Characterization of the neutralizing epitopes of VP4 of the Gottfried strain of porcine rotavirus

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Introduction

Rotaviruses are a major etiological agent of acute gastroenteritis in humans, as well as in other mammalian and avian species. Rotaviruses, members of the family Reoviridae, are nonenveloped particles with icosahedral symmetry. Mature viral particles have 11 segmented genome of double-stranded RNA enclosed by triple-layered protein capsids. The core contains VP1, VP2 and VP3, inner capsid consists of VP6, viral major group antigen, and outer capsid is composed of glycoprotein VP7 and protease-sensitive protein VP4. Two outer capsid proteins VP4 and VP7 elicit antibodies capable of virus neutralization and are candidates for vaccine development. The neutralization epitopes of the outer capsid protein VP4 of a porcine rotavirus (Gottfried strain) were studied by using neutralizing monoclonal antibodies(N-MAbs). VP4, nonglycosylated spike protein performs several important functions associated with cell attachment, penetration, hemagglutination, neutralization, virulence and host range determanation. In addition VP4 is implicated in membrane permeabilization. Proteolytic cleavage of the VP4 into VP8* and VP5* proteins is required for rotavirus infectivity and for rotavirus-induced membrane permeabilization.

Methods and Materials

In the present study, VP4-specific neutralizing monoclonal antibodies(N-MAbs) against the Gottfried strain(G4/P2B) of porcine rotavirus were used to characterize the neutralizing epitopes of VP4. Eight N-MAbs which were specific for the VP4 of the Gottfried strain were used for analyzing the antigenic sites of VP4. Three different approaches were used for this analysis : i) testing the serological reactivity of each N-MAb against different P types of human and animal rotaviruses ii) analyzing N-MAb-resistant viral antigenic variants and iii) performing a nucleotide sequence analysis of the VP4 gene of each of the viral antigenic variants generated. The reactivities of these N-MAbs with distinct serotype of animal rotaviruses(Porcine rotavirus OSU(G5/P9), bovine rotaviruses NCDV (G6/P6) and B223(G10/P8)) and symptomatic(Wa(G1/P1A), DS-1(G2/P1B), M(G3/P1A), and VA70(G4/P1A)) and asymptomatic human rotaviruses(M37(G1/P2A), 1076(G2/P2A), McN13(G3/P2A), and ST-3(G4/P2A)) were examined by plaque reduction virus neutralization(PRVN) tests. The PRVN test was performed in plastic six-well plates containing MA104 cell monolayers to determine titers of the N-

MAbs as previously described(Kang et al., 1993). Viral antigenic variants resistant to the VP4-specific N-MAbs were selected by a modification of procedures previously described(Taniguchi et al, 1988) and analysed by fluorescence focus neutralization(FFN) test. In addition, the nucleotide changes and the deduced amino acid changes within VP4 responsible for resistance of variants were examined by single stand conformational polymorphism(SSCP) and nucleotide sequence analysis.

Results and Discussion

The cross-reactivity of the N-MAbs specific for VP4 of the Gottfried strain with various human and animal rotaviruses, determined by the PRVN test, is shown in Table 1. Four different reactivity patterns were recognized. Three N-MAbs(24B9, 23G10, and 26A2) were cross-reactive and reacted with symptomatic and asymptomatic human rotaviruses. The other three N-MAbs(30H5, 32B3, and 29B3) reacted with symptomatic human rotaviruses and 21A1 was only reacted with Gottfried strain. It is suggested that these patterns were associated with similarity of P serotype between Gottfried strain and human rotaviruses, rather than animal viruses. The patterns of reactivity between the panel of VP4-specific N-MAbs and eight viral antigenic variants are summarized in Fig 1. Four antigenic sites(I, II, III, and IV) were determined by cross-neutralization tests. Consquently it is identified that the antigenicsite III appeared to be partially overlapping with antigenic site I and II, respectively. The SSCP patterns of Gottfried stain and antigenic variant v-24B9 are shown in Fig. 2. The SSCP pattern of four of the five fragments, B, C, D, and E, except one fragment, A showing altered mobility, are identical. This result shows that the mutations responsible for resistance of variant exist within fragment I. Nucleotide changes and the corresponding deduced amino acid changes detected in the viral antigenic variants are summarized in Table 2. Consequently there exist at least four antigenic sites, three sites on VP5* and one site on VP8*, responsible for neutralization associated with VP4 of otaviruses. Cross-reactive neutralizing antigenic site was identified on VP5*(aa385 or 392) and VP8*(aa70). The aa641 on VP5* was identified as P2 serotype-specific site and the aa109 on VP8* was determined as Gottfried strain-specific site. The results of this study are consistent with the studies reported previously which represented that VP8* possessed the major antigenic sites for serotype specificity and while VP5* contained sites responsible for cross-reactivity among different VP4 and showed that peptide B(aa84-180 of VP8*) contained VP4 serotype and subtype specificities, whereas peptides A(aa1-102)and C(aa150-246) exhibites cross-reactivity among VP4(Larralde et al., 1992, Taniguchi et al., 1988, Kobayashi et al., 1990) The aa641 on VP5* responsible for P2 serotype specificity and Gottfried strain-specific site, aa109 on VP8* was reported first in this study.

Table 1. PRVN titers of Gottfried VP4-specific MAbs against homologous virus, symptomatic and asymptomatic human and animal rotaviruses

Groups	MAbs (Ascites)	Titers against rotaviruses										
		Gottfried (G4/2B)	Symptomatic human rotaviruses (G/P serotype)				Asymptomatic human rotaviruses (G/P serotype)				Animal rotaviruses (G/P serotype)		
			Wa (G1/1A)	DS-1 (G2/1B)	M (G3/1A)	VA70 (G4/1A)	M37 (G1/2A)	1076 (G2/2A)	McN13 (G3/2A)	ST-3 (G4/2A)	OSU (G5/9)	NCDV (G6/6)	B223 (G8/8)
	23G10	15,700	1,100	24	370		52		100	50	<4	<4	<4
1	24B9	13,700	1,500	32	415	16	60	33	330	84	<4	<4	<4
	26A2	8,250	620	61	345	20	25	74	290	50	<4	<4	<4
						17		35					
	29B3	320	16	256	25		<4		<4	<4	<4	<4	<4
2	32B3	215	9	180	35	24	<4	<4	<4	<4	<4	<4	<4
	30H5	285	6	150	25	18	<4	<4	<4	<4	<4	<4	<4
						20		<4					
3	21A1	1,290	<4	<4	<4		<4		<4	<4	<4	<4	<4
						<4		<4					
4	16D2	1,450	<4	<4	<4		7		4	36	<4	<4	<4
						<4		40					

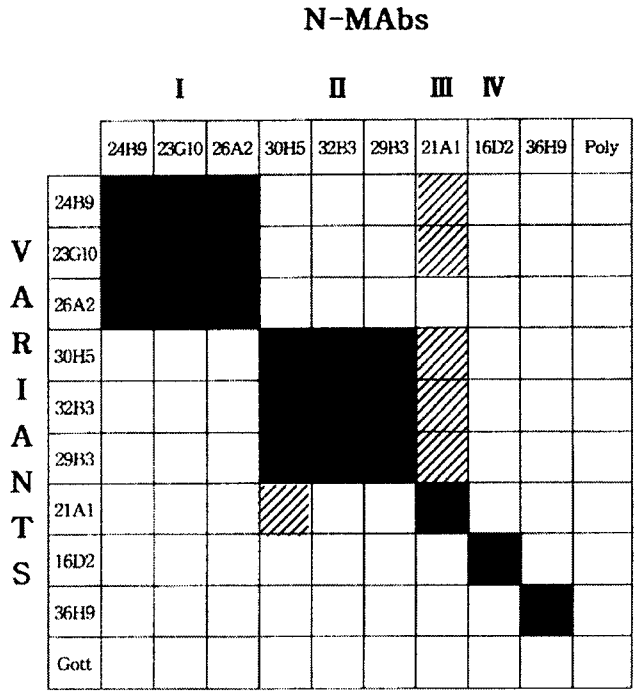

Figure 1. Antigenic mapping of VP4 of the Gottfried strain of porcine rotavirus. A panal of VP4-specific N-MAbs was tested for neutralization withparent virus(Gott) and N-MAbs-resistant viral escape mutants by FFN test. The viral escape mutants was designated as resistant(■), partially resistant(▨) or sensitive(□) against each N-MAbs. 36H9 : VP7-specific MAb, Poly : polyclonal porcine anti-Gottfried serum

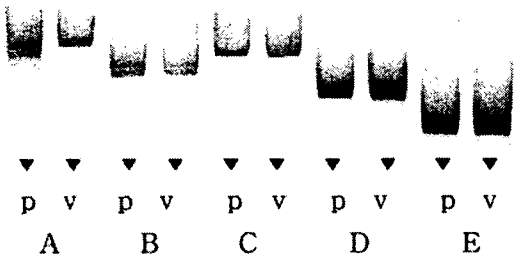

Figure 2. Single-strand conformational polymorphism(SSCP) analysis of rotavirus VP4

p : parent virus(Gottfried strain)

v : N-MAb-resistant viral escape mutant

Escape mutant	Codon change	Amino acid change (Position)
v-24B9	GCA - ACG	Ala - Thr(392)
v-23G10	GCA - ACG	Ala - Thr(392)
v-26A2	GAC - AAC	Asp - Asn(385)
v-30H5	CAG - CGG	Glu - Arg(70)
v-32B3	CAG - CGG	Glu - Arg(70)
v-29B3	CAG - CGG	Glu - Arg(70)
v-21A1	GAA - GGA	Glu - Gly(109)
v-16D2	GAA - AAA	Glu - Lys(641)

Table 2. Nucleotide and amino acid sequence changes found in the viral escape mutants selected with VP4-specific neutralizing monoclonal antibodies(N-MAbs)

Acknowledgements

Reference

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