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Antigenic and Genomic Diversity of Human Rotavirus VP4 in Two Consecutive Epidemic Seasons in Mexico

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In the present investigation we characterized the antigenic diversity of the VP4 and VP7 proteins in 309 and 261 human rotavirus strains isolated during two consecutive epidemic seasons, respectively, in three different regions of Mexico. G3 was found to be the prevalent VP7 serotype during the first year, being superseded by serotype G1 strains during the second season. To antigenically characterize the VP4 protein of the strains isolated, we used five neutralizing monoclonal antibodies (MAbs) which showed specificity for VP4 serotypes P1A, P1B, and P2 in earlier studies. Eight different patterns of reactivity with these MAbs were found, and the prevalence of three of these patterns varied from one season to the next. The P genotype of a subset of 52 samples was determined by PCR. Among the strains characterized as genotype P[4] and P[8] there were three and five different VP4 MAb reactivity patterns, respectively, indicating that the diversity of neutralization epitopes in VP4 is greater than that previously appreciated by the genomic typing methods.

Group A rotaviruses are a leading cause of severe diarrhea in the young of humans and animals (16). Both of the rotavirus surface proteins, VP4 and VP7, are able to induce neutralizing antibodies; hence, the serotypic specificity of these viruses is dual and is termed G and P for VP7 and VP4, respectively (4, 11, 14). Rotavirus serotypes were originally defined on the basis of neutralization assays with hyperimmune sera, and later it was shown that such specificity depends mostly on VP7 (G serotypes) (4, 11). More recently, P serotypes have been defined by neutralization assays with sera hyperimmune to baculovirus-expressed VP4 proteins or to reassortant viruses (4, 8, 11). At least 10 G serotypes (G1 to G6, G8 to G10, and G12) and seven P serotypes (P1A, P1B, P2A, P3, P4, P5, and P8) have been found among human rotaviruses (HRVs) (4, 7, 12).

By sequencing the VP4-coding gene, eight genomic P types (genotypes) have been defined among HRVs, and these genotypes have been further shown to correspond to some of the described P serotypes. In addition to nucleotide sequencing, dot blot hybridization- and PCR-based assays have also been used to determine the VP4 genotypes (6, 17).

The availability of neutralizing monoclonal antibodies (NtMAbs) specific for different VP7 serotypes has allowed extensive epidemiological studies to be carried out, and these studies have identified serotypes G1 to G4 as being the epidemiologically relevant serotypes worldwide. On the other hand, knowledge about the diversity of P serotypes among circulating HRV strains is scarce, due to the lack of readily available VP4-typing polyclonal sera, as well as the lack of monoclonal antibodies (MAbs) specific for different VP4 serotypes; therefore, P-genotyping methods have been used as a surrogate for serotyping.

In the several genotyping studies conducted worldwide, genotypes P[4] (associated with a VP7 protein with G2 specificity) and P[8] (associated with the G1, G3, or G4 VP7 protein) have emerged as the most frequent genotypes, altogether representing about 95% of the typeable strains (7). In those studies, the single strain most frequently found was serotype P[8], G1, followed by P[8], G4; P[4], G2; and P[8], G3 (7). These surveys are providing relevant information about the prevalence of rotavirus P genotypes; however, the nonserological typing methods used do not necessarily reflect the antigenic diversity of the protein. In fact, exceptions to the correlation between P genotypes and the serologically defined P serotypes have been reported (10, 11, 15, 21), indicating the need for antigenic characterization, in addition to genomic typing, of the VP4 proteins of circulating HRV strains.

Recently, neutralizing anti-VP4 MAbs directed to HRV strains having serotype P1A, P1B, and P2A specificities have been produced and evaluated as serotype-specific reagents (3, 22). In this study, using these MAbs we have characterized the antigenic diversity and variability of the VP4 protein associated with HRV strains circulating during two epidemic seasons in three different geographic regions of Mexico. We found that the antigenic diversity of VP4 is greater than that suggested by

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the genomic typing methods and that the prevalent antigenic VP4 type may vary from one epidemic season to the next.

MATERIALS AND METHODS

Stool specimens. A total of 1,091 and 605 stool specimens from infants with acute diarrhea were collected during two consecutive rotavirus epidemic seasons, October 1994 to March 1995 and October 1995 to March 1996, respectively. The infants included in the first year of the study had been admitted for acute diarrhea to hospitals or outpatient clinics in three geographic regions of Mexico: the central region (Oral Rehydration Unit, Hospital Infantil de México, and Iztapalapa Outpatient Clinics 15 and 31, Instituto Mexicano del Seguro Social [IMSS] in Mexico City; Outpatient Clinic of Ministry of Health, in Tlaxcala, Tlaxcala; and Hospital of the University of San Luis Potosí in San Luis Potosí, San Luis Potosí), the northeastern region [Hospitals 4, 6, and 17, IMSS, in Monterrey, Nuevo León), and the southeastern region (Oral Rehydration Unit, O'Horán Hospital, and Clinics 12, 17, and 59 and "El Fénix," IMSS, in Mérida, Yucatán). The same clinics participated in the second year of the study, with the exception of the clinics in the central region of Mexico, where fecal samples were collected only from patients in the Hospital Infantil de México and the Hospital of the University of San Luis Potosí.

Rotavirus screening. Fecal specimens were initially screened for rotavirus either by a rapid (15-min) enzyme-linked immunosorbent assay (ELISA; Meridian Laboratories) or by silver staining of viral double-stranded RNA segments separated by gel electrophoresis (9) and were later confirmed to be positive by an ELISA (Dako Co.) known to be sensitive and specific for rotavirus detection (5). Of the 1,091 stool specimens obtained during the first season, 593 (54%) were positive for rotavirus, while in the second season 323 of 605 (53%) were positive. Of all these samples, 309 from the first year and 261 from the second year were characterized in this study. The samples were chosen so that similar numbers of samples from each of the three geographic regions were studied.

Rotavirus VP4-typing nomenclature. The nomenclature described in a review by Estes (4) is followed in this report. Rotavirus VP4 has been classified on the basis of both genomic (genotype) and antigenic (serotype) characteristics. Since the correlation between VP4 (P) serotypes and genotypes is not completely established, both classification criteria are used to describe rotaviruses. P genotypes are included within brackets, while P genotypes with open numbers are restricted to serotypes. Thus, the full description of human rotavirus Wa strain would be P1A[8], G1. Rotaviruses for which only the P genotype [in addition to the VP7 (G) serotype] has been determined would be described, for instance, as P[8], G3 or P[4], G2.

MAbs. Five serotype-specific VP7 MAbs were used: serotype G1, MAbs KU-4 (27) and 5E8 (22); serotype G2, MAb 1C10 (22); serotype G3, MAb 159 (25, 26); and serotype G4, MAb ST-2G7 (27). In addition, the cross-reactive VP7 MAb 129 (25, 26) was also used. To characterize the VP4 protein, five MAbs were used: F45:4 (hereafter referred to as F45), derived from the serotype P1A strain F45 (3); 1A10, derived from the serotype P1A strain Wa (23); RV5:2 (hereafter referred to as RV5), derived from the serotype P1B strain RV5 (3); and HS6, derived from the serotype P2 strain ST3 (23). These VP4 MAbs had previously been shown to be specific for HRV strains having the same serotype as that of the immunizing virus, P1A, P1B, or P2, when assayed by ELISA (3, 23).

VP7-serotyping ELISA. The G-serotyping ELISA was carried out as described previously (22). A virus was assigned to a specific serotype when the optical density at 410 nm (OD_{410}) 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.

VP4-typing ELISA. The VP4-typing ELISA was performed as described previously (23). MAbs were used to capture viral antigen, and bound antigen was detected with an equivolumetric mix of rabbit antisera hyperimmune to Wa, DS1XRRV, RRV, and ST3 rotaviruses. A MAb was considered to react with a virus when the OD_{410} value for that MAb was at least 0.4. A second and/or a third MAb was also considered to interact with the same virus strain when it reacted with an OD_{410} value higher than 0.2 and was also higher than one-half of the value for the MAb with maximum reactivity. An OD_{410} of 0.4 was chosen as the cutoff value for the MAb with the highest reactivity in order to be able to include low-level reactivity with multiple MAbs.

Identification of P genotypes by reverse transcription-PCR. The identification of genomic types P[4], P[6], P[8], and P[9] was done as described by Gentsch et al. (6).

Statistical analysis. Comparisons of the proportions of HRV G serotypes and the VP4 MAb patterns of reactivity were performed by the chi-square test or Fisher's exact test.

RESULTS

Temporal and geographic distributions of HRV G serotypes. Three hundred nine and 261 rotavirus-positive specimens collected in three geographic regions of Mexico during epidemic seasons of 1994–1995 and 1995–1996, respectively, were characterized (Table 1). In the 1994–1995 season, G3 was the prevalent serotype in the central region of Mexico, while in the other two regions, G1 and G3 viruses were frequently detected ($P < 0.0001$). In the second epidemic season, serotype G1 HRVs were predominant in all geographic regions studied.

TABLE 1. Distribution of human rotavirus G serotypes during two epidemic seasons, 1994–1995 and 1995–1996, in three geographic regions of Mexico

Geographic region	Epidemic season	No. of specimens with the indicated VP7 serotype[a]							
		G1	G2	G3	G4	NI			Total
						Mix	VP7(+)	VP7(−)	
Central	1994–1995	3	—	74	—	—	2	6	85
	1995–1996	65	4	11	1	16	4	35	136
Northeast	1994–1995	38	—	32	1	7	—	13	91
	1995–1996	41	—	—	—	—	1	11	53
Southeast	1994–1995	38	11	70	—	5	2	7	133
	1995–1996	24	14	—	2	3	8	21	72
Total	1994–1995	79	11	176	1	12	4	26	309
	1995–1996	130	18	11	3	19	13	67	261

[a] NI, the G serotype was not identified; Mix, specimens that reacted with more than one VP7 serotype-specific MAb; VP7(+), specimens that reacted only with the cross-reactive VP7 MAb 129; VP7(−), specimens that were not reactive with any of the VP7 MAbs used, including MAb 129; —, no specimen of the indicated G serotype was detected.

The relative frequency of serotypes, considering the two seasons together, was similar in the southeastern and central regions: the most prevalent serotype in both areas was G3 (36% for the regions combined), followed by G1 (31%), G2 (7%), and G4 (<1%). In contrast, in the northeastern region the predominant serotype was G1 (55%), followed by G3 (22%) and G4 (<1%), with no serotype G2 viruses being detected. This difference in the relative frequency of serotypes between regions reached statistical significance ($P < 0.0001$). Furthermore, by splitting by season, in the southeastern and central regions combined, a clear shift in the predominant serotype between the 1994–1995 and the 1995–1996 periods was observed; in the first season the most common serotype was G3 (66%), as opposed to the G1 serotype (43%) in the second season ($P < 0.0001$). In contrast, in the northeastern region, G1 was the most prevalent serotype during both seasons.

Diversity and variation of HRV VP4 antigenic types. To test if the VP4 protein varied from one epidemic season to the next, as was found with VP7, we antigenically characterized the viruses with NtMAbs that have been proposed to be VP4 serotype specific (3, 23). We used MAbs 1A10 and F45, proposed to recognize P1A strains; MAb RV5, proposed to recognize P1B viruses; and MAb HS6, suggested to interact with serotype P2A rotaviruses.

Overall, eight different VP4 MAb patterns of reactivity were detected among the HRVs characterized; for simplicity these patterns were named A to H (Table 2). With the exception of one serotype G3 HRV specimen that reacted with MAb HS6 (pattern H), the serotype G1 and G3 HRV strains collectively had five patterns of reactivity with the VP4 MAbs (patterns A to E). Considering both epidemic seasons combined, VP4 pattern A was by far the most frequently occurring pattern among the G3 strains, while patterns A and B seemed to be equally distributed among serotype G1 viruses ($P < 0.0001$), with the other patterns being represented less frequently. Among the serotype G2 specimens, three VP4 patterns were detected (patterns C, F, and G), with patterns F and G representing all but

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TABLE 2. Relationship between the patterns of reactivity with VP4 NtMAbs and the G serotype of HRV specimens collected during two epidemic seasons, 1994–1995 and 1995–1996, in Mexico

VP7 serotype[a]	Season	No. (%) of specimens that reacted with the indicated MAb[b]									
		F45, 1A10 (A[c])	F45 (B)	1A10 (C)	F45, 1A10, RV5 (D)	F45, RV5 (E)	1A10, RV5 (F)	RV5 (G)	HS6 (H)	Neg[d]	Total
G1	1994–1995	32 (40)	30 (38)	2 (3)	3 (4)	1 (1)	—[e]	—	—	11 (14)	79
	1995–1996	45 (35)	22 (17)	3 (2)	8 (6)	4 (3)	—	—	—	48 (37)	130
G2	1994–1995	—	—	—	—	—	5 (46)	5 (46)	—	1 (9)	11
	1995–1996	—	—	1 (6)	—	—	5 (28)	9 (50)	—	3 (17)	18
G3	1994–1995	101 (57)	11 (6)	6 (4)	14 (8)	1 (1)	—	—	1 (1)	42 (24)	176
	1995–1996	2 (17)	2 (17)	3 (25)	—	—	—	—	—	4 (33)	11
G4	1994–1995	—	—	—	—	—	—	—	—	1 (100)	1
	1995–1996	—	—	2 (67)	—	—	—	—	—	1 (33)	3
Total	1994–1995	133 (50)	41 (15)	8 (3)	17 (6)	2 (1)	5 (2)	5 (2)	1 (0.4)	55 (21)	267
	1995–1996	47 (29)	24 (15)	9 (6)	8 (5)	4 (3)	5 (3)	9 (6)	—	56 (34)	162

[a] The results for 141 viruses whose G serotype could not be identified are not included here.
[b] The P serotypes of the viruses used to generate the VP4 Nt-MAbs are P1A for MAbs 1A10 and F45, P1B for MAb RV5, and P2A for MAb HS6.
[c] For simplicity during their reference in the text, the VP4 MAb patterns found were designated A to H.
[d] Neg, specimens that were not reactive with any of the MAbs used.
[e] —, no specimen reacting with the indicated VP4 MAbs was detected.

one of the characterized G2 strains. VP4 pattern C was the only one shared by serotype G1 to G4 viruses.

Seven of the eight VP4 patterns detected were present in the two seasons analyzed (Table 2). In general, the distribution of these patterns was similar during both years; only the frequency of HRV strains with pattern A showed a decrease (from 50 to 29%) in the two consecutive seasons ($P < 0.0001$). Likewise, the analysis of the temporal variation in the VP4 antigenic types according to their association with G serotypes showed minor differences, except for the VP4 proteins with reactivity patterns A, B, and C among serotype G3 viruses. The prevalence of viruses with pattern A decreased from 57 to 17% in the two consecutive seasons, while the prevalence of viruses with patterns B and C increased from 6 to 17% and from 4 to 25%, respectively ($P < 0.0001$).

Including the results for both epidemic seasons, 262 specimens (61%) reacted with either or both MAbs F45 and 1A10, 14 specimens (3%) reacted with MAb RV5, and one specimen (0.2%) reacted with MAb HS6 (Table 2). Interestingly, 41 specimens (10%) reacted with the P1B-derived MAb RV5 as well as with one or both of the P1A-derived MAbs (MAbs F45 and 1A10).

Diversity of the VP4 gene and correlation with the VP4 MAb reactivity. To understand the apparent paradox of the existence of HRV-containing specimens that were reactive with MAbs raised against rotaviruses of different P-serotype specificities, we investigated the correlation between the VP4 genomic type and the reactivity with VP4 MAbs. A representative sample of 52 specimens that included all the different NtMAb patterns of reactivity detected was genotyped by PCR. All HRV strains having patterns of reactivity A, B, D, and E were found to be genomic type P[8] (Table 3). Like the specimens that were recognized only by MAb RV5 (pattern G), the strains having pattern F were all genomic type P[4]. The strains with pattern C could be either genotype P[4] (one G2 strain) or genotype P[8] (two G3 strains). The single strain reacting with MAb HS6 (pattern H) was, as expected, genomic type P[6]. No strain was genomic type P[9]. These results indicate that the epitopes recognized by MAbs 1A10 and RV5 can be present in both P[4] (presumably P1B) and P[8] (presumably P1A) strains. Of relevance, among the strains characterized as P[4] and P[8], there were three and five different VP4-MAb patterns of reactivity, respectively (Table 3), indicating that the diversity of neutralization epitopes in VP4 is greater than that previously appreciated by the genomic typing methods.

Prevalence of rotavirus genomic P types. The correlation between the VP4 MAb patterns of reactivity and the genomic P types, along with the observation that among specimens

TABLE 3. Relationship between the patterns of reactivity with VP4 NtMAbs and the genomic P types of HRV-positive specimens

VP4 genomic type[a]	No. of specimens that reacted with the indicated MAbs[b]								
	F45, 1A10 (A[c])	F45 (B)	1A10 (C)	F45, 1A10, RV5 (D)	F45, RV5 (E)	1A10, RV5 (F)	RV5 (G)	HS6 (H)	Total
P[8]	27	7	2	7	2	—[d]	—	—	45
P[4]	—	—	1	—	—	2	3	—	6
P[6]	—	—	—	—	—	—	—	1	1
Total	27	7	3	7	2	2	3	1	52

[a] All genotype P[8] strains had a G1 or G3 VP7 serotype specificity; all P[4] viruses were serotype G2; the single P[6] strain belonged to serotype G3. These findings are in agreement with the known association between G serotypes and genomic P types.
[b] The P serotypes of the viruses used to generate the VP4 NtMAbs are P1A for MAbs 1A10 and F45, P1B for MAb RV5, and P2A for MAb HS6.
[c] For simplicity during their reference in the text, the VP4 MAb patterns found were designated A to H.
[d] —, no specimen having the indicated pattern of reactivity with the VP4 MAbs and genomic P type was identified.

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having a VP4 pattern C the serotype G2 viruses are genotype P[4] while other G serotypes are type P[8], allowed us to infer the prevalence of the different genomic P types in the epidemic seasons studied. Three hundred fifteen strains whose G serotype could be determined and which were reactive with at least one VP4 MAb were used to make this inference. Genomic type P[8] was the most prevalent in all three geographic regions, totaling from 62.5 to 100% of the specimens for different regions and epidemic seasons, while genomic type P[6] was the least prevalent (less than 1%). The prevalence of genomic type P[4] was highly variable; it was absent from the northeastern region in both epidemic seasons, while in the central and southeastern regions its prevalence increased from 0 to 6.5% and from 10.3 to 37.5%, respectively, in the consecutive seasons analyzed.

The results of this study are in agreement with the findings from previous surveys carried out in several countries, including Japan, Brazil, the United States, and South Africa, where it was found that P[8] is the genomic type with the highest prevalence, followed by P[4] as the second most prevalent virus and with other types, like P[3], P[6], and P[9], being detected less frequently (7). However, important deviations from this general pattern have recently been described. In a study carried out in India, it was found that genotype P[6] strains with G1, G2, G3, G4, and G9 specificities represented 43% of the typeable strains, with P[6], G9 being the most prevalent virus (24).

DISCUSSION

In this investigation we have characterized the antigenic variability of the VP4 protein from HRV strains isolated during two epidemic seasons in different regions of Mexico. This variability was correlated with the VP7 serotypes and the VP4 genotypes of the viruses studied.

As has been previously observed in Mexico (2, 22, 28) and has also been documented in many other studies around the world (1, 7, 16), we observed a change in the prevalent G rotavirus serotype from one season to the next. Serotype G3 viruses were the most prevalent during the first epidemic season, while during the second season they were superseded by serotype G1 strains. Also, the relative predominance of either of these two G serotypes during a single epidemic season was different among the geographic regions analyzed.

Since VP4 is an important inductor of neutralizing antibodies in natural infections of children (20, 29) and it segregates independently of VP7 (13), we investigated if, like VP7, it changes over time. To characterize the antigenic changes in the VP4 protein we used VP4 NtMAbs that had previously been described as P serotype specific (3, 23). The use of these MAbs allowed the recognition of eight different patterns of reactivity among the specimens studied, and the relative frequency of three of these VP4 patterns (all associated with G3 VP7 proteins) was found to change from one season to the next. It is, however, difficult to draw conclusions from the observed VP4 variability, since the reactivities of the viruses with the VP4 NtMAbs were not all-or-nothing events (see below), and, in addition, the number of G3 viruses collected during the first year was small compared with the number analyzed in the second year (11 versus 176). More studies are needed to confirm if the observed variability in VP4 indeed occurs and to determine if the putative changes of VP4 over time are independent of the associated VP7 protein or are just the consequence of the variability in the VP7 protein. Also, the significance of the potential variation in VP4 for the induction of protective immunity should be investigated.

In this study, the interaction of an HRV strain with more than one VP4 MAb was scored as positive when the second or third MAb recognized the virus with at least one-half the reactivity obtained with the MAb with the highest reactivity. The same twofold difference criterion has been used to determine the G-serotype specificity with VP7 MAbs (22). By changing this criterion, considering as significant reactivities that were at least one-fourth or one-eighth the value of the MAb with the highest reactivity, we found that the virus strains reacted with a wider spectrum of MAbs (data not shown), indicating that there is a difference in the degree rather than in the absolute reactivity of the P[8] and P[4] specimens with the MAbs tested. The high cross-reactivity observed with these VP4 MAbs is consistent with the known level of serologic cross-reactivity of VP4, even for the most serotypically diverse regions of the molecule (8, 18, 19).

The reactivities of the VP4 MAbs in our particular format were found not to be entirely restricted to a given VP4 genomic type. MAb 1A10 was found to react with 80% of P[8] strains and 50% of P[4] strains, while RV5 reacted with 20% of P[8] strains and 83% of P[4] strains. In a previous study, analysis of a limited number of rotavirus-positive stool samples showed that when hyperimmune sera matched to the VP7 serotype of the strains being characterized were used as capture antibodies, MAbs F45, ST3:3, and RV5 specifically recognized strains with inferred genotypes P[8], P[6], and P[4], respectively. However, when a mixture of hyperimmune sera to different G serotypes was used as the capture antibody in that study, a large proportion of the samples was found to cross-react with more than one MAb (3). Thus, the degree of the serotype cross-reactivity of the VP4 MAbs seems to depend on the specificity of the hyperimmune antiserum used in the assay for capture or detection of virus antigen.

The multiplicities of the VP4 MAb patterns of reactivity observed within P[8] and P[4] strains suggests that the diversity of neutralization epitopes on VP4 is greater than that suggested by genomic typing methods. These observations, together with the inconsistencies found between VP4 genotyping and VP4 serotyping, indicate that to fully understand the antigenic diversity and variability of VP4 and its relevance for the induction of protection, both serotyping and genotyping of HRVs should be carried out. In this regard, the MAbs used in this investigation appear to be useful for the antigenic characterization of the viruses, although their use as serotyping reagents still requires further investigation.

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REFERENCES

1. **Bishop, R. F.** 1994. Natural history of human rotavirus infections, p. 131–167. *In* A. Z. Kapikian (ed.), Viral infections of the gastrointestinal tract, 2nd ed. Marcel Dekker, Inc., New York, N.Y.
2. **Contreras, J. F., G. E. Menchaca, L. Padilla-Noriega, R. S. Tamez, H. B. Greenberg, S. López, and C. F. Arias.** 1995. Heterogeneity of VP4 neutralization epitopes among serotype P1A human rotavirus strains. Clin. Diagn. Lab. Immunol. **2:**506–508.
3. **Coulson, B. S.** 1993. Typing of human rotavirus VP4 by an enzyme immu-

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noassay using monoclonal antibodies. J. Clin. Microbiol. **31:**1–8.
4. **Estes, M. K.** 1996. Rotaviruses and their replication, p. 1625–1655. *In* B. N. Fields, D. N. Knipe, P. M. Howley, R. M. Chanock, J. L. Melnick, T. P. Monath, B. Roizman, and S. E. Straus (ed.), Virology, vol. 2. Raven Press, New York, N.Y.
5. **Flewett, T. H., C. F. Arias, L. F. Avendano, A. Ghafoor, M. M. Mathan, L. Mendis, K. Moe, and R. F. Bishop.** 1989. Comparative evaluation of the WHO and DAKOPATTS enzyme-linked immunoassay kits for rotavirus detection. Bull. W. H. O. **67:**369–374.
6. **Gentsch, J. R., R. I. Glass, P. Woods, V. Gouvea, M. Gorziglia, J. Flores, B. K. Das, and M. K. Bhan.** 1992. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J. Clin. Microbiol. **30:**1365–1373.
7. **Gentsch, J. R., P. A. Woods, M. Ramachandran, B. K. Das, J. P. Leite, A. Alfieri, R. Kumar, M. K. Bhan, and R. I. Glass.** 1996. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J. Infect. Dis. **174**(Suppl. 1)**:**S30–S36.
8. **Gorziglia, M., G. Larralde, A. Z. Kapikian, and R. M. Chanock.** 1990. Antigenic relationships among human rotaviruses as determined by outer capsid protein VP4. Proc. Natl. Acad. Sci. USA **87:**7155–7159.
9. **Herring, A. J., N. F. Inglis, C. K. Ojeh, D. R. Snodgrass, and J. D. Menzies.** 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J. Clin. Microbiol. **16:** 473–477.
10. **Hoshino, Y., R. W. Jones, and A. Z. Kapikian.** 1997. Serotypic characterization of VP4 of vervet monkey rotavirus (RV) SA11 by neutralization, p. 165, abstr. W38-11. *In* Abstracts of the 16th Annual Meeting of the American Society for Virology.
11. **Hoshino, Y., and A. Z. Kapikian.** 1994. Rotavirus antigens, p. 179–227. *In* R. F. Ramig (ed.), Rotaviruses, vol. 185. Springer-Verlag, Berlin, Germany.
12. **Hoshino, Y., and A. Z. Kapikian.** 1994. Rotavirus vaccine development for the prevention of severe diarrhea in infants and young children. Trends Microbiol. **2:**242–249.
13. **Hoshino, Y., M. M. Sereno, K. Midthun, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1985. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82:**8701–8704.
14. **Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Flores, and A. Z. Kapikian.** 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. J. Infect. Dis. **149:** 694–702.
15. **Isa, P., and D. R. Snodgrass.** 1994. Serological and genomic characterization of equine rotavirus VP4 proteins identifies three different P serotypes. Virology **201:**364–372.
16. **Kapikian, A. Z., and R. M. Chanock.** 1996. Rotaviruses, p. 1657–1708. *In* B. N. Fields, D. N. Knipe, P. M. Howley, R. M. Chanock, J. L. Melnick, T. P. Monath, B. Roizman, and S. E. Straus (ed.), Virology, vol. 2. Raven Press, New York, N.Y.
17. **Larralde, G., and J. Flores.** 1990. Identification of gene 4 alleles among human rotaviruses by polymerase chain reaction-derived probes. Virology **179:**469–473.
18. **Larralde, G., B. G. Li, A. Z. Kapikian, and M. Gorziglia.** 1991. Serotype-specific epitope(s) present on the VP8 subunit of rotavirus VP4 protein. J. Virol. **65:**3213–3218.
19. **Matsui, S. M., E. R. Mackow, and H. B. Greenberg.** 1989. Molecular determinant of rotavirus neutralization and protection. Adv. Virus Res. **36:**181–215.
20. **Menchaca, G., L. Padilla-Noriega, M. Méndez-Toss, J. F. Contreras, F. I. Puerto, H. Guiscafré, F. Mota, I. Herrera, R. Cedillo, O. Muñoz, R. Ward, Y. Hoshino, S. López, and C. F. Arias.** 1998. Serotype specificity of the neutralizing-antibody response induced by the individual surface proteins of rotavirus in natural infections of young children. Clin. Diagn. Lab. Immunol. **5:**328–334.
21. **Nakagomi, O., Y. Isegawa, Y. Hoshino, Y. Aboudy, I. Shif, I. Silberstein, T. Nakagomi, S. Ueda, J. Sears, and J. Flores.** 1993. A new serotype of the outer capsid protein VP4 shared by an unusual human rotavirus strain Ro1845 and canine rotaviruses. J. Gen. Virol. **74:**2771–2774.
22. **Padilla-Noriega, L., C. F. Arias, S. López, F. Puerto, D. R. Snodgrass, K. Taniguchi, and H. Greenberg.** 1990. Diversity of rotavirus serotypes in Mexican infants with gastroenteritis. J. Clin. Microbiol. **28:**1114–1119.
23. **Padilla-Noriega, L., R. Werner-Eckert, E. R. Mackow, M. Gorziglia, G. Larralde, K. Taniguchi, and H. B. Greenberg.** 1993. Serologic analysis of human rotavirus serotypes P1A and P2 by using monoclonal antibodies. J. Clin. Microbiol. **31:**622–628.
24. **Ramachandran, M., B. K. Das, A. Vij, R. Kumar, S. S. Bhambal, N. Kesari, H. Rawat, L. Bahl, S. Thakur, P. A. Woods, R. I. Glass, M. K. Bhan, and J. R. Gentsch.** 1996. Unusual diversity of human rotavirus G and P genotypes in India. J. Clin. Microbiol. **34:**436–439.
25. **Shaw, R. D., M. D. Stoner, M. K. Estes, and H. B. Greenberg.** 1985. Specific enzyme-linked immunoassay for rotavirus serotypes 1 and 3. J. Clin. Microbiol. **22:**286–291.
26. **Shaw, R. D., P. T. Vo, P. A. Offit, B. S. Coulson, and H. B. Greenberg.** 1986. Antigenic mapping of the surface proteins of rhesus rotavirus. Virology **155:** 434–451.
27. **Taniguchi, K., T. Urasawa, Y. Morita, H. B. Greenberg, and S. Urasawa.** 1987. Direct serotyping of human rotavirus in stools by an enzyme-linked immunosorbent assay using serotype 1-, 2-, 3-, and 4-specific monoclonal antibodies to VP7. J. Infect. Dis. **155:**1159–1166.
28. **Velázquez, F. R., J. J. Calva, M. L. Guerrero, D. Mass, R. I. Glass, L. K. Pickering, and G. M. Ruiz-Palacios.** 1993. Cohort study of rotavirus serotype patterns in symptomatic and asymptomatic infections in Mexican children. Pediatr. Infect. Dis. J. **12:**54–61.
29. **Ward, R. L., M. M. McNeal, D. S. Sander, H. B. Greenberg, and D. I. Bernstein.** 1993. Immunodominance of the VP4 neutralization protein of rotavirus in protective natural infections of young children. J Virol. **67:** 464–468.

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