JOURNAL OF VIROLOGY, Aug. 1988, p. 2978–2984
0022-538X/88/082978-07$02.00/0

Vol. 62, No. 8

Sequence of the Fourth Gene of Human Rotaviruses Recovered from Asymptomatic or Symptomatic Infections

MARIO GORZIGLIA,* KIM GREEN, KAZUO NISHIKAWA, KOKI TANIGUCHI, RONALD JONES, ALBERT Z. KAPIKIAN, AND ROBERT M. CHANOCK

Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

Received 10 February 1988/Accepted 19 April 1988

The complete nucleotide sequence of the fourth gene of symptomatic (Wa, DS-1, P, and VA70) and asymptomatic (M37, 1076, McN13, and ST3) rotaviruses of serotype 1, 2, 3, or 4 was determined by the dideoxy chain termination method. In each strain, the fourth gene, which encodes the outer capsid protein VP3, is 2,359 base pairs in length and has 5′- and 3′-noncoding regions of 9 and 25 nucleotides, respectively. The gene has a single long open reading frame of 2,325 base pairs that is capable of coding for a protein of 775 amino acids. A total of 14 N-terminal and 12 C-terminal amino acids are completely conserved or almost completely conserved, respectively, among nine human rotavirus VP3 genes that have been sequenced. In addition, there is conservation of arginine at the two trypsin cleavage sites as well as conservation of clusters of amino acids in different regions of the two VP3 cleavage products, VP8 and VP5. Three distinct forms of VP3 were identified among the nine human rotavirus strains analyzed. Three symptomatic rotaviruses (serotypes 1, 3, and 4) possess highly related VP3 genes (92.2 to 97% nucleotide identity). Two symptomatic serotype 2 rotaviruses possess VP3 genes which are even more closely related to each other (98.6% nucleotide identity) and only moderately related to the aforementioned VP3 genes of serotypes 1, 3, and 4 (87.4 to 88.2% nucleotide identity). The four asymptomatic rotaviruses, which constitute the third group, possess highly related VP3 genes (95.5 to 97.5% nucleotide identity) which are distinct from those of the virulent rotaviruses (73 to 74.8% nucleotide identity). At 91 positions in the protein sequence of VP3, an amino acid is conserved among the asymptomatic rotaviruses, while a different amino acid is conserved among the symptomatic rotaviruses. Notably, five regions are conserved among the symptomatic rotaviruses, while a different set of sequences are conserved among the asymptomatic rotaviruses. It is possible that some or all of these regions of sequence dimorphism may be responsible for the difference in virulence of these two groups of human rotaviruses. There are 13 regions in the VP3 protein sequence which exhibit the greatest variability; the majority of these variable regions are observed between amino acids 106 to 192. These regions may represent potential antigenic sites related to heterotypic rotavirus neutralization.

Rotaviruses possess two outer capsid proteins, designated VP7 and VP3, which are now known to be independent neutralization antigens (15, 23). VP7, which has a molecular weight of 37,000, is encoded by genomic RNA segment 8 or 9 (14), whereas VP3, which has a molecular weight of 85,000, is encoded by genomic RNA segment 4 (22). VP3 is responsible for the restriction of growth of fastidious rotaviruses in tissue cultures (14) and for hemagglutinating activity (17). In addition, VP3 appears to be associated with the virulence of simian rotavirus SA-11 and bovine rotavirus neonatal calf diarrhea virus in the mouse model of experimental rotavirus infection (24). Trypsin cleavage of VP3 which yields two polypeptides, VP8 and VP5, with molecular weights of 27,000 and 58,000, respectively, is required for the activation of infectivity (6, 7, 25). In a recent study of simian rotavirus SA-11, the larger cleavage product, VP5, was shown to be a mixture of two polypeptides which differed only in the presence of six extra amino acids at the N-terminal region of the longer polypeptide, indicating the existence of two closely located trypsin cleavage sites (20). These observations suggest that VP3, and particularly its cleavage sites, may play an important role in the pathogenesis of infection and disease.

Rotaviruses are now considered to be the single most important etiologic agents of severe diarrhea in infants and young children worldwide (5, 19). However, within the past few years, rotavirus strains have been recovered from newborn nurseries in which virus has persisted and in which most affected infants have failed to develop significant symptoms (26, 28). Also infection of gnotobiotic calves with some bovine rotaviruses does not cause disease (3, 27). The degree of virulence is not serotype specific, because each of the four distinct human rotavirus serotypes has been associated with both symptomatic and asymptomatic infections in infants and children (16). In a previous study with RNA-RNA hybridization, a marked conservation of sequence was observed in the fourth gene of rotavirus strains recovered from newborn infants with asymptomatic infections in nurseries in which virus persisted (9). Furthermore, the deduced amino acid sequence of the 5′-terminal 30% of VP3 (which includes the VP8 cleavage product, the cleavage site, and the N terminus of the other cleavage product, VP5) of symptomatic and asymptomatic human rotaviruses representing each of the four serotypes indicated a high degree of homology (96% or more) among the asymptomatic viruses, while the homology between the asymptomatic and symptomatic strains was considerably lower (68 to 72%) (12). In the present study, we extended our previous (12) examination of asymptomatic (M37, 1076, McN13, and ST3) and symptomatic (Wa, VA70, P, and DS-1) human rotaviruses by completing a sequence analysis of the remaining region of

* Corresponding author.

TABLE 1. Percent VP3 amino acid (or nucleotide) homology among five symptomatic and four asymptomatic human rotavirus strains

Human rotavirus strain (serotype)	% Amino acid (nucleotide) homology of:								
	Wa	DS-1	RV-5	P	VA70	M37	1076	McN13	ST3
Symptomatic									
Wa (1)		89.7 (87.6)	89.2 (87.4)	94.1 (92.3)	96.8 (97.0)	77.8 (73.9)	77.0 (74.4)	77.0 (74.2)	76.8 (74.4)
DS-1 (2)			98.5 (98.6)	90.5 (87.7)	89.4 (88.2)	76.8 (72.9)	75.7 (73.2)	75.7 (72.9)	75.7 (73.3)
RV-5 (2)				90.2 (87.5)	89.2 (88.0)	76.4 (73.0)	75.4 (73.5)	75.4 (73.1)	75.4 (73.4)
P (3)					93.2 (92.2)	76.9 (74.2)	76.4 (74.6)	76.4 (74.6)	76.4 (74.7)
VA70 (4)						77.3 (74.2)	76.4 (74.6)	76.5 (74.6)	76.3 (74.8)
Asymptomatic									
M37 (1)							95.1 (95.5)	96.6 (96.6)	96.3 (97.0)
1076 (2)								96.8 (97.0)	95.7 (96.3)
McN13 (3)									97.4 (97.5)
ST3 (4)									

VP5 in an attempt to better understand the molecular basis for attenuation of the nursery strains of rotavirus.

MATERIALS AND METHODS

Viruses and cells. Four pairs of asymptomatic and symptomatic human rotaviruses were used for sequence analysis of the VP3 gene. Each pair (symptomatic and asymptomatic) represented a different serotype: Wa and M37 (serotype 1); DS-1 and 1076 (serotype 2); P and McN13 (serotype 3); and VA70 and ST3 (serotype 4). All viruses were propagated in MA-104 cells in the presence of 10 μg of trypsin per ml.

In vitro transcription and dideoxynucleotide sequencing. Single-shelled particles were purified (12), and single-stranded plus-strand sense RNA transcripts were prepared as described by Flores et al. (8). Dideoxynucleotide sequencing of these RNA transcripts was performed by the method previously described (12). Synthetic oligonucleotides 18 bases long were used to prime cDNA synthesis by reverse transcription from the VP3 gene plus-strand sense RNA transcript, beginning at its 3′ end. The minus-strand sense initial primer 5′-GGTCACATCCTGGATGAC-3′ corresponded to the sequence of a symptomatic human rotavirus serotype 2 strain, RV5, determined by Kantharides et al. (18). Using this primer, we were able to effectively extend only the cDNA synthesis of transcripts of the symptomatic serotype 2 strain DS-1. With transcripts of symptomatic rotavirus Wa (serotype 1) or asymptomatic rotavirus 1076 (serotype 2), extension proceeded only 20 to 30 nucleotides, from which point it was necessary to prepare a new primer. To complete the 3′-end sequence of the symptomatic serotype 1, 3, and 4 strains as well as of each of the asymptomatic strains, we used a plus-strand sense 18-mer oligonucleotide corresponding to a sequence 50 nucleotides upstream of the 3′ end of a symptomatic serotype 1 strain (Wa) or an asymptomatic serotype 2 strain (1076) to prime the minus strand of genomic double-stranded RNA as a template. Subsequently, oligonucleotide primers 18 nucleotides long which were specific for the symptomatic or asymptomatic rotaviruses were used at intervals of about 200 to 300 nucleotides to sequence the plus strand of the symptomatic serotype 1, 2, 3, and 4 strains and of each of the four asymptomatic strains.

RESULTS

In a previous study (12), sequence analysis of the VP3 gene of symptomatic and asymptomatic rotaviruses was initiated at a position corresponding to 114 nucleotides downstream of the preferred cleavage site of simian rotavirus SA-11 (20). In the present study, the complete sequence of this gene was obtained by extension of its 3′ end with a primer that was complementary to the 3′ end of the VP3 gene of rotavirus serotype 2 strain RV-5 (18). The complete nucleotide sequence of the VP3 gene of each of the eight rotavirus strains is 2,359 base pairs in length, with 5′- and 3′-noncoding regions of 9 and 25 nucleotides, respectively. The gene contains a single long open reading frame of 2,325 base pairs that is capable of coding for a protein of 775 amino acids (Fig. 1).

A comparison of the predicted VP3 amino acid sequences of the four symptomatic strains analyzed and of another symptomatic serotype 2 strain (RV-5) previously sequenced by Kantharides et al. (18) indicated a high degree of homology (93.2 to 96.8%) among the Wa, P, and VA70 strains and a somewhat lower degree of homology (89.2 to 90.5%) between these strains and the two serotype 2 strains, DS-1 and RV-5 (Table 1); however, homology between the two serotype 2 strains, DS-1 and RV-5, was very high (98.5%). The four asymptomatic neonatal rotaviruses (M37, 1076, McN13, and ST3) exhibited 95.5% or more homology within the group, while homology to symptomatic human rotavirus strains was significantly lower, ranging from 75.4 to 77.8%. Amino acid homology for symptomatic and asymptomatic human rotaviruses within the same serotype also ranged from 75.7 to 76.4%.

The deduced VP3 amino acid sequences of the nine human rotavirus strains were compared to define regions which were conserved or variable between symptomatic and asymptomatic strains as well as among strains within each of these two groups of rotaviruses. The regions of conservation or variability are shown in Fig. 1.

A comparison of the deduced amino acid sequences of five symptomatic and four asymptomatic rotavirus strains indicated that 31 regions 3 to 62 amino acids in length are completely or almost completely conserved among both asymptomatic and symptomatic rotaviruses. These regions include 14 N-terminal amino acids and 12 C-terminal amino acids which are completely conserved or almost completely conserved, respectively (Fig. 1). Cysteine is conserved at positions 215, 317, 379, and 773 in all strains studied, while an additional cysteine is present at position 266 in the human asymptomatic strain M37. Twenty-three proline residues are conserved in all strains. These conserved prolines may have a major influence on the conformation of VP3 because

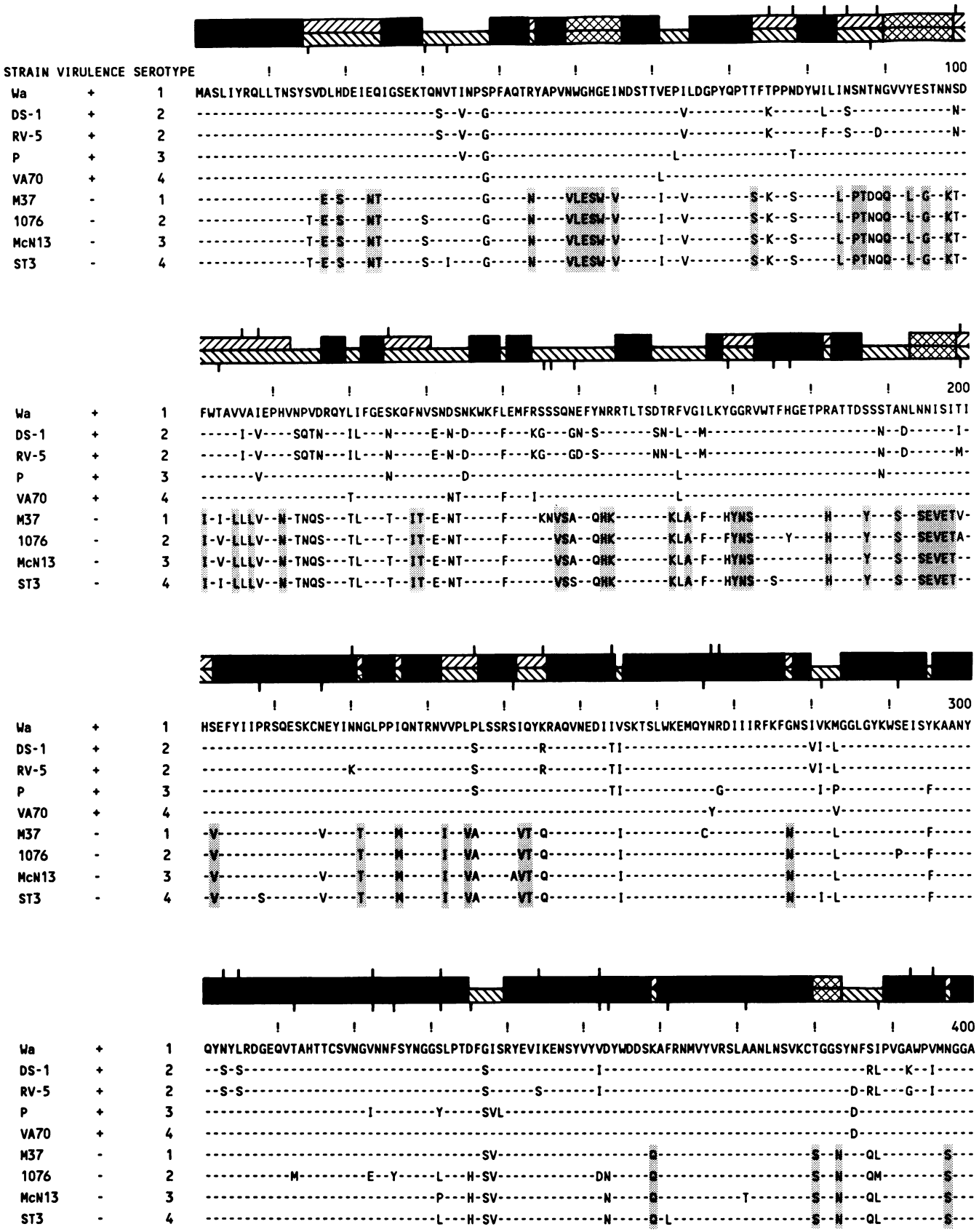

FIG. 1. Comparison of the amino acid sequences of VP3 of five symptomatic (+) and four asymptomatic (−) human rotaviruses. Symbols: ■, region conserved or highly conserved among all strains; ▨, region highly conserved among symptomatic strains; ▧, region highly conserved among asymptomatic strains; ▩, region in which a different sequence is conserved among symptomatic and asymptomatic strains. Blank areas indicate regions variable among asymptomatic strains. Shaded sequences are conserved in all the symptomatic strains, while a different amino acid is conserved at each of these sites in the asymptomatic strains. Amino acid substitutions in symptomatic or asymptomatic strains are indicated by small vertical lines or T, respectively. The published sequence of RV-5 (18) is presented for the purpose of comparison.

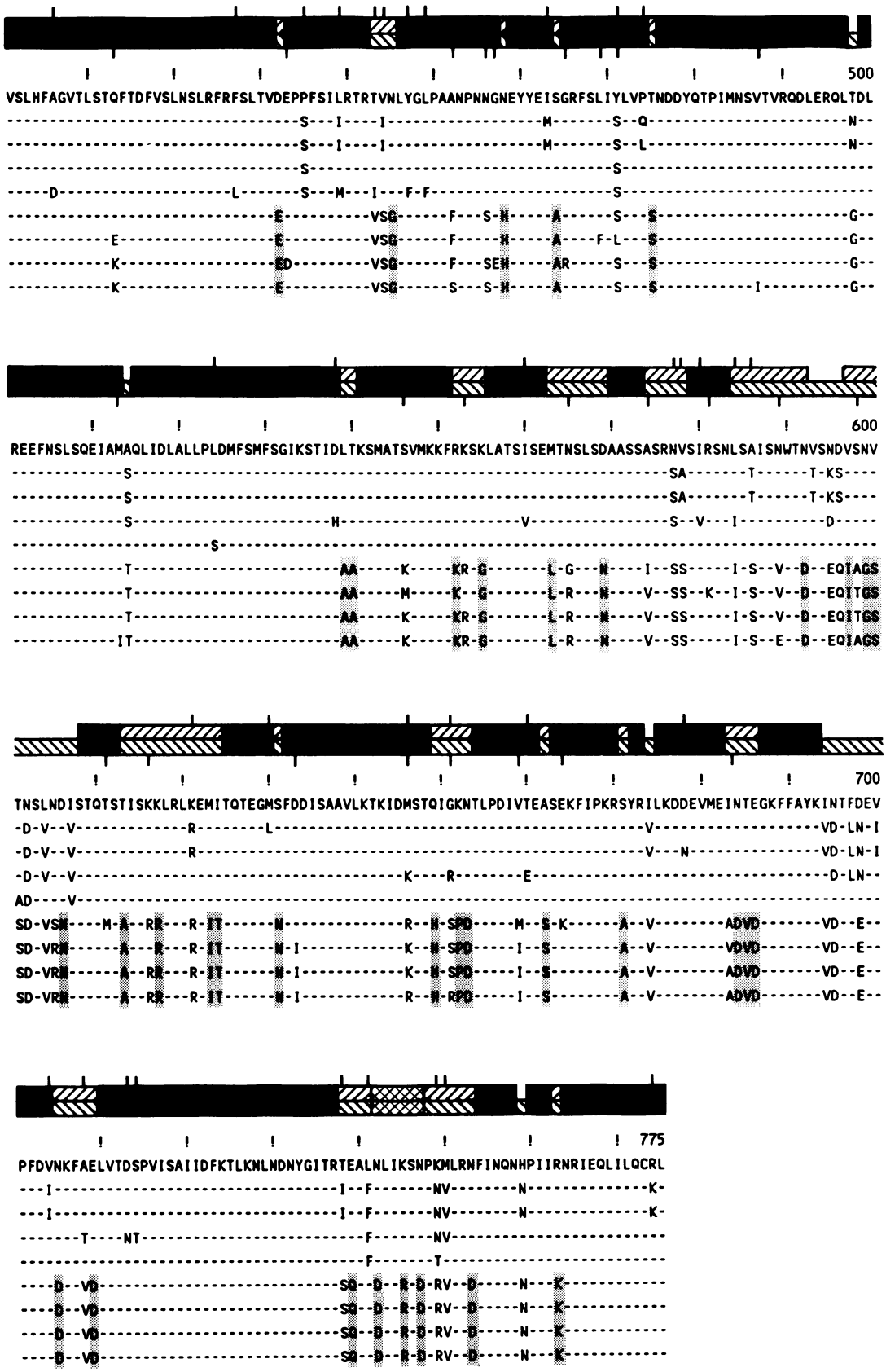

FIG. 1—*Continued*

proline is known to distort three-dimensional structure. The most abundant amino acid is serine, and 56 residues are conserved in all strains. A total of 16 serines are present between residues 561 and 617 of symptomatic strains, while 19 serines are present in asymptomatic strains in the same region.

A total of 20 regions ranging from 3 to 28 amino acids in length are conserved or highly conserved in VP3 of symptomatic rotaviruses, while 27 regions ranging from 3 to 32 amino acids in length are conserved or highly conserved in VP3 of asymptomatic rotaviruses (Fig. 1). Ninety-one amino acid positions in VP3 are conserved among the four asymptomatic rotaviruses, while a different amino acid is conserved at each of these sites in the five symptomatic rotaviruses (Fig. 1). Of these 91 symptomatic and asymptomatic VP3-specific amino acid residues, 64 are distributed in 20 discrete regions. One of these regions includes the connecting peptide, i.e., the region between the two cleavage sites (arginine 240 and arginine 246). Also, the connecting peptide of the symptomatic rotaviruses contains an additional potential cleavage site at position 245 (lysine or arginine) which could serve as an alternative site for the action of a trypsin-like protease, thus facilitating proteolytic processing of these viruses (Fig. 1).

There was more sequence divergence among symptomatic VP3s than among asymptomatic VP3s (Table 1). Sequence divergence among asymptomatic VP3s usually represented discrete single amino acid differences or two consecutive amino acid differences at most. In contrast, each of the VP3s of symptomatic strains of serotype 1, 2, 3, or 4 had 1 to 11 unique regions of sequence which extended 3 to 11 residues. Among the symptomatic viruses, the VP3s of Wa (serotype 1) and VA70 (serotype 4) were most closely related, and this degree of relatedness was similar to that observed among the asymptomatic strains. The asymptomatic strains possessed five regions in VP3 that were unique to these viruses and not shared with symptomatic strains, which possessed a different set of shared unique sequences in these regions.

There are nine regions of sequence which are highly or completely conserved among symptomatic serotype 1, 3, and 4 strains (i.e., residues 106 to 108, 113 to 116, 144 to 154, 160 to 166, 189 to 192, 303 to 305, 387 to 395, 436 to 442, and 593 to 596), while a divergent sequence is present in these regions in the two symptomatic serotype 2 strains. Three of these nine regions (i.e., residues 303 to 305, 387 to 395, and 436 to 442) are shared between the symptomatic serotype 1, 3, and 4 strains and the asymptomatic strains (Fig. 1).

DISCUSSION

RNA-RNA hybridization (8) and our sequence analysis indicate that at least three different VP3 genes are present among the human rotaviruses. Either a Wa-like (serotypes 1, 3, and 4) or a DS-1-like (serotype 2) VP3 gene is present in rotaviruses that cause diarrhea in infants and children (10, 33, 34). A third form of VP3 gene is present in rotaviruses of serotype 1, 2, 3, or 4 that cause asymptomatic infections in neonates (1, 2, 4, 26). These observations suggest that VP3 plays a role in the virulence of human rotaviruses. Also, studies with reassortants have shown that the VP3 gene of simian SA-11 and bovine neonatal calf diarrhea rotavirus strains is related to virulence in an experimental animal model (24).

A comparison of the predicted VP3 amino acid sequences of asymptomatic and symptomatic rotaviruses indicated 75.4 to 77.8% identity. This relatively low percentage of homology may reflect a different evolutionary origin of the fourth gene of these strains. Amino acids conserved among both groups of rotaviruses are clustered in 31 regions scattered along the entire VP3 molecule, but the majority are located in the amino terminus of VP5, between amino acids 246 and 538. These conserved regions may play a critical role in the biological activity of rotaviruses but are not likely to be important determinants of virulence. Also, some of the variable regions which are known to correspond to antigenic sites are probably not involved in determining virulence. For example, antigenic mutants of rhesus rotavirus selected with VP3-specific neutralizing monoclonal antibodies directed against variable regions do not demonstrate altered virulence in mice (29). In addition, several cross-reactive neutralizing monoclonal antibodies (N-MAbs) raised against VP3 of symptomatic human strain KU neutralized the four asymptomatic strains included in this study (K. Taniguchi et al., submitted for publication). For these reasons, it is likely that other regions in human rotavirus VP3 play a major role in virulence or attenuation. Significantly, there are 20 regions that are conserved or highly conserved among the symptomatic strains, while different regions are conserved or highly conserved among the asymptomatic strains. The majority of these regions are located in the amino- and carboxy-terminal portions of VP3. Among these sequences, a likely candidate for a role in virulence is the connecting peptide (amino acids 241 to 246), which contains an extra potential cleavage site (amino acid 245) in each of the symptomatic rotavirus VP3 sequences analyzed.

In view of the recent evidence that VP3 plays a role in determining serotype specificity (15, 23, 32), we searched for discrete regions of VP3 amino acid sequence that are divergent among the four serotypes. This approach was successfully used with the other major outer capsid glycoprotein, VP7. Seven regions of VP7 were found to contain serotype-specific sequences (11, 13). There are 13 variable regions of VP3 among the five symptomatic rotaviruses analyzed, but none of these regions are divergent among all four serotypes. This observation is in agreement with the finding that the currently available VP3 N-MAbs do not distinguish symptomatic strains of serotype 1, 3, or 4 (30). However, recent studies have identified a difference between the neutralization specificity of symptomatic serotype 2 strain VP3 and the neutralization specificities of VP3s of symptomatic strains of serotypes 1, 3, and 4 (31). Furthermore, a comparison of the VP3 amino acid sequence of the symptomatic serotype 1, 3, and 4 strains with that of the symptomatic serotype 2 strains showed that there are several unique regions among serotype 2 strains which are divergent from those of the other serotypes. One or more of these regions may play a role in serotype 2 and serotype 1, 3, and 4 VP3 specificities. Consistent with this view is the recent observation that a DS-1 antigenic mutant selected with a serotype 2-specific N-MAb sustained an amino acid substitution at residue 392, which is within one of these divergent regions, i.e., residues 387 to 395 (Taniguchi et al., submitted). Other variable regions are located between amino acids 106 and 192. These regions contain few amino acids divergent from those of the VP3s of symptomatic serotype 1, 3, and 4 strains, while there is considerable divergence from the corresponding regions of the two symptomatic serotype 2 strains. Significantly, the region from amino acids 73 to 202 also exhibits considerable divergence in two serotype 3 simian rotavirus strains, SA-11 and rhesus rotavirus (12). Also, five of seven antigenic mutants of simian rotavirus SA-11 selected with SA-11 hyperimmune antiserum sustained one or more amino

acid substitutions within this region (Gorziglia et al., manuscript in preparation). Recently, Mackow et al. (21) indicated that five different N-MAbs raised against rhesus rotavirus VP3 selected rhesus rotavirus variants with amino acid substitutions at positions 87 to 89, 100, 114, 148 to 150, and 188. These N-MAbs exhibited limited neutralization of heterotypic rotavirus strains. Our sequence analyses are consistent with this observation and suggest that amino acid divergence in the VP8 region among human rotaviruses may also be responsible for strain-specific rotavirus neutralization.

A comparison of the VP3 amino acid sequence of asymptomatic strains representing the four human rotavirus serotypes with that of symptomatic strains of serotypes 1, 3, and 4 shows that some regions (i.e., regions 303 to 305, 387 to 395, and 436 to 442) are conserved or highly conserved among these viruses. This observation is consistent with the finding that VP3 possesses some cross-reactive neutralization epitopes (29). In contrast, the region from amino acids 73 to 202 is unique among the four asymptomatic strains, suggesting that this sequence may contain epitopes specific for asymptomatic strains.

Although the three families of VP3 genes described in this study are distinct, it is also clear that they have a common ancestry, as indicated by the following observations: (i) there are 31 conserved or highly conserved regions; (ii) both arginines at the cleavage sites are conserved; (iii) four cysteines and 23 prolines are present at the same location; and (iv) some VP3 epitopes have a common location in all human rotaviruses.

ACKNOWLEDGMENTS

We thank P. Collins and M. Hill for preparation of oligonucleotide primers and S. Chang and L. Jordan for editorial assistance.

LITERATURE CITED

1. **Albert, M. H., L. E. Unicomb, G. L. Barnes, and R. F. Bishop.** 1987. Cultivation and characterization of rotavirus strains infecting newborn babies in Melbourne, Australia, from 1975 to 1979. J. Clin. Microbiol. **25**:1635–1640.
2. **Bishop, R. F., G. L. Barnes, E. Cipriani, and J. S. Lund.** 1983. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. N. Engl. J. Med. **309**: 72–76.
3. **Bridger, J. C., and D. H. Pocock.** 1986. Variation in virulence of bovine rotaviruses. J. Hyg. **96**:257–264.
4. **Chrystie, I. L., B. Totterdell, M. H. Baker, J. W. Scopes, and J. F. Banatvala.** 1975. Rotavirus infections in a maternity unit. Lancet **ii**:79.
5. **Davidson, G. P., R. F. Bishop, R. R. W. Townley, I. H. Holmes, and B. J. Ruck.** 1975. Importance of a new virus in acute sporadic enteritis in children. Lancet **i**:242–246.
6. **Espejo, R. T., S. Lopez, and C. Arias.** 1981. Structural polypeptides of simian rotavirus SA-11 and the effect of trypsin. J. Virol. **37**:156–160.
7. **Estes, M. K., D. Y. Graham, and B. B. Mason.** 1981. Proteolytic enhancement of rotavirus infectivity: molecular mechanism. J. Virol. **39**:879–888.
8. **Flores, J., H. B. Greenberg, J. Myslinski, A. R. Kalica, R. Wyatt, A. Z. Kapikian, and R. M. Chanock.** 1982. Use of transcription probes for genotyping rotavirus reassortants. Virology **121**:288–295.
9. **Flores, J., K. Midthun, Y. Hoshino, K. Green, M. Gorziglia, A. Z. Kapikian, and R. M. Chanock.** 1986. Conservation of the fourth gene among rotaviruses recovered from asymptomatic newborn infants and its possible role in attenuation. J. Virol. **60**: 972–979.
10. **Gerna, G., N. Passarani, M. Battaglia, and E. Percivalle.** 1984. Rapid serotyping of human rotavirus strains by solid-phase immune electron microscopy. J. Clin. Microbiol. **19**:273–278.
11. **Gorziglia, M., Y. Aguirre, Y. Hoshino, J. Esparza, I. Blumentals, J. Askaa, M. Thompson, R. I. Glass, A. Z. Kapikian, and R. M. Chanock.** 1986. VP7 serotype-specific glycoprotein of OSU porcine rotavirus: coding assignment and gene sequence. J. Gen. Virol. **67**:2445–2454.
12. **Gorziglia, M., Y. Hoshino, K. Midthun, A. Buckler-White, I. Blumentals, R. Glass, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1986. Conservation of amino acid sequence of VP8 and cleavage region of 84-KDa outer capsid protein among rotaviruses recovered from asymptomatic neonatal infection. Proc. Natl. Acad. Sci. USA **83**:7039–7043.
13. **Green, K. Y., K. Midthun, M. Gorziglia, Y. Hoshino, A. Z. Kapikian, R. M. Chanock, and J. Flores.** 1987. Comparison of the amino acid sequences of the major neutralization protein of four human rotavirus serotypes. Virology **161**:153–159.
14. **Greenberg, H. B., J. Flores, A. R. Kalica, R. G. Wyatt, and R. Jones.** 1981. Gene coding assignments for growth restriction, neutralization and subgroup specificities of the W and DS-1 strains of human rotavirus. J. Gen. Virol. **64**:313–320.
15. **Hoshino, Y., M. M. Sereno, K. Midthun, J. Flores, A. Z. Kapikian, and R. M. Chanock.** 1985. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc. Natl. Acad. Sci. USA **82**: 8701–8704.
16. **Hoshino, Y., R. G. Wyatt, J. Flores, K. Midthun, and A. Z. Kapikian.** 1985. Serotypic characterization of rotaviruses derived from asymptomatic human neonatal infection. J. Clin. Microbiol. **21**:425–430.
17. **Kalica, A. R., J. Flores, and H. B. Greenberg.** 1983. Identification of the rotaviral gene that codes for hemagglutination and protease-enhanced plaque formation. Virology **125**:194–205.
18. **Kantharidis, P., M. L. Dyall-Smith, and I. H. Holmes.** 1987. Marked sequence variation between segment 4 genes of human RV-5 and simian SA-11 rotavirus. Arch. Virol. **93**:111–121.
19. **Kapikian, A. Z., H. W. Kim, R. G. Wyatt, W. L. Cline, J. O. Arrobio, C. D. Brandt, W. J. Rodriguez, D. A. Sack, R. M. Chanock, and R. H. Parrott.** 1976. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. N. Engl. J. Med. **294**: 965–972.
20. **Lopez, S., C. F. Arias, J. R. Bell, J. H. Strauss, and R. T. Espejo.** 1985. Primary structure of the cleavage site associated with trypsin enhancement of rotavirus SA-11 infectivity. Virology **144**:11–19.
21. **Mackow, E. R., R. D. Shaw, S. M. Matsui, P. T. Vo, M. Dang, and H. B. Greenberg.** 1988. The rhesus rotavirus gene encoding protein VP3: location of amino acids involved in homologous and heterologous rotavirus neutralization and identification of a putative fusion region. Proc. Natl. Acad. Sci. USA **85**:645–649.
22. **Mason, B. B., D. Y. Graham, and M. K. Estes.** 1983. Biochemical mapping of the simian rotavirus SA-11 genome. J. Virol. **46**: 413–423.
23. **Offit, P. A., and G. Blavat.** 1986. Identification of the two rotavirus genes determining neutralization specificities. J. Virol. **57**:376–378.
24. **Offit, P. A., G. Blavat, H. B. Greenberg, and F. H. Clark.** 1986. Molecular basis of rotavirus virulence: role of gene segment 4. J. Virol. **57**:46–49.
25. **Offit, P. A., F. H. Clark, W. G. Stroop, M. E. Twist, and A. S. Plotkin.** 1983. The cultivation of human rotavirus, strain "Wa," to high titer in cell culture and characterization of the viral structural polypeptides. J. Virol. Methods **7**:29–40.
26. **Perez-Schael, I., G. Daoud, L. White, G. Urbina, N. Daoud, M. Perez, and J. Flores.** 1984. Rotavirus shedding by newborn children. J. Med. Virol. **14**:127–136.
27. **Pocock, D. H.** 1987. Characterization of rotavirus isolates from sub-clinical infected calves by genome profile analysis. Vet. Microbiol. **13**:27–34.
28. **Rodger, S. M., R. F. Bishop, C. Birch, B. McLean, and I. H. Holmes.** 1981. Molecular epidemiology of human rotaviruses in Melbourne, Australia, from 1973 to 1979, as determined by

electrophoresis of genome ribonucleic acid. J. Clin. Microbiol. **13**:272–278.

29. **Shaw, R. D., P. T. Vo, P. A. Offit, B. S. Coulson, and H. B. Greenberg.** 1986. Antigenic mapping of the surface proteins of Rhesus rotavirus. Virology **155**:434–451.
30. **Taniguchi, K., Y. Morita, T. Urasawa, and S. Urasawa.** 1987. Cross-reactive neutralization epitopes on VP3 human rotavirus: analysis with monoclonal antibodies and antigenic variants. J. Virol. **61**:1726–1730.
31. **Taniguchi, K., S. Urasawa, and T. Urasawa.** 1985. Preparation and characterization of neutralizing monoclonal antibodies with different reactivity patterns to human rotaviruses. J. Gen. Virol. **66**:1045–1053.
32. **Urasawa, S., T. Urasawa, and K. Taniguchi.** 1986. Genetic reassortment between two human rotaviruses having different serotype and subgroup specificities. J. Gen. Virol. **67**:1551–1559.
33. **Wyatt, R. G., W. D. James, E. H. Boh, K. W. Theil, L. W. Said, A. R. Kalica, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1980. Human rotavirus type 2: cultivation *in vitro*. Science **207**:189–191.
34. **Wyatt, R. G., H. D. James, Jr., A. L. Pittman, Y. Hoshino, H. B. Greenberg, A. R. Kalica, J. Flores, and A. Z. Kapikian.** 1983. Direct isolation in cell culture of human rotaviruses and their characterization into four serotypes. J. Clin. Microbiol. **18**:310–317.