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Identification of the Human Herpesvirus 6 Glycoprotein H and Putative Large Tegument Protein Genes

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Determination of the nucleotide sequences of two molecular clones of human herpesvirus 6 (HHV-6) (strain GS) and comparison with those of human cytomegalovirus (HCMV) has allowed the identification of the genes for the glycoprotein H (gH) and the putative large tegument protein of HHV-6. Two molecular clones of fragments of HHV-6, the *Bam*HI-G fragment (7,981 bp) of the clone termed pZVB43 and a *Hin*dIII fragment (8,717 bp) of the clone termed pZVH14, represent approximately 10% of the HHV-6 genome (16,689). An open reading frame within the *Bam*HI-G fragment was designated the gH gene of HHV-6 because of the extensive sequence similarity of its predicted product (79,549 Da) to the HCMV gH gene product. The predicted product (239,589 Da) of an open reading frame within clone pZVH14 showed homology to the predicted product of the proposed gene of HCMV representing the large tegument protein. Computer analyses indicated a closer relationship of the predicted peptides of these HHV-6 genes to those of HCMV than to those of the other human herpesviruses Epstein-Barr virus, herpes simplex virus type 1, and varicella-zoster virus. The gH gene was more conserved among the human herpesvirus group, while significant sequence similarity of the tegument gene could be found only with that of HCMV. The data reported here with one conserved gene (gH) and a more divergent gene (tegument) support previous reports that HHV-6 and HCMV are more closely related to each other than to the other well-characterized human herpesviruses.

Human herpesvirus 6 (HHV-6) (initially named human B-lymphotropic virus) (3) was originally isolated from a number of patients with AIDS and other lymphoproliferative disorders (43). It was distinguished from other human and animal herpesviruses by immunologic and nucleic acid homology criteria and by morphology (5, 19, 43). Since its isolation in 1986, numerous instances of virus isolation from patients with various diseases and normal donors have been reported worldwide (2, 24, 28, 42, 49, 50). HHV-6 infection commonly occurs early in life, and the virus is the etiological agent for roseola infantum (exanthem subitum) (48, 50). HHV-6 is also etiologically linked with approximately 12% of heterophile-negative and Epstein-Barr virus (EBV)- and human cytomegalovirus (HCMV)-negative infectious mononucleosis (4, 37, 47). In a variety of fresh lymphoma and leukemia tissues tested, HHV-6 sequences could be detected in three B-cell lymphomas and in tissues obtained from a patient with T-cell leukemia (16, 18) but not in a large number of tissues from other similar cases.

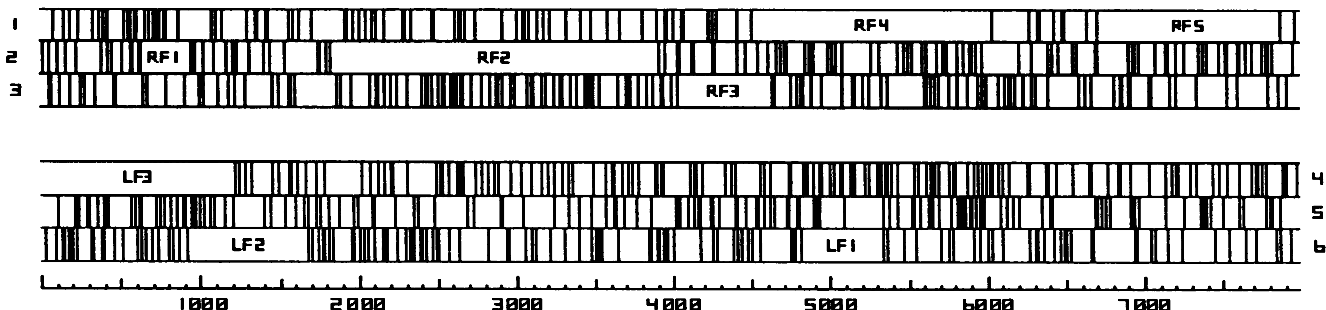

FIG. 1. Termination codon scan of the DNA sequence of the HHV-6 clone pZVB43 *Bam*HI-G fragment. The potential open reading frames of >300 bp were labeled by a two-letter code (RF = right reading frame and LF = left reading frame).

Since HHV-6 can infect CD4$^+$ cells (31), which are also the target of human immunodeficiency virus (HIV) infection, it has been proposed that HHV-6 may play a cofactor role in the progression to AIDS (30). Recent studies have shown that HIV type 1 (HIV-1) expression is elevated in cells dually

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FIG. 2. Nucleotide sequence of the HHV-6 clone pZVB43 *Bam*HI-G fragment and translation of all reading frames of greater than 300 bp or 100 amino acids with an initiator methionine residue. The HHV-6 putative gH peptide begins at nucleotide position 1829. The hydrophobic leader and transmembrane regions of this peptide are overlined, and the potential glycosylation sites are underlined.

infected with HIV-1 and HHV-6 in vitro (30) and that HHV-6 infection can upmodulate HIV-1 long terminal repeat-directed gene expression in vitro (12, 15), as has been reported for other human DNA viruses (EBV, HCMV, and herpes simplex virus [HSV]) and human T-cell lymphotropic virus type I (10, 13, 20, 35, 36, 39, 40, 45). Furthermore, HHV-6 infection was recently shown to upmodulate expression of CD4, a receptor for HIV-1, and thus could expand the range of cells susceptible to HIV-1 infection (29). However, other investigators reported that HHV-6 suppressed HIV-1 replication in vitro (6, 25, 38). A lack of correlation between HHV-6 infection and the course of HIV-1 infection in vivo,

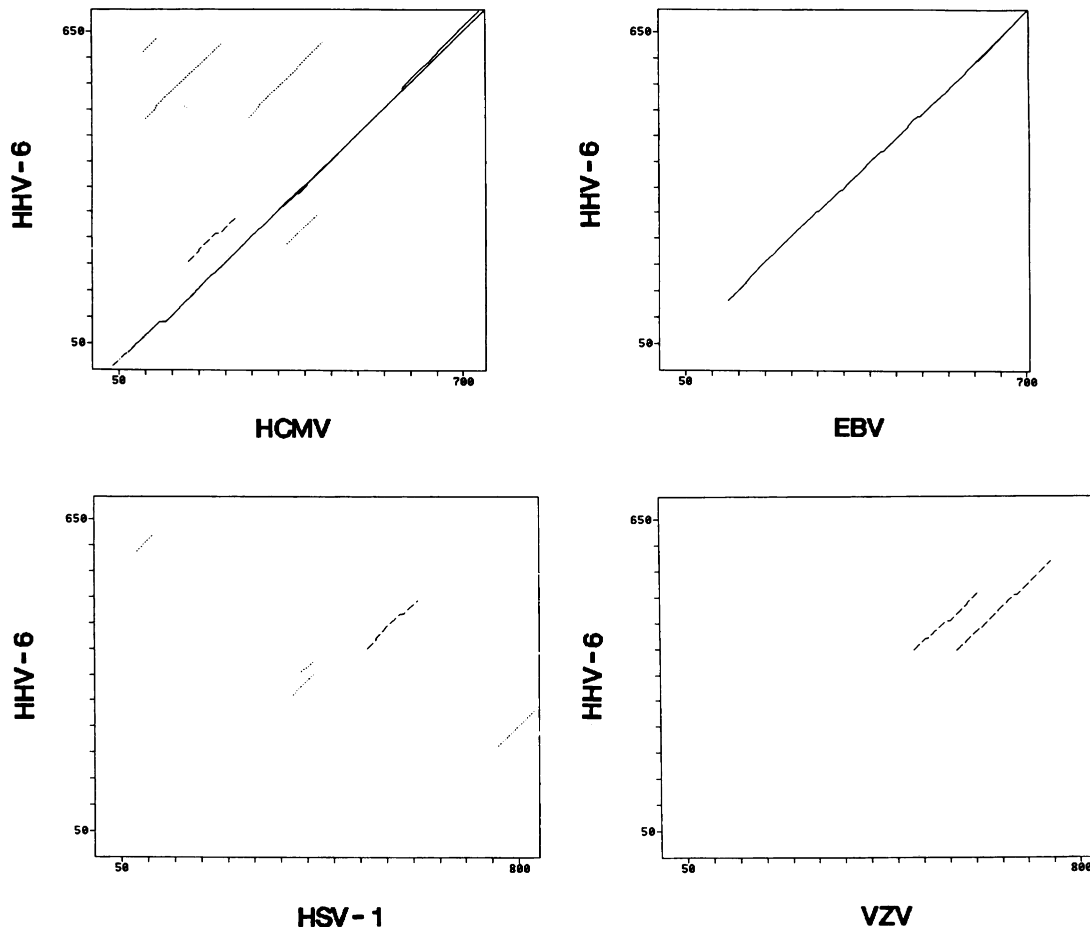

FIG. 3. Homology of the HHV-6 putative gH to the gH peptides of other human herpesviruses. Shown are the LFASTA comparisons with HCMV, EBV, HSV-1, and VZV.

in which HHV-6 antibody titers were not different in patients who progressed to AIDS and those who had a delayed onset of AIDS, was also reported (46).

It must be pointed out that both Carrigan et al. (6) and Lusso et al. (30), using the GS isolate of HHV-6, did observe enhanced cell lysis in HHV-6- and HIV-1-coinfected cells.

HHV-6 contains a linear double-stranded DNA genome of approximately 170,000 bp, arranged as a long unique region

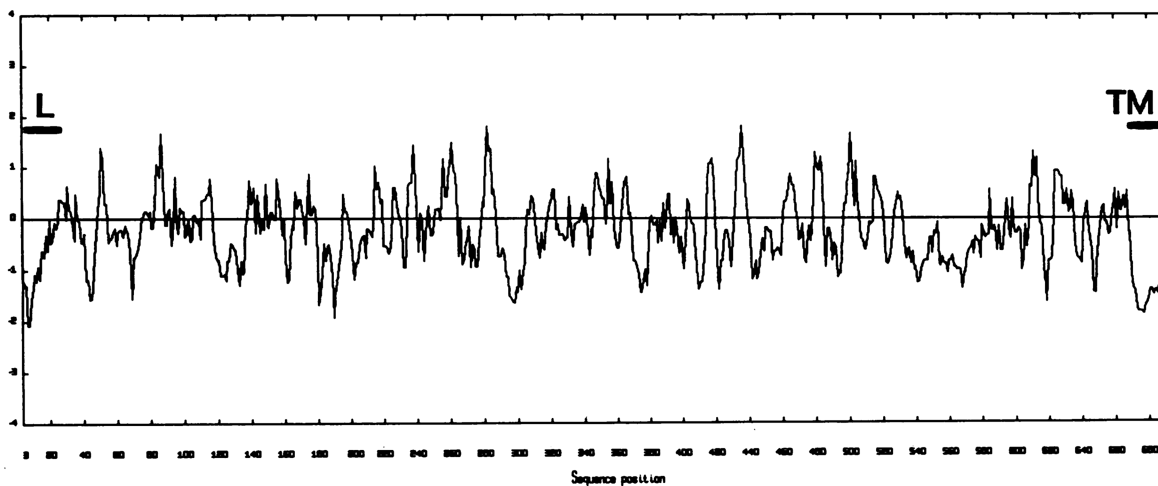

FIG. 4. Hydrophobicity profile of the HHV-6 putative gH peptide. The hydrophobic leader (L) region and the hydrophobic transmembrane domain (TM) are marked by thick lines.

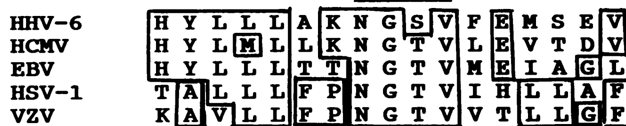

FIG. 5. Alignment of the potential glycosylation site within the gH genes of the human herpesviruses. The regions conserved among more than one virus are boxed.

flanked by variable-length directly repeated sequences (17, 26, 32). To further characterize this genome, we have determined the nucleotide sequences of two molecular clones of HHV-6 (isolate GS). One of these clones (8 kb) contained an open reading frame which was assigned as the glycoprotein H (gH) gene because of the extensive similarity of its predicted peptide to the gH (UL75) gene product of HCMV. HHV-6 gH was more distantly related to the gH genes of the other human herpesviruses. Another open reading frame contained in the second clone (8.7 kb) was similar to the UL48 gene of HCMV and is most likely the large tegument protein gene. The findings presented here are consistent with those previously published that showed a closer genetic relationship of HHV-6 to HCMV than to the other human herpesviruses (11, 23).

Virus was concentrated by continuous-flow centrifugation from supernatants of HHV-6 (isolate GS)-infected HSB2 cells (1). The viral DNA was isolated by cesium chloride gradient centrifugation as previously described (17).

A *Bam*HI library was constructed by using plasmid pIBI31 (International Biotechnologies, Inc.) as previously described (17). Clones were identified by their hybridization to HHV-6-infected HSB2 cell DNA but not to uninfected HSB2 cell DNA. Clone pZVB43 contained two inserts, the *Bam*HI-G and *Bam*HI-L fragments. Clone pZVH14, which contains an 8.7-kb *Hin*dIII fragment of HHV-6, was previously described (19). DNA sequencing was performed by the Maxam-Gilbert method (33) or by using Sequenase protocols (U.S. Biochemicals) on M13 single-stranded DNA (44). Both strands of all subclones were sequenced. Open reading frame, translation, and hydrophobicity analyses were performed with the SAP sequence analysis package developed by Universal Biotechnology, Inc. DNA and peptide similarity searches were performed with the PFASTA and LFASTA programs included in the Intelligenetics program package (version 6.0; Intelligenetics, University of Wisconsin Biotechnology Center) by utilizing the GenBank and PIR data bases (14, 27).

A *Bam*HI plasmid library was constructed from purified HHV-6 DNA. One plasmid clone contained two fragments which hybridized specifically to HHV-6-infected cell DNA. These were the *Bam*HI-G and *Bam*HI-L fragments, as determined by their correspondence in size when run in parallel with *Bam*HI-restricted HHV-6 DNA (17). The *Bam*HI-G fragment (clone pZVB43) was 7,981 bp in length and had a G+C content of 40.3%. A termination codon scan of the sequences showed eight possible open reading frame regions of 300 bp or more, as depicted in Fig. 1. Five of these, RF1 to RF5, were found in the right reading frame, and three, LF1 to LF3, were found in the opposite strand, or the left reading frame. All of these regions potentially encoded peptides of 100 or more amino acids in length, with all except RF1 having an initial methionine residue, as shown in the translated sequence (Fig. 2). A search of the GenBank data base with the DNA sequences of this fragment was performed but yielded no statistically significant matches. In contrast, a search of the PIR data base with the predicted translation product from one open reading frame region, RF2 (bp 1817 to 3911) (Fig. 2), showed significant sequence identity to the HCMV gH (UL75) gene product (7). This HHV-6 peptide was 694 residues long and had a calculated molecular mass of 79,549 Da and an estimated pI of 6.528. An identity of 23.9% was found in a 685-residue overlap of HHV-6 and HCMV gH peptides with an optimized score of 860. As shown in Fig. 3, LFASTA analysis demonstrated that sequence similarity with HCMV gH extended throughout the entire region. For EBV gH (BXLF2), the 24.2% identity in a 314-amino-acid overlap (optimized score, 267) was lower than that for the HCMV gH and the HHV-6 gH peptides. The identity was lowest with gH peptides of varicella-zoster virus (VZV) and HSV type 1 (HSV-1) at 21.8% for a 124-amino-acid overlap (optimized score, 64) and 17.4% for a 310-amino-acid overlap (optimized score, 69), respectively.

The carboxyl-terminal portions of the human herpesvirus gH peptides were more conserved, as found in previous analyses (9, 34). This is shown by the diagonal toward the right for HSV and VZV compared with HHV-6 in Fig. 3. The hydrophilicity profile of the HHV-6 peptide was similar to those of the peptides of the other four human herpesviruses (HCMV, HSV-1, EBV, and VZV) (34) in that a hydrophobic leader sequence was found from residues 1 to 23 and a predicted hydrophobic transmembrane region extended from residues 668 to 694 (Fig. 4). Fourteen potential N-glycosylation sites of the consensus sequence N-X-S/T were noted, as underlined in Fig. 2. This is in contrast to 6, 6, 4, and 10 potential N-glycosylation sites found in the gH proteins of HCMV, HSV-1, EBV, and VZV, respectively. One of the potential N-glycosylation sites of HHV-6 gH

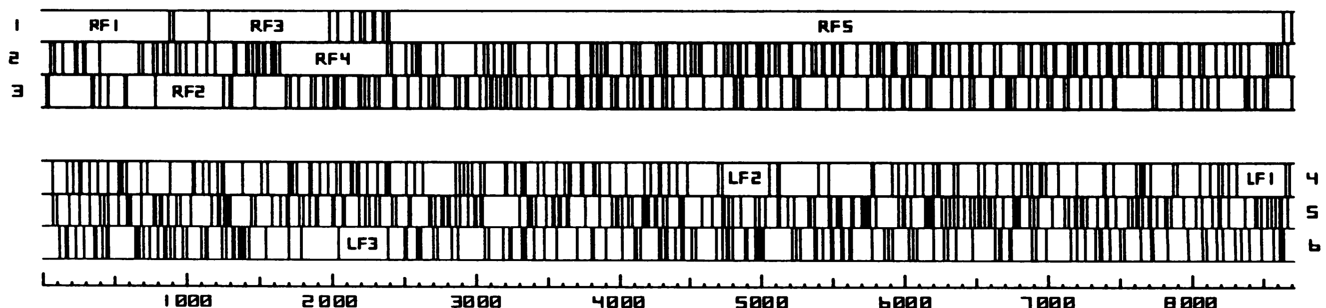

FIG. 6. Termination codon scan of the DNA sequence of the HHV-6 clone pZVH14 *Hin*dIII fragment. (See legend to Fig. 1.)

[illegible]

[illegible]

FIG. 7. Nucleotide sequence and translation of the HHV-6 clone pZVH14 sequences showing open reading frames of greater than 100 amino acids with initiator methionine residue.

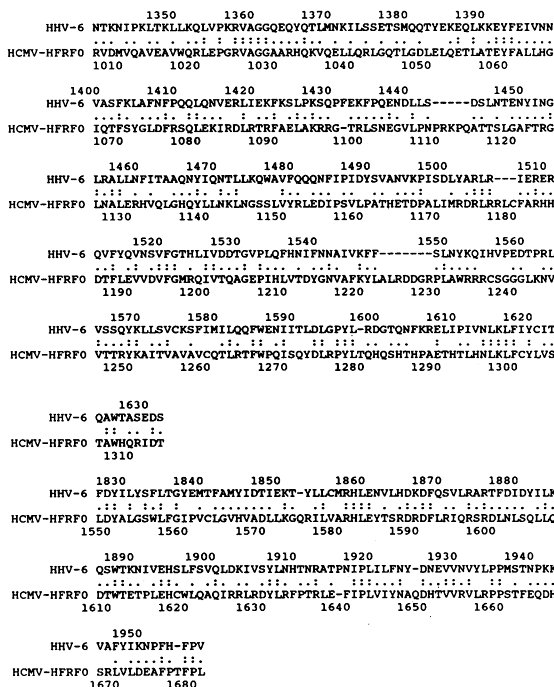

FIG. 8. Alignment of the most-conserved regions of the HHV-6 pZVH14 RF5- and HCMV UL48 (HFRF0)-encoded putative large tegument peptides.

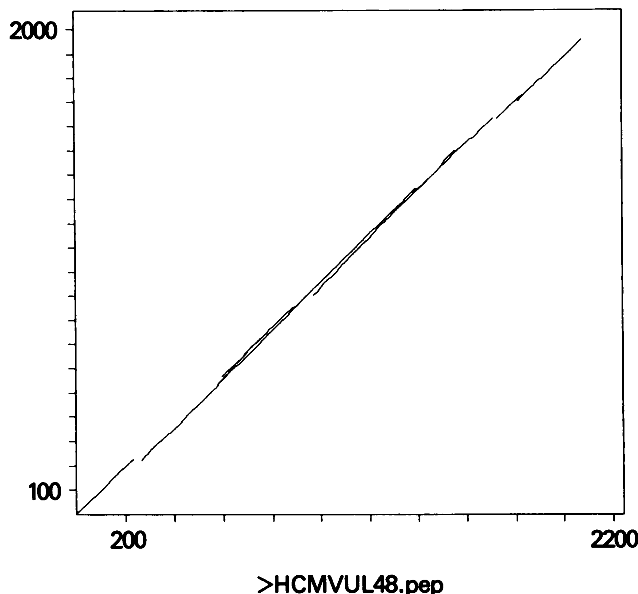

FIG. 9. Homology between the putative large tegument proteins of HHV-6 (clone pZVH14, RF5) and HCMV (>HCMVUL48.pep) as revealed by LFASTA analysis.

protein at position 652 was highly conserved among the other four human herpesviruses, although HHV-6 contained a serine residue at position 654 instead of the threonine found in HCMV, HSV-1, EBV, and VZV gH proteins (34) (Fig. 5). Like the gH proteins of the other four herpesviruses (EBV, HCMV, VZV, and HSV), the peptide sequence of HHV-6 gH has a short carboxyl-terminal cytoplasmic region and four cystine residues near the potential transmembrane region.

Analysis of clone pZVH14, an 8.7-kb *Hin*dIII fragment clone of HHV-6 (19), showed that it contained 8,717 nucleotides with a G+C content of 39.9%. Seven possible reading frames were found, as depicted in the termination codon scan shown in Fig. 6. Five potential reading frames (RF1 to RF5) of 300 bp or more were located in the right-hand reading frame, and three (LF1 to LF3) were located in the opposite strand. Peptides of 100 amino acid residues or more containing initial methionine residues were found in all of the frames except LF1 and LF3 (Fig. 6). A search of the GenBank data base with the DNA sequence found no significant matches. By using the predicted translation products of these reading frames and the PIR data base, a significant score was found between the RF5 peptide (bp 2413 to 8647 [Fig. 6]) and the predicted peptide of the HCMV UL48 open reading frame (previously termed the HFRF0 open reading frame) (7, 22). The regions with the most significant scores are shown in Fig. 7. The HHV-6 peptide was 2,077 residues in length and had a calculated molecular mass of 235,000 Da and an estimated pI of 6.5. A computer comparison (Fig. 8) using the LFASTA program showed a homology score of 14.8% over the carboxyl-terminal regions of these peptides, with an optimized score of 620. The solid diagonal line in Fig. 9 suggests that the homology extends throughout the peptides. No significant homology was noted with peptides of other human herpesviruses. Searches performed with the HCMV peptide also showed no significant homology scores with peptides of the other herpesviruses (22). The UL48 gene product is thought to be the HCMV large tegument protein, since its size is comparable to the large tegument protein encoded by the BOLF-1 gene of EBV (22).

There are AT-rich regions upstream of the coding sequences, at positions 1747 to 1756 (Fig. 2) for the putative HHV-6 gH-coding region in pZVB43 and at positions 2331 to 2337 and 2388 to 2398 (Fig. 7) for the tegument-coding region in pZVH14 RF5, which may serve as promoters. Potential polyadenylation sites of the AATAAA consensus were found at position 3913 of gH and at position 8704 of pZVH14. A consensus TATAAA is located just upstream of this sequence at 8676.

Analysis of the nucleotide sequences of two molecular clones of HHV-6 and of the predicted amino acid sequences of two large open reading frames in these clones suggests that they correspond to the putative gH and the large tegument protein genes of HHV-6, on the basis of their sequence similarities to the HCMV gH and large tegument gene products. Recent sequence determination of the major capsid antigen region of HHV-6 (isolate U110) (11, 23) has shown a close genetic relationship between HHV-6 and HCMV, both in the extent of their homology scores and in the overall shared orientation of specific open reading frames. The data presented here extend the homology to two additional genes of HHV-6. The homology of the HHV-6 gH is observed throughout the human herpesviruses. The finding of extensive homology between the HCMV tegument (UL48) peptide and the pZVH14 RF5 putative tegument peptide (Fig. 9) is consistent with previous studies which revealed a close genetic relationship of HHV-6 and HCMV, especially in that both the HCMV and HHV-6 gene products lack detectable homology to the respective gene products of other human herpesviruses (11, 23).

Because HHV-6 is lymphotropic and mainly infects a

subset of T and B cells in culture (1–3, 28, 31, 49), it has been proposed (28) that HHV-6 be classified as a gammaherpesvirus (8) along with EBV, a B-cell-tropic virus, and herpesvirus saimiri, a T-cell-tropic virus (41). This is in contrast to HCMV, a member of the betaherpesvirus group (41), which productively infects host species-derived fibroblasts. Although the complete genetic structure of HHV-6 has not yet been determined, previous Southern blot hybridization studies (11) and DNA sequence analysis of the putative gH and tegument proteins reported here and the major capsid antigen region reported previously (23) indicate a closer phylogenetic relationship of HHV-6 to HCMV than to other human herpesviruses, except that a GGGTTA repeat sequence is shared by the DNA of HHV-6 and that of Marek's disease herpesvirus (21). These results support the suggestion that HHV-6 be tentatively classified as a betaherpesvirus rather than a gammaherpesvirus or perhaps that it not yet be subclassified.

The role of the HHV-6 putative gH and tegument protein in virus architecture, viral infection, and host immune response remains to be ascertained. The gH gene product of HCMV is thought to function in fusion events early in the process of infection. Certain monoclonal antibodies against HCMV gH protein capable of neutralizing viral infection have been described elsewhere (9). It is reasonable to assume that the putative gH protein described in this report plays a similar role in HHV-6 infection. The detailed information presented here may facilitate the development of molecular and immunologic tools for delineating the events in virus infection and studying aspects of host immune response.

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