 1989), which was also found between SA11 and K9 (Table 4). Such minor VP7 sequence diversity between SA11 and K9 is considered to be related to the lower functional adaptability of K9-VP7 gene to the SA11 genetic background.

Although specific gene segment(s) that influence the adaptability of VP7 gene has not been identified, it appears that VP4 gene may be associated with the assortment of VP7 gene, since some reports described the presence of evident configurational interaction between VP4 and VP7, including alteration of antigenic epitopes on these proteins (Chen et al., 1992; Xu and Woode, 1993, 1994; Lazdins et al., 1995). It is conceivable that SA11-VP4 or certain other viral protein fits better with G3-VP7 than VP7 from other G serotypes.

In the coinfection with SKR1 and G3 rotaviruses, NSP1 gene was also preferentially selected together with VP7 gene from G3 viruses in the genetic background of SA11 (Kobayashi et al., 1996b). However, in the present study, predominant selection of K9-VP7 gene was observed by using the reassortant without NSP1 gene from K9. Hence, NSP1 gene may not necessarily be associated with VP7 gene selection, although NSP1 has been implicated to play a role in packaging of RNA (Mitchell and Both, 1990; Hua et al., 1993).

In the case of RRV-TV administration, some reports described predominant multiplication of

Table 4
Amino acid difference in VP7 sequences between G3 rotaviruses K9 and SA11

	Amino acid position (residue number) in VP7 sequence																				
	9	16	18	22	29	37	47	48	49	65	66	91	123	139	147	212	213	221	242	304	309
SA11	V	I	L	I	I	L	F	L	R	I	A	T	N	V	T	A	T	A	A	D	V
K9	I	F	F	M	M	F	L	I	K	A	D	S	D	I	A	V	S	T	T	N	A
Regions*	VR1	VR1	VR1	C	VR2	VR3	VR3	VR3	VR3	VR4	VR4	VR5	VR6	C	VR7	VR8	VR8	VR8	VR9	C	C

VR1 to VR9 represent variable regions of amino acid sequence in VP7 among different G serotypes, while C indicates conserved region (Green et al., 1989). Signal peptide cleavage occurs between amino acid number 50 and 51 during maturation of VP7 protein in a cell.

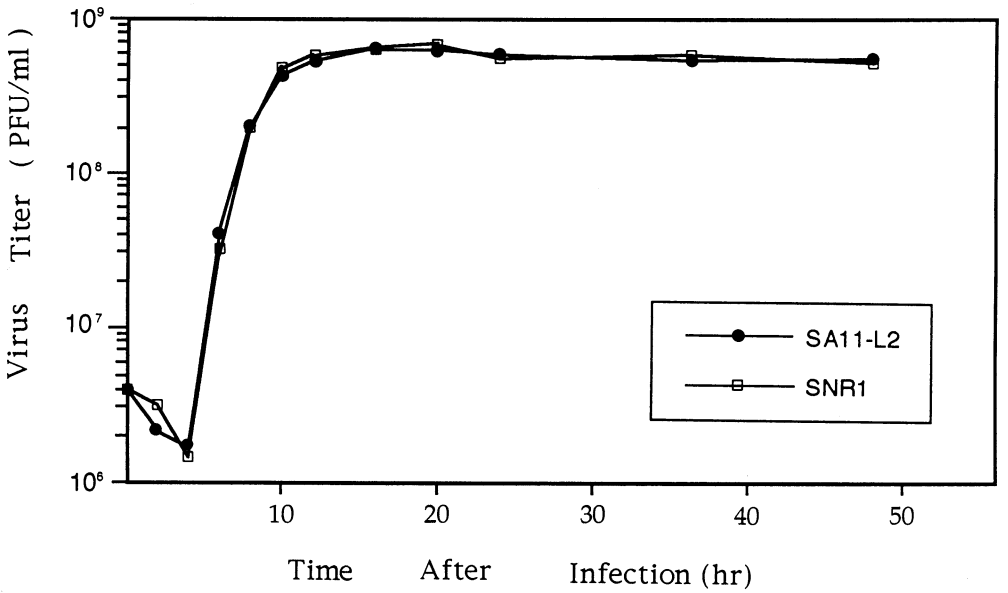

Fig. 3. Single-step growth curves of SA11-L2 and SNR1.

G3 component (RRV) in feces of vaccinees (Perez-Schael et al., 1990; Kobayashi M. et al., 1994). Considering the in vitro findings of our present and previous studies, it seems that the predominance of RRV in some RRV-TV trials might be caused in part by the preferential selection of homologous G3-VP7 gene in the genetic background of RRV. Further, our results seem to indicate that such uneven growth of multivalent vaccine components may not be improved by employing a new reassortant with heterologous G3-VP7 gene instead of RRV. Rather, it may be preferable to adjust doses for each vaccine component as had been attempted (Flores et al., 1990, 1993) or to employ another set of VP7 gene reassortants whose recipient virus belongs to non-HRV G serotype (Clark et al., 1996).

Viruses with rearranged genome have been isolated from chronically infected or immunodeficient children and from culture fluid of rotavirus after serial passage at high m.o.i. (Desselberger, 1996). In the present study, rearranged genome was detected in one clone from coinfection of SA11 and SNR1. A putative rearranged genome was also detected in virus clones isolated from mixed infection of rotaviruses previously (Kobayashi et al., 1993). Therefore, it is suggested that mixed infection or infection at high m.o.i. conditions of rotaviruses may have facilitated the emergence of rearranged genes, although its mechanism is unknown. Further sequence analysis of the rearranged genome may contribute to understand the molecular events and significance of this phenomenon.

References

Bernstein, D.I., Glass, R.I., Rodgers, G., Davidson, B.L., Sack, D.A., 1995. Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children. J. Am. Med. Assoc. 273, 1191–1196.

Chen, D., Estes, M.K., Ramig, R.F., 1992. Specific interactions between rotavirus outer capsid proteins VP4 and VP7 determine expression of a cross-reactive, neutralizing VP4-specific epitope. J. Virol. 66, 432–439.

Clark, H.F., Offit, P.A., Ellis, R.W., Eiden, J.J., Krah, D., Shaw, A.R., Pichichero, M., Treanor, J.J., Borian, F.E., Bell, L.M., Plotkin, S.A., 1996. The development of multivalent bovine rotavirus (strain WC3) reassortant vaccine for infants. J. Infect. Dis. 174, S73–S80.

Conner, M.E., Matson, D.O., Estes, M.K., 1994. Rotavirus vaccines and vaccination potential. Curr. Top. Microbiol. Immunol. 185, 285–337.

Desselberger, U., 1996. Genome rearrangements of rotaviruses. Arch.Virol. 12 (Suppl.), 37–51.

Flores, J., Kapikian, A.Z., 1992. Rotavirus vaccines. In: Ellis, R.W. (Ed.), Vaccines: New Approaches to Immunological Problems. Butterworth-Heinemann, Stoneham, MA, pp. 255–288.

Flores, J., Greenberg, H.B., Myslinski, J., Kalica, A.R., Wyatt, R.G., Kapikian, A.Z., Chanock, R.M., 1982. Use of transcription probes for genotyping rotavirus reassortants. Virology 121, 288–295.

Flores, J., Perez-Schael, I., Blanco, M., White, L., Garcia, D., Vilar, M., Cunto, W., Gonzalez, R., Urbina, C., Boher, J., Mendez, M., Kapikian, A.Z., 1990. Comparison of reactogenicity and antigenicity of M37 rotavirus vaccine and rhesus-rotavirus-based quadrivalent vaccine. Lancet 336, 330–334.

Flores, J., Perez-Schael, I., Blanco, M., Rojas, A.M., Alfonzo, E., Crespo, I., Cunto, W., Pittman, A.L., Kapikian, A.Z., 1993. Reactogenicity and immunogenicity of a high-titer rhesus rotavirus-based quadrivalent rotavirus vaccine. J. Clin. Microbiol. 31, 2439–2445.

Gentsch, J.R., Woods, P.A., Ramachandran, M., Das, B.K., Leite, J.P., Alfieri, A., Kumar, R., Bhan, M.K., Glass, R.I., 1996. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J. Infect. Dis. 174, S30–S36.

Gorziglia, M., Hoshino, Y., Buckler-White, A., Blumentals, I., Glass, R., Flores, J., Kapikian, A.Z., Chanock, R.M., 1986. Conservation of amino acid sequence of VP8 and cleavage region of 84-kDa outer capsid protein among rotaviruses recovered from asymptomatic neonatal infection. Proc. Natl. Acad. Sci. USA 83, 7039–7043.

Green, K.Y., Midthun, K., Gorziglia, M., Hoshino, Y., Kapikian, A.Z., Chanock, R.M., Flores, J., 1987. Comparison of the amino acid sequences of the major neutralization protein of four human rotavirus serotypes. Virology 161, 153–159.

Green, K.Y., Hoshino, Y., Ikegami, N., 1989. Sequence analysis of the gene encoding the serotype-specific glycoprotein (VP7) of two new human rotavirus serotypes. Virology 168, 429–433.

Hoshino, Y., Kapikian, A.Z., 1994. Rotavirus antigens. Curr. Top. Microbiol. Immunol. 185, 179–227.

Hua, J., Mansell, E.A., Patton, J.T., 1993. Comparative analysis of the rotavirus NS53 gene: conservation of basic and cysteine-rich regions in the protein and possible stem-loop structures in the RNA. Virology 196, 372–378.

Kapikian, A.Z., Hoshino, Y., Chanock, R.M., Perez-Schael, I., 1996. Efficacy of a quadrivalent rhesus rotavirus-based human rotavirus vaccine aimed at preventing severe rotavirus diarrhea in infants and young children. J. Infect. Dis. 174, S65–S72.

Kobayashi, M., Thompson, J., Tollefson, S.J., Reed, G.W., Wright, P.F., 1994. Tetravalent rhesus rotavirus vaccine in young infants. J. Infect. Dis. 170, 1260–1263.

Kobayashi, N., Lintag, I.C., Urasawa, T., Taniguchi, K., Saniel, M.C., Urasawa, S., 1989. Unusual human rotavirus strains having subgroup I specificity and 'long' RNA electropherotype. Arch. Virol. 109, 11–23.

Kobayashi, N., Taniguchi, K., Urasawa, T., Urasawa, S., 1993. Efficient production of antigenic mosaic reassortants of rotavirus with the aid of anti-VP4 and anti-VP7 neutralizing monoclonal antibodies. J. Virol. Methods 44, 25–34.

Kobayashi, N., Kojima, K., Taniguchi, K., Urasawa, T., Urasawa, S., 1994. Genotypic diversity of reassortants between simian rotavirus SA11 and human rotaviruses having different antigenic specificities and RNA patterns. Res. Virol. 145, 303–311.

Kobayashi, N., Taniguchi, K., Kojima, K., Urasawa, T., Urasawa, S., 1995. Preferential selection of VP7 gene from a parent rotavirus strain (SA11) in sequential passages after mixed infection with SA11 and SA11-human rotavirus single-VP7 gene-substitution reassortants. Arch. Virol. 140, 775–781.

Kobayashi, N., Okada, J., Taniguchi, K., Urasawa, T., Urasawa, S., 1996a. Selection of rotavirus VP7 gene in the genetic background of simian rotavirus SA11: implications for rotavirus reassortant vaccine development. Antiviral Res. 31, 185–190.

Kobayashi, N., Taniguchi, K., Kojima, K., Urasawa, T., Urasawa, S., 1996b. G (VP7) serotype-dependent preferential VP7 gene selection detected in the genetic background of simian rotavirus SA11. Arch. Virol. 141, 1167–1176.

Lazdins, I., Coulson, B.S., Kirkwood, C., Dyall-Smith, M., Masendycz, P.J., Sonza, S., Holmes, I.H., 1995. Rotavirus antigenicity is affected by the genetic context and glycosylation of VP7. Virology 209, 80–89.

Matsui, S.M., Mackow, E.R., Greenberg, H.B., 1989. Molecular determinant of rotavirus neutralization and protection. Adv.Virus Res. 36, 181–214.

Midthun, K., Kapikian, A.Z., 1996. Rotavirus vaccines: an overview. Clin. Microbiol. Rev. 9, 423–434.

Mitchell, D.B., Both, G.W., 1990. Conservation of a potential metal binding motif despite extensive sequence diversity in the rotavirus nonstructural protein NS53. Virology 174, 618–621.

Nishikawa, K., Hoshino, Y., Taniguchi, K., Green, K.Y., Greenberg, H.B., Kapikian, A.Z., Chanock, R.M., Gorziglia, M., 1989. Rotavirus VP7 neutralization epitopes of serotype 3 strains. Virology 171, 503–515.

Perez-Schael, I., Blanco, M., Vilar, M., Garcia, D., White, L., Gonzalez, R., Kapikian, A.Z., Flores, J., 1990. Clinical studies of a quadrivalent rotavirus vaccine in Venezuelan infants. J. Clin. Microbiol. 28, 553–558.

Ramig, R.F., Ward, R.L., 1991. Genomic segment reassortment in rotaviruses and other reoviridae. Adv.Virus Res. 39, 163–207.

Rennels, M.B., Glass, R.I., Dennehy, P.H., Bernstein, D.I., Pichichero, M.E., Zito, E.T., Mack, M.E., Davidson, B.L., Kapikian, A.Z., 1996. Safety and efficacy of high-dose rhesus-human reassortant rotavirus vaccines-report of the national multicenter trial. Pediatrics 97, 7–13.

Taniguchi, K., Urasawa, T., Morita, Y., Greenberg, H.B., Urasawa, S., 1987. Direct serotyping of human rotavirus in stools by an enzyme-linked immunosorbent assay using serotype 1-, 2-, 3- and 4-specific monoclonal antibodies to VP7. J. Infect. Dis. 155, 1159–1166.

Xu, Z., Woode, G.N., 1993. Studies on the role of VP4 of G serotype 10 rotavirus (B223) in the induction of the heterologous immune response in calves. Virology 196, 294–297.

Xu, Z., Woode, G.N., 1994. Studies on the influence of the VP7 gene on rotavirus replication. Virology 198, 394–398.