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Heterogeneity of VP4 Neutralization Epitopes among Serotype P1A Human Rotavirus Strains

JUAN F. CONTRERAS,[1]* GRISELDA E. MENCHACA,[1] LUIS PADILLA-NORIEGA,[2] REYES S. TAMEZ,[1] HARRY B. GREENBERG,[3] SUSANA LÓPEZ,[2] AND CARLOS F. ARIAS[2]

Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Manuel Barragán y Pedro de Alva, San Nicolás de los Garza, Nuevo León C.P. 66450,[1] and Departamento de Genética y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Colonia Miraval, Cuernavaca, Morelos 62271,[2] Mexico, and Division of Gastroenterology, Stanford University School of Medicine, Stanford, California 94305, and Veterans Administration Medical Center, Palo Alto, California 94304[3]

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We have used serotype-specific VP4 and VP7 neutralizing monoclonal antibodies (Nt-MAbs), as well as subgroup (SG)-specific MAbs, to characterize by enzyme immunoassay rotavirus strains isolated from diarrheic infants in the city of Monterrey, Mexico, from July 1993 to March 1994. Of a total of 465 children studied, 140 were rotavirus positive, including 3 patients infected with non-group A rotaviruses. The SG and VP7 (G) serotype specificities could be determined for 118 (84%) of the 140 rotavirus-positive stool specimens; 4 rotavirus strains were serotype G1 and SGII; 1 strain was serotype G2 and SGI+II; 112 strains were serotype G3 and SGII; 1 strain was serotype G3 and SGI; and none of the strains was serotype G4. Fifty-eight specimens, representing the 13 different group A rotavirus electropherotypes detected, were chosen for VP4 (P) serotyping. Of these, 48 (83%) strains reacted with the P1A serotype-specific Nt-MAb 1A10. None of the strains reacted with the serotype P2-specific Nt-MAbs tested. Not all viruses that reacted with Nt-MAb 1A10 were recognized by Nt-MAbs 2A3 and 2G1, which also recognize P1A strains, indicating heterogeneity of neutralization epitopes among serotype P1A human rotaviruses. This heterogeneity could be relevant for the specificity of the VP4-mediated neutralizing antibody immune response and indicates the need for antigenic characterization, in addition to genomic typing, of the VP4 proteins of circulating human rotavirus field strains.

Group A rotaviruses are the single most important cause of severe dehydrating diarrhea in children under 3 years of age (10). These viruses are an important cause of infant mortality in developing countries and of infant morbidity in developed countries (12). Live vaccines have been tested in humans in different areas of the world and with different age groups (2, 11, 26). These trials have yielded inconsistent results, presumably in part because the most prevalent serotype of rotavirus infecting vaccinated communities has varied (2, 11, 26), suggesting the need for homotypic immunization for protection against rotavirus disease. Therefore, a more detailed knowledge of the prevalence and diversity of different rotavirus serotypes in different geographical areas will be important for the development of effective rotavirus vaccines.

The surface of rotavirus is formed by two proteins, VP7 and VP4. The antibody response to these proteins has the ability to neutralize the infectivity of the virus in vitro as well as in vivo (13, 14, 23), and the specificities of these antibodies to neutralize different rotavirus strains have been used to classify rotaviruses into various serotypes. Since both surface proteins induce neutralizing antibodies, the viruses can be classified on the basis of either VP7 (G serotypes) or VP4 (P serotypes).

On the basis of VP7, 14 different serotypes have been identified so far (9). Nine of these serotypes have been found in rotaviruses isolated from humans, although four of them (G1 to G4) appear to account for the great majority of infections (3, 29). VP4 from group A rotaviruses has been classified into at least 15 genomic types by hybridization and/or sequence analysis (9, 21); 7 of them have been found in human rotaviruses (HRVs), and 6 of these P genomic types have been confirmed to represent different antigenic types (P serotypes), as determined by neutralization with hyperimmune sera directed to the VP4 protein (9, 21). Of the six HRV P serotypes, five have been associated with symptomatic infections (P1A, P1B, P3A, P3B, and P4), and one has been associated with asymptomatic infections (P2).

The availability of neutralizing monoclonal antibodies (Nt-MAbs) specific for different VP7 serotypes permitted extensive epidemiological studies to define the diversity and prevalence of the G serotypes of HRV strains in many countries, including Mexico (3, 15, 25). On the other hand, although apparently the principal neutralizing antibody response is directed to VP4 in a natural infection (5, 18, 27, 28), knowledge about the diversity of P serotypes in circulating HRV strains is scarce. A few initial studies have been recently conducted to characterize the VP4 genes of HRV field strains by hybridization or PCR analysis (7, 19, 20, 22, 24, 30). These studies are providing important information about the prevalence of the different rotavirus P genomic types; however, this typing method does not necessarily reflect the antigenic diversity of the protein. Recently, two groups reported the isolation of the first VP4-directed Nt-MAbs that can recognize strains of different P serotypes (6, 17). Using a panel of such VP4-specific Nt-MAbs, as well as VP7-specific MAbs, we have characterized by enzyme-linked immunosorbent assay the G and P serotypes of HRV strains isolated from diarrheic children in the city of Monterrey, located in northeast Mexico.

Children under 2 years of age who either had been admitted

* Corresponding author. Mailing address: Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Manuel Barragán y Pedro de Alva, Apartado Postal 46 "F," San Nicolás de los Garza, Nuevo León C.P. 66450, Mexico. Phone: (52-8) 376-4319. Fax: (52-8) 352-0504.

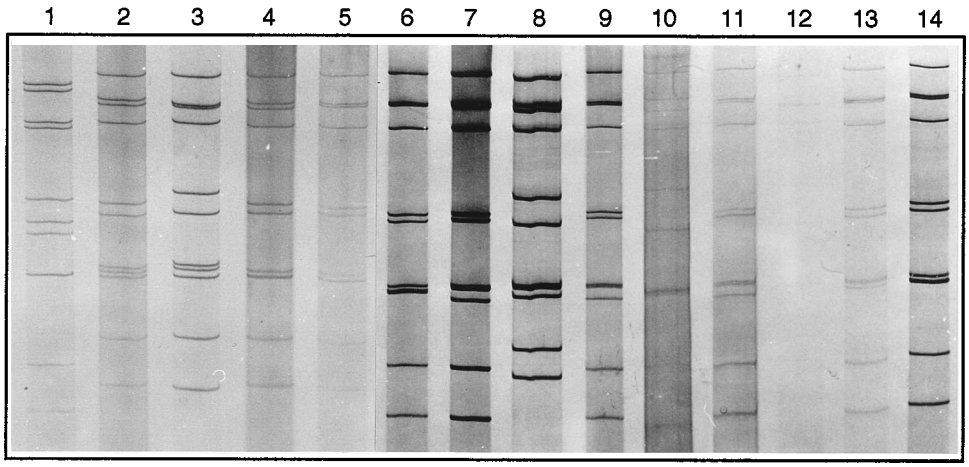

FIG. 1. Polyacrylamide gel electrophoresis analysis of HRV double-stranded RNAs extracted from stool specimens collected during the study. Lanes 1 to 14 show the representative 14 different electropherotypes detected. Lane 1 shows the non-group A rotavirus electropherotype found in three sample specimens. Lanes 6 to 14 were run in a separate gel.

with diarrhea to three hospitals of the Instituto Mexicano del Seguro Social in Monterrey or had been treated at the emergency wards of these hospitals were included in this study. Fecal samples were tested for the presence of rotavirus by silver staining of viral double-stranded RNA segments separated by gel electrophoresis (8). The subgroup (SG) and G serotype specificities were determined as described previously (15). The VP7 serotype was identified with Nt-MAbs KU4 (serotype G1), 2F1 (serotype G2), 4F8 (serotype G3), and ST-2G7 (serotype G4). To confirm the presence of VP7, cross-reactive MAb 129 was included. SGI and SGII were identified with MAbs 255/60 and 631/9, respectively. The VP4 serotype was determined with Nt-MAb 1A10, which is specific for serotype P1A strains; Nt-MAb 2A3, which recognizes serotype P1A strains but cross-reacts with some serotype P2 strains; and Nt-MAb 2G1, which in its initial characterization reacted with most serotype P1A strains but also with the serotype P3 strain K8 and some P2 strains (17). Two Nt-MAbs (HS6 and HS11) specific for serotype P2 were also used (17).

A total of 465 children with acute gastroenteritis were enrolled from July 1993 to March 1994. Of these children, 140 (30%) were rotavirus positive; the frequency of infection peaked in December, when 61 (50%) of the children with gastroenteritis were positive for this virus. Electrophoretic analysis of the viral genomic RNA showed the presence of 13 distinct group A and 1 non-group A (three strains) rotavirus electropherotypes (Fig. 1). All but one of the group A rotavirus strains had a long electropherotype. The SG could be determined for 118 (86%) of the 137 group A rotavirus strains detected: 116 long-electropherotype strains were SGII, 1 strain with a long electropherotype was found to be SGI, and the 1 rotavirus with a short electropherotype reacted with both SGI and SGII MAbs (SGI+II). All of the subgrouped samples were also recognized by VP7-specific MAb 129, and in all these cases the G serotype could be determined: 113 strains were serotype G3, 4 were serotype G1, and 1, the virus with the short electropherotype, was found to be serotype G2 (Table 1). To study the epitope diversity of the VP4 protein, 58 of the 118 HRV strains characterized as described above, representing the 13 group A rotavirus electropherotypes observed, were chosen. Forty-eight (83%) of the 58 samples were recognized by at least two Nt-MAbs: 41 strains reacted with the three serotype P1A Nt-MAbs 1A10, 2A3, and 2G1; 6 strains reacted only with Nt-MAbs 1A10 and 2A3; and 1 strain reacted only with Nt-MAbs 1A10 and 2G1 (Table 2). No strain was recognized by Nt-MAb HS6 or HS11. Ten rotavirus strains (nine serotype G3 and SGII; one serotype G2 and SGI+II) were not recognized by any of the serotype-specific VP4 Nt-MAbs used, although nine of them (including the serotype G2 strain) did react with the cross-reactive VP4 Nt-MAb YO-2C2, suggesting that these viruses could either represent a novel P serotype or belong to a known P serotype not recognized by the MAbs used, for instance, serotype P1B, as is probably the case for the serotype G2 strain. Determination of the P genomic types of these strains should help to answer this question.

We did not observe cross-reactivity of the serotype P2 Nt-MAbs HS6 and HS11 with any of the 48 serotype P1A strains found, thus indirectly confirming the serotype P2 specificity of the epitopes recognized by these MAbs, which have been mapped around amino acids 72 and 217 in VP8, respectively (16). In contrast, the serotype P2-specific Nt-MAbs described

TABLE 1. SG and G serotype specificities of rotavirus strains isolated in Monterrey, Mexico

SG	No. of strains of serotype:				Total no. of strains
	G1	G2	G3	G4	
SGI	0	0	1	0	1
SGII	4	0	112	0	116
SGI+II	0	1	0	0	1
Total	4	1	113	0	118

TABLE 2. VP4 epitope heterogeneity in HRV strains and correlation with G serotype specificity

Serotype	No. of strains reacting with MAbs:			Total no. of strains tested
	1A10, 2A3, and 2G1	1A10 and 2A3	1A10 and 2G1	
G1	2	1	1	4
G3	39	5	0	44
Total	41	6	1	48

by Coulson (6) were found to have some cross-reactivity with serotype P1A strains.

So far, the studies conducted to determine the diversity of VP4 genomic types in HRV field strains have indicated that the most frequently observed VP4 gene allele is the genomic type P8 (which has been correlated with serotype P1A), ranging in frequency from 52 to 93% (7, 19, 20, 22, 24, 30). In this first study of P serotypes, an overall predominance of the serotype P1A strains was observed, representing 83% of the typeable strains.

The fact that not all rotavirus strains recognized by MAb 1A10 were recognized by MAbs 2G1 and 2G3 indicates that there is epitope heterogeneity within the serotype P1A VP4 proteins. Since all of these MAbs have neutralizing activity, this epitope heterogeneity could be a determinant for the specificity of the VP4-mediated neutralizing antibody immune response. This will ultimately depend on the immunodominance of the various VP4 neutralization epitopes in a natural infection. These findings are in agreement with previous studies by our group in which it was found that the specificity of the immune response observed in primary rotavirus infections is an intrinsic property of the virus strain, and this may vary from G serotype to G serotype but also from strain to strain within a given G serotype (1). It is therefore relevant to study the diversity and distribution of the VP4 epitopes within the various P serotypes identified among HRVs and to evaluate the VP4 epitope-specific immune responses of rotavirus-infected children. The epitope diversity within a given P serotype cannot be revealed by the genomic methods for typing VP4; therefore, it is important to conduct epidemiological studies using the currently available serotype-specific and cross-reactive VP4 MAbs and to isolate new sets of MAbs for the fine antigenic characterization of the VP4 serotypes thus far identified in HRV strains.

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