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Similarity of the Outer Capsid Protein VP4 of the Gottfried Strain of Porcine Rotavirus to That of Asymptomatic Human Rotavirus Strains

M. GORZIGLIA,* K. NISHIKAWA, Y. HOSHINO, AND K. TANIGUCHI

Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

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Genomic segment 4 of the porcine Gottfried strain (serotype 4) of porcine rotavirus, which encodes the outer capsid protein VP4, was sequenced, and its deduced amino acid sequence was analyzed. Amino acid homology of the porcine rotavirus VP4 to the corresponding protein of asymptomatic or symptomatic human rotaviruses representing serotypes 1 to 4 ranged from 87.1 to 88.1% for asymptomatic strains and from 77.5 to 77.8% for symptomatic strains. Amino acid homology of the Gottfried strain to simian rhesus rotavirus, simian SA11 virus, bovine Nebraska calf diarrhea virus, and porcine OSU strains ranged from 71.5 to 74.3%. Antigenic similarities of VP4 epitopes between the Gottfried strain and human rotaviruses were detected by a plaque reduction neutralization test with hyperimmune antisera produced against the Gottfried strain or a Gottfried (10 genes) × human DS-1 rotavirus (VP7 gene) reassortant which exhibited serotype 2 neutralization specificity. In addition, a panel of six anti-VP4 monoclonal antibodies capable of neutralizing human rotaviruses belonging to serotype 1, 3, or 4 was able to neutralize the Gottfried strain. These observations suggest that the VP4 outer capsid protein of the Gottfried rotavirus is more closely related to human rotaviruses than to animal rotaviruses.

Many human and animal rotaviruses bear common antigenic determinants. For example, the Gottfried strain of porcine rotavirus shares subgroup and serotype specificities with certain human rotavirus strains. The Gottfried strain belongs to rotavirus subgroup II, as do four of the six human rotavirus serotype reference strains (6). Alignment of the amino acid sequence of the VP6 gene (responsible for subgroup specificity) of Gottfried virus with the VP6 gene of several subgroup I and II rotavirus strains indicated that the Gottfried virus exhibits a high degree of amino acid homology (98%) with subgroup II rotaviruses such as the Wa human strain, while amino acid homology between the Gottfried strain and subgroup I rotaviruses was somewhat lower (92 to 93%) (4). The Gottfried virus is similar, if not identical, to serotype 4 of human rotavirus on the basis of neutralization with hyperimmune antisera (8). In addition (5), the amino acid sequence of its outer capsid neutralization protein, VP7, which is responsible for serotype specificity, is 95.1% homologous with that of VP7 of a serotype 4 human asymptomatic rotavirus strain, ST3.

Another important outer capsid rotavirus antigen, VP4, is encoded by gene 4. Antibodies directed to this protein inhibit viral hemagglutination, neutralize rotavirus in vitro, and passively protect mice against challenge with homologous as well as some heterologous rotaviruses (6, 13). The location of amino acids involved in VP4-mediated heterotypic neutralization has been identified in human and animal rotaviruses (9, 14). Also, VP4 appears to be associated with virulence of rotaviruses in experimentally infected mice (12) and may play a role in the attenuation of naturally occurring asymptomatic human neonatal strains (2, 3).

In this study, we investigated the relationships of the VP4 outer capsid protein of Gottfried virus to various human and animal rotaviruses at the molecular and antigenic levels. To study these relationships, we determined the nucleotide sequence of the gene encoding the VP4 of Gottfried virus and compared this sequence and its deduced amino acid sequence with the corresponding sequences of the VP4 gene of selected human and animal rotavirus strains. Antigenic relationships were also studied by employing hyperimmune antiserum produced against Gottfried virus and against a single-gene-substitution Gottfried × human DS-1 rotavirus reassortant, which derived only its VP7 gene from the human strain. In addition, a panel of VP4 cross-reactive neutralizing monoclonal antibodies (NMAb) directed against human rotavirus strains of serotype 1, 3, or 4 was used for antigenic characterization (15).

The porcine rotavirus Gottfried strain was originally isolated from the intestinal contents of a suckling pig with diarrhea (1) and was kindly supplied by E. H. Bohl. An established cell line of fetal rhesus monkey kidney cells, MA104, was used for virus propagation. Viral mRNAs were produced from single capsid particles as described previously (3), and the total mRNA was hybridized to a series of primers 18 nucleotides in length. The nucleotide sequence of the Gottfried VP4 gene was determined by the dideoxy-chain termination method with avian myeloblastosis virus reverse transcriptase as described elsewhere (3).

The Gottfried VP4 nucleotide sequence is 2,359 bases in length and contains one long open reading frame beginning 10 nucleotides from the 5′ end and terminating with a single stop codon 22 bases from the 3′ end (Fig. 1). The encoded VP4 protein contains 775 amino acids (Fig. 2). This was unexpected, since in contrast to human rotavirus VP4 genes, all animal rotavirus VP4 genes (except for those of Gottfried virus) thus far sequenced (rhesus rotavirus [RRV], SA11, Nebraska calf diarrhea virus, and OSU) possess an extra nucleotide triplet, CAA, at nucleotide positions 382 to 384 (9–11).

The deduced amino acid sequence of the Gottfried VP4 was compared to that of the human and animal rotavirus strains, and the percent sequence identity was calculated. In

* Corresponding author.

FIG. 1. Complete nucleotide sequence of the VP4 gene of Gottfried. Underlined bases indicate the positions of the initiation and termination codons.

previous studies it was observed that there are three distinct families of VP4 among human rotavirus strains (2, 3). They are serotype 1, 3, or 4 symptomatic rotaviruses (Wa, P, and Va70) (93.2 to 96.8% amino acid identity); symptomatic serotype 2 strain DS-1 (89.4 to 90.5% amino acid identity to strains in group 1); and asymptomatic rotaviruses of serotype 1, 2, 3, or 4 (M37, 1076, McN13, and ST3) (95.1 to 97.4% amino acid identity among strains in the group but only 75.4 to 77.8% amino acid identity to strains in group 1 or 2). Among the animal rotaviruses RRV, SA11, Nebraska calf diarrhea virus, and OSU, VP4 amino acid homology ranges from 81.1 to 87.8% and the highest homology in this group is between the two simian strains, RRV and SA11. The overall VP4 amino acid homology of animal rotaviruses to Gottfried virus ranges from 71.5 to 74.3%, whereas the amino acid homology of Gottfried virus to the symptomatic and asymptomatic human rotavirus strains ranges from 76.9 to 77.8% and 87.1 to 88.1%, respectively. Thus, the VP4 protein of Gottfried virus is more closely related to the VP4 of the asymptomatic neonatal human rotaviruses than to the VP4 of symptomatic human or animal rotaviruses. This suggests that the Gottfried strain and the asymptomatic human strains might have diverged from a common ancestor more recently than did the virulent human rotaviruses or the animal rotaviruses included in our survey.

VP4 appears to play an important role in the pathogenesis of infection and disease. A recent study has shown that several regions of VP4 are conserved among human symptomatic rotavirus strains, while a different set of sequences is conserved among human asymptomatic rotaviruses (2, 3). It has been suggested that among these sequences, a likely candidate for a role in virulence is the connecting peptide (amino acids 241 to 246), which is the region in which VP4 is cleaved in the presence of trypsin to VP5 and VP8. This region contains an extra potential cleavage site (amino acid 245) in human symptomatic rotaviruses (Fig. 2). Gottfried VP4 has only two potential cleavage sites, both of which are identical in location to the corresponding sites on the VP4 of human asymptomatic strains. Moreover, five of the six amino acids of the connecting peptide are identical to those of the asymptomatic human strains. The only divergent amino acid is a valine in place of isoleucine, which represents a conservative change. This finding suggests that the cleavage peptide and cleavage sites may not be related to

TABLE 1. Antigenic and immunologic characterization of porcine rotavirus Gottfried VP4 protein by plaque reduction neutralization (PRN)

Rotavirus strain	Serotype	Virulence	Reciprocal of 60% PRN antibody titer of guinea pig hyperimmune antiserum to:	
			Gottfried strain	Reassortant[a]
Wa	1	+ (human)	640	640
DS-1	2	+ (human)	<80	40,960
P	3	+ (human)	640	640
Va70	4	+ (human)	10,240	640
M37	1	− (human)	640	640
1076	2	− (human)	640	10,240
McN13	3	− (human)	640	640
ST3	4	− (human)	20,480	640
Gottfried	4	+ (porcine)	20,480	10,240
33-1-1	2	—[b]	2,560	40,960

[a] Gottfried (10 genes including VP4) × DS-1 (VP7), 33-1-1; single-VP7-gene-substitution reassortant which derived only the VP7 gene from the human DS-1 strain (serotype 2) and the remaining 10 genes from the porcine Gottfried strain. Hyperimmune serum to the DS-1 strain neutralizes only human rotaviruses which share the same VP7 serotype 2 specificity.

[b] —, Not known.

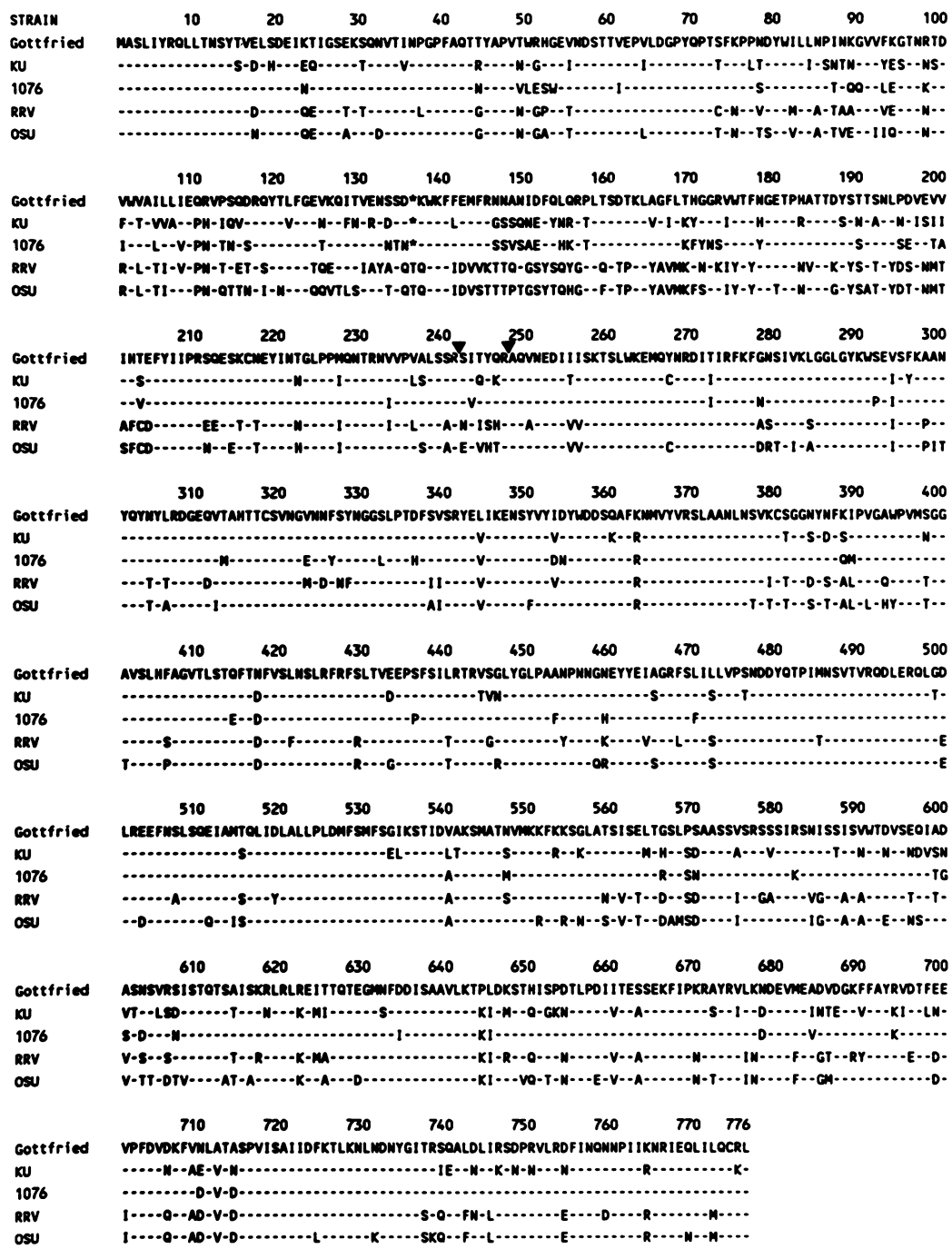

FIG. 2. Comparison of the amino acid sequences of the VP4 of porcine Gottfried, human symptomatic KU, human asymptomatic 1076, simian RRV, and porcine OSU strains. Symbols: ▼, cleavage sites; ★, amino acid deletion.

virulence, since Gottfried virus induces symptomatic infection in pigs. However, it is possible that the valine substitution in Gottfried VP4 may be related to the virulence of this virus. It has been demonstrated that certain symptomatic human strains can infect and in some cases cause diarrhea in piglets. We are currently in the process of infecting piglets with asymptomatic human strains to determine whether these strains are virulent or attenuated for these animals.

We also analyzed the antigenic relationships between the Gottfried virus and the symptomatic and asymptomatic human rotavirus strains by neutralization with a hyperimmune serum to Gottfried virus and reassortant 33-1-1, which derived only its VP7 gene from human rotavirus DS-1 and its remaining genes from Gottfried virus. With the latter serum, the antigenic reactivities of VP4 and VP7 could be dissociated. Antiserum to Gottfried virus (serotype 4 via its VP7) neutralized to a high titer the human rotaviruses which share the same VP7 specificity (i.e., serotype 4; strains VA70 and ST3). This antiserum also neutralized to a high titer the reassortant 33-1-1, which possessed the VP4 but not the VP7

TABLE 2. Reactivity of porcine rotavirus Gottfried with anti-VP4 cross-reactive NMAbs as determined by neutralization[a]

Virus strain (serotype)	Titer of indicated NMAb[b]					
	KU-4D7 (433)	KU-6B11 (392 or 439)	YO-1E6 (439)	YO-1S3 (392)	YO-2C2 (305)	ST-1F2 (392)
Gottfried (4)	6,400	25,000	6,400	25,000	6,400	1,600
1076 (2)	200	6,400	12,800	25,000	6,400	12,800
RRV (3)	200	3,200	200	1,600	200	200

[a] Neutralizing antibody titers are expressed as the reciprocal of the highest dilution able to neutralize 60% of infectious virus as determined by plaque assay. The homologous titer of NMAbs to KU (serotype 1), YO (serotype 3), or ST3 (serotype 4) was 25,000 in each case (15).

[b] Number in parentheses indicates the position at which a single amino acid substitution occurred in mutants selected with the corresponding NMAb (14).

of Gottfried. The Gottfried antiserum also recognized, but at a lower titer, the asymptomatic human strains M37, 1076, and McN13, which belong to serotypes 1, 2, and 3, respectively, as well as the symptomatic human strains Wa and P (serotypes 1 and 3). However, the serum failed to neutralize the symptomatic DS-1 strain (serotype 2). Thus, hyperimmune antiserum against the Gottfried strain recognized antigenic sites on VP7 at the highest titer but also neutralized most of the other strains at a lower titer. In an attempt to dissect the neutralization response with respect to VP7 and VP4 specificity, we also tested each rotavirus with hyperimmune serum to the single-gene-substitution reassortant 33-1-1 (Table 1). This antiserum neutralized to a high titer the serotype 2 viruses DS-1 and 1076, as expected from the serotype 2 VP7 specificity of these viruses. Gottfried virus was also neutralized to a high titer via its VP4. In addition, the antiserum against the reassortant neutralized the appropriate symptomatic and asymptomatic strains to the same level as the Gottfried antiserum did, suggesting that these relationships were mediated by VP4. Thus, it appears that some VP4 epitopes common to these human rotaviruses are also shared by the porcine Gottfried strain.

Cross-reactive neutralization among heterotypic rotaviruses is mostly attributable to antigenic sites on VP4. In a previous study with a panel of six VP4 NMAbs (KU-4D7, KU-6B11, YO-1E6, YO-1S3, YO-2C2, and ST-1F2) produced from symptomatic strains of serotypes 1 (KU) and 3 (YO) or the asymptomatic strain of serotype 4 (ST3), we observed that each NMAb reacted with each of these three types of symptomatic human strains (15). The same cross-reactive NMAbs, except KU-4D7, neutralized asymptomatic human strains which included a representative of each of the four human rotavirus serotypes (14, 15). In contrast, only NMAb KU-6B11 neutralized the bovine Nebraska calf diarrhea virus and simian SA11 strains (14). We therefore examined the reactivities of these NMAbs with the Gottfried strain as well as human strain 1076 (asymptomatic, serotype 2) and simian strain RRV. The Gottfried strain was neutralized to a significant titer by all six NMAbs, and the human asymptomatic 1076 strain was neutralized by five of the six NMAbs as noted above, whereas the simian RRV strain reacted significantly with only two of the NMAbs (Table 2). These results suggest that the VP4 of the human strains shares more neutralization epitopes with the Gottfried strain VP4 than with those of other animal strains. Also, it appears that there is limited diversity of VP4 among different serotypes of human rotaviruses and the Gottfried strain. Moreover, antigenic mutants resistant to the above-mentioned cross-reactive NMAbs sustained amino acid substitutions in the VP5 region of VP4 at amino acids 305, 392, 433, or 439 (Table 2) (14). It is noteworthy that at such positions there was a correlation in amino acid sequence between Gottfried virus and human rotaviruses (Fig. 2). On the other hand, the VP8 region of the VP4 protein does not appear to be extensively involved in heterotypic rotavirus neutralization (9). Specifically, the region of VP8 between amino acids 99 and 192 has been identified as the region of greatest variability on VP4 (2, 3). The Gottfried strain shows a unique amino acid sequence in this region and thus may contain strain-specific neutralization sites (Fig. 2).

Various studies suggest that the VP4 protein is an important immunogen that stimulates neutralizing antibodies during rotavirus infection (7, 16). Furthermore, studies with animals indicate that these antibodies are associated with resistance to rotavirus challenge (7). Our observations suggest that the Gottfried strain should be evaluated further for its potential usefulness as a donor of its VP4 gene for the generation of reassortant vaccine viruses.

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