Serological and genomic characterization of L338, a novel equine group A rotavirus G serotype

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A group A rotavirus designated L338 was isolated from the faeces of a diarrhoeic foal and was compared to 11 standard G serotype strains of group A rotaviruses by cross-neutralization. It was clearly distinct from serotypes G1 to G11 and thus representative of a novel rotavirus G serotype tentatively designated G13. The nucleic acid sequence of the virion protein 7 (VP7) coding region was determined and the deduced amino acid sequence compared to published sequences. Within VP7 regions A and B, L338 was clearly distinct from serotypes G1 to G12 (excluding G7 which has not been sequenced), but region C was very similar to those of G3 and G8. This further questions the significance of region C in determining serotype specificity of at least three distinct rotavirus G serotypes.

Introduction

The major neutralization antigen of group A rotaviruses is the major outer coat protein, virion protein 7 (VP7) (Greenberg et al., 1983). As this protein is glycosylated, serotypes of this antigen are now referred to as G serotypes, to distinguish them from serotypes of the minor outer coat protein, virion protein 4, which are referred to as P serotypes (Hum et al., 1989). Twelve G serotypes have been characterized among isolates from various mammalian and avian species on the basis of cross-neutralization with hyperimmune sera (Hoshino et al., 1984; Ruiz et al., 1988; Green et al., 1989; Snodgrass et al., 1990; Urasawa et al., 1990). A 20-fold difference between two viruses is generally accepted as indicative of distinct G serotypes.

The sequence of the gene encoding VP7 has been determined for 11 G serotype rotaviruses. By sequencing neutralizing monoclonal antibody escape mutants, serotype specificity has been correlated with three restricted regions (A, B and C) of 11 to 15 amino acid residues in length (Dyall-Smith et al., 1986; Taniguchi et al., 1986, 1988; Mackow et al., 1988). A further six regions of divergence between serotypes have been identified by sequence comparison, but the significance of these regions to antigenicity is unknown (Green et al., 1989). This is especially so for the three regions between residues 1 and 50, which are probably not present in the mature protein following cleavage at residue 51 (Stirzaker et al., 1987). The deduced amino acid sequences in regions A, B and C have been used with some success to predict G serotypes (Green et al., 1987). However, the discovery of unexpected similarity between different serotypes in one of these regions (Green et al., 1989; Hum et al., 1989), and the characterization of a porcine rotavirus VP7 with dual serotype specificity (Nagesha et al., 1990) have suggested caution in interpretation of the findings.

Only three equine group A rotaviruses have been characterized serotypically; two were found to be serotype G3 and one serotype G5 (Hoshino et al., 1983a, b, 1987). Although over 60% of equine rotaviruses are G3, more than 30% of field samples cannot be typed with currently available reagents (unpublished data), indicating the probable existence of novel G serotypes. The aim of this study was to characterize a rotavirus isolated from a diarrhoeic foal which was not able to be typed by standard serotyping ELISAs, and to determine whether it was distinct from other known group A rotavirus G serotypes.

Methods

Isolation and cell culture adaptation of virus. Equine rotavirus strain L338 was identified in the faeces of a 2-day-old thoroughbred foal with diarrhoea by PAGE and silver staining (Herring et al., 1982). The virus was isolated in rolled MA-104 cell monolayer cultures in the presence of 1 μg/ml trypsin and was plaque-purified three times before characterization.
Standard G serotype strains. Standard strains representing each of 11 G serotypes were grown in MA104 cell cultures in the presence of 1 µg/ml trypsin: Wa (G1), DS-1 (G2), RRV (G3), ST-3 (G4), OSU (G5), UK (G6), Ch-2 (G7), 69M (G8), WI-61 (G9), B223 (G10) and YM (G11) (Hoshino et al., 1984; Matsumoto et al., 1985; Clarke et al., 1987; Ruiz et al., 1988). Serotype G12 (Taniguchi et al., 1990b) was not available for this study.

Production of hyperimmune antiserum. Hyperimmune antisera to each of the standard strains and to L338 were raised in rabbits from a rotavirus-free rabbit colony. Each rabbit was confirmed to be free of rotavirus antibody before vaccination.

The vaccine was prepared by centrifugation of rotavirus from clarified (5000 g for 1 h at 4 °C) cell culture lysates at 80000 g for 1 h at 4 °C and resuspending the pellet in 50 mm-Tris–HCl pH 7-6 at a 100-fold greater concentration than the original lysate. The virus was inactivated in 0-16 M-formaldehyde for 1 h at 37 °C then 14 h at 4 °C. An emulsion of equal volumes of the antigen and adjuvant was used to inoculate the rabbits. Each rabbit received 1 ml of vaccine intramuscularly; the primary inoculation was in Freund’s complete adjuvant, and the boosting inoculation 4 weeks later was in Freund’s incomplete adjuvant.

Cross-neutralization tests. Serum neutralization tests were performed using the microdilution fluorescent focus reduction method, with the endpoint determined as a 60% reduction in foci.

Sequencing of the VP7 gene. Viral transcript was produced with purified single-shelled particles (Flores et al., 1982). The sequence of the L338 VP7 gene transcript was determined by the dideoxynucleotide chain-termination method using reverse transcriptase with synthetic oligonucleotide primers complementary to the VP7 mRNA of rhesus rotavirus or NCDV rotavirus (Gorziglia et al., 1986b).

Results

The results of the cross-neutralization tests between L338 and the standard rotavirus strains are shown in Table 1. The neutralization titres of L338 antiserum to all 11 of the standard strains had titres against L338 at least 20 times less than against the homologous strain.

Table 1. **Comparison of equine rotavirus L338 with standard serotype strains by cross-neutralization**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Wa</th>
<th>DS-1</th>
<th>RRV</th>
<th>ST-3</th>
<th>OSU</th>
<th>UK</th>
<th>Ch-2</th>
<th>69M</th>
<th>WI-61</th>
<th>B223</th>
<th>YM</th>
<th>L338</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wa (G1)</td>
<td>5120</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>20</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>160</td>
<td>&lt;10</td>
<td>20</td>
<td>&lt;10</td>
</tr>
<tr>
<td>DS-1 (G2)</td>
<td>&lt;10</td>
<td>10240</td>
<td>20</td>
<td>&lt;10</td>
<td>20</td>
<td>20</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>40</td>
<td>&lt;10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>RRV (G3)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>20480</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
<td>1280</td>
<td>20</td>
<td>20</td>
<td>&lt;10</td>
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<tr>
<td>ST-3 (G4)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>20480</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>80</td>
<td>10240</td>
<td>&lt;10</td>
<td>10</td>
<td>80</td>
<td>640</td>
<td>&lt;10</td>
</tr>
<tr>
<td>OSU (G5)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>UK (G6)</td>
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<td>&lt;10</td>
<td>&lt;10</td>
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<td>&lt;10</td>
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<td>10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Ch-2 (G7)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<td>10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td>69M (G8)</td>
<td>&lt;10</td>
<td>20</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>640</td>
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<td>10240</td>
<td>&lt;10</td>
<td>20</td>
<td>&lt;10</td>
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<tr>
<td>WI-61 (G9)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<td>20</td>
<td>&lt;10</td>
<td>10240</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td>B223 (G10)</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<td>&lt;10</td>
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<td>&lt;10</td>
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<td>&lt;10</td>
<td>&lt;10</td>
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<td>&lt;10</td>
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<tr>
<td>YM (G11)</td>
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<td>20</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>640</td>
<td>20</td>
<td>10</td>
<td>&lt;10</td>
<td>40</td>
<td>5120</td>
<td>5120</td>
<td>&lt;10</td>
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<tr>
<td>L338</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>40</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>1280</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

is shown in Fig. 1 along with the deduced amino acid sequence of VP7. The L338 VP7 sequence has two potential glycosylation sites, at amino acids (aa) 69 and 237, both of which occur commonly in other serotypes. It exhibits changes at six amino acid residues which have been identical in other VP7 sequences (aa 13, 14, 22, 23, 93 and 265), but only the changes at aa 22 (I to T) and aa 265 (G to R) are non-conservative.

In the consensus sequence the substitution at aa 22 alone would create a potential glycosylation site, but the accompanying unusual change at aa 20 (consensus N to D) in L338 prevents this. These changes suggest that the function of the hydrophobic H1 region (aa 6 to 23) is probably inhibited by glycosylation at aa 20, and that such an inhibition of function has a significant effect on the viability of the virus. The precise role of the H1 region is unknown, although this region can act as a signal peptide for transport to the endoplasmic reticulum (Whitfield et al., 1987).

The three serotype-specific regions of L338 are compared to those of the 11 sequenced G serotypes in Fig. 2. With the exception of region C, where L338 showed a high degree of sequence similarity with G3 and G8 serotypes, the sequence of L338 was quite distinct from those of the other G serotypes. The L338 sequence also varied from other G serotypes in the six other regions in which variability between G serotypes has previously been observed. The proportion of identical nucleotides in the coding sequence, and the proportion of identical amino acid residues between L338 and all other published G serotype sequences are shown in Fig. 3.

Discussion

L338 appears to be distinct, both serologically and in the deduced amino acid sequence of its VP7, from other G serotypes described previously. Sequence comparisons
### Fig. 1. The nucleic acid sequence of the coding region of equine rotavirus VP7 gene and the deduced amino acid sequence. Amino acid substitutions for residues conserved in all other sequenced VP7 genes are indicated with an asterisk. Potential glycosylation sites are underlined. The 3' end and the first two bases at the 5' end of the gene were not determined.

### Virus G type Strain

<table>
<thead>
<tr>
<th>Virus</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>L338</td>
<td>NEVSVSELNDSSWKT</td>
<td>VKYSTELQLDI</td>
<td>LTTDTETFEEVATL</td>
</tr>
<tr>
<td>Wa</td>
<td>T-ASTQI- - GD- - DS</td>
<td>M - DQS- E - M</td>
<td>Q - NVDS - MI - EN</td>
</tr>
<tr>
<td>S2</td>
<td>A - AKN- - TS- - E - E</td>
<td>MR - DNTSE - V</td>
<td>K - - VD - - I - SS</td>
</tr>
<tr>
<td>RRV</td>
<td>T - AAT - I - N - D</td>
<td>M - DAT - - M</td>
<td>- - - - A - - - - A</td>
</tr>
<tr>
<td>ST-3</td>
<td>S - APTQIS- - TE- - D</td>
<td>IRFVSGEE - - - M</td>
<td>Q - N - A - T - DS</td>
</tr>
<tr>
<td>UK</td>
<td>V - ASN - IA - TE - D</td>
<td>M - DSE - - M</td>
<td>- - I - NPD - - T - T</td>
</tr>
<tr>
<td>69M</td>
<td>V - VET - IA - S - D</td>
<td>M - VAE - - M</td>
<td>- - N - NE - - A</td>
</tr>
<tr>
<td>61A</td>
<td>V - VET - IA - S - D</td>
<td>M - VAE - - M</td>
<td>- - N - NE - - A</td>
</tr>
<tr>
<td>WI-61</td>
<td>A - AATQI- - TE- - D</td>
<td>M - DSE - - M</td>
<td>- - I - NPD - - T - T</td>
</tr>
</tbody>
</table>

**Fig. 2.** Comparison of the deduced amino acid sequence of L338 in antigenic regions of A (aa 87 to 101), B (aa 141 to 151) and C (aa 208 to 221) to those of other G serotypes. Amino acid residues in other serotypes identical to those in L338 are shown by a dash. From data in Elleman et al. (1983), Richardson et al. (1984), Gunn et al. (1985), Mason et al. (1985), Gorziglia et al. (1986a), Green et al. (1987), Ruiz et al. (1988), Green et al. (1989), Reddy et al. (1989) and Taniguchi et al. (1990a, b).
between L338 and Ch-2 could not be made as the RNA sequence of the VP7 gene of G7 rotavirus has not been reported. However, serological comparison of these viruses clearly demonstrated the absence of any cross-reaction. Similarly, serological comparison of L338 and L26 was not possible as the L26 (G12) isolate was not available for this study. However, comparison of the L338 VP7 coding sequence with that of L26 showed that their RNA and, more significantly, amino acid sequences were less similar to each other than they were to several other G serotypes. Furthermore, in the antigenic regions A, B and C their deduced amino acid sequences were quite distinct. This warrants describing L338 as the prototype of a novel G serotype, tentatively G13. The growing number of group A rotavirus G serotypes renders the task of assigning new isolates to a serotype increasingly difficult, especially in laboratories where access to standard strains of veterinary origin is restricted by quarantine regulations. The determination of the sequence of each serotype enables comparisons to be made more easily, but serological comparisons are still necessary to establish the significance of observed differences in any apparently novel serotypes. This requirement is partially met by the availability of monoclonal antibodies specific for each serotype, although the occurrence of monotypes among G4 (Gerna et al., 1988) and the diversity of G3 (Nishikawa et al., 1989) limits their general applicability. Furthermore the occurrence of monoclonal antibodies able to neutralize more than one G serotype (Mackow et al., 1988) suggests that it is not possible to predict that a given monoclonal antibody will not neutralize or bind to a new serotype.

In this study we observed significant neutralization of G5 rotavirus (OSU) by 69M (G8) antisera and of G3 (RRV) and G11 (YM) rotaviruses by G10 (B223) antisera. The cross-reaction between 69M antisera and G5 virus was possibly due to P serotype as it was not seen with bovine G8 rotavirus antisera (data not shown). However we have confirmed the cross-reaction between G3 and G10 viruses using two different G10 viruses, six different G3 viruses and their respective antisera. Antisera against some serotype 3 viruses were able to neutralize serotype 10 viruses at low levels (unpublished data). Serotypes 5 and 11 showed low-level two-way cross-neutralization. Although Ruiz et al. (1988) observed this, it was at a lower level than in our study. These two viruses have identical region B sequences (Fig. 2) and this may partially explain the observed cross-reaction. Additionally these two porcine viruses may share the same P serotype.

Although the overall amino acid sequence similarity between L338 and other serotypes is relatively low (71.9% to 81.6%) compared to that between serotypes G1 to G12 (excluding G7 which has not been sequenced) (72.5% to 88.7%), the similarity between L338, G3 and G8 in antigenic region C is high (85.7% to 92.9%). This similarity does not appear to be reflected in immune responses to these viruses. Cross-neutralization between the three G serotypes is minimal and monoclonal antibodies A10/N3, RV-3:1 and A11/M9, all known to
select escape mutants with changes in the C region of G3, failed to neutralize L338, as found by Hum et al. (1989) for G8. Hum et al. (1989) suggested that carbohydrate bound to the potential glycosylation site at aa 238 in G8 may shield the C region from antibody. However, although this potential site occurs in L338, it also occurs in several G3 isolates, some of which are recognized by monoclonal antibody YO-1E2 which selects escape mutants in region C (Nishikawa et al., 1989). It seems likely that region C plays only a contributory role to the serotypic epitopes, and is strongly influenced by one of the other regions, probably A, as suggested by studies of monoclonal antibody escape mutants (Dyall-Smith et al., 1986). It is tempting to speculate that such regions of similarity, although not recognized by hyperimmune sera raised in naive animals, may be significant in the heterotypic response observed in experienced animals, where vaccination with a single serotype produces a broad response across all serotypes (Brüssow et al., 1988; our unpublished results). The residues in region C may be minor contributors to the immunodominant epitopes that define serotypic specificity, but also major contributors to minor epitopes which are shared by all serotypes and which become more apparent with repeated exposure. The greater interserotypic sequence similarity of region C compared to regions A and B suggests that it is the most probable site of the hypothetical common epitopes.

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References


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