Amino acids involved in distinguishing between monotypes of rotavirus G serotypes 2 and 4

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Neutralizing monoclonal antibodies (N-MAbs) to serotype G2 and G4 rotaviruses were used to study intrasertype variation by selection and characterization of N-MAb-resistant antigenic variants and reaction of N-MAbs with prototype rotavirus strains. Two G2-specific N-MAbs reacted with G2 rotaviruses $2, DS-1, RV-5 and RV-6 but not with 1076. Sequence analysis of the gene encoding VP7 of 1076 virus showed that the differences in amino acid sequence between 1076 virus and the other G2 strains at position 147, 213 and 217 correlated with the loss of N-MAb reactivity. Rotavirus variant mutation mapping data suggested that the amino acid difference at position 213 was likely to be of greatest importance. Rotavirus 1076 was defined as monotype b within G2 strains, whereas S2, DS-1, RV-5 and RV-6 belong to monotype a. The molecular basis for G4 subtypes/monotypes was also studied. The monotype G4b N-MAb 3A3 selected an antigenic variant with an amino acid mutation at position 96, whereas variants of the G4a-reactive N-MAb ST-3:1 showed a mutation at position 94, which produced a new, utilized glycosylation site. Neutralization by N-MAb ST-3:1 was also affected by amino acid changes at position 96. Reactions with these N-MAbs show that serotype G2 viruses can be divided into monotypes and confirm the observation that serotype G4 rotaviruses can be subdivided into subtypes/monotypes a and b. The G2 monotypes relate to differences at particular amino acids within antigenic region C and possibly region B, whereas antigenic region A is most important for G4 monotype differentiation.

Introduction

The two rotavirus outer capsid proteins, VP4 (encoded by gene 4) and VP7 (encoded by gene 7, 8 or 9) elicit neutralizing and protective antibodies. VP4 forms the spikes projecting from the virion surface (Prasad et al., 1988) and is associated with protease enhancement of viral infectivity, growth restriction in culture, viral virulence and protease-sensitive or P serotypes.

Glycoprotein (G) serotypes are defined using antisera or neutralizing monoclonal antibodies (N-MAbs) reactive with VP7, and are more clearly distinguishable serologically than P types. A full understanding of the antigenic diversity of rotaviruses is necessary for rotavirus vaccine development, particularly as at least nine of the known G types have been recorded in humans and types 1 to 4 occur frequently.

Nucleotide sequence analysis of rotavirus variants selected for resistance to neutralization by VP7-specific MAbs, has led to the definition of six antigenic regions on VP7: region A (aa 87–101); region B (aa 142–152); region C (aa 208–221); region D (aa 291); region E (aa 189–191) and region F (aa 238–242) (Coulson & Kirkwood, 1991; Dunn et al., 1993; Dyall-Smith et al., 1986; Kirkwood et al., 1993; Lazdins et al., 1995). All antigenic regions except D correspond to regions of sequence on VP7 which are divergent between rotavirus serotypes (Glass et al., 1995; Green et al., 1987). All are widely separated on the linear sequence, but may participate in conformation-dependent neutralization, either as a single domain or as multiple domains.

Antigenic variation between the neutralization epitopes of VP7 of rotaviruses of the same serotype has also been described. Coulson (1987) showed that G1 strains reacted variably in ELISA with G1-neutralizing polyclonal and MAbs, and termed these antigenic variants 'monotypes'. G1 monotype (a, b or c) is related to the presence of particular amino acids in antigenic regions A, B or C (Coulson & Kirkwood, 1991).
Two subtypes of G4 rotaviruses have been distinguished with cross-adsorbed polyclonal antisera using solid-phase immune electron microscopy (Gerna et al., 1985). Strains designated subtype A (ST-3-like) and subtype B (VA70-like) also were clearly distinguishable as monotypes by ELISA using N-MAbs raised to ST-3 and VA70 viruses (Gerna et al., 1988a, b, 1989). Comparisons of deduced aa sequences of VP7 from prototype and field strains have suggested that G4 monotypic differences may result from aa substitutions in region A (aa 96) and/or C (aa 212 or 217) (Green et al., 1992; Palombo et al., 1993). However, the epitopes necessary for neutralization of virus by the N-MAbs have not been mapped.

Variable reactivities of G2-specific N-MAbs with G2 rotaviruses by ELISA have been reported (Raj et al., 1992; Ward et al., 1991) suggesting that monotypes may exist within G2 viruses as well. Recently, we have mapped the mutations selected in virus variants resistant to five G2-specific N-MAbs to antigenic regions A, C and E (Lazdins et al., 1995).

In this study, the existence of G2 monotypes is proposed and the amino acids important in distinguishing G2 and G4 monotypes have been examined, using N-MAbs developed in our laboratories. N-MAbs to G2 rotavirus RV-5 were reacted with a panel of cultivable rotaviruses, including reference G2 viruses RV-5, DS-1, S2 and 1076. Sequence analysis of the gene encoding VP7 of G2 rotavirus 1076 and comparison of the VP7 gene sequence with that of the other G2 reference strains enabled identification of monotypes within G2 rotaviruses and identification of amino acids likely to be important in the determination of G2 monotypes.

G4 monotypes were studied by the selection and characterization of antigenic variants resistant to the N-MAbs specific for subtype/monotype 4A and 4B. Sequence analysis of the gene encoding VP7 of the human G4 rotavirus Hosokawa assisted in identification of amino acids involved in determination of G4 monotypes.

**Methods**

*Viruses.* The human G2 rotaviruses RV-5 and RV-6 were isolated in Melbourne (Albert & Bishop, 1984). The origins of human G4 rotaviruses ST-3, VA70, Hosokawa (Hoso) and human G2 rotaviruses DS-1 and S2, have been described (Coulson et al., 1986). The Australian porcine rotavirus BEN144 was isolated by Nagesha et al. (1989). Rotavirus G2 strain 1076 was provided by Dr B. Tufvesson (Trelleborg Hospital, Trelleborg, Sweden) and Dr K. Green (NIH, Bethesda, Maryland, USA). All viruses were propagated in MA104 cells with trypsin, as described previously (Coulson et al., 1986).

*N-MAbs.* N-MAb ST-3:1 (raised to VP7 of ST-3 virus) is subtype 4A-specific and has been described previously (Coulson et al., 1986). N-MAbs F45:1, F45:2 (raised to VP7 of G9 virus F45) and N-MAbs RV-5:1, RV-5:3, RV-5:4, RV-5:5, N3 (raised to VP7 of RV-5 virus) have also been described, characterized and mapped to antigenic regions of VP7 by selection of antigenic variants by the N-MAbs (Coulson et al., 1986; Kirkwood et al., 1993; Lazdins et al., 1995). N-MAb 3A3 (raised to VP7 of VA70 virus) has been shown to be subtype 4B-specific (Gerna et al., 1988b). N-MAbs ST-3:1 and RV-5:3 have been used extensively to serotype field strains of rotaviruses (Bishop et al., 1991; Coulson et al., 1987; Gerna et al., 1988a, b, 1989; Raj et al., 1992; Ward et al., 1991) as at working dilutions of 1:2000, they have proven to react by ELISA in a serotype-specific, rather than monotype-specific, manner (Coulson et al., 1987). N-MAb F45:1 has been used to serotype G9 rotavirus field strains (unpublished data).

N-MAbs ST-3:2 and ST-3:4 were produced by fusion of splenocytes from mice immunized intraperitoneally with P3-X63-Ag8 653 (clone 653) cells as described previously (Coulson et al., 1985, 1986). Partially purified ST-3 rotavirus grown with a trypsin level of < 0.5 μg/ml was used as immunogen (Coulson, 1993). Hybridoma cell screening, subcloning, ascites production and N-MAb purification were carried out as described previously (Coulson et al., 1985). Determination of N-MAb titres in mouse ascites fluid was done by fluorescent focus reduction neutralization (FFN) assay (Coulson et al., 1985) in which the titre at which a 50% reduction in numbers of fluorescent foci occurred was taken as the endpoint. N-MAbs were titrated by direct binding ELISA, in which N-MAbs were reacted with polystyrene-adsorbed, partially purified virus (Coulson, 1993) and some purified G4 N-MAbs were also titrated by serotyping ELISA, by reaction with virus attached to the solid phase via a rabbit hyperimmune antiserum to ST-3 virus (Coulson et al., 1987). The specificity of N-MAbs ST-3:1, ST-3:2 and ST-3:4 for VP7 was determined by their FFN titres with rotaviruses ST-3, SA11 and the reassortant virus ST-3 × SA11, which has all RNA segments derived from SA11 except that coding for VP7, which came from ST-3. Unless stated, all N-MAbs were used as ascites fluids.

*Antigenic variant selection.* Antigenic variants resistant to N-MAbs ST-3:1 (VST-3:1) and 3A3 (V3A3) were selected using rotaviruses ST-3 and VA70, respectively, as parents. Selection of variants of ST-3 and reassortant virus ST-3 × SA11 by N-MAbs ST-3:2 and ST-3:4 was also attempted although repeated selections were unsuccessful, as has occurred occasionally previously (Lazdins et al., 1995). Variants were selected and plaque-purified three times as described previously (Kirkwood et al., 1993). In each instance, four variants resistant to each N-MAb were derived from two independent selections, and were all titrated by FFN assay. All variants selected with each N-MAb showed similar neutralization resistance patterns with the appropriate N-MAb panel.

*RT-PCR amplification of RNA and nucleotide sequencing.* RNA purification and RT-PCR of the RNA segment encoding VP7 of Hoso, 1076, parental viruses and all antigenic variants were conducted as previously described by Kirkwood et al. (1993). Direct thermal cycling dideoxynucleotide chain-termination sequencing analysis was performed using the fmol DNA sequencing system (Promega) and specific oligonucleotide primers. These primers were selected to permit the determination of the nucleotide sequence of the antigenic regions A, B, C, D, E and F for Hoso, 1076, variant viruses and parent strains. The VP7 gene of two variant clones was sequenced, as the four variants from each N-MAb selection which were tested gave similar neutralization resistance patterns with the appropriate N-MAbs. In all cases, at least 70% of the gene encoding VP7 was sequenced and both variant clones showed identical single mutations. We and others have selected variants with more than 20 of our VP7-reactive N-MAbs to date, and have found only single amino acid mutations when homologous parent virus is used (Coulson & Kirkwood, 1991; Dyall-Smith et al., 1986; Kirkwood et al., 1993; Lazdins et al., 1995).
Results

Antigenic regions on VP7 which correlate with neutralization of 1076 virus by G2 N-MAbs

The reciprocal FFN titres of five G2-specific N-MAbs with G2 viruses are shown in Table 1. All the N-MAbs neutralized G2 rotaviruses DS-1, RV-5 and RV-6 to similar high titres. All of the N-MAbs except N3 neutralized S2 virus to lower titres. N-MAbs RV-5:3 and RV-5:4 neutralized 1076 virus at a very low level, just above the positive/negative cut-off. The direct ELISA titres of the N-MAbs with the entire panel of rotaviruses, including G types 1–6, 8–10 and 12, paralleled the FFN titres (data not shown).

These results showed that the 1076 and S2 strains both differed in neutralization epitopes from DS-1, RV-5 and RV-6 viruses and that 1076 virus might be a monotype within G2 rotaviruses. In order to determine the molecular basis of these differences between 1076, S2 and the other G2 viruses, the nucleotide sequence of the gene encoding the VP7 of 1076 virus was determined for the antigenic regions A to F. The deduced amino acid sequence was compared with that of other G2 viruses and with neutralization by N-MAbs RV-5:3 and RV-5:4 (Table 2).

Neutralization by N-MAbs RV-5:3 and RV-5:4 correlated with the presence of threonine at aa 147 (region B), asparagine at aa 213 (region C) and isoleucine at aa 217 (region C). The 1076 rotavirus showed amino acid differences from RV-5 and DS-1 at each of these positions, and was not detected by the N-MAbs. The S2 virus showed a single amino acid difference at aa 213 only, and was detected by the N-MAbs at a low level only.

Neutralization and binding specificities of the G4 N-MAbs

The FFN titres of the N-MAbs with G4 rotaviruses are shown in Table 3. Each N-MAb showed a distinct neutralization profile. N-MAb ST-3:1 neutralized all human G4 rotaviruses but not the porcine strain, BEN144. N-MAbs ST-3:2 and ST-3:4 neutralized ST-3 and BEN144 viruses, with ST-3:4 also neutralizing strain Hosu. N-MAb 3A3 neutralized parent subtype 4B strain VA70 only.

The binding titres of the N-MAbs to virus measured by direct ELISA (data not shown), generally paralleled the neutralization titres. In particular, reciprocal ELISA titres with the panel of mammalian rotaviruses of G

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Table 1. FFN titres of G2-specific N-MAbs with G2 rotaviruses

<table>
<thead>
<tr>
<th>Reciprocal titre of N-MAb*</th>
<th>RV-5:4 (94, 213)</th>
<th>RV-5:3 (96, 213)</th>
<th>N3 (94)</th>
<th>RV-5:5 (190)</th>
<th>RV-5:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV-5, RV-6, DS-1</td>
<td>3 x 10^-6 - 10^4†</td>
<td>6 x 10^4 - 8 x 10^4†</td>
<td>1 x 10^3 - 3 x 10^3†</td>
<td>2 x 10^3 - 9 x 10^3†</td>
<td>1 x 10^1†</td>
</tr>
<tr>
<td>S2</td>
<td>5 x 10^4†</td>
<td>7 x 10^4†</td>
<td>2 x 10^4†</td>
<td>2 x 10^4†</td>
<td>9 x 10^4†</td>
</tr>
<tr>
<td>1076</td>
<td>100</td>
<td>600</td>
<td>2 x 10^9</td>
<td>4 x 10^1</td>
<td>4 x 10^1</td>
</tr>
</tbody>
</table>

* FFN titres of all N-MAbs with a large panel of mammalian rotaviruses of G types 1, 3–6, 8–10, 12 and P genotypes 2–11, 13–15 were negative (<100).
† Numbers in brackets indicates position of amino acid change selected by the N-MAbs in the VP7 of antigenic variants of RV-5 (first position) and in antigenic variants of the reassortant rotavirus RV-5 x SA11 (second position), Lazdins et al. (1995).
‡ Titres from Coulson et al. (1986).

Table 2. Comparison of amino acid sequences in VP7 antigenic regions of G2 rotaviruses with neutralization by N-MAbs RV-5:3 and RV-5:4

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sensitivity to N-MAbs*</th>
<th>Amino acid sequence of antigenic region†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B (aa 142–152)</td>
</tr>
<tr>
<td>RV-5, DS-1</td>
<td>2+</td>
<td>M R Y D N T S E L A</td>
</tr>
<tr>
<td>S2</td>
<td>1+</td>
<td></td>
</tr>
<tr>
<td>1076</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* From Coulson et al. (1986) and Table 1.
† The VP7 amino acid sequence of 1076 virus was determined for aa 24 to 115, aa 142 to 223 and aa 236 to 304. The amino acid sequence of the given rotavirus strain was obtained from the following references: RV-5 (Dyall-Smith & Holmes, 1984); DS-1 and S2 (Green et al., 1987). No amino acid sequence differences between rotaviruses RV-5, DS-1, S2 and 1076 were observed in antigenic regions A, D, E and F.
Table 3. FFN titres of G4-reactive N-MAbs with G4 rotaviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Serotype and subtype</th>
<th>ST-3:1</th>
<th>ST-3:2</th>
<th>ST-3:4</th>
<th>3A3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-3</td>
<td>4A</td>
<td>$1 \times 10^7$</td>
<td>$6 \times 10^4$</td>
<td>$&gt; 1 \times 10^6$</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>Hoso</td>
<td>4A†</td>
<td>$1 \times 10^7$</td>
<td>130</td>
<td>$3 \times 10^8$</td>
<td>400</td>
</tr>
<tr>
<td>VA70</td>
<td>4B</td>
<td>$8 \times 10^7$</td>
<td>100</td>
<td>&lt; 100</td>
<td>$2 \times 10^8$</td>
</tr>
<tr>
<td>BEN144</td>
<td>4</td>
<td>&lt; 100</td>
<td>$1 \times 10^4$</td>
<td>$4 \times 10^8$</td>
<td>&lt; 100</td>
</tr>
</tbody>
</table>

* FFN titres of N-MAb ST-3:1 with a large panel of mammalian rotaviruses have been published (Coulson et al., 1986). Reciprocal titres of N-MAbs ST-3:2 and ST-3:4 with the panel (comprising strains of G types 1, 3-6, 8-10, 12 and P genotypes 2-11, 13-15) were all negative (< 100).
† Determined in this study.
‡ Titres from Coulson et al. (1986).

Table 4. Neutralization of ST3, VA70 and their variant viruses by the selecting N-MAb panel, and other cross-reactive N-MAbs

<table>
<thead>
<tr>
<th>Virus</th>
<th>Reciprocal FFN titre with N-MAbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-3</td>
<td>ST-3:1</td>
</tr>
<tr>
<td>ST-3</td>
<td>$1 \times 10^6$</td>
</tr>
<tr>
<td>VST-3:1</td>
<td>200*</td>
</tr>
<tr>
<td>VA70</td>
<td>$8 \times 10^3$</td>
</tr>
<tr>
<td>V3A3</td>
<td>250</td>
</tr>
</tbody>
</table>

* Single underlining indicates variants that became sensitive, double underlining indicates those that became resistant.

The epitopes detected by N-MAbs ST-3:1 and 3A3 are non-identical but overlapping and that the antigenic determinants recognized by N-MAbs F45:1 and F45:2 in the antigenic C and F regions, respectively (Kirkwood et al., 1993) are related to that detected with N-MAb 3A3. Interestingly, in the reverse of the usual resistance pattern, V3A3 was sensitive to N-MAb ST-3:4, even though the parent virus VA70 was totally resistant to neutralization by N-MAb ST-3:4.

The single nucleotide changes detected in the antigenic variants when their sequences were compared with those of the parent viruses were at position 331 (G → A) in VST-3:1 and at position 337 (A → C) in V3A3. VST-3:1 showed an aa mutation at position 94, from serine to asparagine, producing a new potential glycosylation site of the type N-X-T. Western blot analysis of this virus showed an upward mobility shift of the VP7 of VST-3:1 compared with ST-3 virus, showing that this new potential site is utilized (data not shown). V3A3 showed a substitution of threonine for asparagine at aa 96. This resulted in an A region amino acid sequence identical to that of ST-3 and Hoso viruses.

A comparison of the amino acid sequences of the antigenic regions of G4 rotaviruses and variants with the virus sensitivity to N-MAbs ST-3:1, 3A3 and ST-3:4 is


types 1, 3–6, 8–10 and 12 were all < 1000 and considered to be negative. However, N-MAb ST-3:4 showed a reciprocal titre by direct ELISA of $1 \times 10^6$ with VA70, but did not neutralize this virus, and N-MAb 3A3 bound to BEN144 (reciprocal titre: $3 \times 10^5$) without neutralizing it.

In the sensitive serotyping ELISA format (data not shown) both the G4A and G4B-specific N-MAbs cross-reacted with viruses of the other monotype. However, the G4A N-MAbs ST-3:1 and ST-3:4 reacted to 100-fold higher reciprocal titre with G4A viruses ($6 \times 10^6$) than with the G4B strain VA70 ($6 \times 10^4$), and N-MAb 3A3 reacted to 30-fold higher reciprocal titre with VA70 ($1 \times 10^6$) than with G4A strains ($3 \times 10^4$).

Location of antigenic sites on VP7 recognized by G4 N-MAbs

The neutralization resistance patterns of variant rotaviruses VST-3:1 and V3A3 with the VP7 N-MAbs specific for G4 viruses and two N-MAbs cross-reactive between VP7 of G9 and G4 rotaviruses (F45:1, F45:2) are shown in Table 4. The VST-3:1 was resistant only to the selecting antibody. However, V3A3 was resistant to N-MAbs ST-3:1, F45:1 and F45:2. This suggests that the epitopes detected by N-MAbs ST-3:1 and 3A3 are non-identical but overlapping and that the antigenic determinants recognized by N-MAbs F45:1 and F45:2 in the antigenic C and F regions, respectively (Kirkwood et al., 1993) are related to that detected with N-MAb 3A3. Interestingly, in the reverse of the usual resistance pattern, V3A3 was sensitive to N-MAb ST-3:4, even though the parent virus VA70 was totally resistant to neutralization by N-MAb ST-3:4.

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A comparison of the amino acid sequences of the antigenic regions of G4 rotaviruses and variants with the virus sensitivity to N-MAbs ST-3:1, 3A3 and ST-3:4 is
Monotypes in rotavirus G types 2 and 4

In this study, monotypes within G2 rotaviruses have been described and the molecular basis of G2 and G4 monotypes has been studied.

Two G2-specific N-MAbs, including one (RV-5:3) routinely used for G-typing field strains of rotavirus, failed to react with 1076 rotavirus. In earlier studies (Lazdins et al., 1995) these N-MAbs selected variants with single mutations in antigenic region A from homologous parent virus RV-5, at aa 94 (RV-5:3) and at aa 96 (RV-5:4). However, variants selected by these N-MAbs from the reassortant rotavirus RV-5 × SA11, which had all genes from SA11 except the gene encoding VP7 which was from RV-5, showed either the same region A mutation, or a single amino acid change at aa 213 in region C (Lazdins et al., 1995). The mutation selected at aa 213 in the variants (asparagine to serine) was identical to the difference observed at aa 213 between RV-5 rotavirus (asparagine) and 1076 rotavirus (serine). Rotavirus 1076 was identical in sequence with the other G2 viruses in region A, but differed in sequence at aa 147, 213 and 217. This suggests that asparagine at aa 213 was most critical for reaction with these N-MAbs, but that aa 147 and 217 might also be involved. We define monotype 2a rotaviruses, such as RV-5, DS-1, S2 and RV-6, to be those which react with N-MAbs RV-5:3 and RV-5:4, and monotype 2b viruses, like 1076, to be those which show reciprocal titres of less than 1000 by FFN, and less than 4000 by direct ELISA with these N-MAbs.

Rotavirus S2, although classified as monotype 2a, showed reduced titres with these N-MAbs, which may relate to the particular aa 213 difference between S2 and RV-5, DS-1 and RV-6 viruses. S2 had the conservative substitution of aspartic acid for the asparagine in RV-5, DS-1 and RV-6, whereas 1076 showed a non-conservative substitution of serine at aa 213.

N-MAb RV-5:3 was shown previously to be un-
reactive with three of 23 culture-adapted G2 rotaviruses from Bangladesh, probably due to epitope changes (Ward et al., 1991). This, and the effectiveness of N-MAb RV-5:3 in serotyping G2 rotaviruses in many studies (Coulson et al., 1987; Gerna et al., 1988a, b, 1989) suggests that G2b rotaviruses have been uncommon. However, serotype G2 rotaviruses have been detected (causing recent epidemics of rotavirus gastroenteritis in Alice Springs and Darwin in the Northern Territory of Australia) which fail to react with N-MAbs RV-5:3 and RV-5:4 (B. S. Coulson, R. F. Bishop & P. Masendycz, unpublished observations). Sequence analysis of the VP7 gene in these viruses is underway.

Neutralization by the human G4A-specific N-MAb ST-3:1 was affected by aa mutations at positions 94 and 96. The subtype 4B N-MAb 3A3 required asparagine at aa 96 for neutralization, whereas the G4A-specific N-MAb ST-3:4 required threonine at aa 96. The overlap in epitopes recognized by these N-MAbs is therefore most likely to relate to aa 96. The conversion of the antigenic region A of G4B virus VA70 to that of G4A viruses ST-3 and Hosu by a single amino acid change in V3A3 at aa 96, led to neutralization of V3A3 by G4A reactive N-MAb ST-3:4. Thus, conversion from one monotype to another may occur with a single amino acid change. Our data is consistent with that from a previous VP7 sequence comparison between G4A and G4B strains, which showed that subtype A and B strains have different, conserved amino acids at positions 96, 212 and 217 (Green et al., 1992). We have extended these observations to show that the amino acids at positions 94 and 96 are the ones most critical to monotype determination.

Some cross-reactivity of both G2 and G4 monotype-specific N-MAbs by ELISA within their G type was observed in this study. However, N-MAbs ST-3:1, ST-3:4, 3A3, RV-5:3 and RV-5:4 all reacted to at least 16-fold higher titre with one monotype over the other. By titration with the N-MAbs, or absorbance ratio calculation, monotyping of stool-derived G2 and G4 rotavirus strains is feasible and has been achieved for G4 strains (Gerna et al., 1988b).

It could be argued that changes other than those observed in the sequenced regions could influence the reactivity with neutralizing antibodies. However, most escape mutants sequenced so far in VP7 had all their changes in the regions A to F investigated here, even after selection with polyclonal antibodies (Gorziglia et al., 1990).

The clinical significance of monotypes and subtypes is unclear. However, changes in rotavirus serotype 1 and 4 monotypes have been associated with rotavirus seasons of unusually prolonged duration in hospitalized children in Melbourne (Coulson, 1987; Bishop et al., 1991; Palombo et al., 1993). It may be that rotavirus monotype changes have a similar epidemiological effect to that of antigenic drift in influenza viruses. Further monitoring of the seasonal occurrence of rotavirus G1, 2 and 4 monotypes causing gastroenteritis, in a range of geographical locations, is needed to answer this question.

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