A new serotype of the outer capsid protein VP4 shared by an unusual human rotavirus strain Ro1845 and canine rotaviruses

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The VP4 protein of human rotavirus (HRV) strain Ro1845 and canine rotavirus strains K9 and CU-1 exhibited greater than 98% amino acid identity within their group, but showed less identity with VP4 proteins of other HRV and animal rotavirus strains, the simian rotavirus strain RRV VP4 being most similar to them (90% amino acid identity). To exclude the possibility that these three strains were members of the RRV VP4 serotype P3, neutralization studies were performed using antisera to reassortant viruses containing the VP4 gene from each of Ro1845, CU-1 and RRV. The result established close antigenic similarity among the VP4 proteins of Ro1845, K9 and CU-1 and revealed only a marginal degree of similarity between the VP4 proteins of these three strains and that of strain RRV. These sequence and serological data suggest that the VP4 proteins of Ro1845, K9 and CU-1 represent a new P serotype which we propose to assign P13.

Group A rotaviruses, family Reoviridae, genus Rotavirus, have been recovered from a number of animal species and are the single most important aetiological agent of severe diarrhoea in infants and young children (Kapikian & Chanock, 1990). The genome of rotavirus consists of 11 segments of dsRNA contained within a double-layered capsid (Estes & Cohen, 1989). The surface of the outer capsid is composed mainly of a glycoprotein, VP7, encoded by gene segment 7, 8 or 9, depending on the strain (Estes & Cohen, 1989). The other outer capsid protein, VP4, the product of gene segment 4, forms spike-like structures that protrude from the outer surface of the virion and functions as the viral haemagglutinin (Estes & Cohen, 1989). Both proteins are involved in virus neutralization and segregate independently (Hoshino et al., 1985; Offit & Blavat, 1986). It is therefore important to classify rotavirus strains for their VP4 serotypes as well as VP7 serotypes. The VP7 serotype is termed the G (glycoprotein) serotype and the VP4 serotype the P (protease-sensitive protein) serotype (Estes & Cohen, 1989). Thus far, 12 P serotypes have been described based on differences in the VP4 gene sequence (Estes & Cohen, 1989; Qian & Green, 1991; Hardy et al., 1992, 1993), although the definition of any serotype should be based on the results of neutralization assays.

Human rotavirus (HRV) strain Ro1845 (serotype G3 and subgroup I) was isolated from an Israeli baby with diarrhoea who had a history of contact with a puppy prior to the diarrhoeal episode (Aboudy et al., 1988; Nakagomi et al., 1992) and was shown by RNA–RNA hybridization to be genetically related to canine and feline rotavirus (CRV and FRV) strains (Nakagomi et al., 1990). Since strain Ro1845 was shown to possess the haemagglutinin capable of agglutinating erythrocytes from various animal species, we determined the nucleotide sequence of the gene encoding the VP4 protein of Ro1845 together with those of CRV strains K9 and CU-1. This study shows that a highly conserved VP4 protein shared by strains Ro1845, K9 and CU-1 represents a new P serotype, which we propose to assign P13.

HRV strain Ro1845 (serotype G3, subgroup I) (Aboudy et al., 1988) and CRV strains K9 (Fulton et al., 1981) and CU-1 (Hoshino et al., 1983) (both serotype G3, subgroup I) were grown in MA104 cells. Single-shelled particles were prepared from the infected cell culture harvest by caesium chloride density equilibrium ultracentrifugation.

Single-stranded RNAs (mRNAs) were prepared by in vitro transcription of the genomic dsRNAs as described...
Table 1. Comparison of nucleotide (nt) and deduced amino acid (aa) sequence identity between the VP4 genes of strains Ro1845, K9 and CU-1, and various group A rotaviruses carrying distinct VP4 alleles

<table>
<thead>
<tr>
<th>Strain</th>
<th>P type</th>
<th>Identity with strain indicated (%)</th>
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<tbody>
<tr>
<td></td>
<td>nt</td>
<td>aa</td>
</tr>
<tr>
<td>Ro1845</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>K9</td>
<td>97</td>
<td>98</td>
</tr>
<tr>
<td>CU-1</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>C486 (bovine)</td>
<td>P1</td>
<td>76</td>
</tr>
<tr>
<td>SA11</td>
<td>P2</td>
<td>76</td>
</tr>
<tr>
<td>RRV</td>
<td>P3</td>
<td>81</td>
</tr>
<tr>
<td>RV-5</td>
<td>P4</td>
<td>71</td>
</tr>
<tr>
<td>UK</td>
<td>P5</td>
<td>70</td>
</tr>
<tr>
<td>Gottfried</td>
<td>P6</td>
<td>70</td>
</tr>
<tr>
<td>OSU</td>
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<td>76</td>
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<tr>
<td>KS</td>
<td>P8</td>
<td>70</td>
</tr>
<tr>
<td>AU-1</td>
<td>P9</td>
<td>69</td>
</tr>
<tr>
<td>69M</td>
<td>P10</td>
<td>75</td>
</tr>
<tr>
<td>B223</td>
<td>P11</td>
<td>62</td>
</tr>
<tr>
<td>H-2</td>
<td>P12</td>
<td>75</td>
</tr>
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previously (Nakagomi et al., 1989). Full-length copies of the VP4 genes of strains K9 and Ro1845 were prepared from mRNA by reverse transcriptase (RT)-PCR with 5' and 3' consensus primers (BegG4 and EndG4) as described previously (Isegawa et al., 1992a). For the determination of both K9 and Ro1845 VP4 sequences, DNA fragments generated by RT–PCR were directly sequenced by a modification of the dideoxynucleotide-chain termination method as described previously (Isegawa et al., 1992a, b). On the other hand, the CU-1 VP4 sequence was determined by a modification of the above method, as described by Air (1979), using as templates, mRNA generated by in vitro transcription and genomic dsRNA (for the 3' end sequence). The CU-1 VP4 sequence was also confirmed by sequencing cDNA clones which were obtained by using the Clean-Amp kit (Bethesda Research Laboratories).

Three reassortants, RRV x DS-1, CU-1 x DS-1 and Ro1845 x DS-1, were made such that their VP4 gene (encoding P serotype) was derived from each of simian rotavirus (SRV) strain RRV, and CU-1 and Ro1845, whereas the VP7 gene (encoding G serotype) was derived from the DS-1 parent. These reassortants were produced as described previously (Hoshino et al., 1985) and were plaque-purified three times.

Serotyping was carried out by plaque-reduction neutralization in MA104 cells in 6-well culture plates as described (Hoshino et al., 1984). An antibody titre was expressed as the reciprocal of the highest dilution of antisemum neutralizing 60% or more of the input virus. Hyperimmune antisera against each of the reassortants, RRV x DS-1, CU-1 x DS-1 and Ro1845 x DS-1, and strain DS-1, were prepared in guinea pigs with multiple subcutaneous injections of partially purified virus particles emulsified with either Freund's complete adjuvant (for priming) or Freund's incomplete adjuvant (for subsequent immunizations).

The Ro1845 VP4 sequence was determined to be 2362 nucleotides in length and contained an open reading frame capable of encoding a protein of 776 amino acids. This VP4 sequence was highly similar to that of strains K9 and CU-1 (greater than 98% amino acid identity) (Table 1), indicating that these VP4 genes belong to the same VP4 gene allele. By contrast, this degree of similarity was not observed between these VP4 proteins and those of other HRV and animal rotavirus strains thus far sequenced. An identity value of 90% was calculated between the VP4 of SRV strain RRV and that of these three strains (Table 1).

To exclude the possibility that the VP4 proteins of Ro1845, K9 and CU-1 were members of the RRV VP4 serotype which has been established as P serotype 3 according to the numbering system of Estes & Cohen (1989), neutralization studies were performed using hyperimmune antisera raised against reassortants. Antiserum to Ro1845 x DS-1, which derived its VP4 gene from Ro1845 and its VP7 gene from DS-1, neutralized Ro1845, K9 and CU-1 at the same high titre. This neutralization was considered to be mediated by their VP4 protein because antiserum to DS-1 (in which both VP4 and VP7 genes are different from Ro1845) neutralized Ro1845, K9 and CU-1 at much lower titres (16- to 32-fold lower) (Table 2). Similarly, antiserum to CU-1 x DS-1, which derived its VP4 gene from CU-1 and its VP7 gene from DS-1, neutralized Ro1845, K9 and CU-1, at comparably high titres, again via their VP4. By contrast, these two antisera neutralized RRV at much lower titres (one-eighth to one-sixteenth of the strains possessing homologous VP4 proteins). Correspondingly, antiserum to RRV x DS-1, which derived its VP4 gene from RRV and its VP7 gene from DS-1, neutralized Ro1845, K9 and CU-1 and K9 at much lower titres (16- to 32-fold lower) (Table 2). These data clearly showed that the antigenicity of the VP4 proteins of Ro1845, K9 and CU-1 strains were very similar, and that RRV VP4 had a marginal antigenic relationship with these three rotavirus strains.

Prediction of a new P serotype by amino acid identities is consistent with neutralization data for HRV and porcine rotavirus strains (Gorzigilia et al., 1990a, b). For example, Gorzigilia et al. (1990a) have reported that viruses of the same group sharing more than 93% amino acid identity exhibit close antigenic relatedness as judged by neutralization assays. Furthermore, they showed that strains which shared equal to or less than 89% amino acid identity exhibited lesser degrees of antigenic relatedness in accordance with predicted amino acid homology.
Such precedents have provided a molecular basis for predicting that the VP4 proteins of strains Ro1845, K9 and CU-1 represent a new P serotype distinct from those of other HRV and animal rotavirus strains. Given that bovine rotavirus (BRV) strain B223 and equine strain H-2 have been shown to possess a unique VP4 protein by amino acid sequence analysis (Hardy et al., 1992, 1993), it is reasonable to assign the VP4 proteins of B223 and H-2 to P11 and P12, respectively, and to assign those of Ro1845, K9 and CU-1, to P13.

The prediction that the VP4 proteins of strains Ro1845, K9 and CU-1 represent a new P serotype was substantiated by neutralization assays with antisera to reassortant viruses containing the VP4 gene from each of Ro1845, CU-1 and RRV. Reassortants and antisera to them have been used successfully to determine the role played by each of VP7 and VP4 proteins in complex antigenic relationships among rotavirus strains (Hoshino et al., 1985; Gorziglia et al., 1990; Matsuda et al., 1990; Snodgrass et al., 1992).

The nucleotide sequence identities between Ro1845 and K9 or CU-1 (96.4% and 95.6%, respectively) were as high as that between K9 and CU-1 (97.6%) although Ro1845 was derived from a human baby and K9 and CU-1 were derived from dogs. The observation may be best explained by relatively recent transmission of a CRV to a human host. This is consistent with our previous hybridization and haemagglutination studies (Nakagomi et al., 1990, 1992). The occurrence of a highly conserved VP4 gene sequence in rotaviruses of different host species was reported recently for the VP4 sequence carried by HRV strain AU-1 and FRV strain FRV-1 (Isegawa et al., 1992a) and for the VP4 sequence carried by HRV strains I321 and 116E, and BRV B223 (Das et al., 1993; Gentsch et al., 1993). Similarly, high sequence conservation was reported for the VP7 gene of HRV A64 and BRV B223 (Beards et al., 1992) and for the VP7 gene of HRV I321 and BRV B223 (Das et al., 1993). These sequencing studies, together with previous RNA–RNA hybridization studies (Nakagomi & Nakagomi, 1989; Nakagomi et al., 1990; Dunn et al., 1993), provide strong support for the assertion that animal rotaviruses can infect and spread within humans under natural conditions.

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### References


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