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Diversity of Rotavirus Serotypes in Mexican Infants with Gastroenteritis

LUIS PADILLA-NORIEGA,[1,2,3]* CARLOS F. ARIAS,[4] SUSANA LÓPEZ,[4] FERNANDO PUERTO,[5] DAVID R. SNODGRASS,[6] KOKI TANIGUCHI,[7] AND HARRY B. GREENBERG[1,2]

Division of Gastroenterology, Stanford University, Stanford, California 94305[1]; Veterans Administration Medical Center, Palo Alto, California 94304[2]; Unidad de Investigación Clínica en Enfermedades Infecciosas y Parasitarias, Instituto Mexicano del Seguro Social, Mexico City 04000,[3] Centro de Investigación sobre Ingeniería Genética y Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos 62271,[4] and Departamento de Patología Tropical, Centro de Investigaciones Regionales, Universidad Autónoma de Yucatán, Mérida, Yucatán 97240,[5] Mexico; Moredun Research Institute, Edinburgh, Scotland[6]; and Department of Hygiene and Epidemiology, Sapporo Medical College, Sapporo 060, Japan[7]

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One hundred thirty-two stool specimens from infants with rotavirus gastroenteritis hospitalized in two Mexican cities (Mexico City and Mérida) were examined by serotype- and subgroup-specific enzyme immunoassays. Among them, 38 (29%) were serotype 1, 15 (11%) were serotype 2, 13 (10%) were serotype 3, 22 (17%) were serotype 4, none was serotype 5 or 6, and 44 (33%) could not be serotyped. By subgrouping, 121 specimens were characterized as follows: 24 (18%) were subgroup 1, 97 (74%) were subgroup 2, and none had both subgroup specificities. While serotype 1 rotavirus predominated in the Mexico City area for 4 consecutive years (1984 to 1987), serotype 4 predominated in Mérida during the single epidemic season studied (1985). These data demonstrate that all four primary human rotavirus serotypes circulated in Mexico, with serotype 1 being the most prevalent. The seroneutralization responses of 14 of the 22 patients infected with serotype 4 strains had been previously studied. Of these 14 infants, 11 appeared to have primary infections, as indicated by absence of neutralizing antibodies in the acute-phase sera and their young age (8 months on average) at the time of illness. Seven patients seroresponded to serotypes 1 and 4; two seroresponded to serotypes 1, 3, and 4; three seroresponded to serotype 1; and two had low-level seroresponses to serotype 3 or 4. These data indicate that heterotypic neutralizing antibody responses occur frequently following infection with serotype 4 rotaviruses.

Group A rotaviruses are the single most important cause of severe dehydrating diarrhea in children under 3 years of age (20). These viruses are an important cause of infant mortality in developing countries and of infant morbidity in developed countries (20).

Among group A rotaviruses, nine different serotypes have been identified on the basis of cross-neutralization assays with hyperimmune sera prepared in antibody-negative animals (7, 19, 23, 40), and six of them (serotypes 1 to 4, 8, and 9) have been found in humans, although little is known about the epidemiological importance of the last two serotypes, which have only recently been described (7, 23).

Live vaccines derived from bovine (serotype 6) and simian (serotype 3) rotaviruses have been tested in humans in different areas of the world and in different age groups (5, 6, 9, 16, 21, 37, 38). These trials have yielded inconsistent results, presumably in part because the most prevalent serotype of rotavirus infecting vaccinated communities has varied (36). The planning and evaluation of rotavirus vaccine trials in the future clearly require more detailed knowledge of the prevalence and temporal distribution of different serotypes in the particular geographic area where the vaccines are evaluated.

Several sets of serotype-specific monoclonal antibodies (MAbs) to VP7 have been produced and used in enzyme-linked immunoassays (ELISAs) for detection of rotavirus serotypes 1 to 4 (8, 17, 29, 32). Furthermore, for at least one set of serotyping reagents, consisting of MAbs KU-4, S2-2G10, YO-1E2, and ST-2G7, the specificity of the reagents has been confirmed by comparison with results obtained in a conventional neutralization test (34). The availability of specific serotyping reagents has allowed some epidemiological studies to be performed in which large numbers of human rotavirus strains were tested (1, 11, 35). However, more studies are needed worldwide, especially in developing countries, where administration of successful vaccines would be of most benefit.

Both homotypic and heterotypic serological responses have been observed in rotavirus infections of seronegative infants (3, 28, 41). However, there is very limited information which correlates the serotype of the infecting virus with the specificity of the neutralizing immune response (4, 13).

To learn about the epidemiology of various rotavirus strains in Mexico, we determined the frequency of infection with human rotavirus serotypes 1 to 4, subgroups 1 and 2, and electropherotypes (long and short) in stool specimens from infants with diarrhea hospitalized in two Mexican cities. Previously, paired sera from some of the patients included in this study had been titrated by using a focus reduction neutralization assay to rotavirus serotypes 1, 3, and 4 (28), thus allowing us to correlate the serotype of the infecting virus with the specificity of the neutralizing immune response that follows infection.

MATERIALS AND METHODS

Stool specimens. Stool specimens from 132 infants known to be positive for rotavirus by RNA electrophoresis or electron microscopy were studied. The infants had been

* Corresponding author.

admitted for acute diarrhea to two hospitals in Mexico City, Hospital del Niño, Desarrollo Integral de la Familia, and Hospital Infantil, between May 1984 and October 1987 or to the Hospital General O'Haran in Mérida, Yucatán, Mexico, between June and December 1985. The patients had limited, if any, exposure to large domesticated animals such as swine and cattle.

MAbs. Three VP7-specific MAbs used in this study, 5B8, 5E8, and 1C10, have not been previously described. 5B8 was derived from a mouse immunized with porcine rotavirus OSU, while 5E8 and 1C10 were derived, respectively, from mice immunized with rhesus rotavirus reassortants D × RRV and DS1 × RRV (24). All three MAbs neutralize and inhibit the hemagglutination of the homologous strain to high titers. MAbs were prepared and characterized as previously described (30), by using inhibition of hemagglutination as the screening method.

Strain identification was as follows: serotype 1, MAbs KU-4 (32), 5E8, 2C9 (29), and KU-6A11 (25); serotype 2, MAbs S2-2G10 (32), 1C10, and 2F1 (29); serotype 3, MAbs YO-1E2 (32), 159 (30), and 4F8 (29); serotype 4, MAb ST-2G7 (32); serotype 5, MAb 5B8; serotype 6, MAb UK7/IC3 (31). To detect the presence of VP7, cross-reactive MAb 129 was used (30). In addition, MAbs 255/60 (specific for subgroup 1) and 631/9 (specific for subgroup 2) were used (15, 39).

All of the serotype-specific MAbs and cross-reactive MAb 129 were tested by serotyping ELISA (as described below) with a panel of control tissue culture-grown rotaviruses belonging to serotypes 1 to 6, 8, and 9 (strains KU, DS1, ITO, ST3, OSU, NCDV, 69M, and WI61, respectively). MAb 129 reacted, as expected, with all of the viruses tested, while among the other serotyping MAbs, all but one (YO-1E2) reacted in a serotype-specific manner. Besides reacting with serotype 3 strain ITO, MAb YO-1E2 reacted with serotype 5 strain OSU. The usefulness and specificity of this MAb for detection of human serotype 3 strains has already been documented (32, 34). It has also been shown to react with some porcine strains (27); however, these strains were not tested with a serotype 5-specific reagent. Of the two animal rotavirus-specific MAbs used in this study, 5B8 reacts specifically by ELISA with a wide variety of serotype 5 porcine strains (H. B. Greenberg, unpublished data; L. Saif, personal communication) and UK7/IC3 reacts specifically with both prototype serotype 6 strains (UK and NCDV) and with most serotype 6 strains found in the field (31).

Serotyping ELISA. The serotyping ELISA was done as previously described (29), with the following modifications. Immulon II (Dynatech Laboratories, Inc., McLean, Va.) microtiter plates were coated with 1:1,000 to 1:8,000 dilutions of a MAb from ascites in phosphate-buffered saline (PBS) containing 0.05% sodium azide (PBS-Az). After overnight incubation at 4°C, the plates were washed twice with PBS-Az and blocked with 5% fetal bovine serum in PBS-Az for 12 to 24 h at 4°C (all further washings were done with PBS-Az, and all incubations were done in 5% fetal bovine serum in PBS-Az, unless otherwise indicated). The plates were then washed twice and incubated for 2 h at 37°C with 50 μl of 1% stool suspensions. After being washed four times, guinea pig hyperimmune anti-rhesus rotavirus serum was added and incubated for 1 h at 37°C. The plates were then washed four times and incubated for 1 h at 37°C with an alkaline phosphatase–anti-guinea pig immunoglobulin G conjugate (Kirkegaard & Perry Laboratories, Gaithersburg, Md.). The plates were then washed four times, the substrate (1 mg of *p*-nitrophenylphosphate per ml in 1 mM $MgCl_2$–1% diethanolamine buffer [pH 9.8]) was added, and the plates were incubated at 37°C until the A_{410} of the controls was approximately 1.0. Each stool sample had its own blanks, which were run in two noncoated wells. As positive controls, tissue culture-grown rotavirus or rotavirus reassortants (24) D × RRV, DS1 × RRV, RRV, ST3 × RRV, OSU, and NCDV (representing serotypes 1 to 6, respectively) were used. A virus was assigned to a specific serotype when the A_{410} value with the MAb corresponding to that serotype was higher than 0.2 and at least twice as high as the value corresponding to any other serotype.

Subgrouping ELISA and electropherotyping. Subgrouping ELISA (39) and electropherotyping (18) were performed as previously described.

RESULTS

Comparison of serotyping MAbs. The ELISA with MAbs KU-4, S2-2G10, YO-1E2, and ST-2G7 has already been validated as a serotyping method with field isolates (32, 34). We recorded the results obtained with this reference panel and compared them with those obtained with a test panel consisting of nine serotype-specific MAbs to serotypes 1 (5E8, 2C9, and KU-6A11), 2 (1C10 and 2F1), 3 (159 and 4F8), 5 (5B8), and 6 (UK7/IC3). Because this test panel did not contain a serotype 4 reagent, MAb ST-2G7 was incorporated as well. The comparison was made possible by running both panels simultaneously and using the same detection antibody.

Among the 132 rotavirus-positive specimens assayed, we serotyped 8 with only the reference panel (6 serotype 1 and 2 serotype 2), 11 with only the test panel (1 serotype 1, 2 serotype 2, and 8 serotype 3), and 69 with both panels. There was an absolute coincidence in all 69 specimens that were serotyped with both panels; i.e., the specificities were identical. The results that follow represent the combined results obtained with both panels.

When used with field specimens, the various serotyping MAbs clearly differed in typing efficiency. For MAbs to serotype 1, 2, or 3, a comparison of their relative serotyping efficiencies was made by calculating the ratio of the number of samples recognized by one MAb versus the number of samples recognized by all MAbs specific for the same serotype (Table 1). Because only a single serotype 4 MAb was available, such a comparison could not be made for this reagent. Similarly, since none of the isolates reacted with MAb 5B8 or UK7/IC3, the efficiency of these reagents could not be measured. Among the antibodies to serotype 1, KU-4 was the most efficient, followed by 5E8, whereas KU-6A11 and 2C9 appeared to recognize epitopes which are present in only a small fraction of the serotype 1 rotaviruses studied. All three MAbs specific for serotype 2 had approximately the same efficiency, whereas among the MAbs to serotype 3, 159 had relatively higher efficiency than either 4F8 or YO-1E2.

Serotype analysis. Of the 132 rotavirus-positive specimens examined, 38 (29%) were determined as serotype 1, 15 (11%) were serotype 2, 13 (10%) were serotype 3, 22 (17%) were serotype 4, and none was either serotype 5 or 6. The remaining 44 specimens (33%) could not be serotyped, but 12 of them were recognized by cross-reactive MAb 129, which was used to detect VP7.

Relationship among subgroups, electropherotypes, and serotypes. One hundred twenty-one stool samples (92%) were subgrouped by ELISA; of these, 24 (18%) were classified as subgroup 1, 97 (74%) were subgroup 2, and none had both

TABLE 1. Efficiencies of MAbs specific for serotypes 1 to 3 for detection of rotaviruses from stools by ELISA in relation to those of other MAbs with the same serotype specificities

MAb	Serotype specificity	Efficiency[a]
KU-4	1	0.97 (37/38)[b]
5E8	1	0.84 (32/38)
KU-6A11	1	0.03 (1/38)[c]
2C9	1	0.03 (1/38)[c]
S2-2G10	2	0.86 (13/15)[d]
1C10	2	0.80 (12/15)[e]
2F1	2	0.80 (12/15)
159	3	1.00 (13/13)
4F8	3	0.53 (7/13)
YO-1E2	3	0.38 (5/13)[f]

[a] Ratio of the number of stool samples recognized by one MAb versus the number of samples recognized by other MAbs with the same serotype specificity.
[b] KU-4 failed to recognize one strain detected only by 5E8.
[c] The strains recognized by these MAbs were also detected by both KU-4 and 5E8.
[d] S2-2G10 failed to recognize two strains detected only by 1C10 or 2F1.
[e] 1C10 failed to recognize three strains detected only by S2-2G10 (two strains) or 2F1 (one strain).
[f] Five strains detected by YO-1E10 were also detected by both 4F8 and 159.

subgroup specificities. Of 120 specimens that were electropherotyped (91%), 21 (16%) had short patterns and 99 (75%) had long patterns.

Of the 97 specimens classified as subgroup 2, 93 had long patterns and the remaining 4 did not have enough RNA for electropherotyping. Of the 24 subgroup 1 rotaviruses found, 23 had short RNA patterns and 1 did not have enough RNA to be typed. All of the short-electropherotype and/or subgroup 1 strains that were serotyped (n = 14) were serotype 2, and all of the long-electropherotype and/or subgroup 2 strains that were serotyped (n = 73) were serotype 1, 3, or 4.

Temporal distribution of rotavirus serotypes. The frequencies of individual serotypes in Mexico City and in Mérida, Yucatán, are shown in Fig. 1. In Mérida, serotype 4 was prevalent during the single epidemic season analyzed and only one serotype 2 and two serotype 1 strains were detected. In Mexico City, serotypes 1, 3, and 4 were identified throughout the study period and serotype 2 was detected only from May to November 1984 and from August 1986 to January 1987.

Correlation between the serotype of an infecting rotavirus and the neutralizing immune response. We have previously reported the existence of different serotype-specific seroresponse patterns in Mexican infants infected with rotavirus (28). In Mérida, the prevalent pattern was the response to both serotypes 1 and 4, while in Mexico City, the prevalent response was to serotype 1. In this study, the serotypes of the infecting rotavirus strains were determined for 22 of the 36 infants previously studied (Table 2). Of the 14 infants infected with serotype 4 rotavirus strains, 7 seroresponded to serotypes 1 and 4; 2 seroresponded to serotypes 1, 3, and 4; 3 seroresponded to serotype 1; and 2 showed low-level responses to serotype 3 or 4. Among six infants infected with serotype 1 rotavirus, two seroresponded to serotype 1, one seroresponded to serotypes 1 and 4, one seroresponded to serotypes 1 and 3, and two did not respond to serotype 1, 3, or 4. The remaining two patients were infected with serotype 2 or 3 rotavirus, but they did not seroresponse to any of the three serotypes tested.

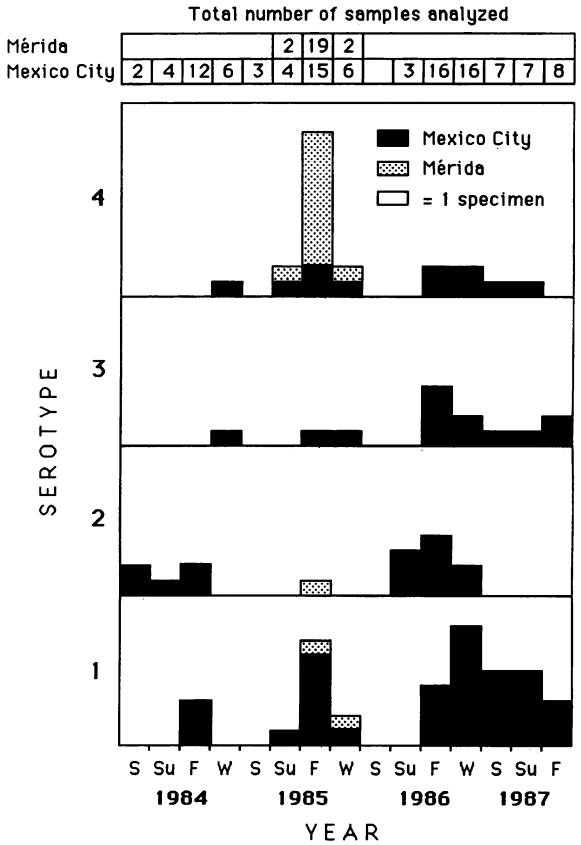

FIG. 1. Relative frequencies of serotypes 1 to 4 of human rotaviruses in stool specimens collected in Mexico City and Mérida. S, Spring; Su, summer; F, fall; W, winter.

DISCUSSION

We compared the efficiencies of two panels of VP7-specific MAbs for serotyping of rotavirus-positive stool samples obtained from symptomatic Mexican infants. The specificity of one panel of reference reagents has been previously studied by using field specimens (32, 34). The specificities of all of the type 1 to 3 reagents from both panels appeared to be similar, if not identical, in this study. When type 1 to 4 MAbs were included in the analysis, in no case was there a disagreement in serotype assignment between the two panels when a serotype assignment was made. However, some MAbs differed markedly in the sensitivities with which they detected the homologous strains. This is not likely to be simply a matter of antibody titer or affinity, since all of the MAbs reacted similarly with the control viruses included in each assay. The differences between reagents is more likely a reflection of the so-called "monotype" phenomenon, whereby some serotype-specific MAbs react with only a subset of strains from a particular serotype (8). This must be true of MAbs 2C9 and KU-6A11, which failed to react with most of the serotype 1 strains in this study. It is also probably true for MAbs 4F8 and YO-1E2, which reacted with only a portion of the serotype 3 strains. Whenever possible, the use of more than one MAb specific for each serotype can help to compensate for the differences in efficiency shown by these reagents.

The serotype specificities of 88 (67%) of the 132 rotavirus-positive samples were determined. In the Mexico City area, 74 (68%) of 109 samples were serotyped; serotype 1 viruses

TABLE 2. Correlation in rotavirus-infected infants between the serotypes of viruses from stools (determined by ELISA with VP7-specific MAbs) and serotype-specific seroresponses (determined by neutralization) to serotype 1 (Wa), 3 (SA11), and 4 (ST3) rotavirus strains

Patient designation	Serotype of infecting rotavirus	Seroresponse (serotype[s])[a]	Age (mo)
MEX425	1	+	17[b]
MEX437	1	+	18
MEX421	1	1	7[b]
YUC16	1	1	12[b]
MEX457	1	1, 3	26
YUC14	1	1, 4	7[b]
YUC3	2	+	3[b]
MEX361	3	+	12[b]
YUC21	4	1	7[b]
MEX362	4	1	3[b]
MEX471	4	1	9[b]
YUC17	4	3	5
YUC13	4	4	15[b]
MEX430	4	1, 4	11
YUC8	4	1, 4	6[b]
YUC9	4	1, 4	6[b]
YUC11	4	1, 4	5[b]
YUC15	4	1, 4	12[b]
YUC20	4	1, 4	3[b]
YUC24	4	1, 4	3[b]
YUC6	4	1, 3, 4	23[b]
YUC18	4	1, 3, 4	10

[a] Increase of fourfold or more in neutralizing titer. Actual titers in reference 28. +, Serum samples that did not show a seroresponse by neutralization to any of the rotavirus strains tested but did show a seroresponse when total rotavirus antibodies were measured (28).

[b] Children without preexisting antibodies to rotavirus in their acute-phase serum specimens.

predominated (33%), followed by serotypes 2 (13%), 3 (12%), and 4 (10%). Rotaviruses of serotypes 1, 3, and 4 cocirculated throughout the study, while serotype 2 viruses appeared in two nonconsecutive epidemic seasons. This apparently cyclic behavior of serotype 2 viruses is in agreement with the observation made by Espejo et al. in 1977 to 1978 (10), when there was a marked shift in the prevalent electropherotype from one year to the other (long in 1977 and short in 1978). On the other hand, the apparent cocirculation in a single geographic setting of several rotavirus serotypes, with predominance of serotype 1, has been described in places as distant as Italy, Australia, the Central African Republic, Venezuela, Brazil, India, and Japan (1, 2, 11–13, 22, 26, 35).

In contrast to the results obtained in Mexico City, most (n = 11) of the 14 specimens from Mérida that were serotyped during the single epidemic season analyzed (of 23 specimens studied) were serotype 4. The observation that two different serotypes predominated at the same time in two different regions of the same country is important in the context of vaccine planning, since a monovalent rotavirus vaccine might well be expected to induce different levels of protection under these circumstances.

Despite the use of several MAbs specific for serotypes 1 to 3, 44 of the rotavirus-positive samples could not be serotyped. Twelve of these were recognized by the cross-reactive MAb used to detect VP7. These untypeable strains could still belong to serotypes 1 to 4 but not be recognized by the panel of antibodies used, or they might represent serotypes other than the ones tested in this study. Available data indicate that human rotavirus serotype 8 or 9 or other unknown serotypes are quite rare. It is of note that we failed to identify any human rotavirus strain with serotype 5 or 6 specificity. These types, which are prevalent in pigs and cattle, respectively, have not been found in humans. It is likely that relatively powerful genetically based host range barriers restrict serotype 5 and 6 viruses from infecting and spreading in humans. Thirty-seven (84%) of the untypeable strains were collected in 1984 or 1985, while only seven (16%) were from 1986 or 1987. If all of the specimens had been fresh and not subjected to several cycles of freezing and thawing, presumably the percentage of untypeable strains would have been lower. Unicomb et al. (33) also reported differences in serotyping success in relation to sample storage time.

In this study, there was an absolute coincidence of subgroup 1 specificity, the short electropherotype, and serotype 2 specificity when a serotype designation was made. Similarly, there was coincidence among the long electropherotype, subgroup 2, and serotype 1, 3, or 4. Although several exceptions to these associations have recently been identified (14) and in view of the ease with which rotaviruses reassort in vitro, it is remarkable how infrequently such exceptions appear to occur. The genetic restrictions governing reassortment of human rotavirus strains in vivo require further investigation.

There was a high association (50%) between infection with serotype 4 rotavirus and double seroresponse to serotypes 1 and 4. Of the seven patients with double seroresponses following serotype 4 rotavirus infection, six were likely to have primary infections, since (i) none of them had detectable neutralizing antibodies to serotype 1, 3, or 4 in their acute-phase sera and (ii) they were as young as 3 months old (two patients) and on average less than 6 months old. We conclude that heterotypic neutralizing antibody responses occur frequently following infections with serotype 4 rotaviruses. Although it is impossible to prove that some of the infants in this study had not undergone silent rotavirus infections previously, this seems unlikely to be true of all of the patients with heterotypic responses, especially the very young infants. The molecular basis of the heterotypic response and the effect of such a response on subsequent immunity is obviously of great importance for future vaccine initiatives. Although both VP4 and VP7 contain heterotypic domains, most of the information available indicates that VP4 is responsible for this cross-reactive neutralizing antibody.

Heterotypic neutralizing seroresponses have been previously observed in rotavirus infections of seronegative children (3, 4, 13, 28). Gerna et al. (13), by using solid-phase immune electron microscopy, identified the serotypes of rotaviruses infecting 38 infants and the neutralizing antibody responses to serotypes 1, 2, and 4 in their convalescent-phase sera. When the infecting rotavirus was serotype 1 or 2, the infants developed specific seroresponses, but when the infecting rotavirus was serotype 4, there was a heterotypic response to serotype 1 in addition to the homotypic response. Chiba et al. (4) reported heterotypic neutralizing immune responses in outbreaks of rotavirus gastroenteritis caused by serotype 3 rotaviruses. All but one of the infants seronegative for serotypes 1 and 4 also acquired antibodies to these heterotypic viruses. Future studies should be directed at determining whether infection with specific viral strains predisposes to a heterotypic response and, if so, what the molecular basis of this response is.

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