Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7155–7159, September 1990
Medical Sciences

Antigenic relationships among human rotaviruses as determined by outer capsid protein VP4

(rotavirus VP4 expression/rotavirus VP4 serotypes/rotavirus VP8/rotavirus VP5)

MARIO GORZIGLIA, GISELA LARRALDE, ALBERT Z. KAPIKIAN, AND ROBERT M. CHANOCK

Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Contributed by Robert M. Chanock, June 12, 1990

ABSTRACT **cDNA clones representing the VP4 gene of symptomatic human rotavirus strain KU (VP7 serotype 1) or DS-1 (VP7 serotype 2) or asymptomatic human rotavirus strain 1076 (VP7 serotype 2) were constructed and inserted into a baculovirus expression vector under the control of the polyhedrin promoter. The resulting recombinants expressed the appropriate authentic VP4 rotavirus outer capsid protein. Guinea pigs immunized with these VP4 proteins developed antibodies that neutralized infectivity of the rotavirus from which the immunizing VP4 was derived. These antisera were then used in neutralization tests to define the extent and distribution of VP4 antigenic polymorphism among human rotaviruses. Three distinct serotypes and one subtype of the VP4 outer capsid protein were identified among 17 human rotavirus strains that had previously been assigned to five distinct VP7 serotypes. For the most part, VP4 serotype segregated independently of VP7 serotype. Ten strains of human rotavirus that were associated with symptomatic infection and that exhibited VP7 serotype 1, 3, 4, or 9 specificity, each possessed a VP4 of the same serotype and subtype, designated VP4 serotype 1A. Both symptomatic human rotavirus strains with VP7 serotype 2 specificity were related by neutralization to the VP4 serotype 1A strains and were classified as a subtype of VP4 serotype 1—i.e., serotype 1B—since viruses of serotype 1A appeared to be prime strains. Four human rotavirus strains that were recovered from healthy infants in newborn nurseries in which virus transmission persisted over a long interval, belonged to VP7 serotype 1, 2, 3, or 4, but each strain possessed the same VP4 antigenic specificity that was designated VP4 serotype 2. Finally, a single strain of symptomatic human rotavirus of VP7 serotype 1 specificity possessed a unique VP4 that was provisionally classified as VP4 serotype 3 but this remains to be confirmed because neutralization tests were performed in only one direction. Among the 10 rotavirus strains whose VP4 gene was previously sequenced, there was complete concordance between assignment of VP4 serotype by neutralization and classification according to VP4 amino acid homology. Thus, rotaviruses that exhibited a VP4 amino acid homology of 89% or greater belonged to the same VP4 serotype and subtype as determined by neutralization. Finally, evidence was obtained that the serotype-specific domain is located on the VP8 subunit of VP4.**

Rotaviruses are ubiquitous agents that cause diarrheal disease in the young of not only humans but also many animal species (1). Several candidate vaccines against human rotavirus (HRV) disease are currently being evaluated with special emphasis on the VP7 outer capsid protein, which is a major protective antigen (2, 3). At least six different serotypes have been established among HRVs based on the antigenic specificity of VP7 (1). The outer capsid protein, VP4, is also a major protective antigen, but relatively little is known about the antigenic relationships of the VP4 of various HRVs. It has been demonstrated by sequence analysis that at least four alleles of the VP4 gene are present among HRVs (4–7). One allele of VP4 is present on symptomatic strains that are classified by means of VP7 as serotype 1 (strains KU and Wa), serotype 3 (strain P), or serotype 4 (strain VA70). These rotavirus strains, designated as VP4 genetic group 1, possess a highly conserved VP4 protein that exhibits 93–97% amino acid identity. A second allele of VP4 is present in HRVs belonging to VP7 serotype 2 (strains DS-1 and RV-5) that exhibit 98% VP4 amino acid identity. These strains, designated as VP4 genetic group 2, were also recovered from symptomatic infections. Moreover, the VP4s of genetic group 2 are most closely related to the corresponding proteins of group 1, exhibiting an 89–90% amino acid homology. A third allele of VP4 is present in HRV strains that cause asymptomatic neonatal infection and that belong to VP7 serotype 1 (M37), VP7 serotype 2 (1076), VP7 serotype 3 (McN), or VP7 serotype 4 (ST3). The VP4s of these strains (designated VP4 genetic group 3) are highly related, as indicated by 95–97% amino acid identity. The VP4s of the third genetic group exhibit only a 75% amino acid identity with the corresponding VP4 proteins of groups 1 and 2. A fourth allele is present on the HRV K8 strain (belonging to VP7 serotype 1) that appears to have a unique VP4 gene exhibiting only 64–65% amino acid identity with the VP4 proteins of viruses of VP4 genetic group 1, 2, or 3. In this study, three different VP4 alleles present on HRV strains KU (genetic group 1), DS-1 (genetic group 2), and 1076 (genetic group 3) were separately expressed in *Spodoptera frugiperda* (Sf9) cells using the baculovirus vector *Autographa californica* nuclear polyhedrosis virus. Antisera produced in response to immunization with these recombinant VP4 proteins allowed us to define the extent and distribution of VP4 antigenic polymorphism among rotaviruses currently circulating in humans.

MATERIALS AND METHODS

Cells and Viruses. Rotaviruses were grown in MA104 monkey kidney cells as described (4). The following HRV strains were used: symptomatic strains classified according to their VP7 as serotype 1 (strains Wa, KU, and K8), serotype 2 (strains DS-1 and S2), serotype 3 (strains P, Mo, and Yo), serotype 4 (strains VA70, Hosokawa, and Hochi), or serotype 9 (strains Wi61 and F45); and asymptomatic strains that are classified by means of VP7 as serotype 1 (strain M37), serotype 2 (strain 1076), serotype 3 (strain McN), or serotype 4 (strain ST3). *S. frugiperda* Sf9 cells obtained from the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: HRV, human rotavirus.

American Type Culture Collection were grown in Grace's insect cell medium supplemented with 0.3% yeastolate, 0.3% lactalbumin hydrolysate, and 10% fetal calf serum. Wild-type baculovirus (*A. californica* nuclear polyhedrosis virus) and intermediate baculovirus vector pVL-941 were kindly provided by M. Summers (College Station, TX).

Construction, Selection of Baculovirus Recombinants Containing a KU, DS-1, or 1076 Gene 4 cDNA Insert, and Production of Antiserum to Each of the Expressed VP4 Proteins. Single-shelled virus particles of strain KU, DS-1, or 1076, produced by treatment of concentrated virus with EDTA, were purified by CsCl gradient centrifugation (4). Viral mRNA was synthesized *in vitro* by the endogenous transcriptase in viral cores and was freed of double-stranded RNA by precipitation with 2 M lithium chloride (4). The construction of VP4 cDNAs followed the method described previously (8). Briefly, 5 μg of oligonucleotide 5′-CACGGATCCGGTCACATCCTCAATAGC-3′, 5′-CACGGATCCGGTCACATCCTGGATGACATTC-3′, or 5′-CACGGATCCGGTCACATCCTCTATAGA-3′ (primer sequence representing a flanking *Bam*HI site and the complement of the 3′ end of the gene 4 of KU, DS-1, or 1076, respectively) was used to transcribe the first strand of DNA from viral mRNA by reverse transcriptase. After hydrolysis with NaOH (0.3 M, 37°C, 2 hr), cDNA was hybridized with 5 μg of the primer 5′-CACGGATCCGGCTATAAAATGGCTTCGCTC-3′ (the flanking *Bam*HI site and the complement of the 5′ end conserved nucleotide sequence of the VP4 gene) and transcribed with reverse transcriptase. After digestion with *Bam*HI, each of the VP4 cDNAs was separately cloned in the *Bam*HI site of plasmid pTZ18R for amplification. Then, the VP4 gene of KU, DS-1, or 1076 was excised with *Bam*HI and inserted into the unique *Bam*HI site of the intermediate baculovirus vector pVL-941. Transfection and the selection of the recombinants were performed as described (9). The corresponding baculovirus VP4 recombinants were designated as KU-G4, DS-1-G4, and 1076-G4. Guinea pigs were immunized with a lysate of KU, DS-1, or 1076 baculovirus recombinant-infected Sf9 cells as described (9). Following immunization, serum samples were tested for antibodies to VP4 by immunoprecipitation and plaque neutralization assays (10, 11).

Construction, *in Vitro* Transcription, and Translation of the VP8 and VP5 Subunits of VP4 of Rotavirus Strain KU, DS-1, or 1076. The sequences for the VP8 and VP5 subunits of VP4 of HRV strain KU, DS-1, or 1076 were amplified by the polymerase chain reaction (12) using as template the cloned VP4 gene cDNA. Two primers were used to amplify the VP8 subunit of VP4: one, that corresponds to the 5′ end conserved nucleotide sequence of the VP4 gene (see above), and the second, complementary to nucleotides at position 741–712 of the KU VP4 gene (5′-ATACTGTATAGATCTGGATGATAATGATAA-3′) or complementary to nucleotides at position 743–710 of the 1076 VP4 gene (5′-ATAAGTCACAGATCTAGATGATAGTGCAACTGC-3′). Primers complementary to the 3′ end of the KU or 1076 VP8 subunit were designed to include a *Bgl* II restriction site at position 729. A similar primer was not required for DS-1 because its VP4 gene already possesses a *Bgl* II site at this position. For amplification of the VP5 subunit of VP4, oligonucleotides complementary to the 3′ end of each VP4 gene (see above) were used together with oligonucleotide 5′-CACGGATCCACCATGGTTAATGAAGATATTA-3′, which is complementary to the completely conserved sequence at positions 756–771 in the KU, DS-1, or 1076 VP4 gene. The latter primer was designed to substitute alanine for threonine at the amino terminus of VP5 and replace glutamine for methionine at the second position in order to achieve a sequence highly favorable for initiation of translation. This primer also created a *Bam*HI flanking site. After amplification, VP5 cDNA was digested with *Bam*HI, whereas VP8 cDNA was digested with *Bam*HI/*Bgl* II. The resulting products were chromatographed on a NACS column (BRL) and cloned into pTZ18R. 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 sequencing. Transcripts of VP5 and VP8 were produced using a Riboprobe system kit (Promega) following the manufacturers' specifications. *In vitro* translation was performed using rabbit reticulocyte lysate treated with nuclease (Promega). One microgram of RNA and 1 mCi of [^{35}S]-methionine per ml (>800 Ci/mmol; 1 Ci = 37 GBq) were added to 50 μl of reaction mixture. After incubation at 37°C for 1 hr, the samples were immunoprecipitated by guinea pig hyperimmune antiserum to human strain Wa (possessing the same VP4 serotype as KU), DS-1, or M37 (possessing the same VP4 serotype as 1076) or by guinea pig antiserum produced to each of the baculovirus recombinant-expressed VP4 proteins. The translated VP4 subunit proteins were also used to assay for VP5- and VP8-specific antibodies in postinfection serum of a gnotobiotic piglet (61-5) infected with human strain Wa (VP7 serotype 1) (kindly furnished by L. Saif), postinfection serum of a gnotobiotic calf (77-29) infected with strain DS-1 (VP7 serotype 2) (13), or postinfection serum of a gnotobiotic piglet (5+1) infected with M37 strain (VP7 serotype 1) (kindly furnished by L. Saif, Ohio State University).

RESULTS

Expression of Rotavirus VP4 by Baculovirus Recombinants. *S. frugiperda* cells infected with the baculovirus recombinant KU-G4, DS-1-G4, or 1076-G4 expressed a protein of ≈85 kDa that had the same mobility during gel electrophoresis as authentic VP4 produced by MA104 cells infected with the

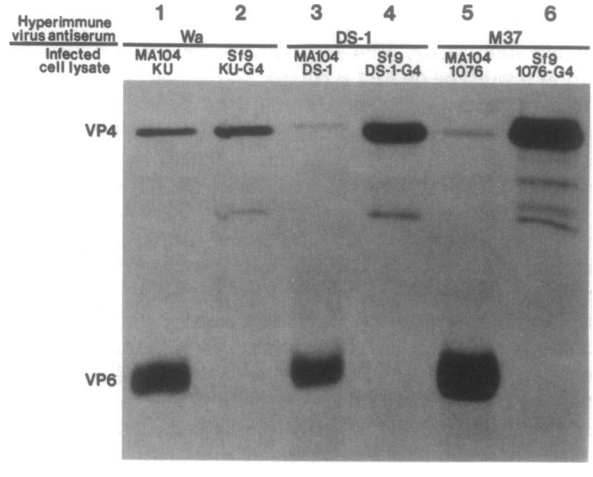

FIG. 1. Expression of VP4 of rotavirus strain KU, DS-1, or 1076 by a baculovirus VP4 recombinant. Hyperimmune antiserum to purified strain Wa virus (that has a VP4 highly homologous in amino acid sequence to KU VP4) was used to immunoprecipitate [^{35}S]methionine-labeled rotavirus protein(s) present in a lysate of MA104 cells infected with strain KU (lane 1) or Sf9 cells infected with a baculovirus recombinant expressing KU VP4 (lane 2). Hyperimmune antiserum to purified virions of strain DS-1 was used to immunoprecipitate [^{35}S]methionine-labeled rotavirus protein(s) present in a lysate of MA104 cells infected with strain DS-1 (lane 3) or Sf9 cells infected with a baculovirus recombinant expressing DS-1 VP4 (lane 4). Hyperimmune antiserum to purified virus of strain M37 (that has a VP4 highly homologous in amino acid sequence to 1076 VP4) was used to immunoprecipitate [^{35}S]methionine-labeled rotavirus protein(s) present in a lysate of MA104 cells infected with strain 1076 (lane 5) or Sf9 cells infected with a baculovirus recombinant expressing 1076 VP4 (lane 6).

rotavirus from which the VP4 gene insert was derived (Fig. 1). The identity of the expressed VP4 proteins was confirmed in this instance by immunoprecipitation with the appropriate guinea pig antiserum to purified virus of rotavirus strain Wa (that has a VP4 highly homologous in amino acid sequence to KU VP4), DS-1, or M37 (that has a VP4 highly homologous in amino acid sequence to 1076 VP4).

The baculovirus recombinant-expressed rotavirus VP4 protein of KU, DS-1, or 1076 was immunogenic because immunization of guinea pigs induced the development of antibodies that immunoprecipitated an 85-kDa protein from a lysate of strain KU, DS-1, or 1076 infected MA104 cells, respectively (Fig. 2). The gel mobility of these proteins was similar to that of authentic VP4 immunoprecipitated by the appropriate virus antiserum.

Antigenic Relationships Among the Baculovirus Recombinant-Expressed VP4 Proteins of HRVs Assayed by Neutralization. Antigenic relationships of the baculovirus recombinant-expressed VP4s were studied by cross-neutralization tests performed with guinea pig antiserum to the VP4 of KU, DS-1, or 1076. Previously, each of these VP4 proteins was shown to belong to a different VP4 genetic group as defined by VP4 amino acid homology (4, 5). Analysis of 17 HRVs revealed three nonoverlapping patterns of neutralization suggesting that there are at least three distinct HRV VP4 serotypes (1, 2, and 3) and one subtype (1B) (Table 1). Antiserum to the KU VP4 neutralized 10 HRVs to high titer (1:1920 to ≥1:2560). These rotaviruses were (*i*) recovered from patients with diarrheal disease and (*ii*) represented four distinct VP7 serotypes, 1, 3, 4, and 9. The KU VP4 antiserum also neutralized two symptomatic strains of VP7 serotype 2 to a high titer that was ≈2-fold lower than that observed for the aforementioned group of viruses. The four asymptomatic strains were neutralized to a significantly lower titer by the KU VP4 antiserum (i.e., 8-fold lower than the homologous titer), whereas the single strain (K8) of the remaining group was not neutralized at all. Antiserum to DS-1 VP4 neutralized the two viruses of the second genetic group (the symptomatic DS-1 and S2 strains) to highest titer, whereas neutralization of the first genetic group was 4- to 8-fold less efficient. The DS-1 VP4 antiserum failed to neutralize viruses of the third or fourth genetic group. Finally, the four asymptomatic neonatal strains formed a homogeneous group that was neutralized to high titer by antiserum against VP4 of one of its member strains (1076). This antiserum neutralized the first and second genetic groups of strains with 16- to 32-fold lower efficiency, whereas the single strain of genetic group 4 (K8) was not neutralized at all.

The VP4 of the fourth genetic group, represented by strain K8, appeared to be the most distinct, differing from the first three VP4 genetic groups by its failure to be neutralized by antisera to any of the baculovirus recombinant-expressed VP4s. The third genetic group was also clearly distinct from each of the other groups as indicated by an 8- to 32-fold reduction in heterologous neutralization titer in reciprocal assays. Finally, the first and second groups appeared to be the most closely related of the four genetic groups because reciprocal differences in neutralizing antibody titer between groups 1 and 2 were only 2-fold in one direction and 4- to 8-fold in the other direction. In cross-neutralization tests involving groups 1 and 2, the group 1 antiserum (KU) exhibited more cross-reactivity than the group 2 antiserum (DS-1). Since the KU strain appeared to be the prime strain, the group 2 viruses were considered to be a subtype of VP4 serotype 1.

Distribution of Serotype-Specific and Cross-Reactive Antigenic Sites on VP4. Previous studies had provided evidence that amino acid homology among distantly related rotavirus VP4 proteins was greater in the VP5 cleavage subunit of VP4 than in the VP8 cleavage subunit (5). Furthermore, neutralizing monoclonal antibodies directed at a site on VP5 exhibited considerable cross-reactivity for heterologous rotaviruses, whereas neutralizing monoclonal antibodies directed at a site on VP8 were observed to be more specific (14, 15). Because of these findings, the VP5 and VP8 cleavage subunits of VP4 were studied to determine if the overall antigenic specificity of these subunits followed the patterns observed for amino acid sequence homology and reactivity with homologous or heterologous monoclonal antibodies.

VP8 or VP5 was transcribed *in vitro* from the appropriate cloned cDNA and translated in a rabbit reticulocyte lysate. The translated VP8 or VP5 was then immunoprecipitated with (*i*) guinea pig hyperimmune antiserum to purified virions of strain Wa (VP4 serotype 1A), DS-1 (VP4 serotype 1B), or M37 (VP4 serotype 2); (*ii*) guinea pig antiserum to baculovirus recombinant-expressed VP4 of KU (VP4 serotype 1A), DS-1 (VP4 serotype 1B), or 1076 (VP4 serotype 2); or (*iii*) serum obtained after infection of a gnotobiotic calf or piglet

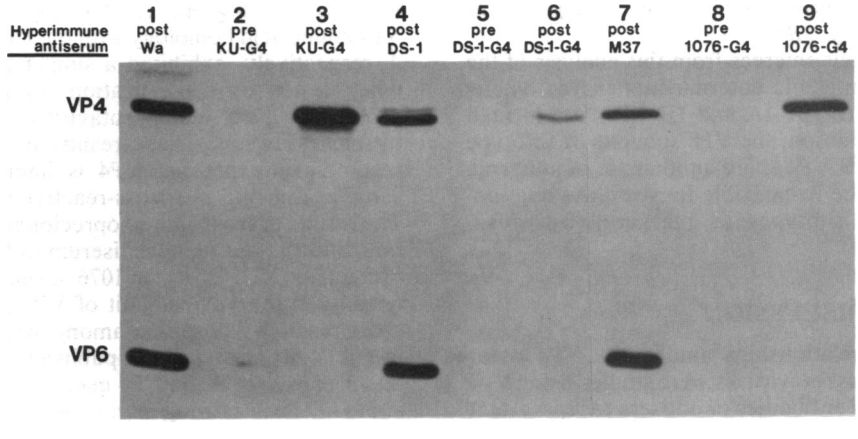

FIG. 2. Characterization of antisera produced to baculovirus recombinant-expressed VP4. Guinea pig antiserum was tested for VP4-specific antibodies by immunoprecipitation of proteins of KU, DS-1, or 1076 present in virus-infected MA104 cells labeled for a short period—i.e., 4 hr with [^{35}S]methionine under conditions that permit detection of primarily VP4 and VP6. KU labeled proteins were immunoprecipitated with guinea pig antiserum to purified Wa virus (that has a VP4 highly homologous in amino acid sequence to KU VP4) (lane 1) or guinea pig serum collected before (lane 2) or after immunization with baculovirus recombinant-expressed KU VP4 (lane 3). DS-1 labeled proteins were immunoprecipitated with guinea pig antiserum to purified DS-1 virus (lane 4) or guinea pig serum collected before (lane 5) or after immunization with baculovirus recombinant-expressed VP4 (lane 6). 1076 labeled proteins were immunoprecipitated with guinea pig antiserum to purified M37 virus (that has a VP4 highly homologous in amino acid sequence to 1076 VP4) (lane 7) or guinea pig serum collected before (lane 8) or after immunization with baculovirus recombinant-expressed 1076 VP4 (lane 9).

Table 1. Antigenic relationships among HRVs observed in neutralization tests employing hyperimmune antisera against baculovirus recombinant-expressed VP4 protein

HRV VP4 serotype*	Symptomatic infection	VP7 serotype	Rotavirus strain	Reciprocal of 60% PRN antibody titer of hyperimmune antisera against expressed VP4 protein of strain		
				KU	DS-1	1076
1A	+	1	KU	≥2560	160	160
		1	Wa	2560	160	160
		3	P	2560	160	160
		3	Yo	2560	160	160
		3	Mo	1920	80	80
		4	Hochi	≥2560	160	80
		4	VA70	2560	160	160
		4	Hosokawa	≥2560	160	80
		9	Wi61	≥2560	160	160
		9	F45	≥2560	160	160
1B	+	2	DS-1	1280	640	160
		2	S2	1280	640	160
2	−	1	M37	320	<80	2560
		2	1076	320	<80	2560
		3	McN	320	<80	2560
		4	ST3	320	<80	2560
3	+	1	K8	<80	<80	<80

PRN, plaque reduction neutralization.
*The three VP4 serotypes and the one VP4 subtype correspond to four genetic groups that were defined previously by the degree of VP4 amino acid sequence homology. VP4 genetic groups 1, 2, 3, and 4 correspond to VP4 serotypes 1A, 1B, 2, and 3, respectively (4–7).

with strain Wa, DS-1, or M37. In tests performed with the VP4 subunits of KU (VP4 serotype 1A), DS-1 (VP4 serotype 1B), or 1076 (VP4 serotype 2), VP5 was immunoprecipitated by each homotypic and heterotypic antiserum (Fig. 3). In contrast, the VP8 of each virus reacted in a more specific manner. For example, the VP8 of strain 1076 was precipitated only with homotypic antiserum. However, M37 hyperimmune antiserum did precipitate the VP8 of strain DS-1, but this heterotypic reaction was significantly less than the homotypic reaction (Fig. 3*A*). Cross-immunoprecipitation of the VP8 subunits of KU and DS-1 with antisera to purified virus (Fig. 3*A*) or sera from an infected piglet or calf (Fig. 3*C*) indicated that KU had a predominantly one-way relationship with DS-1. The exception was the greater immunoprecipitation of KU VP8 by antiserum to baculovirus recombinant-expressed DS-1 VP4 (Fig. 3*B*).

The overall pattern that emerges from this analysis of the VP4 subunits is that antigenic determinants shared among serotype 2 VP4 and serotype 1A and 1B VP4s are located primarily on VP5. In addition, the VP8 subunits of serotype 1A VP4 and serotype 1B VP4 share another set of antigenic determinants that may be responsible for the close relationship of these 2 VP4 subtypes as measured by cross-neutralization.

DISCUSSION

Previously, antigenic relationships among the VP4 outer capsid proteins of various rotaviruses were studied using VP4 neutralizing monoclonal antibodies or antisera to reassortant rotaviruses (14, 16, 17). More recently, the neutralizing antibody response to VP4 was studied using the VP4 recombinant protein of porcine strain OSU (9) and simian strain RRV (18). Those studies indicated that it was possible to distinguish between the VP4 of OSU or RRV as well as the VP4 of other animal and human strains by neutralization assay.

To gain a more detailed understanding of the VP4 antigenic relationships among HRVs, antibodies were induced against the baculovirus recombinant-expressed VP4 protein of human rotavirus strain KU, DS-1, or 1076. The VP4 of these strains represents three of the four distinct alleles identified thus far among HRVs (4–7). Nucleotide sequence analysis indicated that the VP4 genes of strain KU (genetic group 1) and strain DS-1 (genetic group 2) were more closely related to each other than to the VP4 of strain 1076 (genetic group 3). Also, it was observed that the VP4 of K8 virus, the prototype strain of genetic group 4, was the most divergent of the four distinct groups. A direct correlation was observed between VP4 amino acid homology and antigenic relatedness as measured by neutralization. For example, intragroup viruses that share >93% VP4 amino acid homology exhibited 100%, or close to 100%, relatedness by neutralization. In contrast, strain KU from group 1, which shares 89%, 75%, and 64% VP4 amino acid homology with the viruses of groups 2, 3, and 4, respectively, exhibited a similar gradient of relatedness when tested by neutralization. In general, the four VP4 genetic groups of human rotaviruses could be differentiated by neutralization. These results indicate that baculovirus recombinant-expressed VP4 is immunogenic and induces serotype-specific and cross-reactive neutralizing antibodies. The results of cross-immunoprecipitation and reciprocal neutralization assays using antiserum to VP4- or VP5-expressed proteins of KU, DS-1, or 1076 suggest that antigenic determinants in the VP5 subunit of VP4 are responsible for the cross-reactivity observed among strains that belong to different VP4 serotypes (unpublished data). Moreover, sequence analysis of the VP4 gene of antigenic mutants resistant to VP4 neutralizing monoclonal antibodies demonstrated that the amino acids involved in heterotypic neutralization were located in VP5 (14, 15). On the other hand, the correlation of results obtained by reciprocal neutralization and by immunoprecipitation of VP8 using antiserum to VP4 or VP8 suggests that the latter, a subunit of VP4, contains the major antigenic site (or sites) responsible for neutralization of rotaviruses by means of VP4 (unpublished data). Strains that exhibit a high degree of sequence homology in the VP8 subunit (and belong to the same genetic group) show a high

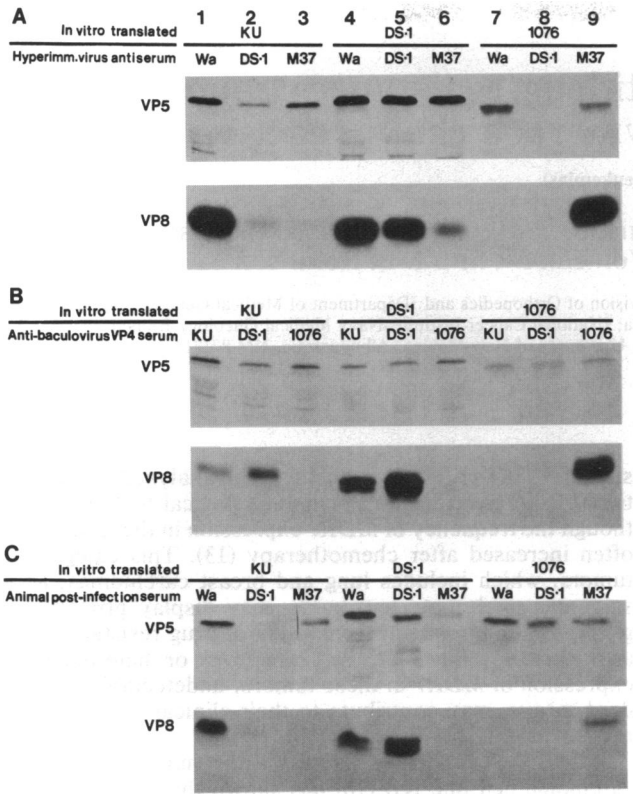

FIG. 3. Immunoprecipitation of VP5 and VP8 subunits of VP4 of rotavirus strain KU, DS-1, or 1076. pTZ18R plasmid containing KU, DS-1, or 1076 VP5 or VP8 cDNA was transcribed into RNA by T7 RNA polymerase and the mRNAs were translated into protein by a rabbit reticulocyte lysate in the presence of [^{35}S]methionine. The VP5 and VP8 proteins of each virus were immunoprecipitated and assayed directly by SDS/12% PAGE followed by fluorography. Lanes 1–3, VP5 and VP8 of KU; lanes 4–6, VP5 and VP8 of DS-1; and lanes 7–9, VP5 and VP8 of 1076. (*A*) Hyperimmune antiserum to purified strain Wa virus was used to immunoprecipitate VP5 and VP8 subunits of VP4 of strain KU (lane 1), DS-1 (lane 4), or 1076 (lane 7). Hyperimmune antiserum to strain DS-1 virus was used to immunoprecipitate VP5 and VP8 subunits of VP4 of strain KU (lane 2), DS-1 (lane 5), or 1076 (lane 8). Hyperimmune antiserum to strain M37 virus was used to immunoprecipitate VP5 and VP8 subunits of VP4 of strain KU (lane 3), DS-1 (lane 6), or 1076 (lane 9). (*B*) Antiserum to baculovirus recombinant-expressed KU VP4 was used to immunoprecipitate VP5 and VP8 subunits of VP4 of strain KU (lane 1), DS-1 (lane 4), or 1076 (lane 7). Antiserum to baculovirus recombinant-expressed DS-1 VP4 was used to immunoprecipitate VP5 and VP8 subunits of VP4 of strain KU (lane 2), DS-1 (lane 5), or 1076 (lane 8). Antiserum to baculovirus recombinant-expressed 1076 VP4 was used to immunoprecipitate VP5 and VP8 subunits of VP4 of strain KU (lane 3), DS-1 (lane 6), or 1076 (lane 9). (*C*) Serum from a gnotobiotic piglet infected with Wa was used to immunoprecipitate VP5 and VP8 subunits of VP4 of strain KU (lane 1), DS-1 (lane 4), or 1076 (lane 7). Serum from a gnotobiotic calf infected with DS-1 was used to immunoprecipitate VP5 and VP8 subunits of VP4 of strain KU (lane 2), DS-1 (lane 5), or 1076 (lane 8). Serum from a gnotobiotic piglet infected with M37 was used to immunoprecipitate VP5 and VP8 subunits of VP4 of strain KU (lane 3), DS-1 (lane 6), or 1076 (lane 9).

level of cross-reactivity by immunoprecipitation and neutralization. This is consistent with prior monoclonal antibody analysis, which suggested that neutralization specificity is mainly determined by the VP8 subunit (14). Strains of genetic groups 3 and 4, which were poorly neutralized by antisera to expressed VP4 protein of strain KU or DS-1, probably have relatively few epitopes in the VP8 subunit that are shared by genetic groups 1 and 2. On the other hand, the VP8 epitopes shared by strains of group 1 and group 2 are probably responsible for their high level of cross-reactivity observed in neutralization tests.

Recent studies indicate that antibodies induced by immunization with VP8 may be used in an ELISA to determine the VP4 serotype of rotavirus isolates. In addition, expressed VP8 subunit may be used to measure VP4 serotype-specific antibodies in sera of animals and humans obtained following rotavirus infection. This type of information should prove helpful in devising more effective strategies for prevention of serious rotavirus disease by immunization. The immunogenicity of the VP4 component of various immunogens, live or inactivated, can now be assayed directly. In addition, since antiserum to the VP4 of KU, the prime strain of VP4 serotype 1, was capable of neutralizing to high-titer viruses belonging to each of the four epidemiologically important human VP7 serotypes, it may be possible to induce immunity in infants to a broad range of HRVs using a vaccine containing a single attenuated rotavirus strain possessing a serotype 1A VP4.

We thank R. Jones for technical assistance and T. Heishman for editorial assistance. G.L. is the recipient of a grant from the World Health Organization.

1. Kapikian, A. Z. & Chanock, R. M. (1990) in *Virology*, eds. Fields, B. N., Knipe, D. M., Chanock, R. M., Hirsch, M. S., Melnick, D. L., Monath, T. P. & Roizman, B. (Raven, New York), 2nd Ed., pp. 1353–1404.
2. Offit, P. A., Clark, H. F., Blavat, G. & Greenberg, H. B. (1986) *J. Virol.* **60,** 491–496.
3. Hoshino, Y., Saif, L. J., Sereno, M. M., Chanock, R. M. & Kapikian, A. Z. (1988) *J. Virol.* **62,** 744–748.
4. Gorziglia, M., Hoshino, Y., Midthun, K., Buckler-White, A., Blumentals, I., Glass, R., Flores, J., Kapikian, A. Z. & Chanock, R. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7039–7043.
5. Gorziglia, M., Green, K. Y., Nishikawa, K., Taniguchi, K., Jones, R., Kapikian, A. Z. & Chanock, R. M. (1988) *J. Virol.* **62,** 2978–2984.
6. Gorziglia, M., Larralde, G. & Kapikian, A. Z. (1990) in *Vaccines 90* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 205–208.
7. Taniguchi, K., Nishikawa, K., Urasawa, T., Urasawa, S., Midthun, K., Kapikian, A. Z. & Gorziglia, M. (1989) *J. Virol.* **63,** 4101–4106.
8. Olmsted, R. A., Elango, N., Prince, G. A., Murphy, B. R., Johnson, P. R., Moss, B., Chanock, R. M. & Collins, P. L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7462–7466.
9. Nishikawa, K., Fukuhara, N., Liprandi, F., Green, K., Kapikian, A. Z., Chanock, R. M. & Gorziglia, M. (1989) *Virology* **173,** 631–637.
10. Fukuhara, N., Yoshie, O., Kitaoka, S. & Konno, T. (1988) *J. Virol.* **62,** 2209–2218.
11. Hoshino, Y., Wyatt, R. G., Greenberg, H. B., Flores, J. & Kapikian, A. Z. (1984) *J. Infect. Dis.* **149,** 694–702.
12. Saiki, R., Scharf, S., Faloana, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230,** 1350–1354.
13. Wyatt, R. G., Greenberg, H. B., James, W. D., Pittman, A. L., Kalica, A. R., Flores, J., Chanock, R. M. & Kapikian, A. Z. (1982) *Infect. Immun.* **37,** 110–115.
14. Mackow, E. R., Shaw, R. D., Matsui, S., Vo, P. T., Dang, M. & Greenberg, H. B. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 645–649.
15. Taniguchi, K., Maloy, W. L., Nishikawa, K., Green, K. Y., Hoshino, Y., Urasawa, S., Kapikian, A. Z., Chanock, R. M. & Gorziglia, M. (1988) *J. Virol.* **62,** 2421–2426.
16. Hoshino, Y., Sereno, M. M., Midthun, K., Flores, J., Kapikian, A. Z. & Chanock, R. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8701–8704.
17. Taniguchi, K., Morita, Y., Urasawa, T. & Urasawa, S. (1987) *J. Virol.* **61,** 1726–1730.
18. Mackow, E. R., Vo, P. T., Broome, R., Bass, D. & Greenberg, H. B. (1990) *J. Virol.* **64,** 1698–1703.