Neutralizing Serum Antibodies to Serotype 6 Human Rotaviruses PA151 and PA169 in Ecuadorian and German Children

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Serum samples from 726 Ecuadorian children who underwent natural rotavirus (RV) exposure were tested for neutralizing serum antibodies against two serotype 6 (ST6) human RV (HRV) isolates from Italy, PA151 and PA169, and two ST6 bovine RV (BRV) isolates, NCDV and UK. Gene 4 was distinct in all four ST6 strains. Ninety-one, 56, 67, and 65 serum samples neutralized HRV PA151 (13%), HRV PA169 (8%), BRV NCDV (9%), and BRV UK (9%), respectively. A total of 44 of the 91 serum samples which neutralized HRV PA151 did not neutralize the other three ST6 RV strains. In addition, we identified three serum samples that neutralized HRV PA151 but none of the six human or four animal RV STs. However, we failed to identify serum samples that neutralized HRV PA169 without neutralizing at least one of the major HRV STs. With a hospital-based serum collection from German children (excluding gastroenteritis patients), we identified 3 out of 197 serum samples tested that neutralized HRV PA151 specifically, whereas none neutralized HRV PA169 exclusively. None of the 71 German infants hospitalized with primary RV gastroenteritis showed a PA151- or a PA169-specific antibody response.

Group A rotaviruses (RV) are a common cause of diarrhea in children and in the young of many animal species, including cattle (19). Serotype (ST) 1, 2, 3, 4, and rarely 8 and 9 RV have been isolated from children (19), whereas ST6, ST10, and occasionally ST8 RV have been isolated from calves (2, 26). On the basis of epidemiological and serological surveys, it was concluded that cross-species infections with RV do not occur between cattle and humans or at least are rare events (4, 11, 20, 24, 25). However, this view was recently challenged by the isolation of two distinct ST6 RV strains (PA151 and PA169) from two Italian RV patients (14). A total of 10 of the 11 genes of both RV isolates, including the gene coding for VP7 protein, were closely related to genes of bovine RV (BRV) strains UK and NCDV. Gene 4, however, which codes for the neutralizing antigen VP4, showed no cross-hybridization with gene 4 of either BRV NCDV or BRV UK, both of which have antigenically distinct VP4 proteins (17, 18, 23). Furthermore, no cross-hybridization between gene 4 of the two human ST6 RV isolates was observed. These observations (14) suggest that both human isolates represent natural reassortants between a BRV and two different RV strains of poorly defined origin that became virulent for infants.

In the present study, we screened serum collections from Ecuadorian and German children for neutralizing antibodies to PA151 and PA169 RV. The Ecuadorian serum collection was investigated previously for neutralizing antibodies to six human and four animal RV STs (STs 1 to 10) (1, 4–6). We found serological evidence that children experienced natural exposure to PA151-like but not to PA169-like RV.

MATERIALS AND METHODS

Serum samples. As described previously (6, 10), 7,798 children were studied in a representative nutritional and health survey of Ecuadorian children of <5 years of age. A representative subset was selected for serum collection. In the present study, 726 serum samples from 0- to 2 1/2- and 4 1/2- to 5-year-old children were analyzed. For comparison, 197 serum samples from newborn to 48-month-old German children and 40 German adults were obtained. They were from a hospital-based serum collection described previously (8). In addition, paired serum samples from 71 German infants hospitalized with a serologically defined primary RV infection were studied (7).

Neutralization test. The sera were screened for neutralizing antibodies to the indicated RV strains at a 1:100 serum dilution in the peroxidase focus reduction test (12) described in detail previously (4).

Viruses. Human RV (HRV) strains PA151 and PA169 with subgroup I specificity and a long RNA pattern were isolated from two children with acute gastroenteritis in Sicily, southern Italy, in the winter season 1987 to 1988, 3 months apart from each other. The HRV isolates were adapted to growth in cell cultures and then characterized by neutralization and by RNA-RNA (Northern blot) hybridization (14). Briefly summarized, cross-neutralization studies using type-specific immune sera to RV STs 1 through 10 showed the antigenic relatedness of the two strains with ST6 bovine strains UK and NCDV. Monoclonal antibodies to VP7 of UK were able to recognize UK and NCDV strains as well as both HRV isolates. Northern blot cross-hybridization studies showed a genetic relatedness of PA151 and PA169 to bovine strains for all genes except gene 4. Gene 4 of PA151 appeared to be genetically related to that of AU228 (a human strain of subgroup I and G3 specificity belonging to a feline genogroup) (21, 22), whereas gene 4 of PA169 appeared to be unique yet related to gene 4 of two recently reported subgroup I HRV strains, one (PA710) with G3 and the other (HAL1271) with G8 specificity.

RESULTS

Ecuadorian children. Serum samples from 726 Ecuadorian children from a group ranging in age from newborn to 30 months old (n = 640) and from an older reference group (56

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to 60 months old, \( n = 86 \) were analyzed for neutralizing antibodies to four different ST6 RV strains. Of these, 67 showed neutralizing antibodies to BRV NCDV and 65 neutralized BRV UK, which shares VP7 (16) but not VP4 antigen with BRV NCDV (17, 18, 23). Forty serum samples neutralized both BRV strains, whereas 25 serum samples neutralized BRV UK but not BRV NCDV and 27 serum samples neutralized BRV NCDV but not BRV UK. We observed an age-related increase in the prevalence of antibodies to BRV NCDV and UK (Fig. 1).

Sera from 56 children neutralized RV PA169, while 23 sera (41%) neutralized both BRV strains concomitantly. Sera from 91 children neutralized RV PA151. Interestingly, only 26 and 40 of these 91 serum samples neutralized in parallel both or at least one of the two BRV strains. Antibodies to RV PA151 and PA169 were observed nearly as frequently in children living in urban areas as they were in children living in rural areas (Table 1). We observed an age-related increase in antibodies to RV PA169 (\( P < 0.001 \), Yates’ corrected chi-square test) but not to RV PA151 (Fig. 1).

Overall, sera from 148 children neutralized at least one of the four ST6 RV strains. Notably, 80 of these 148 serum samples (54%) neutralized only one of the four ST6 RV strains, whereas 24, 30, and 14 serum samples neutralized two, three, or all four of the ST6 strains, respectively. Within the 80 serum samples which neutralized only one ST6 strain, we identified 44 serum samples that neutralized RV PA151 exclusively and 10, 16, and 10 serum samples that neutralized RV PA169, BRV UK, and BRV NCDV, respectively, exclusively. Most of the ST6-reactive sera from children in the first year of life neutralized but a single ST6 strain (81%), whereas in older children 50% of the ST6-reactive serum samples neutralized two or more ST6 strains.

Next, we analyzed the 148 serum samples that neutralized at least one ST6 RV strain for their neutralization specificity to the four major HRV STs (Table 1). The prevalence of ST6-neutralizing sera gradually increased from 1% in sera that neutralized none of the major human STs to 72% in sera that neutralized all four of the major human STs. Forty-five percent of the ST6-reactive serum samples from children in the first year of life neutralized none or only one of the four major HRV STs; this percentage dropped to 16% of sera from children in the second year of life and to 5% of sera from children older than 2 years (Fig. 2).

Interestingly, we identified three serum samples that neutralized HRV PA151 but none of the four major HRV STs (Table 1). One serum sample was from a 9-month-old boy living in an urban, low-altitude area (25 m above sea level) of Ecuador who was suspected of having primary RV infection. The optical density at 450 nm (OD\(_{450}\)) of RV-specific immunoglobulin G (IgG) antibodies and that of IgM antibodies was 0.09 in an enzyme-linked immunosorbent assay (ELISA) (6). The second serum sample was from a 14-month-old girl living in an urban, high-altitude area (2,700 m above sea level). It is probable that she, too, had a primary RV infection (RV-specific IgG and IgM antibody ODs were 0.09).

**TABLE 1. Prevalence of neutralizing antibodies to ST6 HRV PA151 and PA169 in 726 serum samples from Ecuadorian children**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
<th>No. (prevalence) of serum samples neutralizing the RV:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HRV PA151</td>
<td>HRV PA169</td>
</tr>
<tr>
<td>Total children</td>
<td>726</td>
<td>91 (13)</td>
</tr>
<tr>
<td>Urban</td>
<td>395</td>
<td>46 (12)</td>
</tr>
<tr>
<td>Rural</td>
<td>331</td>
<td>45 (14)</td>
</tr>
<tr>
<td>No. of four major HRV STs neutralized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>261</td>
<td>3 (1)</td>
</tr>
<tr>
<td>One</td>
<td>111</td>
<td>12 (11)</td>
</tr>
<tr>
<td>Two</td>
<td>105</td>
<td>14 (13)</td>
</tr>
<tr>
<td>Three</td>
<td>131</td>
<td>33 (25)</td>
</tr>
<tr>
<td>Four</td>
<td>69</td>
<td>29 (42)</td>
</tr>
</tbody>
</table>

\* Prevalence is expressed as a percentage.

**FIG. 1. Prevalence of neutralizing antibodies to ST6 BRV NCDV, BRV UK, HRV PA151, and HRV PA169 in Ecuadorian children in different age groups. Age groups are indicated by the upper age limit (in months). Prevalence (%) is expressed as 100 \( \times \) the number of serum samples with antibodies to the indicated RV divided by the total number of serum samples in the specified age group. \( n \), number of children in each 2-month age interval.**

**FIG. 2. Prevalence of sera from Ecuadorian children that neutralized at least one out of four ST6 RV strains, specified by the number of the four major HRV STs neutralized. Sera that neutralized none ( ), one ( ), two ( ), three ( ), or all four ( ) of the four major HRV STs are indicated. Age groups and prevalence (%) are as for Fig. 1.**
and 0.21, respectively, in the ELISA). The third serum sample was from an 8-month-old girl living in a rural, high-altitude area (3,000 m); RV-specific IgG and IgM antibody ODs were 0.18 and 0.16, respectively, in ELISA. All three serum samples neutralized RV PA151 but none of the other three ST6 RV strains tested. The three serum samples showed no neutralizing antibodies to STs 1 (Wa), 2 (DS-1, S-2), 3 (SA11, Ito), 4 (Hochi), 5 (OSU), 7 (Ch-2), 8 (69M, 678), 9 (Wf61), and 10 (V1005) RV.

**German children.** Serum samples from 197 German children ranging in age from newborn to 48 months and those from 40 adult blood donors were analyzed for neutralizing antibodies to three ST6 RV strains (Table 2). Serum samples from 54 (27%), 25 (13%), and 47 (24%) children neutralized ST6 RV PA151, PA169, and BRV NCDV, respectively (Table 2). Sera from children <4 months of age showed prevalences similar to those of adult sera, indicating passive antibody transfer. Prevalences were lowest in 4- to 12-month-old children, and they increased with age (Table 2).

The following analysis was limited to 4- to 48-month-old children (n = 152) to exclude the effect of passively acquired maternal antibody. Sera from 46 children (30%) neutralized either RV PA151 or RV PA169. A total of 14 serum samples neutralized both strains, 29 serum samples neutralized only RV PA151, and 3 serum samples neutralized only RV PA169. A total of 28 of these 46 serum samples also neutralized BRV NCDV. The 18 serum samples which failed to neutralize BRV NCDV neutralized RV PA151 but not RV PA169. Furthermore, three of them failed to neutralize any of six HRV STs. These three serum samples were from 7- to 8-month-old children hospitalized between September 1984 and July 1985 in Bochum, Germany, for reasons other than gastroenteritis.

**RV gastroenteritis patients.** Acute- and convalescent-phase sera from 71 German children hospitalized with suspected primary RV gastroenteritis (7) were analyzed for fourfold or greater titer increases of neutralizing antibodies to ST6 RV PA151 and PA169 (data not shown). Only two patients (3%) seroconverted to PA169 RV (neutralizing antibody titers increased from <10 to 80 for both patients). Patient 11 also showed fourfold titer increases to ST1 and ST3 (3), and patient 67 showed fourfold titer increases to ST1, ST3, and ST9 RV (1). Titer increases to RV PA151 or BRV NCDV were not observed for these two patients. According to direct serotyping of RV strains in stool samples (3), both patients were infected with a subgroup II, ST1 RV. Only one patient seroconverted to PA151 RV (neutralizing antibody titers were 20 and 160 for acute- and convalescent-phase sera, respectively). Patient 70 also showed fourfold titer increases to ST1 and ST4 RV, as well as to BRV NCDV (neutralizing antibody titers increased from 150 to 810, from 100 to 400, and from <10 to 60, respectively). The stool sample of patient 70 showed a subgroup II RV. The ST was untypeable, probably due to lack of outer shell proteins.

**DISCUSSION**

In the present study, we analyzed the serum antibody response to antigenically distinct ST6 RV isolates in Ecuadorian and German children who experienced natural RV exposure. The two bovine ST6 RV strains, NCDV and UK, used in this study share a highly related VP7 protein, with 97% amino acid homology (16). Northern blot hybridization and preliminary sequence data also showed a close genetic relationship between gene 9 coding for the VP7 protein of the two human ST6 RV strains, PA151 and PA169, and that coding for the VP7 protein of the two BRV strains (14). However, gene sequencing or Northern blot hybridization (14) techniques showed that all four ST6 RV strains differ with respect to gene 4, which codes for the VP4 protein (9). Sera from Ecuador were tested for neutralizing antibody to all four ST6 RV strains. Sera neutralizing several ST6 RV strains should thus identify those children who recognized VP7 epitopes shared between the isolates. Sera that neutralized only one and not the other three ST6 strains should identify those who recognized strain-specific epitopes on VP4 and VP7. However, this issue can be further investigated only by preparation of reassortant RV strains carrying different VP4 and VP7 genes and by using them to examine the neutralizing antibody response.

About half of the ST6-neutralizing serum samples from Ecuador neutralized only a single ST6 RV strain (80 out of 148 serum samples). Within this group of 80 ST6 strain-specific serum samples, we identified 10 serum samples that neutralized HRV PA169 specifically. These 10 serum samples were not more frequently observed than serum samples that neutralized BRV UK (n = 16) or BRV NCDV (n = 10) specifically. The prevalence of PA169-neutralizing sera increased with the number of HRV STs neutralized. In addition, all sera that neutralized HRV PA169 neutralized at least one of the major HRV STs. These observations support the hypothesis that neutralizing antibodies to HRV PA169 in children’s sera reflect more cross-neutralizing antibodies than exposure to PA169-like RV, as has been argued previously for antibodies to BRV NCDV in children’s sera (4).

We identified 44 serum samples that neutralized HRV PA151 but none of the three other ST6 RV strains tested. Thus, 50% of the PA151-neutralizing serum samples apparently did not react with epitopes on VP7 shared between the ST6 RV strains. In addition, we identified three serum samples that neutralized HRV PA151 but none of the six known HRV STs or the other three ST6 strains. Very similar data were obtained with the smaller serum collection from German children. These data indicate that both Ecuadorian and German children experienced exposure to RV strains that share epitopes with HRV PA151. More specifically, these children seem to have experienced exposure to the nonbovine part of HRV PA151. It is tempting to speculate that this immune response is directed against the VP4 protein of HRV PA151. Gene 4 of RV PA151 appears to be unrelated to gene 4 of BRV NCDV, BRV UK, HRV PA169, or any of the gene 4 alleles so far identified among HRV (14).

However, Northern blot hybridization data and preliminary sequence data showed a relationship between HRV PA151 and HRV AU228 at the level of gene 4 (14). HRV AU 228
was isolated from a Japanese child with gastroenteritis (21). It is an unusual isolate, as it belonged to subgroup I, possessed a long RNA pattern, was ST3, and was shown by RNA-RNA hybridization in solution to have a high degree of homology with a feline RV (21, 22). These results indicated transmission of a feline RV to humans and together with other data (13, 15) suggested a role of animal RV in the evolution of human RV. It might be more than a chance observation that HRV PA151, which is also a candidate for transspecies infection, shared a related gene 4 with HRV AU 228 (14). Our serological data indicate that RV strains which share epitopes with HRV PA151 might circulate in the German and Ecuadorian populations. These PA151-related isolates must, however, be rare, as we found that only 1 out of 71 RV gastroenteritis patients showed a fourfold serum antibody increase to HRV PA151. This patient also showed an antibody increase to ST1 and ST4 HRV as well as to BRV NCDV. This child thus appears to have been exposed to ST6 HRV strains to which he had not been previously exposed. Serotyping of cell culture-adapted subgroup 2 human rotavirus strains by neutralization. Immun. 43:722–729.

Acknowledgments

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References