Genetic and antigenic variation of capsid protein VP7 of serotype G1 human rotavirus isolates

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The deduced amino acid sequences of the outer capsid protein, VP7, of serotype G1 rotavirus clinical isolates collected over a 6 year period (1990–1995) in Melbourne, Australia, were examined. Phylogenetic analysis characterized the sequences into two discrete clusters representing two of the four global lineages of human G1 VP7 proteins. Antigenic characterization using a panel of serotype G1-specific neutralizing monoclonal antibodies classified lineage II isolates (1990–1993) as monotype G1a while lineage I isolates were classified as monotype G1b (1993–1995). Examination of the sequences of the neutralization epitope regions of VP7 revealed a particular amino acid substitution at residue 94 in region A (Asp → Ser/Thr) that correlated with lineage and monotype designation. Our results indicated that temporal genetic variation of the VP7 of serotype G1 rotaviruses was associated with changes in the antigenicity of these isolates.

Group A rotaviruses are the most important aetiological agents of severe diarrhoea in infants and young children worldwide (Kapikian & Chanock, 1996). The virus carries a genome of 11 segments of double-stranded RNA (dsRNA) surrounded by a triple-layered capsid consisting of a core, inner capsid and outer capsid layer (Estes, 1996). The outer capsid is composed of two viral structural proteins, VP7 and VP4, which induce the production of neutralizing antibodies and determine the virus G and P serotype specificity, respectively (Estes, 1996). The major neutralization protein, VP7, has been the subject of considerable study and is the main immunogenic component of the first licensed human rotavirus vaccine (Kapikian et al., 1996). Despite extensive research of the VP7 protein, a greater understanding of its molecular epidemiology is required both to monitor the success of candidate rotavirus vaccines and to provide information relevant to the development of future vaccines and/or preventative therapies.

A number of studies of VP7 of rotavirus clinical isolates belonging to the major serotypes (G-types) known to infect humans have indicated the existence of discrete genetic lineages of this protein (Jin et al., 1996; Wen et al., 1997; Maunula & von Bonsdorff, 1998; Piec & Palombo, 1999). Over the last decade, serotype G1 has represented the most common serotype responsible for the hospitalization of children worldwide (Gentsch et al., 1996). Phylogenetic analysis has determined that at least four major global lineages of serotype G1 VP7 occurred within rotaviruses collected from diverse geographical locations (Jin et al., 1996; Maunula & von Bonsdorff, 1998). Although these studies identified particular amino acid substitutions in VP7 (including the major neutralization epitope regions) that correlated with lineage, the antigenic relationships between the different lineages were not examined.

In this report, we describe the changing genetic and antigenic properties of the VP7 protein of serotype G1 of rotavirus strains isolated from children admitted to the Royal Children’s Hospital in Melbourne, Australia, over a 6 year period (1990–1995). We further report that these strains could be classified into two of the four previously described lineages and that they could be discriminated antigenically according to their reactivity to a panel of serotype G1-specific neutralizing monoclonal antibodies (NMAbs).

Faecal samples were collected from children within 48 h of admission with acute gastroenteritis to the Royal Children’s Hospital in Melbourne, Australia, and stored at −70 °C. Each sample was routinely tested for the presence of rotavirus by an in-house enzyme immunoassay (EIA) (Coulson et al., 1987). The VP7 serotype was also determined by EIA (Coulson et al., 1987). Faecal homogenates (10%, w/v) were prepared from samples collected between 1992 and 1995 and identified as containing serotype G1 rotavirus. DsRNA was isolated by phenol–chloroform extraction, purified with hydroxyapatite (Gouvea et al., 1991), electrophoresed in 10% (w/v) polyacrylamide gels and stained with silver nitrate (Dyall-Smith & Holmes, 1984). According to the electrophoretic migration patterns observed, ten samples representing common and less...
common electropherotypes present between 1992 and 1995 were selected for further analysis. The samples selected, in general, represented major serotype G1 electropherotypes that accounted for up to 64% of isolates analysed in a particular year. Only sample 1993C represented a minor electropherotype (< 1% of isolates in 1993). Details of the distribution of electropherotypes are to be published elsewhere. Hence, it is possible that lineages other than those defined below were present in a given year. Characterization of isolates from 1990 and 1991 has been carried out previously (Palombo et al., 1993).

Monotypes of serotype G1 were assigned, according to the classification system of Coulson (1987), to each sample investigated according to their reactivities against a panel of serotype G1-specific NMAbs, RV4:1, RV4:2 and RV4:3, by EIA. These NMAbs have been characterized previously (Coulson et al., 1986). Briefly, microtitre plates were coated with homologous rabbit polyclonal antibody or preimmune immunoglobulins followed by incubation with 10% (w/v) faecal homogenate. NMAbs were reacted at dilutions of 1:3000 (RV4:1), 1:200 (RV4:2) or 1:2000 (RV4:3). Binding of NMAb was detected using anti-mouse immunoglobulins conjugated to horseradish peroxidase (Silenus Laboratories, Australia). The substrate used to detect bound immunoglobulin–enzyme complexes was 3,3′,5,5′-tetramethylbenzidine and the absorbance was measured at 450 nm. A positive reaction against a particular NMAb was observed when the sample displayed an A450 using polyclonal antibody as the coating antibody which was at least twice that obtained with preimmune immunoglobulins (Coulson et al., 1987). Samples that displayed positive reactivities against all three NMAbs were assigned monotype G1a while those samples displaying positive reactivities against all but the NMAb RV4:3 were classified as monotype G1b.

Full-length VP7 cDNA (1062 bp) was generated and amplified from dsRNA by RT–PCR using the method described by Gouvea et al. (1990). The resulting cDNA was electrophoresed in a 1:2% (w/v) low melting point agarose gel prepared with 1 x TAE buffer, excised and purified by binding to silica particles (Bresatec). Purified cDNA was stored at 4 °C.

Direct cycle sequencing of the full-length VP7 gene was carried out using the fmol DNA cycle sequencing kit (Promega) and gene-specific internal nucleotide primers. Primer sequences (5′–3′), their location on the VP7 gene and their orientation are as follows: CAGTGGGATAATACAAAAC (291–310, −); ATGTTATTATATATCCACA (53–72, +); ATGTTTGTATTATCCACTG (291–310, +); ATCAACATCCGGAGAAATCA (572–591, +); AGAATGACGCTGTAATACAA (815–834, +). Sequences have been deposited in GenBank under the accession nos AF043677–AF043682.

Deduced amino acid sequence data were analysed using programs available through the Australian National Genomic Information Service (ANGIS, University of Sydney). Phylogenetic trees were constructed by the TreeView program (Page, 1996) using genetic distances generated by the neighbour-joining method of Saitou & Nei (1987) with ClustalW analysis (Wisconsin package, version 8.1 UNIX, 1995).

According to Jin et al. (1996), VP7 of serotype G1 rotavirus isolates can be classified into four distinct lineages based on amino acid sequences. Similar results were obtained by Maunula & von Bonsdorff (1998). These studies indicated that there was no geographical clustering within the lineages; however, samples of Australian origin were classified into only one lineage [lineage II according to the classification of Jin et al. (1996)]. Utilizing complete VP7 deduced amino acid sequences derived from strains representative of each of these lineages, the 12 samples investigated in this study [10 sequences determined here and two from 1990 and 1991 determined by Palombo et al. (1993)] were phylogenetically analysed. While all samples investigated displayed over 89% amino acid identity, sufficient variation occurred throughout the VP7 sequence for the strains to be classified into two distinct phylogenetic groups (Fig. 1).

Both lineages I and II were present among the Melbourne sample population suggesting that geographical origin was not the sole determinant of lineage grouping, consistent with previous studies. The fact that samples collected between 1990 and 1992 and sample 1993A clustered together into lineage II while the more recent samples clustered to lineage I suggested that temporal genetic variation could lead to rotavirus strains from one geographical location resembling those derived from other regions. In fact, Maunula & von Bonsdorff (1998) have suggested that the lineages may predominate in different parts of the world concurrently. Our results further indicated that while a single lineage may exist in a given geographical location over a defined period of time (e.g. 1990–1992 and...
Table 1. Amino acid sequences of antigenic regions of VP7 and reactivities with serotype G1-specific NMAbs of serotype G1 rotavirus strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Region A (aa 87–101)</th>
<th>Region B (aa 142–152)</th>
<th>Region C (aa 208–221)</th>
<th>Lineage‡</th>
<th>P/N value with NMAb‡</th>
<th>Monotype§</th>
</tr>
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<tr>
<td>1990</td>
<td>TEASTQINDGENKDS</td>
<td>MKYDQNELEDM</td>
<td>QTNNVDSFWMVAEN</td>
<td>II</td>
<td>24 6</td>
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<td>I</td>
<td>8 1</td>
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* Dashes indicate identity to strain 1990 amino acids.
† As determined in Fig. 1.
‡ A virus was considered to have a positive reactivity if its $A_{NMA}$ with a given NMAb using homologous polyclonal antibody as the coating antibody ($P$ value) was at least twice the $A_{NMA}$ value obtained with preimmune immunoglobulins ($N$ value), i.e. a $P/N$ value $> 2.0$. Positive values are in **bold** type.
§ As defined by Coulson (1987).

1994–1995), it is also possible for more than one lineage to co-circulate (e.g. 1993).

Antigenic analysis showed that both monotypes G1a and G1b existed within the sample population. In addition, a correlation between antigenic type (monotype) and genetic type (phylogenetic lineage) was observed. All samples collected between 1990 and 1992 and sample 1993A displayed positive reactivities against all NMAbs defining them as monotype G1a (Table 1). In contrast, samples 1993B, 1993C and the 1994 and 1995 samples did not show reactivity against RV4:3 (Table 1). This pattern defined them as monotype G1b. The sequences of the neutralization epitope regions A (amino acids 87–101), B (142–152) and C (208–221) (Kapikian & Chanock, 1996) were compared, since these have been shown to contain the binding sites of the NMAbs used in this study (Coulson & Kirkwood, 1991). As seen in Table 1, an amino acid substitution at residue 94 (Asp → Ser/Thr) correlated with a change in monotype specificity as well as lineage designation. This site has previously been described by Coulson & Kirkwood (1991) as discriminating between monotypes of serotype G1 rotavirus strains. Our results indicated that, for two lineages (I and II), overall genetic differences correlated with antigenic differences.

Evidence for genetic variation within antigenic region C was observed as the recent isolates (1993C, 1994 and 1995 isolates) contained a substitution at residue 217 (Thr or Arg) compared to the earlier strains (Met). Although this change did not correlate with altered reactivity to NMAbs (strain 1993B has Met at 217 yet is monotype G1b), it may lead to conformational changes in the epitope regions that could be detected by other NMAbs. Other changes in regions A–C were also apparent (Table 1) which might affect the antigenic properties of VP7.

The results presented here indicated that temporal genetic variation of serotype G1 rotavirus clinical isolates has lead to changes in antigenic properties. Moreover, we have shown that previously defined phylogenetic lineages of serotype G1 VP7 could be discriminated antigenically. However, further studies are necessary to elucidate the antigenic relationships between the other lineages. Continued surveillance of rotavirus isolates, together with characterization of strains, is necessary to determine the extent to which genetic and antigenic variation in the virus population will assist in monitoring the success of vaccine strategies and the development of new therapies.

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References


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