GENETIC HOMOGENEITY OF HUMAN SEROTYPE G1 ROTAVIRUSES ISOLATED DURING A SINGLE EPIDEMIC SEASON: IMPLICATIONS FOR VACCINE STRATEGIES

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Summary. – The extent of genetic variation of rotavirus isolates representing the common serotype G1 circulating in Australian urban centres during 1996 was investigated. The sequences of the major outer capsid glycoprotein, VP7, of three isolates from Brisbane, Sydney and Melbourne were determined and found to be highly conserved (>99% nucleotide identity, >98.3% amino acid identity and >99% amino acid similarity). In particular, the sequences of the neutralisation epitope regions were absolutely conserved. These strains and those from other cities were analysed by Northern blot hybridisation using a probe derived from a prototype serotype G1 virus. For all strains, the eleven genomic RNA segments hybridised with the probe indicating that these strains were not derived from genetic reassortment between different rotavirus genogroups. These results suggest that, within a single epidemic season, rotavirus strains circulating in geographically diverse communities share common genetic features.

Key words: rotavirus; epidemiology; reassortment; VP7

Group A rotaviruses are the most important cause of severe diarrhoea in infants and young children worldwide (Kapikian and Chanock, 1996). The virus particle contains 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 layer is composed of two viral structural proteins, VP7 and VP4, which induce the production of neutralising antibodies and determine the virus G and P serotype specificity, respectively (Estes, 1996). The major neutralisation 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). Therefore, 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 preventive therapies. The segmented nature of the rotavirus genome allows for the potential for rapid evolution through the process of reassortment. Random reassortment between viruses of different rotavirus genetic groups, genogroups (Nakagomi and Nakagomi, 1993), occurs readily *in vitro* (Urasawa *et al.*, 1986; Kobayashi *et al.*, 1994), although the extent to which this occurs *in vivo* is unknown. However, the increased number of reports of rotaviruses with unusual genetic properties suggests that this may be a common phenomenon (Palombo *et al.*, 1996).

Over the last decade, the serotype G1 has represented the most common serotype responsible for the hospitalisation of children worldwide (Gentsch *et al.*, 1996). In this report, we describe the genetic features of serotype G1 human rotavirus strains isolated from major urban centres in Australia during 1996. Specifically, we have investigated the level of variation in the major capsid protein, VP7, and used Northern blot analysis to determine the extent of reassortment in these strains.

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Abbreviations: DIG = digoxigenin; dsRNA = double-stranded RNA; RT-PCR = reverse transcription-polymerase chain reaction

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     51       60         70         80         90         100        110        120        130        140        150

RV4  QNYGINLPIT GSMDIVYANS TQEEIFLTST LCLYYPTEAS TQINDGEWKD SLSQMFLTK GWPTGSVYFKE YSSIVDFSVD PQLYCDYNLV LMKYDQNLEL
M149 ----L----- ----TT---- ---G------ ---------- ---------- --------- ---------- ---------- ---------- ----------
B147 ----L----- ----TA---- ---G------ ---------- ---------- --------- ---------- ---------- ---------- ----------
S149 ----L----- -LL-TA---- ---G------ ---------- ---------- --------- ---------- ---------- ---------- ----------

           160        170        180        190        200        210        220        230        240        250

RV4  DMSELADLIL NEWLCNPMDI TLYYYQQSGE SNKWISMGSS CTVKVCPLNT QTLGIGCQTT NVDSFEMVAE NEKLAIVDVV DGINHKINLT TTTCTNRNCK
M149 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----Y----- -----I----
B147 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----Y----- -----I----
S149 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----Y----- -----I----

           260        270        280        290        300        310        320    326

RV4  KLGPRENVAV IQVGGSNVLD ITADPTTNPQ TERMMRVNWK KWWQVFYTIV DYINQIVQVM SKRSRSLNSA AFYYRV
M149 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------
B147 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------
S149 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------
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Fig. 1

Deduced amino acid sequences of mature VP7 protein of serotype G1 rotaviruses

Dashes indicate identity to RV4 amino acids. Overlined sequences indicate the neutralisation epitope regions while potential glycosylation sites (aa 69-71 and 238-240) are in bold.

Faecal samples containing rotavirus were collected from children within 48 hrs of admission to hospital with acute gastroenteritis and stored at - 70°C after transportation to Melbourne. The VP7 serotype was determined by enzyme immunoassay (Coulson *et al.*, 1987). dsRNA was isolated from 10% (w/v) faecal homogenates 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 and Holmes, 1984). According to the electropherotypic migration patterns observed, samples representing the common serotype G1 electropherotypes present in 1996 were selected for further analysis.

Full length VP7 cDNA (1062 bp) was generated and amplified from dsRNA by reverse transcription-polymerase chain reaction (RT-PCR) using the method described by Gouvea *et al.* (1990). The resulting cDNA was electrophoresed in 1.2% (w/v) agarose gels prepared with 1 x TAE buffer (0.04 mol/l Tris-acetate and 0.002 mol/l EDTA, pH 8.0), excised and purified by binding to silica particles (Bresatec, Australia). The 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, USA) and gene-specific internal nucleotide primers. Their sequences and location on the VP7 gene were as follows: 3'-CAGTTGGATAATACAAACAT-5' (nt 291-310); 5'-ATGGTATTGAATATACCACA-3' (nt 53-72); 5'-ATGTTTGTATTATCCAACTG-3' (nt 291-310); 5'-ATCAACAATCGGGAGAATCA-3' (nt 572-591); 5'-AGAATGTAGCTGTAATACAA-3' (nt 815-834).

The nucleotide and deduced amino acid VP7 sequences of three strains, M149, S149 and B147 isolated in Melbourne, Sydney and Brisbane, respectively, showed a high level of conservation. The strains exhibited nucleotide sequence identity of 99.0 – 99.8%, amino acid identity of 98.3 – 99.0%, and amino acid similarity of 99.0 – 99.3%. This high level of conservation is reflected in Fig. 1 which shows the deduced amino acid sequences of the mature VP7 protein (following cleavage of the signal peptide after amino acid 50). Minimal amino acid sequence variation was observed between the clinical isolates and the prototype Australian serotype G1 strain, RV4 (Fig. 1), and between the isolates themselves. In particular, all strains shared identical sequences in the neutralisation epitope regions located on VP7 (Kapikian and Chanock, 1996) and no changes in potential glycosylation sites. Similar levels of conservation have previously been found in strains from Melbourne isolated between 1990 and 1995 (Diwakarla and Palombo, 1999), where isolates from a single year generally exhibited limited diversity. However, the results of the present study further showed that this high degree of conservation extended to strains isolated from geographically diverse locations within Australia during the same year.

To investigate the degree of variation at the whole genome level, isolates collected from major urban centres of Australia (Adelaide, Alice Springs, Brisbane, Melbourne, Perth and Sydney) were analysed by Northern blot hydridisation. The probe was derived from the prototype serotype G1 strain, RV4, which represents one of the two major human rotavirus genogroups (Nakagomi and Nakagomi, 1993). Genomic dsRNA was isolated from RV4, gel-purified and labelled with digoxigenin (DIG) by chemical linking of DIG to RNA using the DIG Chem-Link Reagent (Roche Biochemicals, Germany). The Northern blot analysis and detection of the bound probe was carried out as described previously (Palombo *et al.*, 1998) using dsRNA extracted from the standard strains RV4 and RV5 (serotype G2 virus strain representing the second major human genogroup) and the clinical isolates (Fig. 2). The probe exhibited genogroup specificity as shown by its limited ho-

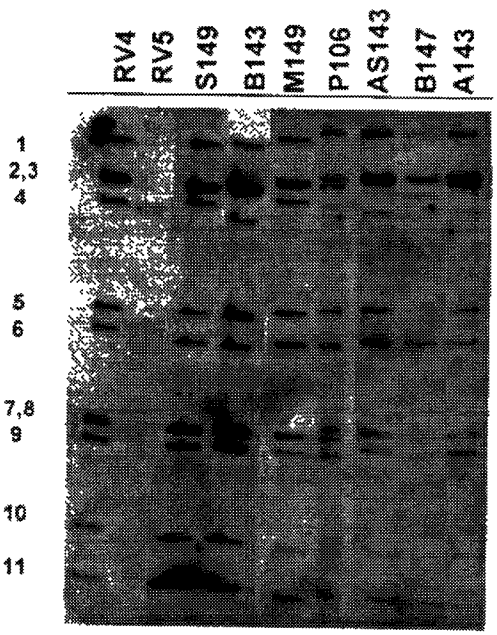

Fig. 2
Northern blot hybridisation analysis of serotype G1 rotaviruses
The investigated strains included prototype viruses RV4 (serotype G1) and RV5 (serotype G2), and serotype serotype clinical isolates. The letter prefixes in designations of the clinical isolates indicate the city of origin, i.e A = Adelaide, AS = Alice Springs, B = Brisbane, M = Melbourne, P = Perth and S = Sydney The numbers on the left indicate the migration order of genomic RNA segments.

mology to RV5 dsRNA. However, all gene segments from the clinical isolates hybridised with the probe indicating that all strains shared genetic homology at the whole genome level. This suggested that serotype G1 rotaviruses exhibit genetic stability and do not readily undergo reassortment with viruses of other genogroups. Therefore, even though variation in the migration patterns of RNA segments was observed (Fig. 2), all segments shared a common genetic background.

The results of this study present important molecular information about the genetic composition of human rotavirus populations. The high level of conservation observed encourages the hope that rotavirus vaccine strategies will prove effective. However, further characterisation of rotavirus isolates including their antigenic properties will help to determine the extent of variation in viruses from different locations. In addition, similar investigation of other rotavirus serotypes that are the target of vaccines is warranted. This information will assist in monitoring the success of current vaccine strategies and the implementation of new strategies.

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