Original Article

Rotavirus Antigenemia and Genomia in Children with Rotavirus Gastroenteritis

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SUMMARY: We investigated group A rotavirus (GARV) antigenemia and genomia in children with rotavirus gastroenteritis. A total of 16 patients (2–29 months old), who received a diagnosis of GARV gastroenteritis using a commercial rapid test, were enrolled in this study. The sera from the patients were tested for the presence of GARV antigen and the VP7 and NSP3 genes using an enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction, respectively. Furthermore, when the VP7 gene was amplified, G type was identified and compared with that of GARV from the fecal samples of the patients. GARV antigen was detected in 12 of 16 serum samples (75.0%). No GARV antigen was found in infants that were 6 months old or younger. Thirteen of 16 serum samples (81.3%) were positive for GARV genes. In cases where both antigen and gene analyses were conducted, either GARV antigens or genes, or both, were detected in all cases. The GARV antigen levels of serum collected at 2 days of illness or more were significantly higher than were the levels in the samples obtained from the 1st day. Furthermore, the ELISA optical density values of patients with convulsion were significantly higher than those of patients without convulsion, suggesting that the antigen level is associated with disease severity.

INTRODUCTION

Rotavirus gastroenteritis is one of the most common forms of viral gastroenteritis in the world. It is a highly communicable illness that is accompanied by vomiting, fever, and abdominal pain, followed by watery diarrhea that may last 5 to 7 days. The diarrheic stool turns yellow to white and is characterized by a strong acidic, fermentative odor. It is a disease that frequently requires inpatient hospital care. There are 7 groups of rotavirus, referred to as A to G. Humans are primarily infected by groups A, B, and C, and most cases of rotavirus gastroenteritis in children are caused by group A rotavirus (GARV). Rotavirus infection is considered a localized infection that targets small intestinal epithelial cells and does not cause viremia. Nonetheless, there have been reports of cases of rotavirus gastroenteritis progressing to rotavirus-associated encephalopathy (1–6), which is characterized by clustered and difficult-to-control seizures (7) or sudden death (8).

There are reports of viral antigens (9–12) and genes (9,13) being found in the sera of GARV gastroenteritis patients. However, only two reports have examined the relationship between the individual clinical symptoms and GARV antigenemia (12) or viremia (13). In these studies, Chiappini et al. (13) did not describe the relationship between the individual clinical symptoms and GARV antigenemia, and Sugata et al. (12) did not investigate the viral genes detected in the sera of patients with GARV gastroenteritis. Recently, we reported the detection of GARV RNA and antigens in serum and cerebrospinal fluid samples from diarrheic children with seizures (7).

The purpose of the present study was to investigate the GARV antigenemia and genomia in children with GARV gastroenteritis. G serotyping (G typing) of the genes identified was performed, and the results were compared with the G type of the virus of fecal origin. In addition, we compared the clinical features and laboratory data to GARV antigen levels.

MATERIALS AND METHODS

Patients: Among the children who exhibited gastroenteric symptoms and visited Nihon University Itabashi Hospital and Itabashi Medical Association Hospital between November of 2002 and April of 2004, 16 inpatients (aged from 2 to 25 months old; 8 males and 8 females), in whom the presence of GARV in the feces was identified by a rapid test and from whom serum samples could be obtained, were chosen as the subjects of the study (Table 1). Sixteen serum samples, collected between days 1 and 7 after the onset of symptoms, were analyzed for the presence of GARV antigen and genes. The system of an institutional review board was not established in our university hospital and Itabashi Medical Association Hospital until 2004 and 2005, respectively, so the informed consent for study participation was obtained from the parents of the patients and recorded on individual clinical charts.

GARV antigen detection: GARV antigens in stools were detected using a commercial kit (Rapidtesta Rota-Adeno; Daiichi Pure Chemicals Co., Tokyo, Japan). GARV antigens were also detected in the serum samples using Rotacon (Meridian Bioscience, Inc., Cincinnati, Ohio, USA) according to the manufacturer’s instructions. Two-fold diluted se-
rum samples (100 µL) were used for the detection of GARV antigen. In this assay, an absorbance of 0.3 or greater was considered to indicate positivity for the antigen according to the report by Fischer et al. (10).

GARV detection by reverse transcription-polymerase chain reaction (RT-PCR): Mossel et al. (14) reported that nonstructural protein (NSP3) was the primary determinant for extraintestinal spread in neonatal mice, so we hypothesized that the NSP3 genes of GARV are the best targets. We focused our investigation on the outer capsid glycoprotein (VP7) and NSP3 genes of GARV, which were detected in the patients’ serum and stool by RT-PCR using specific primers. The positional relationship of the VP7 gene and NSP3 gene was described in a previous report (7). Sixteen patients’ serum and stool by RT-PCR using specific primers. We determined the G type of GARV using RT-PCR when the presence of G1 to G4, G8, and G9 according to the G-type-specific PCR procedure reported previously (16). When the presence of more than one G type was suspected, we reconfirmed the G type by performing PCR using single G-type-specific primers. Reconfirmation tests were performed twice to rule out the possibility of contamination. G serotyping was performed on fecal samples from 10 cases using the RT-PCR method. In two cases, RNA could not be obtained due to insufficient specimen volume. Apart from cases 10, 14, and 15, which were processed during a different year from the other cases (2002–2003), the G types of 10 serum samples were compared to the serotypes of feces from 23 cases collected in the same area.

Clinical features and laboratory data: Clinical features of the patients were examined retrospectively from medical charts. For the purpose of comparing disease severity with rotavirus antigen levels, we examined the association between viral antigen levels and fever (>37.5°C), elevated transaminase levels (alanine aminotransferase [ALT], >30 IU/L; aspartate aminotransferase [AST], >50 IU/L; lactate dehydrogenase; CRP, C-reactive protein; LCH, Langerhans cell histiocytosis.

Statistical analysis: Statistical analysis was performed by Fisher’s exact probability test and the unpaired t test with P < 0.05 considered statistically significant.

RESULTS

Detection of GARV antigens in sera: Based on the ELISA analysis, GARV antigens were detected in 12 of 16 serum samples (75.0%; Table 1). GARV antigen was not detected in infants 6 months old or younger.

Detection of GARV genes: Thirteen of 16 serum samples (81.3%) were positive for GARV genes, as determined by the second round of PCR analysis. Twelve cases showed both VP7 and NSP3 genes, while 1 showed only the NSP3 gene (Table 1).

Correlation between GARV antigen and gene identification: Nine cases were positive for both antigens and genes. Three cases were positive only for antigens, and 4 cases only for the genes. All 4 cases for which only genes were detected

### Table 1. Characteristics of the patients with rotavirus gastroenteritis and detection of serum GARV Ag, VP7 gene and NSP3 gene, and G serotype of GARV in serum and feces

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (mo)</th>
<th>Sex</th>
<th>Underlying disease</th>
<th>Fever &gt;37.5°C</th>
<th>Leukocyte (µ/ml)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>LDH (IU/L)</th>
<th>CRP (mg/dl)</th>
<th>Convulsion</th>
<th>Sampling day</th>
<th>GARV Ag</th>
<th>VP7 gene G type</th>
<th>NSP3 gene</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>F</td>
<td>None</td>
<td>39.7</td>
<td>5,670</td>
<td>50</td>
<td>69</td>
<td>236</td>
<td>0.18</td>
<td>–</td>
<td>1</td>
<td>0.032 (+)</td>
<td>+ (5)</td>
<td>+ (3)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>F</td>
<td>None</td>
<td>37.6</td>
<td>6,300</td>
<td>36</td>
<td>37</td>
<td>249</td>
<td>0.14</td>
<td>–</td>
<td>2</td>
<td>0.048 (+)</td>
<td>+ (1)</td>
<td>+ (1)</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>F</td>
<td>Ohtahara syndrome</td>
<td>37.5</td>
<td>6,100</td>
<td>51</td>
<td>39</td>
<td>244</td>
<td>0.22</td>
<td>–</td>
<td>1</td>
<td>0.054 (+)</td>
<td>+ (1)</td>
<td>+ (1)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>F</td>
<td>None</td>
<td>37.1</td>
<td>8,400</td>
<td>22</td>
<td>17</td>
<td>201</td>
<td>0.69</td>
<td>+</td>
<td>1</td>
<td>0.227 (+)</td>
<td>+ (1)</td>
<td>+ (1)</td>
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<tr>
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<td>39.0</td>
<td>10,020</td>
<td>49</td>
<td>38</td>
<td>345</td>
<td>0.04</td>
<td>–</td>
<td>1</td>
<td>0.657 (+)</td>
<td>+ (3)</td>
<td>+ (3)</td>
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<tr>
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<td>14</td>
<td>M</td>
<td>None</td>
<td>39.3</td>
<td>5,980</td>
<td>61</td>
<td>25</td>
<td>237</td>
<td>0.15</td>
<td>–</td>
<td>3</td>
<td>1.165 (+)</td>
<td>–</td>
<td>+ (3)</td>
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<tr>
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<td>48</td>
<td>31</td>
<td>312</td>
<td>0.33</td>
<td>–</td>
<td>1</td>
<td>2.109 (+)</td>
<td>+ (3)</td>
<td>+ (3)</td>
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<tr>
<td>8</td>
<td>16</td>
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<td>38.7</td>
<td>6,000</td>
<td>56</td>
<td>27</td>
<td>336</td>
<td>0.39</td>
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<td>2</td>
<td>2.799 (+)</td>
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<tr>
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<td>29</td>
<td>277</td>
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<td>+</td>
<td>3</td>
<td>3.290 (+)</td>
<td>–</td>
<td>–</td>
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<tr>
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<td>37.2</td>
<td>9,040</td>
<td>47</td>
<td>45</td>
<td>266</td>
<td>0.05</td>
<td>+</td>
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<td>2.133 (+)</td>
<td>+ (3)</td>
<td>+ (3)</td>
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<td>11</td>
<td>17</td>
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<td>36.9</td>
<td>7,700</td>
<td>51</td>
<td>18</td>
<td>282</td>
<td>0.1</td>
<td>–</td>
<td>7</td>
<td>0.834 (+)</td>
<td>+ (4)</td>
<td>ND</td>
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<tr>
<td>12</td>
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<td>M</td>
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<td>36.7</td>
<td>11,900</td>
<td>35</td>
<td>21</td>
<td>427</td>
<td>0.1</td>
<td>–</td>
<td>1</td>
<td>0.434 (+)</td>
<td>+ (1)</td>
<td>+ (1)</td>
</tr>
<tr>
<td>13</td>
<td>20</td>
<td>M</td>
<td>LCH</td>
<td>37.9</td>
<td>7,700</td>
<td>24</td>
<td>15</td>
<td>297</td>
<td>0.1</td>
<td>–</td>
<td>1</td>
<td>0.574 (+)</td>
<td>–</td>
<td>ND</td>
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<td>37.8</td>
<td>5,600</td>
<td>45</td>
<td>24</td>
<td>236</td>
<td>0.1</td>
<td>+</td>
<td>3</td>
<td>3.946 (+)</td>
<td>+ (1 &amp; 3)</td>
<td>+ (1 &amp; 3)</td>
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<td>40.1</td>
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<td>113</td>
<td>49</td>
<td>637</td>
<td>1.16</td>
<td>+</td>
<td>4</td>
<td>0.308 (+)</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>29</td>
<td>M</td>
<td>None</td>
<td>36.5</td>
<td>4,800</td>
<td>54</td>
<td>30</td>
<td>264</td>
<td>0.23</td>
<td>+</td>
<td>4</td>
<td>4.351 (+)</td>
<td>+ (1)</td>
<td>+ (1)</td>
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</tbody>
</table>

| (7/16)     |        |     |                   |        |                 |           |           |           |             |            | (12/16)     | (12/16)   | (13/16)     |
|------------|--------|-----|-------------------|--------|-----------------|-----------|-----------|-----------|-------------|-------------|-------------|-----------|-------------|-----------|
| 75%        |        |     |                   |        |                 |           |           |           |             |            | 75%         | 81.3%     |             |           |

ND, The G type could not be determined.

GARV, group A rotavirus; Ag, antigen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; LCH, Langerhans cell histiocytosis.
were infants younger than 6 months old (Table 1). In all cases, either the GARV antigen or the gene, or both, were detected.

As a negative control, the same analyses were performed on 2 cases that were negative for the GARV antigen in the feces. Neither the antigen nor the genes was detected in these samples.

**G typing of sera and feces**: G typing of the GARV genes isolated from serum revealed 6 cases of G1, 4 cases of G3, 1 case of G4, and 1 case of mixed G1 and G3 infection. G typing of the GARV genes from feces was also performed in 13 cases. Eleven showed a perfect match between the G type of the feces and the serum of the same patient (Table 1).

**Comparison of serum G types from GARV-infected subjects and from fecal samples obtained during a local epidemic**: Among the 10 serum samples, 6 were of type G1 (60%), 3 were of type G3 (30%), and 1 was of type G4 (10%). In the fecal samples, 13 were of type G1 (54.1%), 1 was of type G2 (4.2%), and 10 were of type G3 (41.7%).

**Association between GARV antigen levels and clinical symptoms and laboratory data** (Tables 1 and 2): ELISA optical density (OD) values in serum samples were compared with the severity of clinical symptoms and laboratory data. OD values of GARV antigen were similar in serum collected from 7 patients sampled on the 1st day (0.58 ± 0.72) than in that of 9 patients without convulsion (0.36 ± 0.67, P = 0.028). However, the OD values of GARV antigen were significantly lower in the serum that was collected from 7 patients sampled on more than the 2nd day (2.10 ± 1.59, P = 0.025). The OD values of GARV antigen were similar in the serum that was collected from 7 patients with elevated AST (≥50 IU/L; 1.36 ± 1.63) than in that of 9 patients with normal levels of AST (<50; 1.49 ± 1.43, P = 0.868). There was no difference in the serum levels of CRP, AST, or ALT, as shown in Table 1.

**DISCUSSION**

In recent years, there have been reports of the isolation of viral antigens and genes from the sera of GARV gastroenteritis patients, and the prevalence of GARV antigens or genes in the blood is estimated to be 60 to 70% (5,9,14). In our data, if the results of antigen and gene detection are examined separately, the rates are similar, with antigenemia occurring in 75.0% of GARV gastroenteritis patients and GARV genes detected in 81.3% of GARV gastroenteritis patients. Nevertheless, when the two methods of detection are combined, the antigen or the genes were present in almost all cases. Therefore, it is likely that invasion of the blood by GARV during the infection occurs commonly. We compared the clinical symptoms and laboratory data with GARV antigen levels and observed a correlation between the GARV antigen levels with the date of specimen collection and the occurrence of GARV-related convulsions, as an indicator of case severity.

Sugata et al., reporting on GARV antigenemia and extraintestinal manifestations in children with GARV gastroenteritis (12), described that rotavirus antigen levels peaked on day 2 of the illness, followed by a gradual decrease in antigen levels to nearly undetectable levels by day 6. In this study, the GARV antigen level in samples collected on more than the 2nd day was significantly higher than that in samples collected on the 1st day, as shown in Table 2. These results indicate that the invasion of GARV into the bloodstream may require at least 2 days. Sugata et al. also showed that the quantity of GARV antigen was significantly higher in serum collected from patients with fever than in that from those without fever (12). Our data are not consistent with their results because there were small numbers of patients without fever in our study (less than 37.4°C). On the other hand, our data showed that the presence of seizure was significantly correlated with serum rotavirus antigen levels. The results of Sugata et al. (12) and our study suggest that GARV antigen levels may be an indicator of disease severity.

In each of the 4 cases that were negative for GARV antigen, but were gene positive, the serum was obtained from infants less than 6 months old. The reason why these cases were negative for the antigen remains unclear. However, it may have been due to age dependence in the defense mechanism of the mucosa of the small intestine against antigen penetration, or to inhibition of the ELISA technique by other factors, such as fetal transfer of maternal antibodies of rotavirus. Serum rotavirus antibodies from maternal blood are near 6 months of age.

Ray et al. reported that antigenemia was associated with infection with G1 strains and with low baseline titers of rotaviral serum antibody (14). However, in our results, there was no correlation between G typing of GARV genes and the detection of antigen. If antigenemia was associated with low baseline titers of rotaviral serum antibody, it contradicts our results of not detecting antigenemia in infants less than 6 months old. In this study, we were unable to test for rotavirus
antibody because we ran out of samples. In the near future, we will try to examine the serum rotavirus antibodies from infants less than 6 months old whose results were negative for GARV antigen but positive for the genes. In this study, there were 3 cases in which genes were not detected in the serum despite positive results for the antigen. This suggests that, in GARV infection, the finding of antigens in the serum may not always indicate the presence of viral particles.

There have been few previous reports on the G types of GARV in the blood or on the relationship between GARV G types in blood and in fecal samples. In our data, we identified various G types in serum and found that the G types of the fecal and serum samples from the same patient were identical. These results suggest that GARV can migrate from the gut into the blood with relative ease. The mechanism of the invasion of GARV into the bloodstream among children suffering from GARV gastroenteritis is not clear. It is important to clarify this mechanism in the future.

The distribution pattern of G types among the viruses from the sera of our patients showed an almost identical pattern to that of GARV G types associated with a local epidemic. This finding implies that it is unlikely that only certain G types are capable of spreading into the blood. Rather, this result suggests that the intestinal virus migrates into the blood unselectively.

In cases where both antigen and gene analyses were conducted, either GARV antigens or genes, or both, were detected in all cases. The G type of the feces and the serum from the same patient were identical. The high levels of ELISA OD may indicate disease severity in patients with convulsion having high levels of GARV in the bloodstream.

REFERENCES