Comparisons of Rotavirus VP7-Typing Monoclonal Antibodies by Competition Binding Assay

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Three sets of neutralizing monoclonal antibodies (MAbs) used to type the outer capsid protein VP7 of four group A rotavirus serotypes (1 through 4) were compared in competition immunoassays. Reciprocal competition was observed for each of the VP7 type 2-, 3-, and 4-specific MAbs. The VP7 type 1 MAbs exhibited variable competition patterns with other VP7 type 1 MAbs. MAb RV4:3, which has been used to recognize antigenic variants within VP7 type 1 strains, showed reciprocal competition with the four VP7 type 3 MAbs (RV3:1, YO-1E2, 4F8, and 159) using a VP7 type 3 virus (SA11) as antigen. MAb 2C9, also prepared against VP7 type 1, reacted with VP7 type 3 strains and competed with a VP7 type 3 MAb, 159, using RRV as antigen. Use of the different sets of VP7 type-specific MAbs in the enzyme-linked immunosorbent assay permitted the recognition of six antigenic variants within VP7 types 1, 2, and 3 among specimens whose VP7 type could not be determined previously with only one set of typing MAbs. These results demonstrate differences of typing ability among these VP7-specific MAbs and emphasize the need to improve the sensitivity of typing systems by incorporating panels of MAbs reacting with several neutralizing epitopes.

Rotaviruses are the most commonly recognized cause of gastroenteritis in children worldwide (9, 19). Rotaviruses have two surface structural proteins, VP4 and VP7, which generate neutralizing antibodies (16, 18, 19, 26, 27). VP7 is the major determinant of serotype specificity (7). Several investigators have prepared serotype-specific neutralizing monoclonal antibodies (MAbs) to VP7 and incorporated these MAbs into typing enzyme-linked immunosorbent assays (ELISAs) to describe the antigenic diversity of circulating rotaviruses (4, 17, 23, 30, 33, 35, 36).

We are interested in understanding the antigenic diversity of circulating rotaviruses, as well as the immune response to antigenic variants. In this study, we compared the three most widely used sets of neutralizing VP7-typing MAbs for their abilities to bind rotavirus particles in competition immunoassays. These panels of MAbs already have been shown to bind differently to sets of circulating human rotaviruses in typing ELISAs (11, 23, 25, 35, 36). Some neutralizing MAbs bind only a subset of viruses (called monotypic variants) within a VP7 type in ELISAs (4, 10). The apparent natural antigenic diversity of circulating rotaviruses has made it questionable whether only one set of suitable reagents can be applied as a standard set of typing MAbs worldwide. Some of the MAbs have been used to measure epitope- and VP7 type-specific antibody responses in vaccine recipients (15, 29). One method for selecting typing MAbs would be to choose MAbs that bind to different sites on VP7. Unfortunately, the mutation site of the gene encoding VP7 in viral variants that escape neutralization by type-specific neutralizing MAbs is known for only some MAbs (6, 22, 31, 32) (see Table 1). We used direct comparisons of the MAbs in competition assays to help improve the selection of MAb sets for typing assays, for studies of the

range of antigenic subtypes circulating in epidemiological surveys, and for the investigation of epitopes involved in protective immunity.

MATERIALS AND METHODS

Virus production and purification. Both laboratory strains and virus from clinical specimens were used as antigen in the competition assays. Cultivable virus strains included human Wa (serotype 1); human S2 (serotype 2); simian SA11 and RRV; human YO, Ito, and P (serotype 3); and human St. Thomas 3 (serotype 4) (20). These viruses were propagated in MA-104 cells in the presence of pancreatin as previously described (8, 20). Each antigen was diluted in the competition assay with homotypic MAb reagents to yield an absorbance 50% of maximal, which was in the range of 0.4 to 0.8 at 414 nm. Because MAb RV4:1 bound the prototype VP7 type 1 virus Wa poorly, for competition assays with MAb RV4:1, three clinical specimens from the year 1989, from the same site (23), and showing the same reactivity pattern with all six VP7 type 1 MAbs were pooled in equal volume to obtain a sufficient quantity of antigen.

Competitive MAb capture ELISA. The competition ELISA was performed as described previously (1) with minor modifications. Three sets of neutralizing MAbs (purified or ascites), RV4:1, RV4:2, RV4:3, RV5:3, RV3:1, and ST3:1; KU-4, S2-2G10, YO-1E2, and ST-2G7; and 5E8, 2C9, IC10, 4F8, and 159, specific for the rotavirus outer capsid protein VP7 of serotypes 1 through 4, were tested (Table 1). We found that all the MAbs could be used as both capture and detector reagents in the competition assay, even though some of these MAbs had been reported previously to be more sensitive or effective (in serotyping tests) as detector antibodies (4). MAb 60-F2D4 was included in the competition assays for comparison because it reacts with a VP7 epitope present on most rotavirus strains (28). Each MAb

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TABLE 1. Properties of VP7 type 1, 2, 3, and 4 and VP7 common MAb used in this study

MAb	Immunizing antigen	VP7 type	Amino acid site (region) ^a	Amino acid change	Isotype	Form tested ^b	Reference(s)
RV4:1	RV4	1a	147 (B)	Asn→His	IgG1	Purified	2b, 4
RV4:2	RV4	1b	213 (C)	Asp→Gly	IgG3	Purified	2b, 4
RV4:3	RV4	1c	94 (A)	Asn→Ser	IgG2B	Purified	2b, 4
KU-4	KU	1	213 (C)	Asp→Gly	IgG3	Purified	32, 33
5E8	D × RRV ^c	1	—	—	—	Ascites	25
2C9	Wa	1	94 (A)	—	IgG1	Ascites	14, 30
RV5:3	RV5	2	—	—	IgG2B	Purified	4
S2-2G10	S2	2	190 (?)	Asp	IgG2A	Purified	15, 33
1C10	DS1 × RRV ^d	2	—	—	—	Ascites	25
RV3:1	RV3	3	211 (C)	Asp→Asn	IgG2B	Purified	4, 6
YO-1E2	YO	3	221 (C)	Ala ^e	IgG2B	Purified	24, 33
4F8	RRV	3	96 (A)	Asn→Asp	IgG1	Purified	22, 30
159	RRV	3	94 (A)	Asn→Lys	IgG1	Ascites	22, 31
ST3:1	ST-3	4	—	—	IgG3	Purified	4
ST-2G7	ST-3	4	—	—	IgG2A	Purified	33
60-F2D4	Wa	VP7com	—	—	IgG1	Purified	28, 31

^a Within VP7, the following three regions exhibit marked variability among VP7 types and are important sites for type-specific neutralizing MAb binding: A, amino acids 87 to 101; B, amino acids 143 to 152; and C, amino acids 208 to 221. The region for S2-2G10 is uncertain. —, not known.

^b Most assays used purified MAbs. Ascites fluid was used when only small amounts were available.

^c Reassortant containing VP7 type 1 from human rotavirus D strain on a background of RRV.

^d Reassortant containing VP7 type 2 from human rotavirus DS1 strain on a background of RRV.

^e More than one amino acid for substitution of alanine has been observed.

was titrated for capture activity with homologous antigen, and reactivity without antigen served as a control. MAbs used for capture in the competition experiments were diluted to yield maximal antigen binding. The protein concentration of each purified MAb used for capture varied as much as 190-fold (Table 2). The specificity of each MAb for prototype virus strains representing each of the human VP7 types 1 through 4 was confirmed. Because the VP7 type 1 MAb RV4:3 detected and captured VP7 type 3 viruses, including serotype 3 clinical specimens (3), competition of MAb RV4:3 was evaluated with both VP7 type 1 and type 3 MAbs. MAb 2C9 also captured VP7 type 3 viruses, including RRV, YO, Ito, and P but not SA11, a cross-reactivity not described previously. Therefore, competition of 2C9 with the VP7 type 3 MAb 159 was also tested with RRV.

For the competition ELISA, flexible polyvinyl microtiter plates (Dynatech Laboratories, Inc., Chantilly, Va.) were coated with 60 µl of the optimal dilution of purified MAb or ascites in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and incubated overnight at 4°C. Simultaneously, in separate tubes, serial twofold dilutions of competing MAb in PBS were mixed with a predetermined concentration of homologous viral antigen and incubated overnight at 4°C. Prototype viral test antigens were infected cell lysates. Some were concentrated by centrifugation through a 40% sucrose cushion and resuspension of the pellet in Tris-buffered saline. After the plates were blocked with 1% bovine serum albumin, the preincubated antigen-antibody complexes were added and incubated overnight at 4°C. After a wash step with PBS containing 0.1% Tween 20 (PBS-T), hyperimmune guinea pig antirotavirus antiserum homologous for the bound antigen (23) was added and incubated at 37°C for 1 h. After another wash step, horseradish peroxidase-conjugated goat anti-guinea pig immunoglobulin G (IgG) antibody (Organon Teknica Corp., West Chester, Pa.) was added and incubated for 1 h at 37°C. Finally, the plates were washed and incubated with ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] (Sigma Chemical Co.) for 30 min at room

temperature, and optical densities at 414 nm were determined with a Titertek Multiskan plate reader (Flow Laboratories, Inc., McLean, Va.).

The first column of the microtiter plate received only substrate and served as a blank, the second column contained antigen homologous to the capture MAb without competing MAb, the third column received antigen diluent, and the remaining columns received serial dilutions of competing MAbs with viral antigen. The first dilution of competing MAb (1:1) was the first dilution to yield maximal competition when the same MAb was used as capture antibody. Competition was scored as positive when a competing MAb reduced the absorbance value by 50% or more of that detected in the wells containing antigen but no competing MAb. In the competition assay, we defined three types of reactions: competition (51 to 100%), partial competition (40 to 50%), and no competition (0 to 39%).

Serotyping ELISA and detection of intratypic antigenic variants among VP7 type 1 and "untypeable" clinical specimens. Stool specimens were serotyped by a MAb-capture ELISA (23). To test whether the different VP7 type 1 MAbs are able to detect antigenic variants of VP7 type 1 strains, we tested 60 fecal extracts representing eight consecutive years (1983 through 1990) and from two geographic locations. Of these, 36 were collected from Houston and the other 24 were from Ohio (23). These specimens were strongly positive with VP7 type 1 MAb KU-4 in the VP7-typing ELISA (23). Another set of 30 samples whose VP7 type was undetermined previously was also tested with the three sets of VP7 type 1- to 4-specific MAbs to detect antigenic variants within VP7 type 1, 2, 3, or 4. A total of 6 of these 30 samples had previously been shown to be strongly reactive with MAb 60-F2D4, and the remaining 24 samples reacted only with a MAb to VP6 (were negative with the VP7 common MAb). Samples reacting with only some of the typing MAbs were compared with previously typed specimens by electrophoresis of phenol-chloroform-extracted rotavirus RNA, as previously described (5).

TABLE 2. Summary of competition assay results

VP7 Type	Capture MAb	Capture MAb concn (μg/well)	Antigen ^a	VP7 MAb to indicated VP7 type used as the competing antibody ^b															
				Type 1				Type 2				Type 3				Type 4		Common	
				RV4:1	RV4:2	RV4:3	KU-4	5E8	2C9	RV5:3	S2-2G10	1C10	RV3:1	YO-1E2	4F8	159	ST3:1		ST-2G7
1	RV4:1	0.15	CS ^c	Yes	Yes	Yes	Yes	Yes	P	— ^d	—	—	—	—	—	—	—	—	No
1	RV4:2	0.54	Wa	Yes	Yes	Yes	Yes	Yes	Yes	—	—	—	—	No	—	—	—	—	No
1	RV4:3	1.9/0.24 ^e	Wa (SA11)	Yes	Yes	Yes (Yes)	P	Yes	Yes	—	—	—	(Yes)	No (Yes)	No (Yes)	(Yes)	—	—	No
1	KU-4	0.47	Wa	Yes	Yes	Yes	Yes	Yes	Yes	—	No	—	—	No	—	—	—	No	No
1	5E8	Ascites ^f	Wa	Yes	Yes	Yes	P	Yes	Yes	—	—	—	—	—	—	—	—	—	No
1	2C9	Ascites	Wa (RRV)	Yes	Yes	Yes	P	Yes	Yes	—	—	—	—	—	—	(Yes)	—	—	No
2	RV5:3	0.02	S2	—	—	—	—	—	—	Yes	Yes	Yes	—	—	—	—	—	—	No
2	S2-2G10	0.18	S2	—	—	—	No	—	—	Yes	Yes	Yes	—	No	—	—	—	No	No
2	1C10	Ascites	S2	—	—	—	—	—	—	Yes	Yes	Yes	—	—	—	—	—	—	No
3	RV3:1	0.47	SA11	—	—	Yes	—	—	—	—	—	—	Yes	Yes	Yes	Yes	—	—	—
3	YO-1E2	0.28	SA11	—	No	Yes	No	No	—	—	No	—	Yes	Yes	Yes	Yes	—	No	No
3	4F8	0.15	SA11	—	—	Yes	No	—	—	—	—	—	Yes	Yes	Yes	Yes	—	—	No
3	159	Ascites	SA11 (RRV)	—	—	Yes	—	—	(No)	—	—	—	Yes	Yes	Yes	Yes	—	—	No
4	ST3:1	3.8	ST3	—	—	—	—	—	—	—	—	—	—	—	—	—	Yes	Yes	—
4	ST-2G7	0.12	ST3	—	—	—	No	—	—	—	No	—	—	No	—	—	Yes	Yes	—
VP7 com	60-F2D4	2.4	** ^g	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes

^a Antigen listed in parentheses indicates competition experiments included as control reactions or to evaluate MABs reacting with more than one VP7 type. CS, clinical specimens.^b Yes, competition was observed as defined in Materials and Methods; No, competition was not observed; P, partial competition.^c Competition with MAb RV4:1 with a pool of three VP7 type 1 CS.^d Not determined.^e The first concentration shown was used for experiments with the first antigen listed, and the second concentration was used for experiments with the second antigen.^f Ascites fluid was used. The specific concentration of each MAB in the ascites fluid is not known.^g Because 60-F2D4 bound each antigen, the antigen used in competition was selected to match that of the competing MAB: CS (RV4:1); Wa (RV4:2, RV4:3, KU-4, 5E8, 2C9); S2 (RV5:3, S2-2G10, 1C10); SA11 (RV4:3, RV3:1, YO-1E2, 4F8, 159); ST3 (ST3:1, ST-2G7); and RRV (2C9, 159).

FIG. 1. Competition between MAb KU-4 and other VP7 type 1 MABs with VP7 type 1 virus Wa as the antigen. Each line represents a competition curve between two MABs in which the first MAB in the following pairs is the capture MAB and the second is the competing MAB, as explained in Materials and Methods: ■, RV4:1 versus KU-4; □, RV4:2 versus KU-4; ◆, RV4:3 versus KU-4; ◇, KU-4 versus KU-4; ▲, 5E8 versus KU-4; and △, 2C9 versus KU-4. The points at the right end of each line indicate the optical density values of wells containing no competing MAB. Variation of "no competing antibody" results occurred because the competition data for each figure were compiled from experiments performed with separate plates and because different virus preparations were required to complete a set of competition experiments.

RESULTS

Competition of MABs prepared against different VP7 types.

Our initial assays showed that, with the exception of MABs RV4:3 and 2C9, none of the MABs tested bound to standard laboratory strains outside the VP7 type against which they were prepared. To confirm that MAB binding in the competition assays showed the same specificity as in the VP7-typing ELISA (23), we performed cross-competition assays among the serotype 1- to 4-specific typing MABs KU-4, S2-2G10, YO-1E2, and ST-2G7, respectively. As expected, only homotypic competition was observed (Table 2).

Competition of each MAB against different MABs to the same VP7 type. Each MAB competed with itself. Each of the VP7 type 2-, 3- and 4-specific MABs competed with the other MABs within its respective VP7 type (Table 2). However, among the six MABs prepared against VP7 type 1, five patterns of competition were apparent. The MABs prepared against type 1 fell into two groups, differentiated by their ability to be competed by MAB KU-4. KU-4 competed with RV4:1 and RV4:2 to the degree that KU-4 competed with itself (Fig. 1). KU-4's competition with MABs RV4:3, 5E8, and 2C9 was consistently less than 50%. This result was

observed whether the pool of clinical isolates or Wa was used as antigen. RV4:1 and RV4:2 were unlike KU-4 in that both competed with RV4:3, 2C9, and 5E8 (Fig. 1 and 2; Table 2). This indicated that the VP7 type 1 MABs might be grouped as follows: RV4:1 with RV4:2; RV4:3 with 2C9 and 5E8; and KU-4 by itself. However, 2C9 differentiated RV4:1 from RV4:2 by competing with RV4:1 partially but competing with RV4:2 fully (80%). RV4:1 also differed from RV4:2 because RV4:1 reacted poorly with VP7 type 1 strain Wa, whereas RV4:2 reacted strongly with this strain. Finally, differences among MABs RV4:3, 2C9, and 5E8 were noted. MABs RV4:3 and 2C9 bound VP7 type 3 viruses and RV4:3 competed with type 3-specific MABs, whereas 5E8 did neither (see below). In summary, then, among the six VP7 type 1 MABs evaluated, only RV4:3 and 2C9 exhibited similar patterns of competition.

Competition of MABs RV4:3 and 2C9 with VP7 type 3 virus. MAB RV4:3 is one of a panel of three MABs that recognize monotypic variants within type 1 viruses (2, 34). This MAB also reacts with VP7 type 3 viruses (3), a finding confirmed in this paper. MAB RV4:3 competed with itself whether Wa or SA11 was used as test antigen. When the type 3 virus SA11

FIG. 2. Competition between MAb RV4:1 and each of the VP7 type 1 MAbs with pooled VP7 type 1 clinical specimens as the antigen. ■, RV4:1 versus RV4:1; □, RV4:3 versus RV4:1; ◆, 2C9 versus RV4:1; ◇, 5E8 versus RV4:1. See the legend to Fig. 1 for further explanation of the symbols and lines.

was used as antigen, reciprocal competition between MAb RV4:3 and the VP7 type 3 MAbs was observed (Fig. 3). Two other MAbs prepared against VP7 type 1, RV4:2 and 5E8, did not compete with the VP7 type 3 MAb YO-1E2 for binding to SA11. MAb 2C9 behaved in a manner similar to

MAb RV4:3. MAb 159 competed with 2C9 for binding to RRV, confirming the cross-reactivity of this MAb. So that we might have a negative control for the competition assays, we also tested whether the VP7 type 3 MAbs (that had not been shown to bind to Wa in ELISA and neutralizing tests)

FIG. 3. Competition between MAb RV4:3 and VP7 type 3 MAbs with VP7 type 3 virus SA11 as the antigen. ■, RV4:3 versus RV4:3; □, RV4:3 versus RV3:1; ◆, RV4:3 versus YO-1E2; ◇, RV4:3 versus 4F8; ▲, RV4:3 versus 159. See the legend to Fig. 1 for further explanation of the symbols and lines.

could compete with VP7 type 1 MAbs. We found that MAbs YO-1E2 and 4F8 did not compete with RV4:2, RV4:3, and KU-4 when Wa was the antigen.

Competition of VP7-typing MAbs with a MAb to a common epitope on VP7. The sites of binding for all of the VP7-typing MAbs are not known. We tested each MAb for its ability to compete with MAb 60-F2D4, a MAb that reacts with an epitope present on the VP7 molecule of many types. VP7 common MAb 60-F2D4 competed with itself with all antigens tested. The type 2- and 3-specific MAbs competed with 60-F2D4 (Table 2). The type 4-specific MAbs did not. Among the MAbs prepared against VP7 type 1, RV4:1 and RV4:2 competed with 60-F2D4, but RV4:3, KU-4, 2C9, and 5E8 did not. All of these competitions were in one direction; 60-F2D4 did not compete with any MAb other than itself.

Intratype antigenic variants among clinical specimens. Sixty clinical specimens from Houston and Ohio which were originally characterized as VP7 type 1 with MAb KU-4 were tested with the other VP7 type 1 MAbs also. These 60 samples were positive with all six of the VP7 type 1 MAbs. A total of 6 antigenic variants were detected among the 30 samples from Houston and Ohio previously designated "un-typeable." Of these 30 samples, 6 could be scored as antigenic variants. One monotype 1d (34) of VP7 type 1 reacting with MAb RV4:3 was detected. Three specimens yielded serotype 2 viruses; one reacted with S2-2G10 and 1C10, while the other two reacted only with 1C10. Two serotype 3 rotaviruses were detected only by MAbs 159 and RV3:1, which appear to detect closely related epitopes (36). These antigenic variants had electropherotypes which differed from those of viruses of the same VP7 type circulating in the same season, which were typed previously (23).

DISCUSSION

We compared in competition assays VP7-typing MAbs to group A rotaviruses prepared by three different groups of investigators. Competition ELISAs permit a more rapid comparison of MAb binding sites than does the sequencing of the altered genes of neutralization escape mutants. In some cases, competitive ELISAs are more informative than comparisons of direct MAb binding to viral antigen (12, 25). Because competition assays require only the binding properties of the immunoglobulin, experiments can be conducted with neutralizing as well as nonneutralizing MAbs.

The competition results suggest that the type-specific epitopes cluster together. For VP7 type 3, the ninth genes of neutralization escape mutants to MAbs RV3:1, YO-1E2, 4F8, and 159 have been sequenced, and mutations induced by the MAbs have been found at amino acids 211, 221, 96, and 94, respectively (Table 2) (6, 22, 24). The first two sites are located within region C and the latter two sites are located within region A of VP7 (6). The finding of complete competition among these MAbs would support previous reports (6) that regions A and C are adjacent to each other on the particle although separated in the linear amino acid sequence. Others have reported nonreciprocal competition between VP7 type 3 MAbs 159 and 4F8 with RRV as antigen (31). Sufficient MAb mapping information for MAbs to VP7 type 2 and type 4 is not available for us to comment upon spatial localization of the binding epitopes of these MAbs.

We observed partial or one-way competition among the VP7 type 1 MAbs as well as for a number of the VP7-typing MAbs with the VP7 common MAb. Differences in MAb affinity sometimes can explain such reactions. If the concentrations of each MAb needed to obtain the optimal binding of

antigen reflected differences in the binding affinity (Table 2), then the partial and one-way competition observed with RV4:3 and KU-4 (and that observed with the VP7 common 60-F2D4 and several other MAbs) may have been due to differences in binding affinity. Minor differences in the degree of competition in some instances may also correlate with known differences in the gene sequence for VP7. For example, we failed to observe reciprocal competition between VP7 type 1 MAb KU-4 and MAbs RV4:3, 2C9, and 5E8. For the pair KU-4 and 2C9, competition with Wa antigen was virtually complete (95%) in one direction; in the other direction, competition was partial (40%). This result contrasts with that of others who have reported reciprocal competition between KU-4 and 2C9 (15). The difference between our results and those of Green et al. may be due simply to differences in the interpretation of competition curves. Our result, that competition between these MAbs is one way, is supported by mapping data; KU-4 induces mutations which map to amino acid 213 (region C) (32), whereas 2C9 induces mutations which map to amino acid 94 (region A) (14). Also, reciprocal competition between KU-4 and RV4:2 was observed, and both induce mutations at residue 213 (2b).

Partial competition (47%) between 2C9 (region A) and RV4:1 (region B) again was observed when 2C9 was used as the competing antibody. This observation confirms that the variable regions A, B, and C are closely related to one another (21). Region A previously has been shown to be close to region C (6). The possibility that region B is adjacent to both region A and region C is supported by the observation that competition between RV4:1 (region B) and another MAb that appears to map to region A (RV4:3) was reciprocal, as was competition between RV4:1 and MAbs RV4:2 and KU-4, which appear to map to region C. Another MAb which appears to map to region B (6) has not been used in competition assays.

Changes in amino acid sequence also may explain observed differences in MAb binding at the same site. MAb RV4:1, produced against serotype 1 strain RV4, induces mutations at residue 147 of region B and reacted weakly with the serotype 1 virus strain Wa. The amino acid sequences of Wa and RV4 are similar in region B except that Wa contains a serine at position 147 rather than an asparagine. Among all VP7 type 2 human rotaviruses, aspartic acid is conserved at position 190, while in VP7 type 1, 3, or 4, this position is occupied by serine (13). VP7 type 2 MAb S2-2G10 is type specific (33), and it has been mapped to amino acid site 190 (12).

The behavior of MAbs RV4:3 and 2C9 is particularly interesting. MAbs RV4:3 and 2C9 differed in their binding to VP7 type 3 viruses. RV4:3 bound strongly to VP7 type 3 viruses, and often this reactivity exceeded that of the VP7 type 3-typing MAbs. MAb 2C9 also bound to VP7 type 3 viruses (such as RRV) but not to SA11, and the reactions were generally, but not always, weak. For comparison, MAb 5E8 binding to VP7 type 3 viruses was not demonstrated. MAb 2C9 has reacted also with a number of VP7 type 3 clinical specimens obtained from the 1990-1991 rotavirus season in Houston. From our competition results, it seems that RV4:3 and 2C9 are able to recognize VP7 type 3-specific epitopes, but not completely. The apparent recognition site of MAbs RV4:3 and 2C9 is at amino acid 94, an asparagine (2a), and this asparagine is conserved among most VP7 type 1 and 3 rotaviruses (13). This might explain the cross-reactivity of MAb RV4:3 and 2C9 with VP7 type 3 viruses. However, the VP7 type 3 MAb 159 also induces mutations at

amino acid 94, yet it was not shown to cross-react with VP7 type 1 viruses. This indicates that other amino acids or the secondary structure of VP7 must affect binding of these MAbs.

We detected one monotypic variant of VP7 type 1 among clinical specimens from Houston and Ohio, and this variant would be classified as monotype 1d (34). The remainder of the tested samples were monotype 1a (4). We detected two different antigenic variants of VP7 type 2 and one of VP7 type 3. One antigenic variant of VP7 type 2 reacted exclusively with MAb 1C10, confirming that this MAb can detect specimens not recognized by MAb S2-2G10 (25, 36). Determination of the nucleotide sequences of these antigenic variants may help identify the amino acid sequences important for antigenic variation within each VP7 type. MAbs 159 and RV3:1 were more likely to detect a VP7 type 3 virus than MAb YO-1E2 (12, 25, 36). Similarly, MAb 5E8 was more efficient in VP7 typing than MAb 2C9 (36).

The findings of our study confirm the need for multiple MAbs for each VP7 type in rotavirus serotyping by ELISA to ensure that antigenic variants within rotavirus serotypes are detected. The finding of cross-reactivity of MAb 2C9 with VP7 type 3 viruses was surprising, as this reaction was previously unreported (14, 25, 30, 36). Our finding of 2C9 reactivity with naturally occurring VP7 type 3 viruses, when others have not noted such reactions, reinforces our impression of the broad antigenic diversity exhibited by naturally occurring rotaviruses. One example of this phenomenon is exhibited by reports of the use of MAb YO-1E2 for detection of VP7 type 3 clinical specimens. MAb YO-1E2 was a poor reagent for characterizing VP7 type 3 viruses in a collection from Bangladesh (36) and was a weak detector of VP7 type 3 viruses in a collection of samples from Mexico (25). On the other hand, YO-1E2 proved to be a suitable reagent for VP7 type 3 detection in samples from Japan (35), Houston, and elsewhere in the United States (11, 23). Naturally occurring VP7 type 1 viruses appear to be highly heterogeneous (2, 23, 34). Identification of heterogeneous VP7 type 1 strains and accumulation of additional nucleotide sequence data will be important in assessing the extent of genetic and antigenic variation in field strains of rotaviruses. Such molecular epidemiology studies of strains collected in specific areas will permit determination of whether there are accumulations of amino acid changes over time in antigenic sites and whether these eventually lead to the production of a new serotype and become problematic in successful vaccine development.

Our results also point out the need to use several MAbs in epitope-blocking assays to measure VP7 type 1-specific immune responses (15, 29). For example, each of the six VP7 type 1 MAbs we tested is likely to give a qualitatively and quantitatively different measurement of the number of responders and achieved titers in epitope-blocking assays. The competition results predict that the heterogeneity of answers will be greater for measurements of immune responses with VP7 type 1 MAbs than for measurements of those with VP7 type 2, 3, or 4 MAbs, among the MAbs tested. Because we observed that MAb RV4:1 reacted weakly with Wa, it is likely that use of this virus strain instead of a vaccine or a field strain may not be appropriate in certain cases. This idea is supported by the observation that more than 80% of children vaccinated with a serotype 1 monovalent vaccine developed an epitope-specific antibody response measured by MAb 2C9, while none of the children responded when measured by MAb KU-4 (15). Thus, the careful selection of both the MAb and the virus strain is crucial for determining

the specific immune response by epitope-blocking assays. The use of multiple MAbs and virus strains in epitope-blocking assays may permit investigation of immune responses influencing susceptibility to reinfections with the same virus type, reasons for vaccine failure, and the nature of heterotypic immunity.

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