Comparison of Enzyme Immunoassay and Reverse Transcriptase PCR for Identification of Serotype G9 Rotaviruses

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While only four globally important rotavirus G serotypes (1 to 4) have been documented, many studies suggest that serotype G9 viruses may be widely distributed and more important than previously recognized. We have evaluated 10 serotype G9 rotavirus-neutralizing monoclonal antibodies (MAbs) directed to VP7, which bind by direct enzyme immunoassay (EIA) to P1A[8], G9 rotaviruses F45, WI61, and AU32, for their ability to recognize the New Delhi G9 rotavirus 116E. Only one MAb (MAb F45:1) bound to P[11], G9 virus 116E to a high titer by EIA. This MAb was incorporated into an indirect EIA for G serotyping, which was validated with prototype cultivable human rotaviruses of G types 1 and 9. The EIA was compared with genotyping by reverse transcriptase PCR (RT-PCR) under code for the determination of the G types of rotaviruses obtained from neonates in New Delhi, India. The sensitivities of RT-PCR and EIA (after two additional freeze-thaw cycles) for the typing of G9 rotaviruses were 91 and 86%, respectively, for 24 culture-adapted rotavirus strains. The untypeable culture-adapted rotavirus samples also were unreactive with VP7 group antigen-reactive MAb 60. After two additional freeze-thaw cycles, only 26 of 42 (62%) of stools containing rotavirus typed as G9 by RT-PCR were positive for G9 rotavirus by EIA. Stools containing rotavirus untypeable by EIA contained significantly less MAb 60-reactive VP7 antigen (P = 0.0001) than the stools containing typeable rotavirus. Thus, RT-PCR genotyping was the more sensitive method for determination of G9 type, but a serotype was readily determined in rotavirus samples containing MAb 60-reactive VP7 antigen by an EIA that incorporates MAb F45:1.

Group A rotaviruses are the major etiologic agents of severe acute diarrhea in infants and young children worldwide (33). Infectious virions comprise six structural proteins in three protein layers enclosing 11 segments of double-stranded RNA (dsRNA). Rotavirus serotype classification is based on differences in antigenic determinants that elicit neutralizing antibodies on the major component of the outer capsid, VP7 (G serotypes), and the spike protein, VP4 (P serotypes), whose proteolytic cleavage activates rotavirus infectivity. VP7 is a glycoprotein encoded by gene segment 7, 8, or 9, whereas VP4 is encoded by gene segment 11. VP7 numbers, with letters used to designate current subtypes. For example, the prototype human rotavirus strain RV-4 is designated P1A[8], G1 (18). In this paper, the G types of rotaviruses for which only the G genotype has been determined also will be indicated with brackets.

Numerous epidemiological studies have shown that G1 ro-
taviruses predominate worldwide as a cause severe rotavirus gastroenteritis, with G2, G3, and G4 strains being responsible for the majority of the residual disease (22). Most P-genotyping studies have shown that the rotaviruses of G1, G3, and G4 are P[8] and that the G2 strains are associated with P[4]. When the P serotypes of these G1 to G4 rotaviruses have been determined, they generally correspond to the genotype determined or to the P type predicted (4, 6, 42), so that, in descending order, the predominant rotaviruses that cause disease are P[8] G1, P[8] G4, P[4] G2, and P[8] G3 (22).

Although rotaviruses of the G9 serotype have been found less often than serotypes G1 to G4, they have been important causes of diarrhea in India (43), Bangladesh (50), and the United States (44). P[8] G9 rotavirus W161 was isolated in Philadelphia, Pa., in 1983 and 1984, and viruses of this RNA electropherotype caused 9% of rotavirus disease at that time (3). In Japan in 1985 and 1986, 12% of cases of rotavirus disease in Yamagata (39) and 52% of cases of rotavirus disease in Osaka (32) were attributed to G9 rotaviruses, of which F45 (P1A[8], G9) was the most commonly detected type in children with diarrhea (43). P[11], G[9] rotaviruses, represented by culture-adapted strain 116E (20), and P[6], G[9] rotaviruses were the predominant strains isolated from neonates in New Delhi, India, between 1986 and 1993 (12). The G serotype of 116E was determined to be G9 by cross-neutralization with hyperimmune antiserum (43) and was representative of the majority of the New Delhi G9 rotaviruses, which also were P[11]. A minority of these G9 rotaviruses were P[6] (12).

Materials and Methods

Viruses. The following prototype cultivable rotavirus origins whose have been described previously (4, 8, 10) were used for the evaluation of the G9-reactive MAbs for serotyping: human reports (RV-4 (P1A[8], G1), RV-5 (P1B[4], G2), ST-5 (P2A[6], G4), Hosokawa (P1A[8], G4), and F45, W161, and AU32 (P1A[8], G9); saimin virus SA11 (P[2], G3); and porcine virus TFR41 (P2B[7], G4). The P[11], G[9] rotavirus 116E was isolated from a New Delhi neonate infected asymptptomatically (13). Rotaviruses were propagated in MA 104 cells in the presence of 1 μg of porcine trypsin (Sigma) per ml following activation with 10 μg of porcine trypsin per ml as described previously (10). Rotaviruses and mock-infected MA 104 cells (for use as a negative control) were partially purified as described previously for EIA antigen (4) for use in direct and serotyping EIA formats.

A panel of 50 stool samples and 24 culture-adapted rotaviruses isolated from a separate set of stools from newborn infants at six government hospitals in New Delhi between 1986 and 1988 and between 1992 and 1993, as well as strains from a longitudinal study (1, 12), were evaluated. Since the serotype of the viruses in almost all of the stools and culture-adapted rotaviruses from which the 74 samples were selected were G[9] by RT-PCR (12), the selection of the samples for study was random. A few samples with non-G[9] rotaviruses were chosen to serve as controls, and the viruses in these samples were representative of the small number of non-G[9] rotaviruses present in this population. G9 rotavirus strain 116E was serotyped by cross-neutralization with hyperimmune antiserum (13) and was representative of the majority of the New Delhi G9 rotaviruses, which also were P[11]. A minority of these G9 rotaviruses were P[6] (12).

MAbs. The derivation and characterization of the VP7-specific, rotavirus-neutralizing MAbs and selection of rotavirus escape mutants with these MAbs have been described previously (5-11, 17, 34-36). Antibody designation, immunoglobulin class, and G-serotype specificity are summarized in Table 1. Antibodies were titrated against prototype cultivable rotaviruses by using a direct EIA, in which partially purified virus or cell control antigen was adsorbed to the solid phase and then serial twofold dilutions of MAbs were added and allowed to bind to immobilized virus. Bound antibody was detected with horse-radish peroxidase (HRP)-conjugated anti-mouse immunoglobulins (Silenus, Melbourne, Victoria, Australia) and then with substrate containing 3,3',5,5'-tetramethylbenzidine (TMB; Sigma Chemical Co., St. Louis, Mo.) (4, 10, 11). MAb F45-1 was also titrated by the serotyping EIA format (see below). Neutralization titers of MAbs against prototype cultivable rotaviruses were determined by fluorescent focus reduction neutralization (FFN) assay, as described previously (7).

Serotyping EIA. The EIA method for G types 1 to 4 has been described previously (6, 11, 49). It was adapted to include G9 specificity by the inclusion of rabbit hyperimmune antisera raised to F45 rotavirus (11) diluted 1 in 4,000 in phosphate-buffered saline (PBS; pH 7.4) to coat the solid phase and MAb F45-1 to detect bound virus. Otherwise, the EIA was performed as described previously. In brief, for each sample to be tested, wells of a microtiter plate were coated separately with rabbit hyperimmune antisera to each of rotavirus G types 1 to 4 and 9. Test samples diluted in PBS containing 0.05% (vol/vol) Tween 20 and 2.5% (wt/vol) skim milk powder (PBS-T-SMP) were added, followed by the addition of purified, serotype-specific MAbs diluted in PBS-T-SMP relative to ascitic fluid as indicated: RV-4:2 (G1-specific), 1 in 1,000; RV-5:3 (G2-specific), 1 in 4,300; RV-3:1 (G3-specific), 1 in 20,000; ST-3:1 (G4-specific), 1 in 4,500; F45-1, 1 in 10,000; F45-9, 1 in 2,000; and W161-1, 1 in 10,000. A VP7-cross-reactive, nonneutralizing MAb, MAb 60 (41, 46), was also included.

### Table 1. Reciprocal FFN and EIA titers of G9 rotavirus-neutralizing MAbs with G9 rotaviruses

<table>
<thead>
<tr>
<th>MAb</th>
<th>Reciprocal FFN titer with 116E</th>
<th>Fold change in FFN titer with 116E over other that to G9 virus</th>
<th>Reciprocal direct EIA titer with 116E</th>
<th>Fold change in EIA titer with 116E over other that to G9 virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>F45:6</td>
<td>9, 5 M</td>
<td>1 x 10^4</td>
<td>-&lt;1,000 to -200</td>
<td>1 x 10^3</td>
</tr>
<tr>
<td>F45:5</td>
<td>9</td>
<td>5 x 10^4</td>
<td>-16 to -60</td>
<td>3 x 10^5</td>
</tr>
<tr>
<td>W161:1</td>
<td>9, 8, 5 M</td>
<td>5 x 10^4</td>
<td>-20 to -4.0</td>
<td>6 x 10^4</td>
</tr>
<tr>
<td>F45:7</td>
<td>9, 5</td>
<td>2 x 10^4</td>
<td>-150 to -50</td>
<td>2 x 10^5</td>
</tr>
<tr>
<td>F45:9</td>
<td>9</td>
<td>1 x 10^4</td>
<td>-10 to -0.5</td>
<td>4 x 10^4</td>
</tr>
<tr>
<td>F45:7</td>
<td>9</td>
<td>&lt;1 x 10^4</td>
<td>&lt;10^4 to &lt;100</td>
<td>5 x 10^5</td>
</tr>
<tr>
<td>RV-3:2</td>
<td>3, 9, 5</td>
<td>G2b</td>
<td>3 x 10^4</td>
<td>-170 to -67</td>
</tr>
<tr>
<td>RV-3:4</td>
<td>3, 9</td>
<td>G1</td>
<td>4 x 10^4</td>
<td>&lt;50 to &gt;4.0</td>
</tr>
<tr>
<td>RV-3:1</td>
<td>9, 4</td>
<td>G2a</td>
<td>&gt;1 x 10^5</td>
<td>1.0 to &gt;5.0</td>
</tr>
<tr>
<td>F45:2</td>
<td>9, 3, 4 A</td>
<td>4 x 10^4</td>
<td>-2.5 to 4.0</td>
<td>8 x 10^5</td>
</tr>
</tbody>
</table>

### Notes

- a Determined with an EIA kit with subclass- and isotype-specific antisera (Commonwealth Serum Laboratories, Parkville, Victoria, Australia).
- b The other G9 rotaviruses tested were F45, W161, and AU32. FFN and EIA titers of MAbs F45:1, F45:2, F45:5, F45:6, F45:7, F45:8, F45:9, and W161:1 with these rotaviruses were determined previously (34).
- c Described previously (7).
A panel of 10 MAbs was evaluated for the ability to bind EIA.

ble prototype rotaviruses by FFN, direct EIA, and serotyping the highest dilution that gave the maximum optical density at single dilution of the MAb was chosen; the dilution chosen was G1 to G3 and G5 rotaviruses. For use in the serotyping EIA, a with the G4 strains Hoso and ST-3, and at a very low titer with representing G types 1 to 5 and 9 (Table 2). This MAb reacted at EIA format with 10 prototype cultivable rotavirus strains rep-
near G types 1 to 5 and 9 (Table 2). This MAb reacted at EIA format with 10 prototype cultivable rotavirus strains rep-

It was diluted 1 in 2,000 in PBS-T-SMP and was used to detect the presence of VP7 antigen in wells coated with antisera to F45 rotavirus. Bound MAbs were detected by addition of HRP-conjugated antimouse immunoglobulins and TMB substrate as described above. Optimal dilutions of reagents were determined by checkerboard titration.

G typing by RT-PCR. Genomic dsRNA was extracted from fecal samples containing rotavirus by the glass powder method (21). Stocks of rotavirus grown in MA 104 cells were frozen and thawed three times and were then clarified by low-speed centrifugation to remove cell debris. The dsRNA in the supernatant was extracted by the phenol-chloroform method, followed by ethanol precipit-

Nonreactive

TABLE 2. Reactivities of MAbs used to distinguish G types 1 to 4 and 9 by serotyping EIA with a panel of cell culture-adapted rotaviruses

<table>
<thead>
<tr>
<th>Rotavirus strain</th>
<th>G serotype</th>
<th>Reciprocal titer of MAb F45:1 in serotyping EIA formata</th>
<th>OD450 with given MAb (MAb G serotype specificity)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F45:1 (9)</td>
<td>RV-4:2 (1)</td>
</tr>
<tr>
<td>116E</td>
<td>9</td>
<td>&gt;1 x 10⁷</td>
<td>1.283</td>
</tr>
<tr>
<td>F45</td>
<td>9</td>
<td>&gt;1 x 10⁷</td>
<td>2.768</td>
</tr>
<tr>
<td>Wi61</td>
<td>9</td>
<td>&gt;1 x 10⁷</td>
<td>0.970</td>
</tr>
<tr>
<td>AU32</td>
<td>9</td>
<td>&gt;1 x 10⁷</td>
<td>0.463</td>
</tr>
<tr>
<td>Hoso</td>
<td>4</td>
<td>2 x 10⁵</td>
<td>2.586</td>
</tr>
<tr>
<td>ST-3</td>
<td>3</td>
<td>2 x 10⁵</td>
<td>0.130</td>
</tr>
<tr>
<td>RV-4</td>
<td>1</td>
<td>5 x 10⁴</td>
<td>0.127</td>
</tr>
<tr>
<td>RV-5</td>
<td>2</td>
<td>7 x 10⁴</td>
<td>0.133</td>
</tr>
<tr>
<td>SA11</td>
<td>3</td>
<td>5 x 10⁴</td>
<td>0.110</td>
</tr>
<tr>
<td>TFR41</td>
<td>5</td>
<td>5 x 10⁴</td>
<td>0.104</td>
</tr>
</tbody>
</table>

a All rotavirus strains tested reacted with MAb F45:1. However, the dilution of MAb F45:1 used to screen samples in the serotyping EIA was higher than its titers with RV-4, RV-5, SA11, and TFR41, so these rotaviruses were not detected in the serotyping EIA with the single optimal dilution of MAb F45:1.

b Positive reactions in the serotyping EIA, in which MAbs were used at a single optimal dilution, are shown in boldface.

With MAb 60, and their OD450 with this MAb showed a significant correlation with their OD450 with the G-typing MAb(s) that gave a positive result(s) (r = 0.7781, P = 0.01). The G1 to G5 rotaviruses all reacted with the G1- to G4-typing MAbs in the expected pattern. These results showed that the virus samples contained native VP7 in sufficient quantity to be serotypeable. MAb F45:1 reacted strongly with all four G9 rotaviruses and the G4 virus Hosokawa, consistent with its ability to neutralize (34) and bind to this rotavirus. MAb F45:1 did not detect the other G4 rotavirus tested, ST-3, probably because the dilution of the purified MAb used (1 in 10,000 relative to ascitic fluid) was only twofold lower than the end-point titer of this MAb with ST-3 (1 in 20,000). No reaction of MAb F45:1 with G1, G2, G3, or G5 rotavirus was detected, consistent with its use at a dilution of 1 in 10,000 in the sero-
typing EIA.

Comparison of MAb EIA and RT-PCR for determination of G types of culture-adapted and stool rotaviruses. The G types of 24 rotavirus strains that were adapted to culture and that were obtained from stools from New Delhi neonates were determined by RT-PCR and EIA with MAb F45:1 for the determination of G9 rotaviruses (Table 3). Since MAb F45:1 binds to some G4 rotaviruses at the dilution used in the serotyping EIA, we used a correlation of reactivity with MAb F45:1 and bind to this rotavirus. MAb F45:1 did not detect the other G 4 rotavirus tested, ST-3, probably because the dilution of the purified MAb used (1 in 10,000 relative to ascitic fluid) was only twofold lower than the end-point titer of this MAb with ST-3 (1 in 20,000). No reaction of MAb F45:1 with G1, G2, G3, or G5 rotavirus was detected, consistent with its use at a dilution of 1 in 10,000 in the sero-
typing EIA.

Statistical analysis. The correlation between MAb EIA reactivities was exam-

TABLE 3. Comparison of MAb serotyping EIA and RT-PCR for determination of G9 types of cell culture-adapted human rotaviruses

<table>
<thead>
<tr>
<th>G genotype determined by RT-PCR</th>
<th>No. of strains with the following G serotype determined by MAb EIA:</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>13 1 1 0 0 0 0 0 0 1</td>
<td>18</td>
</tr>
<tr>
<td>9+3</td>
<td>1 0 0 0 0 0 0 1</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>0 0 0 0 2 2 2 2 2 2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>0 0 0 0 0 1 1 1 1 1</td>
<td>1</td>
</tr>
<tr>
<td>Nonreactive&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 0 0 0 2 2 2 2 2 2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>16 1 1 1 5 2 2 2 2 2</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup> All samples that contained viral antigen typeable by EIA also reacted with MAb 60, whereas none of the samples that contained nontypeable antigen reacted with MAb 60.

<sup>b</sup> No RT-PCR product was visible, even though a product of the expected size from control prototype rotaviruses was obtained.
were also P[11] by RT-PCR. Identical results were obtained by the two VP7 typing methods for 14 (58%) of strains (13 G9 strains and 1 G3 strain). When either method detected a mixture of G9 virus with another type \((n = 3)\), the non-G9 type was not detected in the alternative system. Neither of the viruses typed as G[2] by RT-PCR was typeable by EIA, and neither reacted with MAb 60. Three strains that were typed as G[9] by RT-PCR were untypeable by EIA. Conversely, two viruses that were typed as G9 by EIA were untypeable by RT-PCR. Thus, of the 21 strains typed as G9 by either method, 19 (91%) were typed by RT-PCR and 18 (86%) were typed by EIA. However, inclusion of MAb WI61:1 as well as MAb F45:9 for G9 rotavirus typing for these stool samples. Used individually, MAb WI61:1 and MAb F45:9 were not as suitable as MAB F45:1 for G9 rotavirus typing for these stool samples. Inclusion of MAb WI61:1 as well as MAb F45:1 for G9 typing increased the overall typing rate by EIA from 62 to 74%, although two (6%) of the reactions with MAb WI61:1 \((n = 32)\) may have been false positive since no MAB 60-reactive antigen was detected in these samples. Interpretation of results obtained with MAB WI61:1 may be complicated by the cross-reactivity of this MAB with G5 and G8 rotaviruses (Table 1).

The G types of rotavirus detectable in 50 stool specimens collected from New Delhi neonates were determined by RT-PCR and EIA with MAB F45:1 (Table 4). As for the cultivable viruses, when either method detected a mixture of G9 virus with another type \((n = 2)\), the non-G9 type was not detected in the alternative system. The single G3 rotavirus was typed by both methods, and four stool samples contained virus untypeable by either method. Of the 42 stool samples in which G9 rotavirus was detected by either method, all were positive by RT-PCR, but only 26 (62%) were positive by EIA. All the stool extracts containing viral antigen typeable by EIA also reacted with MAB 60. However, only 12 (63%) of the stool extracts, which contained rotavirus that was typeable or not, that were tested by RT-PCR and whose viruses were not typeable by EIA \((n = 19)\) contained VP7 antigen detectable with MAB 60. The relation between the level of virus reactivity with MAB 60 and success in obtaining a G serotype by EIA (Fig. 2) suggests that MAB 60 reactivity is a marker for the ability to type VP7 by EIA in stools and virus stocks. Virus reactive with this antibody was G typeable by EIA significantly more often both in stools \((P = 0.0001)\) and in culture \((P = 0.008)\).

Since MAB F45:1 detected only 62% of stool rotaviruses genotyped as G9 by RT-PCR, two additional G9-reactive MABs, MABs WI61:1 and F45:9, were evaluated. These were chosen on the basis of their consistently high EIA titers to cultivable G9 rotaviruses, including strain 116E (Table 1). MAB F45:5 was not evaluated because of its low affinity in the EIA. As shown in Table 5, MAB WI61:1 typed 14 (61%) of the 23 stool G9 rotaviruses typed by MAB F45:1, whereas MAB F45:9 typed only 2 (9%) of these stool viruses. MAB WI61:1 also typed as G9 5 of 16 (31%) of stool viruses that contained G9 virus by RT-PCR but that did not react with MAB F45:1. Used individually, MAB WI61:1 and MAB F45:9 were not as suitable as MAB F45:1 for G9 rotavirus typing for these stool samples. 

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
G genotype determined & No. of strains with the following G & Total \\
by RT-PCR & serotype determined by MAB EIA & \\
\hline
9 & 23 & 0 & 1 & 16 & 40 \\
9+1 & 0 & 0 & 0 & 1 & 1 \\
9+2 & 0 & 1 & 0 & 0 & 0 \\
3 & 1 & 0 & 0 & 1 & 1 \\
Nonreactive & 0 & 0 & 0 & 4 & 4 \\
Not tested & 1 & 0 & 0 & 2 & 3 \\
\hline
Total & 25 & 1 & 1 & 23 & 50 \\
\end{tabular}
\caption{Comparison of MAB serotyping EIA and RT-PCR for determination of G9 type in stool extracts containing human rotaviruses}
\end{table}

\footnotesize{\textsuperscript{a} Only 12 (63%) of the stools containing rotavirus typeable or not tested by RT-PCR and not typeable by EIA \((n = 19)\) contained VP7 antigen detectable with MAB 60, whereas all stools that contained viral antigen typeable by EIA also reacted with MAB 60.}

\footnotesize{\textsuperscript{b} See footnote b of Table 3.}

Since MAB F45:1 detected only 62% of stool rotaviruses genotyped as G9 by RT-PCR, two additional G9-reactive MABs, MABs WI61:1 and F45:9, were evaluated. These were chosen on the basis of their consistently high EIA titers to cultivable G9 rotaviruses, including strain 116E (Table 1). MAB F45:5 was not evaluated because of its low affinity in the EIA. As shown in Table 5, MAB WI61:1 typed 14 (61%) of the 23 stool G9 rotaviruses typed by MAB F45:1, whereas MAB F45:9 typed only 2 (9%) of these stool viruses. MAB WI61:1 also typed as G9 5 of 16 (31%) of stool viruses that contained G9 virus by RT-PCR but that did not react with MAB F45:1. Used individually, MAB WI61:1 and MAB F45:9 were not as suitable as MAB F45:1 for G9 rotavirus typing for these stool samples. Inclusion of MAB WI61:1 as well as MAB F45:1 for G9 typing increased the overall typing rate by EIA from 62 to 74%, although two (6%) of the reactions with MAB WI61:1 \((n = 32)\) may have been false positive since no MAB 60-reactive antigen was detected in these samples. Interpretation of results obtained with MAB WI61:1 may be complicated by the cross-reactivity of this MAB with G5 and G8 rotaviruses (Table 1).
DISCUSSION

We have demonstrated that MAb F45:1 can be used to serotype G9 rotaviruses by EIA. It was found that both culture-adapted and fecal rotaviruses assigned to serotype G9 by RT-PCR genotyping were also G9 by EIA, thus confirming that serotype G9 rotaviruses were a common cause of rotavirus infections of neonates in India in 1993. This study is especially timely because of the recent detection of G9 rotavirus in multiple cities of the United States and in Bangladesh, suggesting with earlier studies that G9 strains probably have a global distribution and may be much more prevalent than was previously believed (13, 33, 39, 43, 44, 50). In view of the recent introduction of the tetravalent rehuss-human reassortant rotavirus vaccine (which contains the VP7 antigens of serotypes G1 to G4 only) in the United States, it will be crucial to conduct large-scale surveillance studies for G9 rotavirus to help determine the effectiveness of the vaccine against these novel strains. Such studies would be facilitated by the availability of EIA-based methods for the serotyping of rotaviruses directly from fecal specimens, as genotyping by RT-PCR is an indirect measure of virus serotype.

It was interesting that of 10 G9-neutralizing MAbs, only one proved to be suitable for detection of all four of the prototype cultivable G9 human rotaviruses by EIA. This was primarily because this was the only MAb that did not show reduced neutralization and binding titers with 116E compared with those with the three other viruses. These results suggest that 116E differs antigenically from the three other G9 rotaviruses at positions that affect VP7 at antigenic regions A, B, and F but not region C. It is possible that 116E is a G9 monotype or subtype (5) different from those of the other G9 strains. Comparison of the amino acid sequences of VP7 of 116E, WI61, F45, and AU32 shows that 116E differs from the other G9 viruses at aa 87 (D to G) and aa 100 (A to I) in antigenic region A and at aa 220 (A to T) and aa 221 (S to N) in antigenic region C. Although MAb-resistant variants with mutations at these positions have not been selected in any rotavirus strain studied, these changes may affect antigenicity. Rotavirus 116E also differs from WI61 and F45 in region B at aa 145 (D to T). This produces a new potential glycosylation site, which, if used, could explain the reduced binding of the B-region MAbs to 116E. A change in region F of 116E at aa 242 (T to N) may also explain the reduced binding of MAb F45:2 to 116E.

It is also possible that differences in other structural proteins, particularly VP4, affect the antigenicity of strain 116E VP7. Interactions between VP4 and VP7 of heterologous par-
rotavirus serotyping by EIA is also highlighted by our comparison of MAbs F45:1, W161:1, and MAb F45:9 for the typing of G9 rotaviruses in stools. All three MAbs showed different reactivity patterns. Of the two A-antigenic region MAbs, W161:1 and F45:9, only W161:1 reacted specifically with a significant number of stool specimens. The EIA reactivity of this MAb overlapped that of MAb F45:1, but it also detected G9 virus in some additional stool samples, including two that were not reactive with MAb 60 by EIA. Should this reactivity prove to be specific for G9 rotaviruses, the use of a combination of MAbs F45:1 and W161:1 may improve the sensitivity of EIA for the detection of G9 rotavirus in stool samples. In addition, other G9 MAbs, such as F45:9, bound to strain 116E at a low titer and barely detected this virus (or G9 rotaviruses in stools) when it was used as a detector antibody in the standard EIA format. Evaluation of these MAbs or hyperimmune antiserum to 116E as capture antibodies might also improve the sensitivity of this assay.

Our findings with Indian neonates need to be extended by evaluation of this panel of MAbs for EIA serotyping of G9 rotavirus-containing fecal samples collected in other locations and at other times from both neonates and older children. It will be of special interest to analyze the G9 strains which have been detected recently in the United States, as the introduction of a universal vaccination campaign against rotavirus with the tetravalent rhesus-human reassortant rotavirus vaccine makes it imperative that the effectiveness of this vaccine against type G9 be understood.

The G serotypes of rotaviruses in stools are most easily and inexpensively determined by an EIA with MAbs. However, RT-PCR is particularly useful for obtaining a rotavirus G genotype in the smaller number of stool samples that contain virus that cannot be typed by EIA. As shown in this study, use of a combination of these methods is advisable to combine maximum sensitivity (RT-PCR) and direct serotype determination (MAb EIA) for the typing of G9 rotaviruses in stools.

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REFERENCES


