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Serotype-Specific Epitope(s) Present on the VP8 Subunit of Rotavirus VP4 Protein

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cDNA clones representing the VP8 and VP5 subunits of VP4 of symptomatic human rotavirus strain KU (VP7 serotype 1 and VP4 serotype 1A) or DS-1 (VP7 serotype 2 and VP4 serotype 1B) or asymptomatic human rotavirus strain 1076 (VP7 serotype 2 and VP4 serotype 2) were constructed and inserted into the pGEMEX-1 plasmid and expressed in *Escherichia coli*. Immunization of guinea pigs with the VP8 or VP5 protein of each strain induced antibodies that neutralized the rotavirus from which the VP4 subunits were derived. In a previous study (M. Gorziglia, G. Larralde, A. Z. Kapikian, and R. M. Chanock, Proc. Natl. Acad. Sci. USA 87:7155–7159, 1990), three distinct serotypes and one subtype of VP4 outer capsid protein were identified among 17 human rotavirus strains that had previously been assigned to five distinct VP7 serotypes. The results obtained by cross-immunoprecipitation and by neutralization assay with antisera to the VP8- and VP5-expressed proteins suggest that the VP8 subunit of VP4 contains the major antigenic site(s) responsible for serotype-specific neutralization of rotavirus via VP4, whereas the VP5 subunit of VP4 is responsible for much of the cross-reactivity observed among strains that belong to different VP4 serotypes.

Rotaviruses are the major cause of severe diarrhea in infants and young children in both developed and developing countries (12). Therefore, intensive efforts are being made to develop an effective vaccine. In pursuing this goal, it is essential to gain an understanding of the antigens responsible for inducing protection. Two outer capsid rotavirus proteins, VP7 and VP4, are associated with the induction of neutralizing antibodies (10, 16, 22). The VP7 protein has been well characterized antigenically, and at least six different serotypes have been established among human rotaviruses (HRVs) on the basis of the antigenic specificity of this protein (12). Antigenic relationships among the VP4 outer capsid protein of human and animal rotaviruses have been studied by using VP4 neutralizing monoclonal antibodies (N-MAbs) or antisera to reassortant rotaviruses (10, 13, 21). Moreover, the neutralizing antibody response to VP4 was studied by using the VP4 recombinant protein of porcine rotavirus strain OSU (15) and simian rotavirus strain MMU18006 (14). Recent studies have begun to elucidate the antigenic relationships among HRVs on the basis of the antigenic specificity of the VP4 protein. A VP4 serotype system was recently established by the neutralization technique with guinea pig antisera to the baculovirus recombinant-expressed VP4 protein of HRV strains KU, DS-1, and 1076. The criterion for serotype specificity was a greater than eightfold difference in neutralizing antibody titer (7). Thus, HRV strains recovered from children with symptomatic or asymptomatic infection and previously assigned to five distinct VP7 serotypes (12) were classified as belonging to three distinct VP4 serotypes and one subtype.

In the presence of trypsin, the VP4 protein is cleaved into two polypeptides (VP5 and VP8), resulting in an enhancement of rotavirus infectivity (2, 3). Previous studies indicated that amino acid homology of the VP4 protein among rotavirus strains was greater in the VP5 cleavage subunit of VP4 than in the VP8 subunit (5). Furthermore, sequence analysis of escape mutants selected with N-MAbs indicated that the cross-reactivity was related to a site on VP5 (20). In contrast, N-MAbs directed at a site on VP8 were observed to be more strain specific (13).

In this study, the VP5 and VP8 subunits of three different HRV strains, KU (VP4 serotype 1A), DS-1 (VP4 serotype 1B), and 1076 (VP4 serotype 2), were independently expressed in *Escherichia coli* by using the pGEMEX-1 expression system (Promega). Guinea pig antisera produced in response to immunization with these recombinant VP5 and VP8 proteins were used in cross-neutralization tests to determine the contribution of each of these two subunits to the overall antigenic specificity of VP4. Antigenic determinants on the VP5 subunit of VP4 were responsible for the cross-reactivity observed among strains that belong to different VP4 serotypes. In contrast, it appears that the VP8 subunit of VP4 contains the major antigenic site(s) responsible for the serotype specificity of rotavirus strains via VP4.

MATERIALS AND METHODS

Cells and viruses. The host for recombinant pGEMEX-1 plasmid (Promega) was JM109 cells, which are lysogenic and bear the bacteriophage T7 RNA polymerase gene under the control of the *lac* UV5 promoter. The following HRV strains were used: symptomatic strains classified according to their VP7 as serotype 1 (Wa, KU, and K8), serotype 2 (DS-1 and S2), serotype 3 (P, Mo, and Yo), serotype 4 (VA70, Hosokawa, and Hochi), and serotype 9 (WI61 and F45); and asymptomatic strains that are classified via VP7 as serotype 1 (M37), serotype 2 (1076), serotype 3 (McN), and serotype 4 (ST3). Rotaviruses were grown in MA104 monkey kidney cells as previously described (11).

In vitro transcription and translation of the VP5 and VP8 subunits of the VP4 protein of HRV strains KU, DS-1, and 1076. The nucleotide sequences corresponding to the VP8 and VP5 subunits of the VP4 protein of HRV strain KU, DS-1 or 1076 were amplified by the polymerase chain reaction (17) and subcloned into the unique *Bam*HI site of the

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pTZ18R vector, as previously described (7). For in vitro transcription of the VP5 and VP8 subunits of KU, DS-1, or 1076, plasmids were selected for the correct T7 promoter orientation by restriction enzyme mapping and sequence analysis. Transcripts of VP5 and VP8 were produced with the Riboprobe system kit (Promega) following the manufacturer's specifications. In vitro translation was performed with rabbit reticulocyte lysate treated with nuclease (Promega). One microgram of RNA and 1 mCi of [^{35}S]methionine per ml (>800 Ci/mmol) was added to 50 μl of reaction mixture. After incubation at 37°C for 1 h, samples were immunoprecipitated by guinea pig antiserum to purified virus KU, DS-1, or 1076.

Construction and expression of the VP5 and VP8 subunits of the VP4 protein of rotavirus strain KU, DS-1, or 1076 in *E. coli*. Sequences coding for the VP5 and VP8 subunits of the VP4 gene, cloned in the pTZ18R vector as previously described, were digested with *Bam*HI. The resulting products were chromatographed on a NACS column (Bethesda Research Laboratories) and cloned into the unique *Bam*HI site of the pGEMEX-1 expression vector under the control of the bacteriophage T7 gene 10 promoter (19). The recombinant plasmids were used to transform *E. coli* JM109 (DE3) containing a single copy of the bacteriophage T7 RNA polymerase gene in its genome, which is inducible by isopropyl-β-D-thiogalactopyranoside (IPTG). The correct recombinants were selected by restriction enzyme and sequence analysis.

Partial purification of the VP5 and VP8 subunits of VP4 of HRV strains KU, DS-1, and 1076 from the bacterial overexpression system. JM109 (DE3) cells carrying a recombinant plasmid were grown in 500 ml of LB broth medium in the presence of ampicillin (100 μg/ml) for 3 h at 37°C or until the culture reached an optical density of 1. Then, the cultures were induced with 1 mM (final concentration) IPTG for 5 h at 37°C. Partial purification of the expressed protein was performed essentially as previously described (9). Briefly, IPTG-induced cell cultures were centrifuged at 7,000 × *g* for 5 min at 4°C. The bacterial pellet was resuspended in 10 volumes of lysis buffer containing 50 mM Tris HCl (pH 8.0), 1 mM EDTA, 100 mM NaCl, and lysozyme at a concentration of 1 mg/ml in the presence of 1 mM phenylmethylsulfonyl fluoride. After 20 min at room temperature, the lysates were centrifuged at 5,000 × *g* for 10 min and the pellets were resuspended in an ice-cold solution of 100 mM NaCl, 1 mM EDTA, 0.1% sodium deoxycholate, and 50 mM Tris HCl (pH 8.0) and incubated on ice for 10 min with occasional mixing. The DNA was digested with 10 μg of DNase I per ml in the presence of 8 mM $MgCl_2$ at room temperature for 30 min or until the viscosity disappeared. The protein was recovered by centrifugation at 10,000 × *g* for 10 min at 4°C, and the pellet was resuspended in phosphate-buffered saline (PBS).

Production of antiserum to the expressed VP5 and VP8 subunits of VP4 of HRV strains KU, DS-1, and 1076. Guinea pigs were prebled and then inoculated intramuscularly with a 100-μg/ml concentration of one of the six sonicated pellet suspensions mixed with Freund's complete adjuvant, followed 2 weeks later by a second inoculation of the lysate that had been mixed with Freund's incomplete adjuvant. The same inocula were administered 2 weeks later, and the animals were bled 1 week later. Serum samples were tested for the presence of antibodies by immunoprecipitation using the in vitro-translated VP5 and VP8 subunits of HRV strain KU, DS-1, or 1076 and by plaque reduction neutralization assays, as previously described (4, 11).

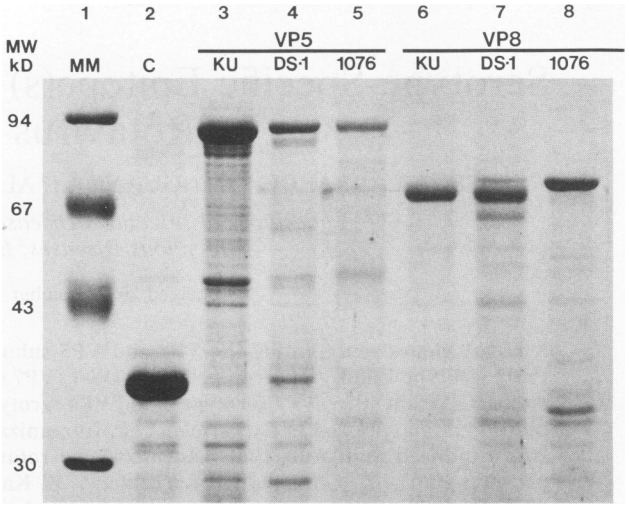

FIG. 1. Expression of VP5 and VP8 subunits of the VP4 protein of rotavirus strains KU, DS-1, and 1076 in *E. coli* cells. pGEMEX-1 plasmids carrying the VP5 or VP8 subunit of KU, DS-1, and 1076 were used to transfect *E. coli* JM109 (DE3). After induction with IPTG, cells were collected by centrifugation and the proteins were partially solubilized as described in Materials and Methods. The proteins were assayed directly by sodium dodecyl sulfate (SDS)–12% polyacrylamide gel electrophoresis (PAGE) and then stained with Coomassie blue. Lane 1, molecular mass markers; lane 2, control T7 gene 10 leader peptide; lanes 3, 4, and 5, VP5 subunits of HRV strains KU, DS-1, and 1076, respectively; lanes 6, 7, and 8, VP8 subunits of HRV strains KU, DS-1, and 1076, respectively.

RESULTS

Expression of the VP5 and VP8 subunits of the VP4 protein of rotavirus strains KU, DS-1, and 1076 in the pGEMEX-1 expression system. JM109 cells (strain DE3) transformed with the recombinant pGEMEX-1 carrying the VP8 or VP5 DNA sequences of HRV strain KU, DS-1, or 1076 expressed a high level of the corresponding VP5 or VP8 subunit of the VP4 protein. Each protein was expressed as a fusion protein with the bacteriophage T7 gene 10 leader peptide. The electrophoretic mobility of these subunits corresponded to the molecular mass of each of these subunits plus 25 kDa; the latter corresponds to the molecular mass of the above-mentioned leader peptide (Fig. 1). The antigenicity of these expressed proteins was confirmed in an enzyme-linked immunosorbent assay (ELISA) with hyperimmune serum to purified virus Wa, DS-1, or M37 (data not shown).

Prior to immunization, sera from guinea pigs were screened for evidence of previous exposure to rotavirus. Each preimmunization serum neutralized the homologous rotavirus strain at a titer of ≤1:30. In addition, these presera failed to immunoprecipitate the VP5 or VP8 subunit of VP4 of the homologous strain KU, DS-1, or 1076 (Fig. 2). As shown in Fig. 3, immunization of guinea pigs with a lysate of *E. coli* cells expressing these proteins induced the production of antibodies that immunoprecipitated the VP4 protein present in MA104 cells infected with the rotavirus strain from which the gene insert was derived. As a control for the VP4 protein in the lysate of infected cells, antisera to specific expressed VP4 proteins were used (Fig. 3, lanes 3, 7, and 11). The VP8 antisera exhibited greater immunoprecipitation activity than the VP5 antisera. Although the VP5 antisera were reactive, as shown in Fig. 4, their low levels of

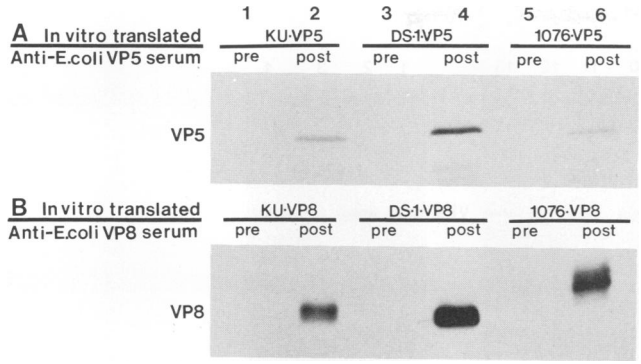

FIG. 2. Immunoprecipitation of VP5 and VP8 subunits of VP4 of rotavirus strain KU, DS-1, or 1076. pTZ18R plasmid containing KU, DS-1, or 1076 VP5 or VP8 cDNA was transcribed into RNA by T7 RNA polymerase, and the mRNA was translated into protein by rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Guinea pig antiserum was tested for VP5- or VP8-specific antibodies by immunoprecipitation of both subunits of VP4 of KU, DS-1, or 1076 and assayed directly by SDS–12% PAGE followed by fluorography. KU-VP5 (A) or KU-VP8 (B) subunit was immunoprecipitated with guinea pig serum collected before (lanes 1) or after (lanes 2) immunization with recombinant-expressed KU-VP5 (A) or KU-VP8 (B). DS-1-VP5 (A) or DS-1-VP8 (B) subunit was immunoprecipitated with guinea pig serum collected before (lanes 3) or after (lanes 4) immunization with recombinant-expressed DS-1-VP5 (A) or DS-1-VP8 (B). 1076-VP5 (A) or 1076-VP8 (B) subunit was immunoprecipitated with guinea pig serum collected before (lanes 5) or after (lanes 6) immunoprecipitation with recombinant-expressed 1076-VP5 (A) or 1076-VP8 (B).

reactivity with VP4 (Fig. 3B) suggest that the expressed VP5 subunit was conformationally different from the VP5 subunit of the native VP4.

Distribution of serotype-specific and cross-reactive sites on VP4. In an attempt to identify the location of serotype-specific domains and cross-reactive sites on the VP4 protein, lysates of *E. coli* expressing the VP5 or VP8 subunit of the VP4 proteins of HRV strain KU, DS-1, or 1076 were used to immunize guinea pigs. The neutralizing activities of these antisera for 17 HRV strains that were previously assigned to three distinct VP4 serotypes and one subtype were then examined (7).

As shown in Table 1, KU-VP8 antiserum neutralized HRVs that shared the same VP4 serotype specificity (1A and 1B) to a high titer (1:960). This antiserum neutralized HRV strains that belong to VP4 serotype 2 or 3 with reduced efficiency, i.e., the titer was six- to eightfold lower than the homologous titer. On the other hand, DS-1-VP8 antiserum neutralized HRV strains that belong to VP4 serotype 1B (the symptomatic DS-1 and S2 strains) to a high titer (1:960), whereas the HRVs that belong to VP4 serotype 1A were neutralized with two- to threefold lower efficiency. The DS-1-VP8 antiserum neutralized HRVs that belong to VP4 serotype 2 or 3 to an even lower titer, one that was six- to eightfold less than the homologous titer. The 1076-VP8 antiserum neutralized the asymptomatic HRV strains that belong to VP4 serotype 2 to a high titer (1:960), whereas HRV strains that belong to VP4 serotype 1A, 1B, or 3 were neutralized 4 to 12 times less efficiently.

Antisera to the VP5-expressed protein of KU, DS-1, or 1076 neutralized the homologous strains to a moderate titer (1:120 to 1:320), but this represents a 4- to 10-fold increase in neutralization titers with respect to the titers of preimmune sera. However, these antisera did not distinguish HRV strains that belong to different VP4 serotypes (Table 1).

The neutralization results correlate with the pattern of immunoprecipitation obtained with antisera to the expressed VP8 subunits of KU, DS-1, and 1076 (Fig. 4). The KU-VP8 subunit was recognized efficiently by the KU-VP8 and by the DS-1-VP8 antisera but at a lower level by the 1076-VP8 antiserum. Similarly, the DS-1-VP8 subunit was recognized by both the homologous antiserum and by antiserum to KU-VP8, confirming the antigenic relationship between these two viruses. In addition, DS-1-VP8 was also recognized by antiserum to 1076-VP8, but at a lower level. Finally, 1076-VP8 was immunoprecipitated by the homologous antiserum but not by the KU or DS-1-VP8 antiserum. The VP5 subunits of KU, DS-1, and 1076 were immunoprecipitated by both homologous and heterotypic VP5 antisera, a result which is in keeping with the neutralization data.

DISCUSSION

Previously, a VP4 serotype classification system was established by neutralization assay with guinea pig antiserum to the VP4 protein of HRV strains expressed by baculovirus recombinants (7). HRV strains that were associated with symptomatic infection and that exhibited VP7 specificities of serotype 1, 2, 3, 4, or 9 each possessed a similar VP4 antigenic specificity and thus were classified as VP4 serotype 1. In addition, human asymptomatic strains belonging to VP7 serotype 1, 2, 3, or 4 each possessed a similar VP4 antigenic specificity and were designated VP4 serotype 2. HRV strain K8, a symptomatic strain of VP7 serotype 1 specificity which possesses a unique VP4, was classified as VP4 serotype 3.

This VP4 serotype classification system of HRVs was established by using antiserum to the complete VP4 protein, which presumably possesses specific as well as cross-reactive neutralization epitopes. Immunodominant epitopes in the VP4 protein appear to be localized in specific regions of its VP8 and VP5 subunits (8). Sequence analysis of neutralization escape mutants selected with N-MAbs suggests that the strain-specific or limited heterotypic rotavirus neutralization epitopes are localized in the VP8 subunit of VP4. In contrast, the cross-reactive neutralization domains appear to be localized in the VP5 subunit (20). In order to demonstrate the direct contribution of each subunit of VP4 to the overall antigenic specificity of this protein, the VP5 and VP8 subunits of three different HRV strains, KU (VP4 serotype 1A), DS-1 (VP4 serotype 1B), and 1076 (VP4 serotype 2), were independently expressed in *E. coli* by using the pGEMEX-1 expression system. Guinea pig antisera produced in response to immunization with these recombinant VP5 and VP8 proteins were used in cross-neutralization tests.

The antigenic relationships of 17 HRV strains that represent five distinct VP7 serotypes were examined by neutralization with antisera to the VP8 subunits. The results were consistent with those obtained previously with antisera to the baculovirus VP4-expressed protein (7). HRV strains that exhibit a high degree of sequence homology in the VP8 subunit and belong to the same VP4 serotype show a high level of cross-reactivity by neutralization. This result strongly suggests that this subunit contains the major antigenic site(s) responsible for the specific neutralization of HRV strains via VP4. With some strains it was not possible to obtain reciprocal eightfold or greater differences in neutralizing antibody titer (the criterion used previously to classify HRV strains into different VP4 serotypes) even

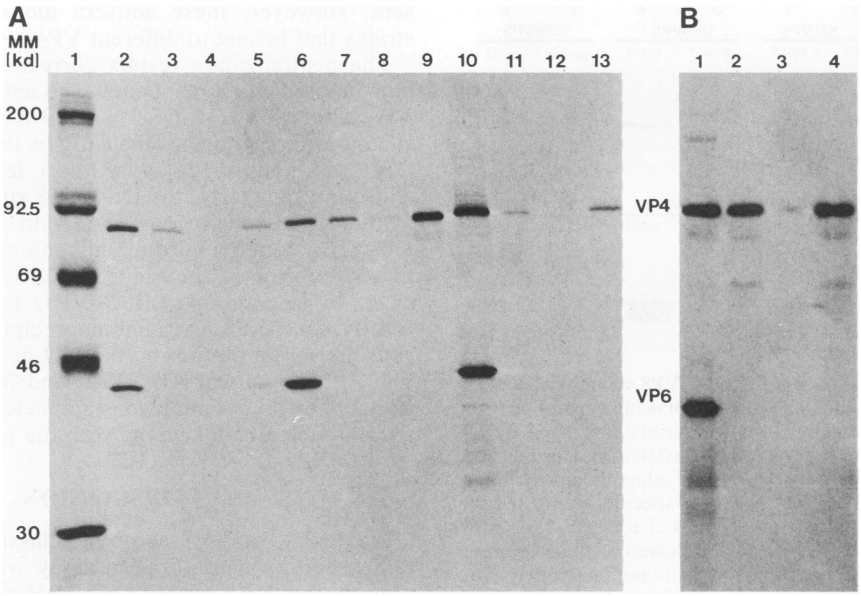

FIG. 3. Characterization of antisera to VP5 and VP8 subunits of VP4 expressed in *E. coli* cells. (A) Guinea pig antisera to VP5 and VP8 subunits of VP4 were tested for VP4-specific antibodies by immunoprecipitation. Proteins of rotavirus strain KU, DS-1, or 1076 were synthesized in virus-infected MA104 cells and labeled for 4 h with [^{35}S]methionine. KU-labeled proteins were immunoprecipitated with guinea pig antiserum to purified Wa virus (VP4 highly homologous in amino acid sequence to KU-VP4) (lane 2), KU-VP4 (lane 3), KU-VP5 (lane 4), or KU-VP8 (lane 5). DS-1-labeled proteins were immunoprecipitated with guinea pig antiserum to purified DS-1 virus (lane 6), DS-1-VP4 (lane 7), DS-1-VP5 (lane 8), or DS-1-VP8 (lane 9). Strain 1076-labeled proteins were immunoprecipitated with guinea pig antiserum to purified M37 virus (lane 10), 1076-VP4 (lane 11), 1076-VP5 (lane 12), or 1076-VP8 (lane 13). Lane 1, molecular masses of ^{14}C-labeled protein standards (Amersham). (B) Guinea pig antisera to the VP5 and VP8 subunits of DS-1-VP4 were tested for VP4-specific antibodies by modifying the washing condition of the immunoprecipitation. The beads of complex protein G-Sepharose-guinea pig antisera-antigen were washed twice with washing buffer 2 (0.1 M NaCl, 0.05 M Tris-HCl [pH 8.0], 0.001 M EDTA, 0.3% SDS, and 0.1% Nonidet P-40) instead of washing buffer 1 (1 M NaCl, 1% sodium deoxycholate, 0.05 M Tris-HCl, 1% Triton X-100, 0.1% SDS, 0.001 M phenylmethylsulfonyl fluoride, and 0.001 M EDTA), which was normally used, and twice with washing buffer 3 (0.05 M Tris-HCl [pH 8.0] and 0.1% Nonidet P-40). DS-1-labeled proteins were immunoprecipitated with guinea pig antisera to purified DS-1 virus (lane 1), DS-1-VP4 (lane 2), DS-1-VP5 (lane 3), or DS-1-VP8 (lane 4).

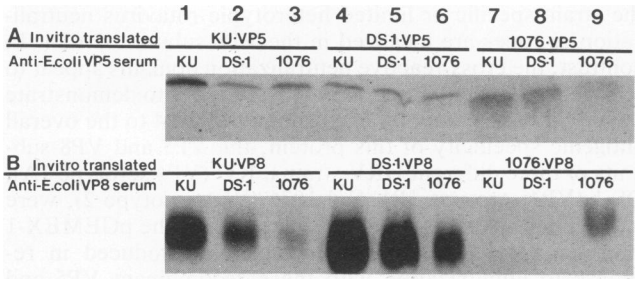

FIG. 4. Cross-immunoprecipitation of VP5 and VP8 subunits of VP4 of rotavirus strains KU, DS-1, and 1076. The VP5 and VP8 proteins of KU, DS-1, and 1076 were synthesized as described in the legend to Fig. 2. After immunoprecipitation, both subunits of VP4 were analyzed directly by SDS–12% PAGE followed by fluorography. Lanes 1 to 3, VP5 and VP8 of KU; lanes 4 to 6, VP5 and VP8 of DS-1; lanes 7 to 9, VP5 and VP8 of 1076. Hyperimmune anti-*E. coli* VP5 (A) and VP8 (B) serum of strain KU was used to immunoprecipitate the VP5 and VP8 subunit, respectively, of VP4 of strains KU (lanes 1), DS-1 (lanes 4), and 1076 (lanes 7). Hyperimmune anti-*E. coli* VP5 and VP8 of strain DS-1 was used to immunoprecipitate the VP5 (A) and VP8 (B) subunit, respectively, of the VP4 of strains KU (lanes 2), DS-1 (lanes 5), and 1076 (lanes 8). Hyperimmune anti-*E. coli* VP5 (A) and VP8 (B) of strains 1076 and M37, respectively, were used to immunoprecipitate the VP5 and VP8 subunit, respectively, of strains KU (lanes 3), DS-1 (lanes 6), and 1076 (lanes 9).

though the strains belonged to different VP4 serotypes as defined previously with VP4 antisera. The VP4 serotype classification system, with VP4 or VP8 antisera, is indeed quantitatively distinct from the serotyping scheme currently in use for rotaviruses (for example, VP7 serotyping is based on ≥20-fold differences in reciprocal neutralizing antibody titer). However, with antisera to VP4 or VP8, three distinct groupings of rotaviruses were observed, and the term serotype was applied to these individual groups. Development of serotyping assays employing specific MAbs to VP8 or VP4 should demonstrate whether the classification of these strains into VP4 serotypes was appropriate. It is of interest that in early serotyping studies of HRVs by neutralization, greater than or equal to fourfold differences in neutralizing antibody titer were considered acceptable for serotype classification (1).

The unexpected high level of cross-reactivity found with antisera to VP8 may be the result of conserved VP8 epitopes that are shared among HRVs which belong to different VP4 serotypes, as evidenced by sequence analysis of the VP8 subunit of several HRV strains (6). These conserved regions may correspond to epitopes responsible for such cross-reactivity. For instance, a synthetic peptide corresponding to one of the conserved regions of VP4, amino acids 228 to 241, of simian rotavirus SA11 was effective in stimulating neutralizing antibodies (18). However, the VP4 gene of antigenic mutants of simian rotavirus SA11 4fM selected with this SA11 4fM polyclonal antiserum did not contain an

TABLE 1. Antigenic relationships among HRVs by using hyperimmune antisera to *E. coli*-expressed VP5 or VP8 subunit

VP4 serotype and HRV strain (VP7 serotype)	Plaque reduction neutralizing antibody titer of antiserum[a] to:					
	VP8			VP5		
	KU	DS-1	1076	KU	DS-1	1076
1A, symptomatic						
KU (1)	960	480	240	120	120	240
Wa (1)	960	320	240	120	160	240
P (3)	960	480	240	240	120	320
Yo (3)	960	480	160	120	320	240
Mo (3)	960	480	240	240	240	240
Hochi (4)	960	480	120	120	120	120
VA70 (4)	960	320	240	120	120	120
Hosokawa (4)	960	480	120	240	240	240
WI61 (9)	960	480	240	120	120	120
F45 (9)	960	480	80	320	120	240
1B, symptomatic						
DS-1 (2)	960	960	240	160	120	320
S2 (2)	960	960	240	320	240	320
2, asymptomatic						
M37 (1)	120	120	960	120	240	160
1076 (2)	120	120	960	120	120	240
McN (3)	160	160	960	120	120	120
ST3 (4)	120	160	960	120	120	120
3, symptomatic, K8 (1)	120	120	120	120	120	120

[a] Each of the preimmunization sera neutralized the homologous rotavirus strain KU, DS-1, or 1076 at a titer of 1:≤30.

amino acid change in this region (8). This suggests that the VP8 subunit possesses conserved regions that contain relatively inaccessible epitopes in the native VP4 protein and that VP8 expressed in *E. coli* may assume a different conformation, thus exposing these epitopes that are recessive in the native VP4.

The low titer of neutralizing antibodies induced by the expressed VP5 subunit made it more difficult to define the independent contribution of the VP5 subunit to the overall antigenicity of VP4. Despite this limitation, antiserum to VP5 was found to be cross-reactive among strains that belong to different VP4 serotypes, indicating that this subunit does contain antigenic determinants that are shared among HRV strains of different VP4 serotype specificities. This agrees with the previous finding that the cross-reactive N-MAbs recognize neutralization epitopes in this subunit (20, 21). The relatively low titer of neutralization obtained with VP5 antisera may result from the altered conformation of the expressed VP5 fusion protein. The bacteriophage T7 leader peptide may modify the solubility and/or the conformational structure of the VP5 subunit, thus affecting the immunogenicity of its neutralization domains. In addition, the VP5 subunit appears to contain fewer neutralization epitopes than the VP8 subunit, as suggested by analysis of antigenic mutants of rotavirus strains RRV, KU, and SA11 4fM. Such mutants were selected with cross-reactive VP4 N-MAbs or polyclonal antisera and only three amino acids (residues 305, 392, and 439) were found to be associated with cross-reactive epitopes of VP5 (8, 20).

In tests performed with neutralizing antibodies to VP8, it was possible to classify various HRV strains into three groups or serotypes that were consistent with previous results obtained with antibodies to the complete VP4. Further studies should demonstrate whether the classification into distinct serotypes on the basis of less than 20-fold differences in antibody titer was appropriate. In this regard, current studies are aimed at producing MAbs to the VP8 subunit of HRV strains which may facilitate VP4 serotyping of HRVs. The availability of specific MAbs may also enable the elucidation of epitopes involved in resistance to rotavirus infection. The results of such studies may be relevant to the development of an effective rotavirus vaccine.

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