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Antibody Response to Serotype-Specific and Cross-Reactive Neutralization Epitopes on VP4 and VP7 after Rotavirus Infection or Vaccination

KOKI TANIGUCHI,[1]* TOMOKO URASAWA,[1] NOBUMICHI KOBAYASHI,[1] MUZAHED U. AHMED,[1]†
NORIAKI ADACHI,[2] SHUNZO CHIBA,[2] AND SHOZO URASAWA[1]

Department of Hygiene[1] and Department of Pediatrics,[2] Sapporo Medical College, Sapporo 060, Japan

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By using a competitive solid-phase immunoassay with serotype-specific and cross-reactive neutralizing monoclonal antibodies directed at VP4 and VP7, we tested the antibody responses to some neutralization epitopes on VP4 and VP7 in individuals infected or vaccinated with rotavirus. Antibody responses to VP7 epitopes of the infecting serotype of virus were found at a high frequency in both infants and children. In contrast, antibody responses to VP4 and heterotypic VP7 were observed only when the individuals possessed antibodies to any serotype of rotavirus in their acute-phase or prevaccination sera.

Rotavirus has two neutralization antigens, VP4 and VP7, in the outer layer of the particle (6, 13). In human rotavirus (HRV), six kinds of serotypes have been defined by cross-neutralization tests by using hyperimmune sera specific to each serotype (13). They include serotypes 1, 2, 3, 4, 8, and 9. The serotype specificity is ascribed mainly to the antigenic specificity of neutralization epitopes on VP7. It has also been suggested that interserotypic relationships exist among different serotypes, since anti-VP7 cross-reactive neutralizing monoclonal antibodies (N-MAbs) could be obtained (3, 21) and since, between serotypes 3 and 8, amino acid sequences in a major antigenic region on VP7 are quite similar to each other (9, 12). VP4 also has an independent antigenic specificity (11, 17). Recent nucleotide sequence analyses implied the presence of at least four types of HRV VP4 (8, 24): VP4 of virulent strains of serotypes 1, 3, and 4; VP4 of virulent strains of serotype 2; VP4 of asymptomatic strains of serotypes 1, 2, 3, and 4; and VP4 of the virulent K8 strain of serotype 1 and other strains with K8-like VP4 (26a). However, the reactivity patterns of N-MAbs with various HRV strains suggest that there still remains some ambiguity in the serotyping of VP4 since several cross-reactive neutralization epitopes are present on different VP4 types (15, 22, 23). Determination of whether serological typing can be applied to VP4 must await further precise serological analysis of VP4.

The antigenic complexity of rotavirus has hampered the development of an effective vaccine. Clinical trials of a rotavirus vaccine developed on the basis of "Jennerian" approaches have been performed worldwide (6, 13). The effectiveness of RRV MMU 18006, a rhesus monkey serotype 3 virus, appears to be serotype specific (14). RIT 4237, a bovine serotype 6 strain, while showing a significant protective effect in some vaccination trials in developed countries, failed to show the effect in developing countries (30). More extensive studies are required for a bovine serotype 6 WC3 virus, although preliminary trials with this virus showed excellent efficacy (2). Recent nucleotide sequence analyses and serological studies with N-MAbs, however, indicated that the VP4 of animal rotaviruses is quite different from that of human strains (6, 8). To evaluate the efficacy of candidate vaccines, it is of value to analyze antibody responses to the two neutralization proteins, especially to the defined epitopes on them. Shaw et al. (18) first applied the competitive enzyme-linked immunosorbent assay (ELISA) for examining the epitope-specific immune responses to rotavirus vaccination. In this study, we examined the antibody responses in patients with rotavirus gastroenteritis as well as in RRV vaccinees by the competitive ELISA with N-MAbs directed to the VP7 serotype-specific or to the VP4 cross-reactive neutralization epitopes.

MATERIALS AND METHODS

N-MAbs. The serotype-specific N-MAbs directed to VP7 used in this study were serotype 1-specific KU-4, serotype 2-specific S2-2G10, serotype 3-specific YO-1E2, and serotype 4-specific ST-2G7. We also used the two cross-reactive anti-VP4 N-MAbs; YO-2C2 neutralizing serotype 1, 3, and 4 strains; and KU-2A neutralizing serotypes 1 through 4. The properties of the N-MAbs listed above have been described previously (14a, 21–25).

Human sera. In three outbreaks (outbreaks 1, 2, and 3) of acute gastroenteritis, paired sera were obtained from six infants, two schoolchildren, and six schoolchildren, respectively. Outbreak 1 occurred in an infant home in March 1982 and affected 25 infants aged 3 to 14 months (1). Outbreak 2, which occurred in January 1977, mainly involved grade schools in the city of Kitami, Japan, and affected more than 53 people, most of whom were over 6 years of age (24). Outbreak 3 occurred in a grade school in Sapporo, Japan, in May 1980. Two hundred twenty-one pupils aged 6 to 8 years were affected in that outbreak. Serotypes of the virus strains isolated in outbreaks 1, 2, and 3 were determined to be 3, 1, and 3, respectively, by neutralization tests with serotype-specific hyperimmune sera. In addition, pre- and postvaccination sera were obtained from five infants in an infant home to whom the RRV vaccine was administered.

The neutralizing antibody titers to rotavirus of these human sera were determined by fluorescent focus neutralization tests (26).

Epitope blocking test. Rotavirus strains (serotype 1, KU;

* Corresponding author.
† Present address: Department of Medicine, Bangladesh Agricultural University, Mymensingh, Bangladesh.

serotype 2, S2; serotype 3, SA11; serotype 4, Hochi) were partially purified by differential centrifugation and fluorocarbon treatment. The immunoglobulin G (IgG) fraction from ascitic fluids was obtained by affinity chromatography with protein A–Sepharose 4B beads (Pharmacia). Purified IgG was biotinylated as described by Shaw et al. (18).

The ELISA for epitope blocking was performed by the procedure described by Shaw et al. (18), with some minor modifications. Polyvinyl microtiter plates were coated with partially purified virus in 10 mM phosphate-buffered saline (PBS; pH 7.4) at 4°C overnight and washed with PBS containing 0.05% Tween 20 (PBST). The plates were blocked with 1% bovine serum albumin in PBST at 4°C overnight and rinsed twice with PBST. Serum samples (serial twofold dilutions starting from 1:10 or 1:20) of 50 μl diluted with PBS containing 2% fetal bovine serum were added and incubated at 37°C for 1 h. Then, 25 μl of biotinylated N-MAb IgG in PBS with 2% fetal bovine serum was reacted at 37°C, and the plates were washed three times with PBST. Streptavidin-peroxidase in PBST (50 μl) was added and incubated at 37°C for 1 h. After the plates were washed four times with PBST, the reaction with *o*-phenylenediamine was allowed to develop for 30 min at room temperature, and then it was stopped by the addition of 25 μl of 3 N sulfuric acid. The optical density was measured at 492 nm with a micro-ELISA reader (EAR; SLT-Labinstrument, Saltzburg, Austria).

The titer was expressed as the reciprocal of the highest dilution in serum which gave an optical density value equal to or less than 50% of the value of control wells, to which no test sera were added. Repeated experiments demonstrated that a twofold rise in titer in paired sera is reproducible.

RESULTS

Antibody responses in the patients were examined by using paired sera from infants and children involved in three outbreaks caused by serotype 1 (outbreak 2) or serotype 3 (outbreaks 1 and 3) rotavirus. The results obtained from the epitope blocking test and the fluorescent focus neutralization test are shown in Table 1. Of the six paired serum specimens from infants involved in outbreak 1, four exhibited more than fourfold seroresponses and one showed a twofold seroresponse to the VP7 epitope of the homologous serotype 3. Patient 2 developed a heterotypic response to serotype 1 and 4 VP7. Responses to the different epitopes on VP4 were found in only two patients who already had preexisting antibodies to serotype 3 VP7. Two schoolchildren from outbreak 2 responded to VP7 epitopes of serotypes 2 and 3 as well as to the serotype 1 VP7 epitope of the infecting serotype. They also showed antibody responses against either YO-2C2 epitope or both epitopes on VP4. Of six paired serum specimens from schoolchildren involved in outbreak 3, three showed at least fourfold seroresponses and two showed twofold seroresponses to serotype 3 VP7. Two patients (patients 13 and 14) showed antibody responses to serotypes 1 and 2, respectively. An antibody response to VP4 epitopes was observed in four patients.

We next tested the seroresponses of the infant vaccinees to whom RRV was administered (Table 2). Two vaccinees developed immune responses to the VP7 epitope of serotype 3, the same serotype as that of the vaccine virus. They also responded heterotypically to the S2-2G10 epitope on VP7 of serotype 2 and to cross-reactive epitopes on VP4 defined by YO-2C2 and KU-2A antibodies. Vaccinees 1 and 4 showed antibody responses to VP7 epitopes of serotypes 1 and 2, respectively, without the development of seroresponses to the homotypic VP7 epitope.

DISCUSSION

Studies on immunity to rotavirus infections in humans are essential for evaluating vaccine-induced protection. While there is a fundamental concept that an immune response in the intestine is essential in affording protection against enteric virus infections, it has been reported that the neutralizing antibody titer in serum of infants correlates well with protection against rotavirus infection (1). In contrast, in a study with adult volunteers, no correlation was found between the antibody level in serum and their protection from infection or illness (28). Interest in the relative degree of contribution of VP4 and VP7 to protective immunity has been increasing. The immune response to each of the two neutralization proteins, VP4 and VP7, has been examined by immunoprecipitation and by using reassortant viruses (20, 29). However, in order to analyze the immune response more precisely, it seems that it is valuable to detect epitope-specific responses to VP4 and VP7. Shaw et al. (18) first applied the competitive binding assay with N-MAbs to examination of the epitope-specific immune response after rotavirus vaccination. In this study, we examined the immune response to the serotype-specific epitope on VP7 and cross-reactive epitopes on VP4 after natural infection as well as after vaccination.

The homotypic response to VP7 was observed at a high frequency. Furthermore, when the acute-phase sera already contained the antibodies to the epitope, a stronger response was detected, probably reflecting the booster effect of the immunization. In contrast, the heterotypic antibody response to VP7 was detected exclusively when the patients or vaccinees had preexisting antibody to any serotype. This was not expected, because epitopes specific to each of the four serotypes are thought to be independent of each other. This phenomenon might be related to the theory of original antigenic sin long known in immunology (4, 7); the molecular mechanism of this phenomenon has not yet been well elucidated. In this regard it seems important to examine the antibody response to the cross-reactive epitope on VP7. However, it was impossible to define the VP7 cross-reactive response in the assay described here because anti-VP7 cross-reactive N-MAb YO-4C2 competed completely with serotype-specific N-MAbs and vice versa (data not shown). This was suggested by previous findings (16, 21) that the cross-reactive YO-4C2 epitope is included in the serotype-specific antigenic site on VP7. The antibody response to cross-reactive epitopes on VP4 was also observed in subjects who possessed preexisting antibody to any serotype. This finding might suggest that the cross-reactive epitopes on VP4 are weakly immunogenic and, therefore, require priming for the antibody response to be elicited. Booster administration of the RRV vaccine might be effective in affording induction of antibodies to cross-reactive epitopes on VP4. Green et al. (10) recently presented similar findings on homotypic and heterotypic immunity against VP4 and VP7 after RRV vaccination. Thus, the immunity patterns found after vaccination and natural infection seem to be essentially the same.

Epitope mappings of rotavirus VP4 and VP7 epitopes have been made for in simian and human rotaviruses (5, 16, 19, 21, 22). In a previous study (16) we showed that five distinct VP7 neutralization epitopes overlap one another and collectively constitute a single large neutralization domain. More re-

TABLE 1. Epitope-specific antibody responses in paired sera from infants and children in outbreaks of diarrhea caused by serotype 1 or serotype 3 HRV

Patient no. (age)	Phase	Epitope-blocking titer						Neutralizing titer[a]
		Anti-VP7				Anti-VP4		
		KU-4 (1)[b]	S2-2G10 (2)	YO-1E2 (3)	ST-2G7 (4)	YO-2C2 (1, 3, 4)	KU-2A (1, 2, 3, 4)	
Outbreak 1 (serotype 3)								
1 (21 mo)	Acute	<10	<10	20	<20	<10	20	16
	Convalescent	<10	<10	40	<20	80	80	1,024
2 (18 mo)	Acute	<10	<10	40	<20	<10	<20	64
	Convalescent	20	<10	≧320	40	≧320	≧320	512
3 (14 mo)	Acute	<10	<10	<10	<20	<10	<20	<16
	Convalescent	<10	<10	40	<20	<10	<20	128
4 (7 mo)	Acute	<10	<10	<10	<20	<10	<20	<16
	Convalescent	<10	<10	40	<20	<10	<20	128
5 (8 mo)	Acute	<10	<10	<10	<20	<10	<20	<16
	Convalescent	<10	<10	80	<20	<10	<20	512
6 (6 mo)	Acute	<10	<10	<10	<20	<10	<20	64
	Convalescent	<10	<10	<10	<20	<10	<20	64
Outbreak 2 (serotype 1)								
7 (11 yr)	Acute	10	<10	<10	<20	<10	<20	<8
	Convalescent	160	40	160	20	10	160	256
8 (14 yr)	Acute	40	<20	40	<20	<10	<20	128
	Convalescent	160	40	≧320	20	40	<20	≧512
Outbreak 3 (serotype 3)								
9 (8 yr)	Acute	<10	<10	10	<20	<10	NT[c]	128
	Convalescent	<10	<10	40	<20	20	NT	2,048
10 (7 yr)	Acute	<10	<10	<10	<20	20	80	128
	Convalescent	<10	<10	<10	<20	20	80	4,096
11 (9 yr)	Acute	<10	<10	40	<20	<10	80	128
	Convalescent	<10	<10	80	20	80	160	4,096
12 (7 yr)	Acute	<10	<10	20	<20	<10	80	256
	Convalescent	<10	<10	40	<20	10	80	4,096
13 (8 yr)	Acute	20	<10	80	<20	<10	40	256
	Convalescent	160	<10	≧320	20	160	80	2,048
14 (8 yr)	Acute	<10	40	<10	<20	<10	40	128
	Convalescent	<10	≧320	20	<20	40	80	1,024

[a] Neutralizing titer was determined by using the strain homologous to the infecting serotype as antigen. (Strain YO for outbreak 1 [serotype 3], strain KU for outbreak 2 [serotype 1], and strain YO for outbreak 3 [serotype 3]).
[b] Numbers in parentheses indicate the serotype(s) which each N-MAb neutralized specifically.
[c] NT, Not tested.

cently, however, another independent neutralization domain on VP7 was identified (14b). These findings imply that the use of more N-MAbs that recognize different epitopes would be helpful in detecting a repertoire of antibodies contained in human sera. In some individuals (Table 1), no antibody response was observed in the ELISA used in this study, despite a significant rise in antibody titers to the infecting virus in fluorescent focus neutralization tests. The sera from such individuals may contain antibodies directed to different epitopes which do not compete with the N-MAb used in this study.

Cross-neutralization and cross-protection in rotavirus immunization and infection have been observed in animals and humans; (i) antisera or convalescent-phase sera from mammals, including humans, immunized or infected with a certain rotavirus strain often neutralized rotaviruses of different serotypes (31, 32), and (ii) heterotypic cross-protection against diarrhea was induced by vaccination with human or animal rotaviruses (2, 27). These phenomena can best be explained by the presence of cross-reactive epitopes on VP4 (15, 22, 23). Indeed, Ward et al. (29) demonstrated that VP4 with limited serotype diversity has a high immunogenicity in

TABLE 2. Epitope-specific antibody responses in paired sera from infants vaccinated with RRV

Vaccinee no. (age [mo])	Vaccination serum specimen	Epitope-blocking titer						Neutralizing titer[a]
		Anti-VP7				Anti-VP4		
		KU-4 (1)[b]	S2-2G10 (2)	YO-1E2 (3)	ST-2G7 (4)	YO-2C2 (1, 3, 4)	KU-2A (1, 2, 3, 4)	
1 (7)	Pre	<10	<10	<20	<20	<10	<20	64
	Post	40	<10	<20	<20	<10	<20	256
2 (5)	Pre	<10	20	<10	<20	<10	<20	64
	Post	10	≧320	80	<20	20	160	512
3 (4)	Pre	<10	<10	<20	<20	<10	<20	32
	Post	<10	<10	<20	<10	<10	<20	256
4 (5)	Pre	<10	<20	<20	<10	<10	<20	32
	Post	<10	80	<20	<20	<10	<20	256
5 (5)	Pre	<10	40	<20	<20	<10	20	64
	Post	10	≧320	160	20	20	160	512

[a] Neutralizing titer was determined by using RRV as the antigen.
[b] Numbers in parentheses indicate the serotype(s) which each N-MAb neutralized specifically.

adult volunteers. In this study, we detected the apparent antibody response to cross-reactive epitopes on VP4, even in vaccinees who were administered RRV, which shares only a limited conserved antigenic region with HRV (8, 22). In addition to the cross-reactive epitopes on VP4, VP7 also seems to play a significant role in heterotypic immunity, since the present study revealed the heterotypic immune response to VP7 serotype-specific epitopes. A time course study of the antibody response after infection with rotavirus strains in an animal model would be useful for dissecting the antibody response in rotavirus infections.

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