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Reassortant Rotaviruses as Potential Live Rotavirus Vaccine Candidates

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A series of reassortants was isolated from coinfection of cell cultures with a wild-type animal rotavirus and a "noncultivatable" human rotavirus. Wild-type bovine rotavirus (UK strain) was reassorted with human rotavirus strains D, DS-1, and P; wild-type rhesus rotavirus was reassorted with human rotavirus strains D and DS-1. The D, DS-1, and P strains represent human rotavirus serotypes 1, 2, and 3, respectively. Monospecific antiserum (to bovine rotavirus, NCDV strain) or a set of monoclonal antibodies to the major outer capsid neutralization glycoprotein, VP_7 (of the rhesus rotavirus), was used to select for reassortants with human rotavirus neutralization specificity. This selection technique yielded many reassortants which received only the gene segment coding for the major neutralization protein from the human rotavirus parent, whereas the remaining genes were derived from the animal rotavirus parent. Single human rotavirus gene substitution reassortants of this sort represent potential live vaccine strains.

Rotaviruses, newly classified members of the reoviridae family, are an important cause of infantile diarrhea in a wide variety of mammalian species including humans. Approximately 40% of severe pediatric diarrheal disease in most parts of the world is caused by rotaviruses (11). Since diarrheal disease is the leading cause of mortality in infants and young children in developing countries, and it is likely that rotaviruses are responsible for a considerable proportion of such fatalities, the development of a rotavirus vaccine represents an important public health priority. Various approaches to this goal have been considered, and two of these, the use of an attenuated human or an animal rotavirus strain as a candidate vaccine, have already been tested in humans (15, 16, 19; A. Z. Kapikian, K. Midthun, Y. Hoshino, J. Flores, R. G. Wyatt, R. I. Glass, J. Askaa, O. Nakagomi, T. Nakagomi, R. M. Chanock, M. M. Levine, M. L. Clements, R. Dolin, P. F. Wright, R. B. Belshe, E. L. Anderson, and L. Potash, *in* R. Lerner, F. Brown, and R. Chanock, ed., *Modern Approaches to Vaccines: Molecular and Chemical Basis of Resistance to Viral, Bacterial, and Parasitic Diseases*, in press; R. G. Wyatt, A. Z. Kapikian, Y. Hoshino, J. Flores, K. Midthun, H. B. Greenberg, R. I. Glass, J. Askaa, M. M. Levine, R. E. Black, M. L. Clements, L. Potash, and W. T. London, *in Proceedings of Conference on Control and Eradication of Infectious Diseases in Latin America*, in press). Animal rotavirus candidate vaccines include bovine rotavirus NCDV and UK strains and rhesus rotavirus (RRV) strain MMU 18006. Vesikari et al. have shown that oral administration of bovine rotavirus NCDV strain induced resistance in infants and young children against moderate or severe diarrheal illness caused by human rotavirus (15, 16). In preliminary studies, bovine rotavirus UK strain and RRV appear to be attenuated. Wyatt et al. administered the UK strain of bovine rotavirus to 11 adult volunteers, 2 with high and 9 with low levels of serum neutralizing antibodies. None of these individuals developed a diarrheal illness, shed the rotavirus in their stools, or demonstrated a seroresponse (Wyatt et al., in press). Kapikian et al. have evaluated orally administered RRV in 31 adults and 21 children with various levels of prevaccination serum neutralizing antibodies to RRV. The vaccine has not induced diarrheal illness in any of the volunteers. However, more than 80% of the vaccinees had a seroresponse to RRV, and approximately half of the vaccinees shed RRV as demonstrated in tissue culture inoculated with stool suspensions (Kapikian et al., in press). Safety testing of RRV in children less than 2 years of age is currently in progress.

Another approach, the development of human-animal rotavirus reassortants as candidate vaccines, takes advantage of the well-known property of the reoviridae to undergo genetic reassortment after mixed infection in cell culture (3). This method was used previously for the cultivation of "noncultivatable" human rotaviruses with a temperature-sensitive (*ts*) mutant of bovine rotavirus as the "rescue" virus.

This report describes the development of reassortants between RRV or wild-type bovine rotavirus UK strain and each of three human rotavirus serotypes represented by the following strains: D (type 1), DS-1 (type 2), and P (type 3). These reassortants offer promise as potential vaccine candidates in that many contain 10 genes from the animal rotavirus parent and a single gene which codes for serotype specificity from the human rotavirus parent. They may well lack pathogenicity for humans because all but one of their genes are derived from an animal virus which appears to be attenuated for humans (Kapikian et al., in press; Wyatt et al., in press). However, they should induce specific neutralizing antibodies to the human virus because they possess the gene which codes for human serotype specificity.

MATERIALS AND METHODS

Animal viruses. Reassortant viruses were derived from coinfection of African green monkey kidney (AGMK) cells

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with wild-type animal rotavirus and "noncultivatable" human rotavirus. Wild-type bovine rotavirus UK strain (1), kindly supplied by G. N. Woode and T. H. Flewett, was crossed with each of the following human rotavirus strains: D (serotype 1), DS-1 (serotype 2), and P (serotype 3). Wild-type RRV strain MMU 18006 (14), kindly supplied by N. J. Schmidt, was reassorted with human rotavirus strains D and DS-1. The bovine rotavirus UK strain was recovered initially in primary calf kidney cells, and RRV strain MMU 18006 was recovered initially in primary cynomolgus monkey kidney cells. These animal virus strains subsequently were plaque purified and passaged in primary AGMK cells. The final passage of RRV and UK virus was in DBS-FRhL-2 (13) and primary calf kidney cells, respectively. The wild-type RRV (strain MMU 18006) used in reassortment experiments with strains D and DS-1 was designated lot RRV-1, whereas the wild-type UK bovine rotavirus used in reassortment with D, DS-1, and P was designated lot BR-3 (final lots were prepared by Flow Laboratories, Inc.).

Human viruses. The D and DS-1 human rotaviruses were originally detected in the stools of children hospitalized with diarrhea. Each was passaged in gnotobiotic calves (18), and stools from infected calves were collected. The P strain was obtained from an infant hospitalized with diarrhea at the Children's Hospital National Medical Center in Washington, D.C., and kindly supplied by R. H. Parrott, H. W. Kim, W. J. Rodriguez, and C. D. Brandt.

Reassortment experiments. The stools containing the "noncultivatable" human rotavirus strains, D, DS-1, and P, were made into 10% suspensions and activated with trypsin (2 to 10 μg/ml); the equivalent of 10 mg of stool was inoculated onto each AGMK cell monolayer of a six-well Costar tissue culture plate. The monolayers were centrifuged at 1,400 × g for approximately 60 min at 37°C. The cultures were washed once, and the animal rotavirus, also pretreated with trypsin (2 μg/ml), was inoculated onto the monolayer at a multiplicity of infection of ca. 1, and the cultures were incubated for 1 h at 37°C. The tissue monolayer was again washed and fed with Eagle minimal essential medium containing glutamine (2 mM), penicillin (250 U/ml), streptomycin (250 μg/ml), aureomycin (25 μg/ml), fungizone (2.5 μg/ml), and trypsin (0.5 μg/ml). Inoculated cultures were then incubated for ca. 36 h at 37°C. Under the culture conditions employed in these studies, the human rotaviruses D, DS-1, and P did not form progeny detectable by plaque assay. Selection for the desired viral reassortants was achieved by exposing the progeny of the coinfected cultures to monoclonal antibodies or hyperimmune antiserum which specifically neutralized the animal rotavirus parent. Guinea pig hyperimmune antiserum prepared against the NCDV strain of bovine rotavirus, as previously described, was used to neutralize the bovine rotavirus UK strain and reassortants bearing the VP_7 of the UK strain (3). This antiserum is highly reactive with the VP_7 protein of the UK virus but does not have neutralizing activity directed at the fourth gene product, VP_3, of UK rotavirus (Y. Hoshino and R. Wyatt, personal communication). A pool of three monoclonal antibodies (954/96/83/10, 954/159/13/38, and 952/3/18/17), amplified in mouse ascites fluid and directed at the neutralization protein, VP_7, of RRV, was used to select against the RRV animal rotavirus parent and reassortants bearing the VP_7 of RRV. These monoclonal antibodies immunoprecipitate the 38,000-dalton glycoprotein VP_7 of RRV and specifically neutralize viruses belonging to serotype 3 (5). The use of antiserum that neutralized VP_7 but not VP_3 of bovine rotavirus UK strain and the use of monoclonal antibodies directed at VP_7 but not VP_3 of RRV may have facilitated the isolation of reassortants as described below. The coinfection growth yield was incubated with the appropriate antiserum or monoclonal antibody ascites mixture diluted 1:100 before being inoculated onto the AGMK cell monolayers. In addition, the antiserum or monoclonal antibody mixture was added to the agarose overlay at a dilution of 1:1,000. The antiserum and the monoclonal antibody pool had neutralization titers (reciprocal) of 40,960 and greater than 81,920, respectively, against the homologous virus. After 4 to 5 days of incubation at 37°C, plaques were picked and these clonal populations were subjected to two further plaque purifications in AGMK cell monolayers. Neutralizing antiserum or a monoclonal antibody mixture was used in the first but not in the second of the two subsequent plaque purifications.

PRNA. The serotype of selected reassortants was determined by plaque reduction neutralization assay (PRNA) as described previously (17). The neutralization titer of a serum to a given virus was defined as the reciprocal of the highest dilution of serum that produced 60% plaque reduction. If the titer fell between two dilutions, the Reed-Muench method was used for calculation.

RNA preparation. RNAs from the reassortant rotaviruses and from each of the parental rotaviruses were used to determine genotype by RNA hybridization. Each reassortant was grown in MA 104 cell monolayers in 2 to 3 wells of a six-well Costar plate. When maximum cytopathic effect was noted, the contents of the wells were pooled and centrifuged at 12,000 × g, and the pellet was extracted with chloroform-phenol to obtain genomic RNAs (9). Parental virion RNAs were prepared by growing a large suspension of wild-type UK bovine rotavirus, RRV, or cultivatable human rotavirus strain DS-1 or P. The crude cell culture fluid was centrifuged at 82,500 × g, and the pellet was resuspended in phosphate-buffered saline. The pellet was then fluorocarbon extracted, and the aqueous phase was pelleted through a 30% sucrose cushion at 155,000 × g. Genomic RNAs were obtained from the pellet by chloroform-phenol extraction. Genomic RNAs were purified from rotavirus D strain by fluorocarbon extraction of a stool from an experimentally infected calf. This material was then pelleted through a sucrose cushion and extracted with chloroform-phenol. ^{32}P-labeled single-stranded RNAs were transcribed from viral cores of each of the parental rotaviruses by the method described by Flores et al. (2), except that newly synthesized transcripts were precipitated with 2 M lithium chloride. Labeled transcripts were stored at −70°C until used.

Genotyping by RNA hybridization. Hybridization of cold, denatured double-stranded viral RNAs to the ^{32}P-labeled single-stranded RNA probes was performed by the method of Kalica et al. (8). RNAs were subjected to electrophoresis in polyacrylamide gel by the method of Laemmli (12). Polyacrylamide gels (10%) were used routinely, and the duration of electrophoresis was ca. 14 h at 20 mA. UK, P, and RRV gene segments 7, 8, and 9 and D gene segments 7 and 8 were extremely difficult to resolve under these conditions.

RESULTS

Wild-type bovine rotavirus (UK strain) × human rotavirus (D, DS-1, or P strain) reassortants. Under the selective pressure of bovine rotavirus NCDV antiserum, 24 bovine rotavirus (UK strain) × human rotavirus (D, DS-1, or P strain) reassortants were isolated (Table 1). Examination of the genotype of these reassortants by RNA-RNA hybridization revealed that 16 of the reassortants derived 10 gene

TABLE 1. Distribution of human rotavirus genes in reassortants derived from coinfection with "noncultivatable" human rotavirus (serotype 1, 2, or 3) and animal rotavirus (wild-type RRV or wild-type or *ts* mutant bovine rotavirus UK strain)[a]

Selection pressure	Animal rotavirus parent	Human rotavirus parent[b]	No. of reassortants tested	No. of reassortants with indicated no. of human rotavirus genes[c]			
				One gene (VP_7)	Two genes (VP_7 and one other gene)	Three genes (VP_7 and two other genes)	Four or more genes (VP_7 and three or more other genes)
Mixture of three monoclones specific for RRV VP_7	Wild-type RRV	D (type 1)	8	4	1 (3)	0	3 (5, 7, 8; 3, 5, 7, 8; 3, 5, 7, 8)
		DS-1 (type 2)	3	0[d]	1 (5)[d]	1 (2 or 3, 5)	1 (1, 5, 6)
Hyperimmune antiserum for bovine rotavirus (NCDV strain) and restrictive temperature in the case of the *ts* mutant UK	Wild-type bovine rotavirus (UK strain)	D (type 1)	9	5	3 (3; 6; 7 or 8)	1 (3, 11)	0
		DS-1 (type 2)	4	2	2 (6; 7)	0	0
		P (type 3)	11	9	2 (3; 11)	0	0
	ts mutant bovine rotavirus (UK strain)	Wa (type 1)	10	0	0	4	6
		W (type 1)	8	0	0	0	8
		DS-1 (type 2)	11	0	0	1	10

[a] Data on reassortants derived from the *ts* mutant bovine rotavirus parent (UK strain) were obtained from Kalica et al. (10) and Greenberg et al. (4). The genotypes of these reassortants are described in the references given.
[b] These viruses did not produce plaques in AGMK or MA 104 cell monolayers.
[c] VP_7 is used to denote the gene coding for the major neutralization protein, VP_7. This indicates the ninth gene of the D, Wa, or W strain, the eighth gene of the DS-1 strain, and the eighth or ninth gene of the P strain. Number(s) in parentheses show origin of gene(s) (in addition to VP_7) derived from human rotavirus parent for each indicated reassortant from this study. Percentage of reassortants with the following numbers of human rotavirus genes: for reassortants derived from wild-type strains, one gene, 57; two genes, 26; three genes, 6; four or more genes, 11; for the reassortants derived from mutant strains, one gene, 0; two genes, 0; three genes, 17; four or more genes, 83.
[d] A single human rotavirus gene substitution DS-1 × RRV reassortant with the DS-1 serotype was isolated subsequently by backcrossing the DS-1 × RRV reassortant with two human rotavirus genes with wild-type RRV.

segments from the bovine rotavirus parent and a single gene segment (the ninth in the case of the D strain, the eighth in the case of the DS-1 strain, and the eighth or ninth in the case of the P strain) from the human rotavirus parent. Seven reassortants derived two genes, and one reassortant derived three genes from their human rotavirus parent strain (Table 1).

Single human rotavirus gene substitution reassortants from the crosses between bovine rotavirus (UK strain) and each of three human rotaviruses (D, DS-1, or P strain) were tested by PRNA (Table 2). One of these reassortants, D × UK (47-1-1), was found to be similar if not identical to the Wa virus, our reference serotype 1 strain, indicating that the ninth gene of human rotavirus D strain codes for serotype specificity. This finding is consistent with previous studies with two other serotype 1 viruses, Wa and W (4, 10). Two other single human rotavirus gene substitution reassortants, DS-1 × UK (66-1-1) and P × UK (22-1-1), were tested by PRNA. The genotype of the latter reassortant is given in Fig. 1. These reassortants were were found to be similar if not identical to DS-1 virus, our prototype serotype 2 virus, and P, our prototype serotype 3 virus, respectively (Table 2). These results indicate that the eighth gene segment of human rotavirus DS-1 strain is associated with serotype specificity,

TABLE 2. Serotype of wild-type animal × human rotavirus reassortants as determined by 60% PRNA[a]

Rotavirus	Gene of human rotavirus parent present in reassortant	Reciprocal of 60% PRNA antibody titer of hyperimmune antiserum to indicated rotavirus						
		Wa (serotype 1)	DS-1 (serotype 2)	P (serotype 3)	ST3 (serotype 4)	RRV (serotype 3)	NCDV (serotype 6)	UK (serotype 6)
D × UK (47-1-1)	Ninth	40,960	<80	<80	<80	160	<80	640
DS-1 × UK (66-1-1)	Eighth	<80	10,240	<80	<80	160	<80	2,560
P × UK (22-1-1)	Eighth or ninth	<80	<80	40,960	<80	10,240	<80	640
D × RRV (6-1-1)	Ninth	40,960	<80	<80	<80	2,560	160	<80
DS-1 × RRV (240-2-1)	Eighth	<80	10,240	<80	<80	640	640	<80
Wa		>81,920[b]	<80	2,560	160	<80	<80	<80
DS-1		<80	10,240[b]	<80	<80	<80	<80	<80
P		640	<80	81,920[b]	640	40,960	<80	<80
ST3		160	<80	<80	40,960[b]	<80	<80	<80
RRV		<80	<80	>81,920	<80	>81,920[b]	640	160
UK		<80	<80	<80	<80	<80	40,960	40,960[b]
NCDV		<80	<80	160	160	160	40,960[b]	10,240

[a] This is a composite table of values derived from seven different tests, each of which involved 12 different viruses and a single hyperimmune antiserum.
[b] Homologous value.

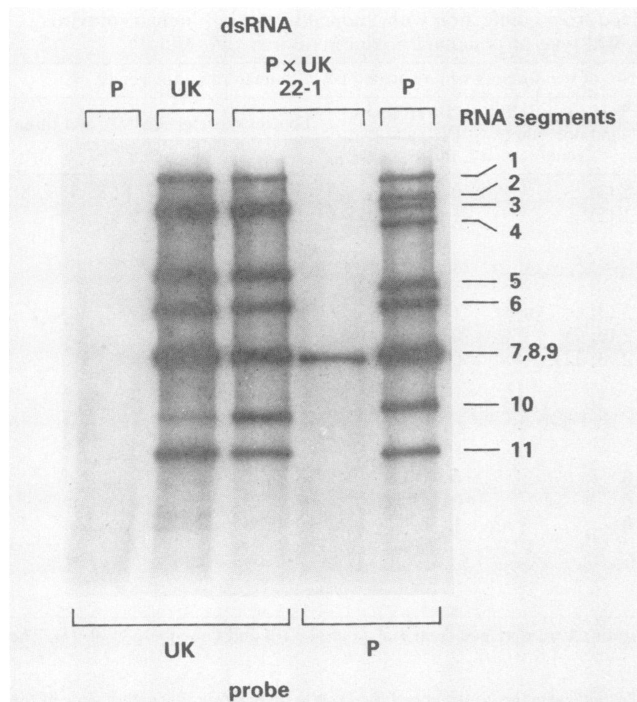

FIG. 1. Analysis of genotype of human rotavirus strain P × bovine rotavirus strain UK reassortant 22-1. Single-stranded ^{32}P-labeled mRNA transcripts of bovine rotavirus UK strain or human rotavirus P strain were hybridized to the genomic RNAs of UK, P, or UK × P reassortant 22-1. The cold double-stranded genomic RNAs were placed in 1 mM EDTA, heated at 100°C for 2 min, and then cooled in ice for 2 to 3 min. Hybridization buffer (100 mM NaCl, 50 mM Tris [pH 8], 0.1% sodium dodecyl sulfate) containing ca. 10,000 cpm of the indicated [^{32}P]RNA probe was added to the denatured cold RNAs, and hybridization was allowed to take place at 65°C for 14 h. Hybridized RNAs were precipitated with 2.5 μg of tRNA and by addition of 2 to 3 volumes of ethanol. After being held overnight at −20°C, the RNAs were pelleted (12,000 × *g*) and taken up in 20 μl of sample buffer and electrophoresed on a 10% polyacrylamide gel at 20 mA for 14 h. In P × UK reassortant 22-1, only one gene in the 7, 8, 9 complex is derived from the human rotavirus parent, whereas the other two in that complex are derived from the bovine rotavirus parent. (Note the increased intensity of the reassortant RNA hybridized to the UK probe as compared with the reassortant RNA hybridized to the P probe in the 7, 8, 9 complex of gene segments). As judged by the distance migrated, the gene derived from the human rotavirus parent is either the eighth or ninth. The remaining genes are derived from the UK parent. Note the absence of homology between the P genomic RNAs and the UK probe.

confirming an earlier observation by Greenberg et al. (4) and the eighth or ninth gene segment of human rotavirus P codes for serotype specificity. Since all of the D × UK and DS-1 × UK reassortants contain the ninth gene of D and eighth gene of DS-1, respectively, it can be assumed that they exhibit the same human rotavirus specificity as their human rotavirus parent. The coding of serotype specificity of the P strain was narrowed to the eighth or ninth gene but could not be specifically assigned to either because these two genes do not separate well by polyacrylamide gel electrophoresis. As a result, all of the P × UK reassortants were tested by PRNA and shown to belong to human serotype 3.

Wild-type RRV × human rotavirus (D or DS-1 strain) reassortants. Under the selective pressure of monoclonal antibodies directed at the VP_7 neutralization protein of RRV, 11 RRV × human rotavirus (D or DS-1 strain) reassortants were isolated (Table 1). Four of these were single human rotavirus gene substitution reassortants, containing only the ninth human rotavirus gene in the case of D × RRV reassortants. A single human rotavirus gene substitution reassortant was not isolated from the original DS-1 × RRV cross. However, such a reassortant with the DS-1 serotype was subsequently isolated by backcrossing a DS-1 × RRV reassortant containing only the fifth and eighth genes of DS-1 with wild-type RRV. Two reassortants derived two genes and five reassortants derived three or more genes from their human rotavirus parent (Table 1). Single human gene substitution reassortants, D × RRV (6-1-1) and DS-1 × RRV (240-2-1) (the latter described in Fig. 2) were tested by PRNA and found to be similar to Wa virus (serotype 1) and DS-1 virus (serotype 2), respectively (Table 2). These results confirm that the ninth gene of human rotavirus D strain and the eighth gene of human rotavirus DS-1 strain are associated with serotype specificity. In addition, some reassortants containing the human rotavirus VP_7 gene coding for serotype as well as one or more other human rotavirus genes were

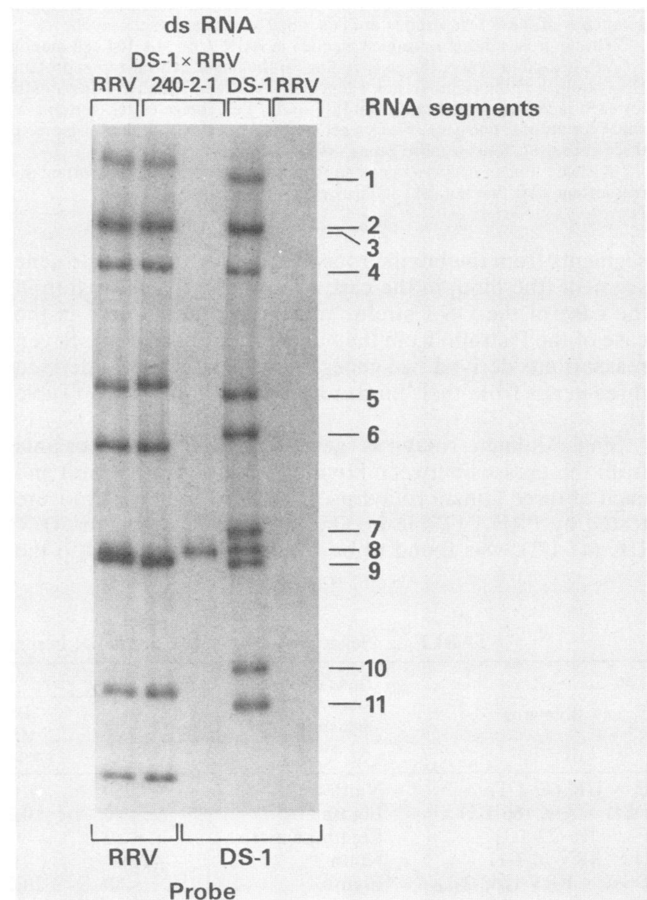

FIG. 2. Analysis of genotype of human rotavirus strain DS-1 × RRV reassortant 240-2-1 by methods similar to those described in Fig. 1. The RNA-RNA hybrids were electrophoresed on a 10% polyacrylamide gel at 20 mA for 14 h. In DS-1 × RRV reassortant rotavirus 240-2-1, only the eighth gene is derived from the human rotavirus parent. The remaining genes are derived from the RRV parent. Hybridization of the ^{32}P-labeled DS-1 probe to the genomic RNAs of RRV fails to show homology.

tested by PRNA and found to exhibit the serotype of their human rotavirus parent (results not shown).

Characteristics of wild-type animal × "noncultivatable" human rotavirus reassortants selected with monospecific neutralizing antiserum or monoclonal antibodies. The reassortants described in this study have the following characteristics in common. They possess the major neutralization protein of the human rotavirus parent, grow to high titer in tissue culture and, in the majority of cases, derive almost all their genes from the animal rotavirus parent. In addition, the bovine rotavirus UK strain and RRV, as well as their single human rotavirus gene substitution reassortants, have been adapted successfully to growth in the diploid cell strain DBS-FRhL-2 (Y. Hoshino and R. G. Wyatt, unpublished data). The DBS-FRhL-2 cell strain, developed at the Division of Biologics Standards (now the National Center for Drugs and Biologics, Food and Drug Administration), was obtained from a fetal rhesus monkey lung (13).

DISCUSSION

Analysis of the reassortants generated in this study reveals a distribution of genotypes that differs from that observed for reassortants derived from crosses between a *ts* mutant of bovine rotavirus UK strain and a "noncultivatable" human rotavirus strain W, Wa, or DS-1 (Table 1). In the latter situation, the non-*ts* human rotavirus parent usually contributes three or more genes to each reassortant (3, 4, 6, 10). In contrast, the reassortants derived from a cross between a wild-type animal rotavirus and a "noncultivatable" human rotavirus frequently (57%) received only one gene from the human rotavirus parent. When a *ts* animal rotavirus was used for production of reassortants with human serotype, we provided selection against the neutralization antigen of the animal rotavirus by incubation with specific antibodies directed at this antigen. We also selected against the *ts* genes of the animal rotavirus parent by incubation of the coinfection growth yield at a temperature restrictive for the *ts* virus parent. In addition, silent mutations in other genes of the mutagenized *ts* animal rotavirus parent may have been responsible for selection against these genes during incubation. When neither rotavirus parent was a *ts* mutant, as in the current study, selection against animal rotavirus genes was provided only by antibodies directed against the gene coding for the neutralization protein of the animal rotavirus parent.

One benefit of using a wild-type animal rotavirus instead of a *ts* animal rotavirus mutant in preparing reassortants for vaccine purposes is that the former has not been exposed to chemical mutagenesis. Thus, the development of silent point mutations, which are often unstable and which can confound genetic analysis of attenuation, would be minimized in reassortants derived from wild-type animal rotaviruses. The advantage of relying exclusively on monospecific neutralizing antiserum or monoclonal antibodies directed against the VP_7 of the animal rotavirus parent to favor the desired serotype is that selection is exerted only against the major neutralization protein of this parent. The other genes of the animal rotavirus parent are thus allowed to outcompete their "noncultivatable" human rotavirus counterparts, and in this manner, reassortants derive all or most of their genes, except for the gene coding for the major neutralization protein, from the animal rotavirus parent.

The analysis by PRNA of single human rotavirus gene substitution reassortants representing human serotypes 1, 2, and 3 enabled us to make gene coding assignments for serotype specificity. The ninth gene of D, the eighth gene of DS-1, and the eighth or ninth gene of P were associated with the major neutralization protein, VP_7, of these human rotaviruses. It is interesting to examine the PRNA results of the bovine × human rotavirus reassortants more closely (Table 2). Although these reassortants are similar, if not identical, to the serotype of their human rotavirus parent, low-level neutralization was noted in the presence of antiserum to bovine UK strain but not in the presence of antiserum to bovine NCDV strain. Likewise, the PRNA of the RRV × human rotavirus reassortants (Table 2) shows that these reassortants are similar, if not identical, to the serotype of their human rotavirus parent. However, low-level neutralization was noted in the presence of antiserum to RRV but not in the presence of antiserum to human rotavirus P strain, although RRV and P strains both belong to human serotype 3. These findings suggest that a viral protein in addition to the major neutralization protein VP_7 plays a role in neutralization. VP_3 is a likely candidate for this minor neutralization protein for the following reasons. First of all, monoclonal antibodies directed at VP_3 of RRV neutralize RRV to a high titer when they are amplified in mouse ascites fluid as described by Greenberg et al. (5). Wyatt and Hoshino made the observation that NCDV antiserum is highly reactive with VP_7 of UK but not with VP_3 of UK, whereas UK antiserum has antibodies directed against both VP_3 and VP_7. In summary, the single human rotavirus gene substitution reassortants described in this study exhibit the serotype specificity of their human rotavirus parent strains. However, they derive their minor neutralization protein, most likely VP_3, from their animal rotavirus parent. This accounts for the low level of neutralization observed when the bovine-human rotavirus reassortants were tested with UK antiserum but not with NCDV antiserum, even though UK and NCDV rotaviruses belong to the same serotype; it also accounts for the low level of neutralization found when the RRV-human reassortants were tested with RRV antiserum but not with P antiserum, even though RRV and P rotaviruses are serotypically identical. In a recent publication, Hoshino et al. have observed some one-way antigenic relationships among certain rotaviruses and suggest that VP_3 may play a role in this phenomenon (7).

The single human rotavirus gene substitution reassortants described in this study represent potential vaccine candidates. The major neutralization protein of these reassortants is derived from the human rotavirus parent, and these viruses should therefore have the desired immunogenicity. It is also likely that the presence of 10 animal rotavirus genes in these reassortants will render such viruses attenuated for humans. This latter supposition is supported by the fact that bovine rotavirus UK and RRV have been administered to susceptible volunteers with a low level of serum antibodies and did not produce illness (Kapikian et al., in press; Wyatt et al., in press). These findings suggest that single human rotavirus gene substitution reassortants may be promising vaccine candidates for use in prevention of human rotavirus disease. The ability of these reassortants to grow in diploid cells may represent an additional advantage in production of vaccine virus because the diploid cell strain DBS-FRhL-2 appears to be free of adventitious agents, in contrast to AGMK cells, which must be carefully screened for endogenous viral contamination.

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