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

KOKI TANIGUCHI,1* TOMOKO URAWA,3 NOBUMICHI KOBAYASHI,1 MUZAHE U. AHMED,4† NORIAKI ADACHI,2 SHUNZO CHIBA,2 AND SHOZO URAWA1

Department of Hygiene3 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 complexity of rotavirus (14). In contrast, 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;
serotype 2, S2; serotype 3, SA11; serotype 4, Hochi) were partially purified by differential centrifugation and fluoro-
carbon 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 1:1 to 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,
Salzburg, 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
seeroresponse 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 examined 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
tenteric 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 im-
une 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 re-

dose 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 prim-
ing 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-

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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

<table>
<thead>
<tr>
<th>Patient no. (age)</th>
<th>Phase</th>
<th>Anti-VP7</th>
<th>Anti-VP4</th>
<th>Neutralizing titera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KU-4</td>
<td>S2-2G10</td>
<td>YO-1E2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1)b</td>
<td>(2)</td>
<td>(3)</td>
</tr>
<tr>
<td>Outbreak 1 (serotype 3)</td>
<td>Acute</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td>1 (21 mo)</td>
<td>Convalescent</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>40</td>
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<tr>
<td></td>
<td></td>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>2 (18 mo)</td>
<td>Convalescent</td>
<td>20</td>
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<td>&lt;10</td>
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<tr>
<td></td>
<td></td>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>3 (14 mo)</td>
<td>Convalescent</td>
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<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>4 (7 mo)</td>
<td>Convalescent</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<td></td>
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<td></td>
<td>Acute</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5 (8 mo)</td>
<td>Convalescent</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>Acute</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>6 (6 mo)</td>
<td>Convalescent</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbreak 2 (serotype 1)</td>
<td>Acute</td>
<td>10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>7 (11 yr)</td>
<td>Convalescent</td>
<td>160</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acute</td>
<td>40</td>
<td>&lt;20</td>
<td>40</td>
</tr>
<tr>
<td>8 (14 yr)</td>
<td>Convalescent</td>
<td>160</td>
<td>40</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Outbreak 3 (serotype 3)</td>
<td>Acute</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
</tr>
<tr>
<td>9 (8 yr)</td>
<td>Convalescent</td>
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<td>&lt;10</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acute</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>10 (7 yr)</td>
<td>Convalescent</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>Acute</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>11 (9 yr)</td>
<td>Convalescent</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acute</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>12 (7 yr)</td>
<td>Convalescent</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acute</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>13 (8 yr)</td>
<td>Convalescent</td>
<td>20</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Acute</td>
<td>&lt;10</td>
<td>40</td>
<td>&lt;10</td>
</tr>
<tr>
<td>14 (8 yr)</td>
<td>Convalescent</td>
<td>&lt;10</td>
<td>40</td>
<td>≥320</td>
</tr>
</tbody>
</table>

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.

Centrally, 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

<table>
<thead>
<tr>
<th>Vaccinee no. (age [mo])</th>
<th>Vaccination serum specimen</th>
<th>Epitope-blocking titer</th>
<th>Neutralizing titer(*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti-VP7</td>
<td>Anti-VP4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KU-4 (1)†</td>
<td>S2-2G10 (2)</td>
</tr>
<tr>
<td>1 (7)</td>
<td>Pre</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>40</td>
<td>&lt;10</td>
</tr>
<tr>
<td>2 (5)</td>
<td>Pre</td>
<td>&lt;10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>10</td>
<td>≥320</td>
</tr>
<tr>
<td>3 (4)</td>
<td>Pre</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td>&lt;10</td>
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<tr>
<td>4 (5)</td>
<td>Pre</td>
<td>&lt;10</td>
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<tr>
<td></td>
<td>Post</td>
<td>10</td>
<td>≥320</td>
</tr>
<tr>
<td>5 (5)</td>
<td>Pre</td>
<td>&lt;10</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>10</td>
<td>≥320</td>
</tr>
</tbody>
</table>

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

ACKNOWLEDGMENTS

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REFERENCES