Rotavirus Isolate W161 Representing a Presumptive New Human Serotype

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A virus (strain W161) representing a presumptive new human serotype was isolated from an 18-month-old child with gastroenteritis admitted to Children’s Hospital of Philadelphia in February 1983. The W161 virus was clearly distinguished by cross-neutralization tests from human rotaviruses of serotypes 1, 2, 3, and 4, human 69M, and representative bovine (NCDV), porcine (OSU), and chicken (Ch2) rotaviruses. Antisera generated in guinea pigs hyperimmunized to W161 virus displayed a partial cross-reactivity with rotaviruses of human serotypes 1, 2, 3, and 4. By means of studies with reassortant rotaviruses, it was presumptively determined that the W161 virus cross-reactive antigenic determinants are localized on the vp3 surface polypeptide coded by gene segment 4. The characteristic RNA genome electropherotype of W161 virus was observed in 5 of 59 cases of infant gastroenteritis detected in 1983 and 1984 but has not been observed subsequently at Children’s Hospital. Serotype W161-specific neutralizing antibodies were observed in a majority of sera of normal adults and infants of <4 or >12 months of age collected in the Philadelphia area. Median antibody titers to W161 equaled or exceeded those to rotaviruses of serotype 1 or 3. Each of seven samples of commercial cow’s milk exhibited neutralizing antibodies to W161 virus at a titer greater than or equal to that to serotype 1 or 3 or bovine (strain NCDV) rotavirus. However, W161 rotavirus did not induce disease or a specific serum-neutralizing antibody response when fed to a caesarean-derived columstrum-deprived newborn calf. W161 rotavirus caused diarrhea in newborn mice with a 50% diarrhea-inducing dose of 107.9 PFU.

Rotaviruses infect virtually all human infants within the first 4 years of life, causing extensive morbidity in all populations and representing one of the leading causes of infant death in the developing world (10). Therefore, there is considerable interest in developing a vaccine against rotavirus (13), and it has been predicted that a reasonably efficacious vaccine would be highly cost-effective (8). Although all group A rotaviruses share cross-reactive antigens primarily localized to inner capsid structural polypeptides (12), specific serotypes may be defined by cross-neutralization tests (18). Two distinct outer capsid polypeptides, vp3 and vp7, are each capable of eliciting serotype-specific virus-neutralizing antibodies (17, 22). Several experimental studies in animals have provided evidence suggesting that serotype specificity plays a major role in governing the efficacy of immunoprotection against rotavirus gastroenteritis (3, 14, 23, 28). Therefore, a complete assessment of extent human rotavirus serotypes is relevant to the design of rotavirus vaccines.

Beards et al. (1) first described distinct human serotypes; human rotavirus isolates were subsequently designated types 1 through 4 as a result of cross plaque-neutralization tests reported by Wyatt et al. (32). A fifth human serotype designated 69M was recently isolated in Indonesia and described by Matsumo et al. (21). We relate here a presumptive additional human serotype described on the basis of isolate W161 recovered from an infant at Children’s Hospital, Philadelphia, Pa. Preliminary cross-neutralization studies with reference polyclonal antisera suggest that W161 rotavirus exhibits a minor cross-reaction with rotaviruses of serotypes 1, 2, 3, and 4, which is expressed only on the vp3 surface polypeptide.

MATERIALS AND METHODS

Rotavirus diagnosis. The polyacrylamide gel technique for detection of rotavirus double-stranded RNA has been described elsewhere (9). In brief, 20 μl of a 5% stool suspension was electrophoresed on a 0.8-mm gel consisting of 12% polyacrylamide gel with a 5% stacking gel. The gel was run in a discontinuous (Laemmli) system for 18 h at room temperature at 10 mA of constant current, removed, and stained with silver by a modification of the method of Herring et al. (16). Enzyme-linked immunosorbent assay (ELISA) for rotavirus in stool was performed by a commercial test (Rotazyme, Abbott Laboratories). Methods employed for electron microscopic analysis of stool and for assay of acute and convalescent serum antibody to rotavirus assessed by ELISA, hemagglutination inhibition, and serum neutralization tests have been described elsewhere (5, 26).

Virus isolation. MA104 cell culture was purchased from M. A. Bioproducts and cultivated in BHK medium (20). Stool was suspended (5% [wt/vol]) in serum-free Eagle minimal essential medium containing 500 U of penicillin per ml, 500 μg of streptomycin per ml, 40 μg of gentamicin per ml, 50 U of nystatin per ml, and 20 μg of trypsin (Flow
Laboratories, Inc.) per ml. The stool suspension was clarified by centrifugation at 2,000 × g for 30 min. Clarified supernatant fluid was incubated with an equal volume of purified trypsin (Sigma Chemical Co.) (10 μg/ml in phosphate-buffered saline) for 60 min at 37°C. Trypsin-treated stool supernatant fluid was inoculated (0.2 ml) into thrice-washed tube cultures of confluent MA104 cells. After absorption of inoculum for 30 min at 37°C, cultures were fed with complete Sato medium (27) and incubated in a roller-tube apparatus at 37°C. Serial passages were performed with suspensions of frozen and thawed whole cultures which were harvested when the entire cell sheet was involved with rotavirus cytopathic effect.

Immune sera. Hyperimmune reference sera were prepared by serial parenteral immunization of guinea pigs as previously described (18). Preparation of immune sera in mice has been previously described (23). In brief, 8-week-old female CD-1 mice were either inoculated orally three times at 2-week intervals with 6 × 10⁶ PFU of virus (oral mice) or inoculated initially with the same dose of virus emulsified in complete Freund adjuvant and inoculated intraperitoneally, followed by a second intraperitoneal dose of the same virus titer emulsified in incomplete Freund adjuvant and administered 6 weeks later (intraperitoneal mice). Immune serum was collected 21 days after the final rotavirus inoculation.

Virus. The method of propagation (26) and the sources of rotavirus reference strain viruses used in comparative studies have largely been described elsewhere: serotype 1, Wa (6); serotype 2, DS-1 (32) and S2 (6); serotype 3, P (32), W178 (2), and SA11 (6); serotype 4, ST3 (32); serotype 5, OSU (18); serotype 6, NCDV (6) and WC3 (6); and serotype 7, WC3 (6). Strains W179 and W180 are serotype 1 rotaviruses isolated from infants with gastroenteritis at Children's Hospital of Philadelphia. Strain 69M rotavirus, distinct from previously designated rotavirus serotypes, was obtained from Shigeo Matsuno, National Institute of Health, Tokyo, Japan.

PRN test. Serum-neutralizing antibody was assessed either by the plaque reduction neutralization (PRN) test previously described by Hoshino et al. (18) (guinea pig serum cross-neutralization tests) or a similar method described by Clark et al. (7) (all other PRN antibody determinations).

Inoculation of newborn mice and calves. Newborn mice were inoculated as described previously (25). CD-1 mouse inoculations demonstrated to be free of rotavirus antibodies detectable by ELISA, and reared in individual isolation pens. Rotavirus was inoculated orally in a volume of 20 to 40 ml within the first 24 h of life. A challenge inoculation of bovine rotavirus strain RO-4 was administered orally at 14 to 18 days after the initial rotavirus inoculation.

RESULTS

Isolation. An 18-month-old girl with a history of chronic failure to thrive was admitted to Children's Hospital on 11 February 1983. Her medical history was notable for omphalocle and congenital heart disease. Despite surgical correction of both clinical conditions, the child continued to have difficulties gaining weight. Upon presentation to the Emergency Department at Children's Hospital, the child was noted to have vomiting and fever and was admitted for dehydration and suspected small bowel obstruction. Epidemiologically, the child had no exposure to animals and did not have an unusual travel history or diet. The child did not attend day care, and no other family members were ill at the time.

Rotavirus was detected in a stool sample collected 3 days postadmission by electron microscopic and polyacrylamide gel analysis for rotaviral RNA (9) but not by ELISA (Rotazyme, Abbott Laboratories). Serum antibody analysis suggested strongly that the infant was seropositive for rotavirus antibody before this infection, as indicated by high concentrations of rotavirus-specific antibody detected by serum ELISA, radioimmuno precipitation tests, and neutralization antibody titers to serotypes 1 and 3 and homotypic virus, all determined in a serum sample collected 1 day after the onset of disease. A marked increase in antibody titer was detected in a serum sample collected 21 days after onset of disease; convalescent ELISA and hemagglutination inhibition titers and titers of neutralizing antibodies to serotype 1 and homotypic (W161) rotavirus were the highest that we have observed in a natural infection (5).

Virus isolation studies were performed with a suspension of stool collected on day 3 after onset of disease. The stool suspension was treated with trypsin, and roller-tube cultures of MA104 cells were infected by the methods reported by Sato et al. (27) and Urasawa et al. (30). Cytopathic effect was observed within 5 days after inoculation. Serial passage into newborn MA104 cells was readily accomplished after the first passage. Clear plaques were induced in MA104 cells. By passage 5, yields of approximately 10⁷ PFU/ml were obtained 3 to 5 days after cell culture inoculation at a multiplicity of infection of 1.0. The virus was given the strain designation W161 based upon the identification number of the patient.

Subgroup. The subgroup of W161 rotavirus was determined to be II by an ELISA system employing monoclonal antibodies (15).

Serotype. The neutralization antigen phenotype (serotype) of W161 was examined in a plaque-reduction neutralization test employing hyperimmune guinea pig antisera to W161 and viruses representing known serotypes of rotavirus (Table 1). Strain W161 rotavirus was not neutralized by antisera to the recognized human serotypes 1, 2, 3, and 4, to the recently described antigenically distinct human rotavirus strain 69M (21), or to animal rotavirus strains serotypes 5, 6, and 7, of porcine, bovine, and avian origin, respectively. However, a minor (1/16) one-way cross-neutralization reaction was represented by neutralization of human rotaviruses of serotypes 1 through 4 by hyperimmune antiserum to W161 virus. This cross-reactivity was not observed with strain 69M human rotavirus or the animal rotavirus serotypes examined.

Indirect evidence of the structural polypeptide localization of the cross-reactive W161 virus neutralization antigen was obtained by assessing the reaction of antisera to W161 virus with two reassortant rotaviruses. Reassortant virus WA × UK, 18-1 containing only gene 9 of serotype 1 Wa virus and reassortant virus DS-1 × UK, 66-1-1 containing only gene 9 of serotype 2 strain DS-1 rotavirus (each reassortant containing 10 segments of bovine strain UK rotavirus) were neutralized by antisera to serotype 1 and 2 rotaviruses, respectively, but not by antisera to W161 virus. This result indicates that the W161 cross-reactive
neutrothection antigen is not the structural polypeptide vp7 (the gene product of gene 9 of strain Wa and of gene 8 of strain DS-1) (12) and is therefore presumably associated with the other rotavirus surface polypeptide vp3, a product of gene segment 4 (12).

Cross-neutralization reactions of W161 virus with human rotavirus of serotypes 1 to 4 and bovine rotavirus strain NCDV were also compared with immune sera prepared in adult mice inoculated parenterally or orally (23). Sera of parenterally inoculated mice indicated that W161 virus is a completely distinct serotype. Although minor reciprocal cross-reactions were detected between W161 virus and each of the other serotypes, particularly when antisera to W161 virus were reacted with heterotypic viruses, none approached the >5% of homologous titer level used to delineate rotavirus serotypes (18).

More extensive cross-reactions were observed with the lower titered antisera induced in orally immunized mice (Table 2). As previously observed with guinea pig-derived antisera, cross-reactions were predominantly one-way, i.e., only detectable when antisera to W161 virus were reacted with heterotypic viruses.

Evidence of cross-reactivity with heterotypic virus was also detected in human infants. In a series of 11 seronegative infants (aged 5 to 11 months) inoculated orally with WC3 bovine-derived rotavirus vaccine (6), a serum-neutralizing antibody response to W161 virus was detected in six (compared with an incidence of response of 8 of 11 to WC3 [serotype 6] and 4 of 11 to SA11 [serotype 3] rotavirus strains).

Natural incidence of antibody to W161. Since W161 appears to represent a new serotype of human rotavirus distinguishable from other known serotypes by the PRN test, it was of interest to determine the naturally occurring incidence of antibody to W161. The observed incidences of antibody to W161 in adult and infant sera are shown in Table 3. Antibody to W161 was detected in 17 of 18 normal adult sera (healthy adult volunteers, residents of Philadelphia, Pa., aged 18 to 63 years). The median antibody titer to W161 was similar to that against SA11 (serotype 3) and greater than that to Wa rotavirus (serotype 1). In 10 of 18 adults, the titer to W161 was higher than that against either Wa or SA11 rotavirus.

The high incidence and titer of antibody to W161 in adults is reflected in the observation that six of seven infants aged 2 to 4 months exhibited antibody to W161; at this age, serum-neutralizing antibody against rotavirus is presumed to most often represent maternally acquired antibody (29). Antibody to rotavirus was infrequently observed at age 5 to 11 months. In sera of infants aged 12 to 26 months collected both in Philadelphia and in Japan, a high incidence of antibody was observed to W161, as well as to Wa and SA11, strain rotaviruses.

It was also of interest to determine whether any evidence of exposure to W161 rotaviruses is present in domestic animals. Inasmuch as we had previously determined that sera and milk of individual cows, as well as commercial milk samples obtained over the counter, often contain neutralizing antibody to rotavirus of human serotypes (Groff and Clark, unpublished data; also reported by Yokken et al. [33], Snodgrass et al. [28], and Ebina et al. [11]), we assayed the neutralizing antibody to W161 in seven commercial milk samples (Table 4). Each contained antibody that neutralized

### Table 1. Antigenic characterization of human rotavirus strain W161 by PRN assay

<table>
<thead>
<tr>
<th>Rotavirus (serotype)</th>
<th>Origin</th>
<th>Reciprocal of 60% PRN antibody titer</th>
<th>Hyperimmune antiserum to indicated rotavirus (serotype)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>W1 (1)</td>
<td>DS-1 (2)</td>
</tr>
<tr>
<td>Wa (1)</td>
<td>Human</td>
<td>81,920</td>
<td>—</td>
</tr>
<tr>
<td>DS-1 (2)</td>
<td>Human</td>
<td>—</td>
<td>40,960</td>
</tr>
<tr>
<td>P (3)</td>
<td>Human</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>ST3 (4)</td>
<td>Human</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>W161</td>
<td>Human</td>
<td>&lt;80</td>
<td>&lt;80</td>
</tr>
<tr>
<td>69M</td>
<td>Human</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>OSU (5)</td>
<td>Pig</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NCDV (6)</td>
<td>Cow</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ch2 (7)</td>
<td>Chicken</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Wa × UK, 18-1</td>
<td>Human × bovine reассortant</td>
<td>81,920</td>
<td>—</td>
</tr>
<tr>
<td>DS-1 × UK, 66-1-1</td>
<td>Human × bovine reассortant</td>
<td>—</td>
<td>40,960</td>
</tr>
</tbody>
</table>

* Homologous values are in boldface type. —, Not tested.

### Table 2. Cross-neutralization reactions between W161 and heterotypic rotavirus serotypes in sera of orally immunized mice

<table>
<thead>
<tr>
<th>Antigen specificity</th>
<th>No. of mice</th>
<th>No. of sera with cross-reactivity to: W161</th>
<th>Wa (1)</th>
<th>S2 (2)</th>
<th>SA11 (3)</th>
<th>W178 (3)</th>
<th>NCDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>W161</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Wa</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S2</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>SA11</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>W178</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>NCDV</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* Greater than 5%. Numbers in boldface are homologous reactions.

### Table 3. Incidence in human sera of neutralizing antibody to rotavirus strains

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of sera</th>
<th>Location</th>
<th>W161 (serotype 1)</th>
<th>Wa (serotype 1)</th>
<th>SA11 (serotype 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>18</td>
<td>Philadelphia, Pa.</td>
<td>17 (805)</td>
<td>14 (250)</td>
<td>15 (688)*</td>
</tr>
<tr>
<td>Infant (mo)</td>
<td>2–4</td>
<td>Philadelphia, Pa.</td>
<td>6 (522)*</td>
<td>3 (&lt;100)</td>
<td>3 (&lt;100)</td>
</tr>
<tr>
<td></td>
<td>5–11</td>
<td>Philadelphia, Pa.</td>
<td>14 (250)</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>12–26</td>
<td>Philadelphia, Pa.</td>
<td>5 (250)</td>
<td>3 (&lt;100)</td>
<td>3 (&lt;100)</td>
</tr>
<tr>
<td></td>
<td>12–23</td>
<td>Japan</td>
<td>5 (1,250)</td>
<td>3 (140)</td>
<td>5 (&gt;1,250)</td>
</tr>
</tbody>
</table>

* PRN antibody titer ≥1:100.

* Median titers extrapolated between individuals at the median bracket as no individual median existed.
TABLE 4. Neutralization of rotavirus by commercial cow’s milk

<table>
<thead>
<tr>
<th>Sample</th>
<th>Titer of neutralization antibody to rotavirus strain:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WI61 (serotype 1)</td>
</tr>
<tr>
<td>A</td>
<td>&gt;1.250</td>
</tr>
<tr>
<td>B</td>
<td>750</td>
</tr>
<tr>
<td>C</td>
<td>170</td>
</tr>
<tr>
<td>D</td>
<td>140</td>
</tr>
<tr>
<td>E</td>
<td>&gt;1.250</td>
</tr>
<tr>
<td>F</td>
<td>600</td>
</tr>
<tr>
<td>G</td>
<td>150</td>
</tr>
</tbody>
</table>

* Samples tested represent seven different brands of commercial pasteurized cow’s milk purchased in Pennsylvania or New Jersey in 1984.

W161 virus to a high titer exceeding that detected against bovine rotavirus strain NCDV or serotype 1 (Wa) or serotype 3 (SA11) rotaviruses. The rotavirus specificity of the milk antibody was confirmed by assay of two milk samples in a radioimmuno precipitation test; each was strongly positive at a dilution of 1:100.

Incidence of WI61 Electropherotype. The WI61 rotavirus RNA electropherotype is illustrated in Fig. 1 in comparison with the two electropherotypes most frequently observed in the Philadelphia area since 1982 (strain W178 [serotype 3] and strain W179 [serotype 1]) and prototypic human- and bovine-derived strains. The WI61 electropherotype was identified in 4 of 45 rotavirus-positive diarrhea stools collected in 1983 and in 1 of 14 positive stools obtained in 1984. The WI61 rotavirus electropherotype was not represented in 7 rotavirus-positive stools obtained in 1982 or in 50 rotavirus-positive stools collected in 1986.

Animal inoculation studies. (i) Newborn mice. Serotype 3 rotaviruses of simian (25) and of human (1) origin have been shown to induce typical clinical and histopathological expression of rotavirus gastroenteritis in newborn mice, with threshold doses of 10^4.5 to 10^6.0 PFU. Serotype 1, 2, and 4 human rotaviruses did not induce disease at maximum test doses of 10^6.0, 10^5.0, and 10^4.3, respectively (2).

Titration of WI61 virus in orally inoculated mice revealed a 50% incidence of gastroenteritis at a dose of 10^7.0 PFU, the highest titer obtainable without concentration of virus. No disease was induced with WI61 doses of less than 10^4.0 PFU.

(ii) Newborn calf. Because of the high incidence of WI61 virus-specific antibody in bovine milk, it was of special interest to determine the infectivity of WI61 virus in the newborn calf model system. A single gnotobiotic calf (Holstein-Fresian), 24 h old, was inoculated with an oral dose of 2.0 x 10^8 PFU of WI61 virus. For comparison, similar calves were inoculated with a human serotype 3 rotavirus isolate (strain W178; dose, 6.0 x 10^6 PFU) and an attenuated strain of bovine rotavirus (NCDV; dose, 2.4 x 10^6 PFU). Each calf was challenged with a virulent strain of bovine rotavirus 14 to 18 days after the primary inoculation.

The WI61 virus-inoculated calf exhibited soft feces on day 4 postinfection and shed rotavirus antigen detected by ELISA only on days 3 and 4. However, no serum (PRN or ELISA) antibody response or fecal antibody response (ELISA) was detected before challenge at 14 days postinfection. After challenge, shedding of rotavirus was observed accompanied by an active serum and fecal antibody response.

The calves inoculated with human serotype 3 and NCDV viruses also shed rotavirus in feces within 3 to 5 days postinfection, the serotype 3 virus infection being accompanied by frank diarrhea. In contrast to the reaction to WI61 virus, each of these calves developed a strong active immune response in serum (>1:4,000 by PRN; ≥1:8 by ELISA) and feces (≥1:80 by ELISA), and neither of these calves shed bovine virus after the challenge inoculation.

DISCUSSION

Strain WI61 human rotavirus has been demonstrated to be a newly recognized serotype by means of cross-neutralization (PRN) tests performed with hyperimmunized guinea pig sera and with sera of orally and parenterally hyperimmunized mice. Using the <5% cross-reactivity threshold by neutralization test accepted for describing nonidentity of human rotavirus serotypes (18), antisera to human serotypes 1 through 4 and 69M did not neutralize WI61 rotavirus. Antisera of guinea pigs hyperimmunized with WI61 exhibited a threshold (1/16 = 6.2%) cross-reaction with serotypes 1 through 4 but not with 69M. Mice orally immunized with WI61 irregularly exhibited partial cross-reactions with rotaviruses of human serotypes 2 and 3, but mice parenterally immunized with WI61 showed no such cross-reactions. Mice orally or parenterally immunized with rotaviruses of serotypes 1 through 3 yielded sera that did not neutralize WI61 rotavirus. We therefore conclude that WI61 represents the sixth recognized serotype of human rotavirus.

A knowledge of the number and relative prevalence of diverse rotavirus serotypes will be important in realizing a thorough understanding of the epidemiology of rotavirus disease. Experience with rotaviruses has been too short and rotavirus serotype surveillance has been too limited to determine whether the emergence of new serotypes (antigenic shift) occurs often or plays an important role in

FIG. 1. RNA electropherotype of strain WI61 rotavirus (lane C). Other rotavirus strains are as follows: lane A, WC3 (bovine); lane B, S2 (serotype 2); lane D, W178 (Philadelphia isolate, 1983) (serotype 3); lane E, W179 (Philadelphia isolate, 1983) (serotype 1); lane F, WIP80 (Philadelphia isolate, 1986) (serotype 1); lane G, Wa (serotype 1); lane H, SA11 (serotype 3).
maintaining rotavirus infection in human populations in which rotavirus-specific antibody is ubiquitous (in adults and infants of >3 years of age [29, 34]).

It is also not yet clear whether rotavirus immunoprophylactic strategies will have to be adapted to diverse serotypes which may vary in relative prevalence over time. Numerous studies performed in experimental animals have suggested that immunoprotection against rotaviral disease is serotype specific (3, 14, 23, 28). Yet clinical trials of bovine-derived rotavirus candidate vaccines of strain NCDV (31) or WC3 (H F. Clark et al., manuscript in preparation) administered to human infants have produced evidence of protection against naturally acquired infections with heterotypic (predominantly serotype 1) rotavirus. On the other hand, Chiba et al. (4) have reported, in studies of a closed orphanage population, that resistance to annually recurring epidemics of serotype 3 rotavirus gastroenteritis was selectively correlated with high titers of homotypic serum-neutralizing antibodies. Clearly the possible need for continued etiologic role of rotavirus candidate vaccines of strain NCDV (31) or WC3 specific (3, 14, 23, 28). Yet clinical trials of bovine-derived rotavirus PRN antibody to rotavirus of strains (K. Green and J. Flores, personal communication). The recent observation that the two rotavirus structural polypeptides vp3 and vp7 play an important role both in eliciting PRN antibodies to rotavirus (17, 22) and in immunoprotection (24) suggests that a new strategy for rotavirus nomenclature that takes each of these independent antigens into account may be required.

The role of W161 in naturally occurring human disease is undetermined. Presumptive evidence based on RNA genome electropherotype suggests that W161 strain rotavirus was responsible for 5 of 59 cases of infant rotavirus disease studied in our hospital in 1983 and 1984. We have not detected the strain W161 electropherotype in 1985 or 1986, but since electropherotypes may vary widely within a single serotype of rotavirus, this observation does not rule out an occasional continued etiologic role of W161 serotype in rotavirus diarrhea.

We have no explanation for the extremely high prevalence and titer of PRN antibody to W161 serotype in human sera and in bovine milk. W161 does not appear to be a major cause of rotavirus infant gastroenteritis, as epidemiological studies in which individual rotavirus isolates have been serotyped have largely implicated serotype 1 or 3 (19). Strain W161 rotavirus appears to be an inefficient immunogen and pathogen in the calf. It is probable that, because of the broad antigenic cross-reactivity of the gene 4-coded polypeptide vp3 product of W161 virus, observed levels of circulating strain-specific PRN antibodies or milk PRN antibodies reflect the accumulated experience of hosts to heterotypic rotaviruses bearing antigenic determinants shared with vp3 of W161 serotype virus. Improved understanding of this question and of the role of W161 serotype rotavirus in human disease will require development of techniques for exhaustive monitor-

ing of the serotypes of all rotavirus isolates associated with human disease in defined populations studied over extended periods of time.

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LITERATURE CITED


