Evidence of conserved epitopes in variable region of VP8* subunit of VP4 protein of rotaviruses of P[8]-1 and P[8]-3 lineages

J.F. CONTRERAS1, G.E. MENCHACA1, R. INFANTE3, C. E. HERNÁNDEZ1, C. RODRÍGUEZ1, R.S. TAMEZ1


Summary. – Although antibody responses to the human rotavirus VP4 protein have been reported, few studies have analyzed the specificity of these responses to the VP8* subunit. This study investigated antibody responses generated against the variable region of the VP4 protein (VP8* subunit) in children infected with rotavirus genotype P[8]. Recombinant VP8* subunit (rVP8*) and truncations corresponding aa 1–102 (peptide A) and 84-180 (peptide B) of rotavirus strains P[8]-1 and P[8]-3 lineages were expressed in Escherichia coli and examined for antibody reactivity using ELISA and Western blot assays. Sera from infected children had IgG antibodies that reacted with full-length rVP8*, peptide A and B of both lineages, with stronger reactivity observed against peptide B. In addition, anti-strain Wa (P[8]-1) and anti-rVP8* (P[8]-3) rabbit polyclonal antiserum reacted against peptide B sequences of both lineages. These data indicate that the VP8* variable region of rotavirus belonging to P[8]-1 and P[8]-3 lineages have conserved epitopes recognized by antibodies elicited during natural infections.

Keywords: antibodies; conserved epitopes; VP8* subunit; VP4 protein; P[8] lineage; rotaviruses

Introduction

Rotaviruses (the species Rotavirus A, the genus Rotavirus, the subfamily Sedoreovirinae, and the family Reoviridae) express two outer proteins (VP4 and VP7) encoded by genes 4 and 9, respectively, which elicit the production of neutralizing antibodies (NtAb) that confer protection against subsequent infectious in children (Jiang et al., 2002). Nucleotide sequences of these two genes are used to classify rotavirus strains into genotypes. To date, 23 G genotypes (VP7) and 32 P genotypes (VP4) have been identified in humans and different animal species (Collins et al., 2010; Okitsu et al., 2011). Worldwide, 80% of human rotavirus strains result from infections with the P[8] genotype (Santos and Hoshino, 2005).

VP4 is an 88 kDa protein necessary for rotavirus penetration into target cells. During this process, VP4 is cleaved by trypsin, producing peptides VP8* and VP5*. Phylogenetic analysis of the VP8* region revealed four P[8] lineages. The amino acid sequence comparisons between lineages revealed 87–91% identity (Maunula and von Bonsdorff, 1998; Cunliffe et al., 2001; Arista et al., 2005). Each P[8] lineage contains a variable region between aa 71–204 that has serotype-specific epitopes (Larralde and Gorziglia, 1992). However, the relationship between serotype and the variable sequences in this region for different lineages is not clear. Therefore, determining the lineage-specificity of antibodies to this region might improve our understanding of humoral responses elicited against P[8] genotypes following natural infections.

To study the antibody response to the VP8* variable region of P[8]-1 and P[8]-3 lineages, truncated peptides corresponding to the VP8* subunit of each lineage were produced, and
antibody specificity present in the sera of rotavirus infected children was assessed by ELISA and Western blot analyses.

Materials and Methods

Stool and serum specimens. Fecal and serum samples (n = 10) were collected from patients <2-years of age during the acute phase of disease, admitted with gastroenteritis to hospitals in Chihuahua City, North Mexico between 2007–2008.

Titration of neutralizing antibodies. Human rotavirus strain Wa (kindly provided by Carlos F. Arias, IBT-UNAM, Mexico) was propagated in MA104 cell as described (Taniguchi et al., 1998). The presence of NiAb to rotavirus strain Wa in the sera of children was determined using the immunochemical focus reduction neutralization test (Arias et al., 1987). The NiAb titer for each serum sample examined was defined as the highest serum dilution where the number of infected cells was reduced by at least 60% compared to infected cells not incubated in the presence of serum.

RT-PCR. RNA was extracted from rotavirus Wa-infected cells and from 20% fecal suspensions obtained from 10 field isolates using the TRI-REAGENT (MRS). A 762 bp amplicon corresponded from 20% fecal suspensions obtained from 10 field isolates presence of serum.

Results

VP8* subunit sequencing analysis.

Amplicons (762 bp) corresponding to the VP8* subunit encoded by gene 4 of prototype rotavirus strain Wa and 10 rotavirus strains collected from infected children were cloned and sequenced. Alignment of the VP8* amino acid sequences of the field isolates showed that they shared 97.2–100% identity and belonged to the P[8] genotype. These isolates, compared to rotavirus strain Wa, had 19–22 amino acid substitutions (91.2–92.4% identity). Sixteen substitutions were localized in the variable region (aa 71–204). Phylogenetic analysis revealed that all field strains clustered to the P[8]-3 lineage whereas rotavirus Wa clustered to the P[8]-1 lineage (Fig. 1).
Fig. 1
Phylogenetic analysis of the deduced VP8* subunit amino acid sequences of P[8] human rotavirus strains

The tree was constructed using the neighbor joining method; bootstrap of 1,000 replications (MEGA v4.1).
Assessment of the antibody responses to the VP8* variable region

To measure the antibody response to the VP8* subunit, sera from infected children were first screened for neutralization activity against rotavirus strain Wa followed by ELISA and Western blot analyses to detect reactivity against the VP8* His-tagged recombinant constructs. We found that 9/10 serum samples examined had detectable levels of NtAb. Specific IgG antibodies reactive against the full-length rVP8* of P[8]-1 and P[8]-3 lineages were also detected in sera with titers ranging between 100-1,600 (Table 1).

Based on these binding data, we focused our analyses to antibody responses recognizing the VP8* variable region (peptide B) and then compared this response to peptide A reactivity (which is the most conserved VP8* subunit). Each serum sample was then analyzed by Western blot for reactivity against peptides A and B of rotavirus strain Wa (P[8]-1 lineage) and rotavirus strain MX08-659 (P[8]-3 lineage). Although peptides from both lineages were detected in all serum samples tested, the reactivity with peptide B was higher than reactivity observed against peptide A (Fig. 2).

Finally, to determine if the serum reactivity observed against peptide B was lineage specific, rabbit hyperimmune antiserum raised against rotavirus strain Wa or against MX08-659 full-length rVP8* was tested by Western blot. These experiments demonstrated that rabbit anti-sera recognized both peptide B of P[8]-1 and P[8]-3 lineages (Fig. 2).

Discussion

Previous studies have described the world-wide circulation of four lineages corresponding to P[8] genotype rotavirus strains. There is an 8.6–13% amino acid sequence divergence between lineages (Maunula and von Bonsdorff, 1998; Cunliffe et al., 2001). Therefore, it is important to determine if amino acid differences between lineages can affect the reactivity of antibodies generated following natural rotavirus infections.

In this report, all field isolates grouped to the P[8]-3 lineage, which is considered the most prominent lineage throughout the world (Ansaldi et al., 2007; Araujo et al., 2007; Rahman et al., 2007; Espinola et al., 2008). We also tested serum from rotavirus-infected children for reactivity against the VP8* subunit of the prototype human rotavirus strain Wa (P[8]-1 lineage) that is similar to the Rotarix vaccine licensed in Mexico since 2004 (Cheuvart et al., 2009). To determine the genetic relationship between these two strains, we analyzed the amino acid sequence of the VP8* subunit of VP4 of the Rotarix vaccine (data not shown). Five substitutions were detected following comparison of the vaccine strain sequence to the non-vaccine Wa strain sequence. This analysis revealed three substitutions, in addition to those reported by Ward et al. (2006). For this reason, we used full-length rVP8* corresponding to the P[8]-1 and P[8]-3 lineages to determine if the antibodies elicited following natural infections reacted against these recombinant proteins.

We detected comparable IgG-titers against both P[8]-1 and P[8]-3 rVP8*, and titers were similar to those reported by Padilla-Noriega et al., who measured the antibody responses to rVP8* from the rehsus rotavirus (RRV) strain using sera from children vaccinated with RRV (Padilla-Noriega et al., 1992). Our results suggested that children infected with P[8] rotavirus strains mounted a homologous immune response to this genotype. In addition, these results were in agreement with our previous findings that demonstrated that a homologous antibody response could be mounted to the P1A serotype VP4 protein following natural infection (Menchaca et al., 1998).

A previous report described that the variable region within the VP8* subunit had serotype-specific epitopes and that this region could be recognized by rabbit antibodies specific to whole human rotavirus (Larralde and Gorziglia, 1992). Therefore, we determined if the sera from infected children contained antibodies specific to the VP8* variable region. This was carried out by examining the reactivity against rVP8* truncated peptides (peptides A and B) of rotavirus strain Wa (P[8]-1 lineage) and MX08-659 isolate (P[8]-3 lineage). Western blot results showed that sera from the infected children examined in this report recognized epitopes present in peptides of both lineages, but that the reactivity against peptide B was stronger than reactivity against peptide A. These results suggested that during a natural rotavirus infection, an immune response against the immunodominant epitopes in the variable region of VP8* subunit is mounted.

To verify that the reactivity against peptide B corresponding to P[8]-1 and P[8]-3 lineages was to conserved epitopes, rabbit polyclonal antiserum generated against either whole

<table>
<thead>
<tr>
<th>Children serum samples</th>
<th>IgG ELISA titers to the VP8* subunit NtAb titers to strain Wa</th>
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<tbody>
<tr>
<td></td>
<td>Strain Wa P[8]-1 lineage MX08-659 isolate P[8]-3 lineage</td>
</tr>
<tr>
<td>MX07-531</td>
<td>200 400 200</td>
</tr>
<tr>
<td>MX07-532</td>
<td>100 100 50</td>
</tr>
<tr>
<td>MX07-554</td>
<td>200 400 50</td>
</tr>
<tr>
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<tr>
<td>MX07-557</td>
<td>200 800 200</td>
</tr>
<tr>
<td>MX08-659</td>
<td>200 200 200</td>
</tr>
<tr>
<td>MX08-674</td>
<td>&gt;1,600 &gt;1,600 100</td>
</tr>
<tr>
<td>MX08-680</td>
<td>&gt;1,600 &gt;1,600 &gt;3,200</td>
</tr>
<tr>
<td>MX08-737</td>
<td>&gt;1,600 &gt;1,600 25</td>
</tr>
<tr>
<td>MX08-740</td>
<td>400 800 &lt;25</td>
</tr>
</tbody>
</table>
Reactivities of IgG antibodies in the sera from rotavirus-infected children with VP8* and peptides A and B of P[8]-1 and P[8]-3 lineages

Western blot analysis. The blotted antigens were reacted with the children’s sera (a-f), antiserum to Wa strain (P[8]-1) (g) and antiserum to VP8 of isolate MX08-659 (P[8]-3) (h), respectively.

rotavirus Wa (P[8]-1 lineage) or against recombinant MX08-659 VP8* (P[8]-3 lineage) were tested in Western blot analyses. Both rabbit serum samples recognized peptide B of P[8]-1 and P[8]-3 lineages, confirming the presence of conserved epitopes in the VP8* variable region across lineages. A possible explanation for the reactivity of the rabbit antisera is that within peptide B (aa 84-180), we found 13 amino acid substitutions between lineages P[8]-1 and P[8]-3, but the positions 106 (V/I), 108 (I/V), 120 (T/N), 121 (I/V), 150 (E/D) and 173 (V/I) had similar biochemical characteristics, suggesting that the antigenicity was not likely to have been significantly altered in the VP8* variable region.

To our knowledge, this is the first report describing the reactivity of antibodies present in the serum from infected children against the variable VP8* region corresponding to different P[8] human rotavirus lineages. Extended studies using recombinant proteins of the four P[8] lineages may be of value in monitoring vaccine efficacy against circulating rotavirus strains.
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References


