Two groups of human herpesvirus 6 identified by sequence analyses of laboratory strains and variants from Hodgkin’s lymphoma and bone marrow transplant patients

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Fifteen human herpesvirus 6 (HHV-6) strain variants were analysed by PCR amplifications, restriction enzyme site polymorphism and sequence analyses. Three DNA regions were chosen for study: a fragment of a variable glycoprotein gene (210 bp), the conserved glycoprotein H (gH) gene complete with intergenic sequences (2381 bp) and the 5' intergenic region with the N-terminal coding sequence of gH up to a polymorphic BamHI site (427 bp). Infected cell DNA from five laboratory reference strains including GS, Ul102, AJ, Z29 and KF were examined together with DNA from peripheral blood lymphocytes infected with HHV-6 reactivated from blood and/or marrow from five bone marrow transplant (BMT) patients. Separate blood and marrow isolates were obtained from four BMT patients.

In addition, HHV-6 sequences were examined directly from one of six Hodgkin’s lymphomas and six B cell proliferations which contained HHV-6 DNA as detected by PCR amplification. The results show two groups of very closely related but heterogeneous strains which correlate with previous groupings by antigenic and restriction site differences. These are variant A strains (including laboratory strains GS, Ul102 and AJ) and variant B strains (including laboratory strains Z29 and KF, the Hodgkin’s lymphoma strain, and the nine BMT patient isolates). Variations between the groups were 4 to 6% in nucleotide sequence and 5 to 8.5% in amino acid sequence. Within each group maximum heterogeneity was observed in different genes. Variant A strains differed by 2.0% in the variable glycoprotein gene sequence whereas variant B strains were identical in this region; conversely, variant B strains differed by 2 to 3% in the gH N-terminal and intergenic sequences whereas variant A strains differed there by less than 0.2%. There was evidence for sequence drift independent of selection: relationships between the groups were shown by analyses of amino acid sequence, coding nucleotide sequence as well as intergenic sequence, and the B variant-specific BamHI site in the gH gene was due to a non-coding nucleotide substitution. There was little evidence for in vivo or in vitro variation: the gH nucleotide sequence from the uncultured lymphoma strain (first variant B gH gene identified) was almost identical to the gH sequence from four BMT isolates, and matched BMT isolates from blood and marrow were identical or with a single nucleotide substitution. The overall variation observed between the HHV-6 strain groups was similar or less than that seen between human cytomegalovirus strains such as AD169 or Towne, but clearly distinct from the much greater divergence between currently designated herpesvirus species.

Introduction

Human herpesvirus 6 (HHV-6) is a recent isolate in the human herpesvirus family (Salahuddin et al., 1986; Downing et al., 1987; Tedder et al., 1987). This virus has a tropism in vitro and in vivo for CD4+ T lymphocytes, although limited replication in vitro has been shown in a number of cell types of lymphoid and non-lymphoid lineage (Lusso et al., 1988; Takahashi et al., 1989; Tedder et al., 1987). A possible site of latent virus DNA has been identified in monocytes or macrophages (Kondo et al., 1991) and initial infection may occur through epithelial cells in salivary glands which can harbour the virus (Fox et al., 1990; Levy et al., 1990a). Primary infection is inapparent or causes a mild skin rash in infants, ‘exanthem subitum’ (Yamanishi et al., 1988; Dewhurst et al., 1992); seroconversion (60% to 90% of...
the population) occurs usually before 1 year of age (Briggs et al., 1988; Okuno et al., 1989).

As with other herpesviruses, HHV-6 infection remains for the lifetime of the host and may be reactivated to cause secondary infections during immunosuppression. As HHV-6 has a tropism similar to that of human immunodeficiency virus (HIV) in infections of CD4+ T lymphocytes and monocytes/macrophages (Yamanishi et al., 1988; Okuno et al., 1989; Levy et al., 1990a; Kondo et al., 1991; Wrzos et al., 1990), it has been suggested that HHV-6 may reactivate and participate in AIDS or cause complications in immunodeficient transplant or cancer patients (Lusso et al., 1989, 1991; Morris et al., 1989; Ward et al., 1989; Okuno et al., 1990; Carrigan et al., 1991). Complications include fatal fulminant hepatitis (Asano et al., 1990) and HHV-6-associated interstitial pneumonitis in bone marrow transplant (BMT) patients (Carrigan et al., 1991). Furthermore, a marrow-suppressive role for HHV-6 has been proposed (Knox & Carrigan, 1992; Drobsky et al., 1992). There has been conflicting evidence for both positive and negative interactions with HIV, although the basis for this discrepancy may lie in strain differences (Ensoli et al., 1989; Carrigan et al., 1990; Levy et al., 1990c; Lusso et al., 1991).

Two groups of HHV-6 strains, A and B or ‘U1102-like’ and ‘Z29-like’, have been proposed on the basis of antigenic differences in reactivity to a subset of HHV-6-specific monoclonal antibodies (MAbs) and variations in restriction enzyme sites (Ablashi et al., 1991; Aubin et al., 1991; Schirmer et al., 1991; Chandran et al., 1992). Laboratory strains GS and U1102 are included in group A, whereas Z29, SF and KF are in group B. Most exanthem subitum isolates from infants have been group B, but strains of both groups, A and B, have been isolated from two patients (Dewhurst et al., 1992). Strains from either group, including GS, U1102, Z29 and SF, have been isolated from adults infected with HIV (Salahuddin et al., 1986; Downing et al., 1987; Lopez et al., 1988; Levy et al., 1990b). Human sera are broadly cross-reactive and cannot distinguish strain groups in immuno-fluorescence tests or radioimmunoassays, although the groups can be differentiated in reactions with certain MAbs, in particular those recognizing envelope glycoproteins (Ablashi et al., 1991; Chandran et al., 1992). There appear to be some growth differences between the groups, although these may be strain-specific. Comparative studies show growth of some isolates in different CD4+ T lymphocytic cell lines: group A isolates in HS2 cells, and group B isolates in MOLT3 cells (Wyatt et al., 1990; Ablashi et al., 1991; Schirmer et al., 1991; Chandran et al., 1992; Dewhurst et al., 1992). It has been suggested that the HHV-6 strain variant groups be reclassified (Schirmer et al., 1991), but the molecular basis of the grouping has not been determined and therefore it is not clear whether this represents variation between strains or new species.

We are working on the U1102 strain of HHV-6 (Downing et al., 1987), for which the only complete restriction enzyme fragment linkage map and set of clones is available (Martin et al., 1991). Sequencing studies of this virus DNA have shown that the organization of conserved genes and their encoded amino acid sequences are more related to those of human cytomegalovirus (HCMV) than those of the other human herpesviruses (Lawrence et al., 1990; Neipel et al., 1991). However, these viruses are distant relations: with the exception of the sequence around the conserved spliced gene (66% identity) (Efstathiou et al., 1988), little nucleotide sequence similarity exists that can be detected by Southern blot hybridization. A similar relationship is observed between varicella-zoster virus (VZV) and herpes simplex virus (HSV) with amino acid identities between aligned sequences of 15% to 59% (McGeoch et al., 1988; Davison & Taylor, 1987). These four virus species have different overall structures, base composition and sizes. In contrast, the prototypes of HHV-6 group A (U1102) and group B (Z29) show in addition to extensive DNA cross-hybridization an identical genome structure, base composition and size. These are AT-rich, 162 kbp genomes bounded by direct terminal repeats (Martin et al., 1991; Lindquester & Pellett, 1991).

In this study we investigated the molecular basis for differences between HHV-6 strain groups by PCR and sequence analysis of 15 variants, including laboratory reference strains from both groups. We also studied, by PCR, strains in two potential patient groups: firstly, low passage isolates from BMT patients and secondly, DNA directly from Hodgkin’s lymphoma, because previous studies have detected virus sequences in this tissue from some patients with this disease (Torrelli et al., 1991). Both variable and conserved genes are examined and the results show overall identity between strain groups of 94 to 96%, which is similar to or less than variation between strains of HCMV or Epstein–Barr virus (EBV) (Chou & Dennison, 1991; Sample et al., 1990).

Methods

**Cells.** Human T cell lines, JHhan and HSB2, and phytohaemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) from an HHV-6 seronegative normal donor were grown in suspension cultures and used to propagate virus isolates. Cells were grown in RPMI-1640 medium (Flow Laboratories) supplemented with 10% fetal calf serum (Myocline, Gibco-BRL), glutamine and antibiotics.

**Viruses.** Fourteen HHV-6 isolates were used for this study. HHV-6 (U1102) was cultivated as described (Martin et al., 1991; Downing et al., 1987). HHV-6 (AJ) (Tedder et al., 1987) was a gift from R. Tedder (Middlesex School of Medicine, London, U.K.). HHV-6 (Z29) (Lopez
et al., 1988) was a gift from P. Pellett (Centers for Disease Control, Atlanta, Ga., U.S.A.). HHV-6 (GS) was a gift from R. Gallo (National Cancer Institute, Bethesda, Md., U.S.A.). HHV-6 (KF) known as DC in Chandran et al. (1992) was isolated from peripheral blood lymphocytes of a patient with chronic lymphocytopenia, propagated as described (Russler et al., 1991). HHV-6 isolates C1, C2, C3, C4, C7, C8 and C9 were isolated from BMT patients with evidence of marrow suppression as described (Drobyski et al., 1992). Isolates C5 and C6 (BA) were from a BMT patient having engraftment failure with disseminated HHV-6 infection (Carri gan et al., 1991). C1 and C2 (UPN 410), C3 and C4 (UPN 399), C5 and C6 (BA strain), and C7 and C8 (NB, UPN 356) were matched sets of marrow and blood HHV-6 isolates from patients after BMT (Drobyski et al., 1992). Patients KF, UPN 410 and BA in addition appeared to have HHV-6-associated pneumonitis (Russler et al., 1991; Car rigan et al., 1991; Drobyski et al., 1992). Isolates C1 to C8 were from patients in the BMT Program, Medical College of Wisconsin, Milwaukee, Wis., U.S.A.; BMT patient JM (isolate C9) was from the Sloan Kettering BMT Program, New York, U.S.A.

Tissue samples. Biopsy samples from 12 B cell proliferations or Hodgkin's lymphoma were collected and stored at -70 °C (Histo pathology Department, Addenbrooke’s Hospital, Cambridge, U.K.).

DNA extractions. Uninfected JHahn, infected T cell lines or PHA blasts were treated with proteinase K and SDS, then extracted with phenol–chloroform as described (Gompels et al., 1992). Tissue samples were minced, treated with Pronase or proteinase K–SDS and extracted with phenol–chloroform.

PCR amplifications. Two primer sets were used to amplify sequences from the genes for conserved glycoprotein gH or a non-conserved glycoprotein (BHLF2) (Gompels et al., 1992): gH, 5' ATA AGA TCT GTT TAT GGA TCC TCA 3', 5' CGG CGT TTA GCT GGA TCC GGA CAA 3'; BHLF2, 5' GAT GGA TCC TCC AAA GGA AGT GTG AAC 3', 5' GAA GGA TCC TTG CCG ATG GCA ATG AGC 3'.

These are at positions 6386 and 4006 (gH) and 3319 and 3110 (BHLF2) of the BamHI H sequence (Gompels et al., 1992). The primers were modified at the underlined nucleotides to create BamHI sites. Amplification reactions with these primers used Taq polymerase (Promega) and deoxyribonucleoside triphosphates (Boehringer Mannheim) with standard buffer conditions and 1.5 to 2.0 mM-MgCl2. PCR thermal cycling reaction conditions were 93 °C for 4 min, then 30 cycles at 93 °C for 30 s, 43 °C for 30 s, 72 °C for 120 s. The gH primers amplified a 2381 bp product; the BHLF2 primers, a 210 bp product. Each PCR reaction included both positive (HHV-6 strain AJ-infected JHahn cell DNA) and negative (uninfected JHahn cells) controls. No evidence of HHV-6 (AJ) DNA PCR contamination was detected; all negative controls were negative and PCR-positive for cellular genes only (not shown).

Sequence analysis. Amplified DNA fragments were digested with BamHI and separated by electrophoresis in an agarose gel. The relevant DNA fragments were purified, then ligated with BamHI-digested, phosphatase-treated M13mp18 vector DNA (Messing, 1983). The sequence was determined from single-stranded M13 clones by the dideoxynucleotide chain termination method (Sanger et al., 1977) using [35S]dATP (Amersham) as radioactive label (Biggin et al., 1983). For gH PCR products from the C1 and C4 isolates, the sequence was determined from both strands. The sequence from all BHLF2 PCR products was determined from both strands. For the 210 bp BHLF2 and 382 bp N-terminal gH fragments, sequences were derived from an M13 universal primer (Promega). For the complete sequence of gH, internal 17-mer primers were used from the following positions in the amplified sequence: 194, 390, 573, 751, 928, 1107, 1296, 1516, 1735, 1938 and 2161 (Fig. 1).

Results and Discussion

To examine HHV-6 strain variation, three regions of DNA were chosen for PCR and subsequent sequence analyses. The first, a conserved gene (gH), was chosen because it is one of the least conserved of a subset of conserved genes in herpesviruses (Davison & Taylor, 1987; Chee et al., 1990). As such it would be suitable both to identify variation and to compare the degree of variation found to that occurring between other herpesviruses and their strains. In addition, this is an important conserved gene because the encoded glycoprotein has roles in the infectivity and immunogenicity of the viruses (Gompels et al., 1988, 1992). Furthermore, variation in gH correlates with variation distributed throughout the genome. Intergenic regions were also amplified with coding sequences to compare the overall drift to possible coding sequence selection. The second region focused on was the variable N-terminal intergenic and coding sequence of gH extending to a B group-specific BamHI site. The third region of DNA was from a non-conserved glycoprotein gene (BHLF2). This sequence is not conserved in HCMV, the closest relation to HHV-6; although HCMV has a positional homologue which also encodes a glycoprotein, the sequences have diverged beyond detectable similarity. Both gH and the variable glycoprotein are encoded by the HHV-6 strain U1102 BamHI H sequence and comparisons to nucleotide sequences from strain GS also show this non-conserved glycoprotein gene to be hypervariable between strains. There is 5-0% difference in an overall conserved sequence, which has less than 0.5% variation (Gompels et al., 1992).

Reference laboratory strains were collected from both the A and B groups which had been identified by antigenic differences and restriction enzyme site polymorphisms (Schirmer et al., 1991; Ablashi et al., 1991). These are U1102-like strains, or group A, including U1102, GS and AJ, and Z29-like strains, or group B, including Z29 and KF. To analyse further strain variants, two sources of DNA were used: one from a Hodgkin's lymphoma and the second from infected PBMCs (less than two passages) from reactivated HHV-6 in BMT patients' blood and marrow after transplantation. The use of these sources avoids complications of PCR contamination because relatively large amounts of virus DNA were shown to be present in selected Hodgkin's lymphomas (three of 25 were HHV-6-positive) (Torrelli et al., 1991) and HHV-6 viraemia in BMT patients (Carrigan et al., 1991). In addition, analysis of the Hodgkin's lymphoma sample allows identification of HHV-6 variation without any influence of passage in cultured cells. Moreover, analyses of the BMT specimens from matched blood and marrow isolates allow the
Fig. 1. Complete HHV-6 gH coding sequence and intergenic sequences of strain U1102 compared to laboratory strains GS and AJ, and to the variant sequence from Hodgkin's lymphoma, sample 9 (L9). The U1102 sequence is from Gompels et al. (1992) and a PCR-amplified product. The GS sequence is from Josephs et al. (1991). AJi and AJii are the sequences of strain AJ from two separate PCR amplification products. Amino acid sequences in bold represent conserved residues in HCMV. The HHV-6 strain sequences are 50% variant, but are 75% divergent from the HCMV amino acid sequence. Primers used are the sequences of strain AJ from two separate PCR amplification products, and coding changes are shown by a star.
and C3 with C4 are matched marrow and blood isolates from BMT underlined; coding changes are shown by a star.

patients UPN 410 (C1 and C2) and UPN 399 (C3 and C4). The Hodgkin's lymphoma (L9) and BMT patients (C1 to C9). C1 with C2

\[ \begin{align*}
\text{CTCC} & \text{L'CGACTC} \text{QGGI} \text{CTGTTGACTCCC} \text{TEACC} \text{GACCGTTC} \text{C} \\
\text{TII} & \text{CTCGC-C} \text{430}
\end{align*} \]

\[ \begin{align*}
\text{L L G} & \text{F} \text{I} \text{T} \text{F} \text{N} \text{•} \text{T} \text{X} \text{N} \text{D} \text{Y} \text{R} \text{I} \text{Y} \text{Q} \text{V} \text{P} \text{K} \text{C} \\
\text{L L R L M V F V L T}. & \text{T} \text{A} \text{C} \text{Y} \text{0 M R P T}. \text{N}
\end{align*} \]

identification of variation \textit{in vivo} in viruses isolated from separate sites.

\textbf{HHV-6 sequences in Hodgkin's lymphoma}

In an ongoing study to be described elsewhere, Hodgkin's lymphomas and other lymphoproliferations were analysed for the presence of herpesvirus sequences (U. Gompels, S. Efstathiou, J. Arno & E. English, unpublished results). One of the 12 samples (sample L9, a mixed cellularity Hodgkin's lymphoma) analysed by PCR for the gH gene was positive for HHV-6-specific sequences. Sample L9 contained a restriction site polymorphism in the gH gene such that \textit{BamHI} digestion of the PCR product yielded two products of 427 bp (N-terminal and intergenic region) and 1954 bp (C-terminal and intergenic region). Both these fragments were cloned and sequenced. The sequence was compared to the sequence derived from multiple PCR/sequencing reactions for the same region in group A strains U1102, GS and AJ. The error rate in this procedure was only a single point mutation (AJi, Fig. 1) in 2381 bp (0.04%). Overall variation between laboratory strains was less than 0.2% whereas variation from the L9 sequence was 4% for the nucleotide sequence and 5% for the amino acid sequence (Fig. 1). The same level of variation was observed in the intergenic sequences, indicating an overall sequence drift. The L9 gH sequence is the first complete herpesvirus gene sequence determined directly from a sample obtained \textit{in vivo}, without any virus tissue culture passage. As such, it was important to determine whether the variation observed was due to the effects from culturing the laboratory strains, the lymphoma or whether the L9 sequence represented another HHV-6 strain variant group. To examine this question, a sequence representing the more variable N-terminal region of gH was analysed in other HHV-6 isolates.

\textbf{HHV-6 sequences in BMT patients}

Preliminary analyses of isolates C1 to C9 from BMT patients show antigenic and restriction site differences to HHV-6 group A laboratory strains U1102 or GS (Carrigan \textit{et al.}, 1991; Drobskyi \textit{et al.}, 1992). These isolates had characteristics more similar to those of group B laboratory strains Z29 or KF. In PCR reactions with the gH primers, a 2.3 kb fragment could be amplified from all infected cell DNA preparations, including those from infections with group B laboratory strains Z29 or KF. Upon digestion with \textit{BamHI} all these isolates and group B laboratory strains yielded two fragments of 1.95 kb and 0.4 kb, like the L9 sequence but unlike group A laboratory strains GS, U1102 or AJ (not shown). The 0.4 kb fragments were cloned and sequenced for strain KF and isolates C1, C2, C3, C4, C6 and C9. These

Fig. 2. N-terminal gH coding and intergenic sequences of strain U1102 compared to laboratory strains (GS, AJ and KF) and variants from Hodgkin's lymphoma (L9) and BMT patients (C1 to C9). C2 and C3 with C4 are matched marrow and blood isolates from BMT patients UPN 410 (C1 and C2) and UPN 399 (C3 and C4). The sequence is shown extending to the polymorphic \textit{BamHI} site, which is underlined; coding changes are shown by a star.
Fig. 3. Portion of the variable glycoprotein gene coding sequence (BHLF2) from laboratory strains (U1102, GS, AJ, Z29 and KF) and a Josephs et al. (1992) and PCR amplification products. The GS sequence is from HHV-6 strains. The dendrograms in Fig. 4, 5 and 6 are produced by sequences from the variable HHV-6 (BHLF2) glycoprotein gene in Fig. 4. Dendrogram showing relationships between nucleotide lengths are proportional to the similarity between nucleotide or amino acid sequences. These are unrooted trees.

Fig. 4. Dendrogram showing relationships between nucleotide sequences from the variable HHV-6 (BHLF2) glycoprotein gene in HHV-6 strains. The dendrograms in Fig. 4, 5 and 6 are produced by the PILEUP program of the GCG package (Version 7.1-UNIX, June, 1992; Devereux et al., 1984). This is based on the multiple alignment program of Feng & Doolittle (1987). The similarity scores for amino acid sequences are indicated on the vertical axis and are based on a weight matrix from amino acid substitutions in related protein families. Overall identities are also shown at branch points. Nucleic acid sequence identities are shown (1.0 = 100%). The vertical branch lengths are proportional to the similarity between nucleotide or amino acid sequences. These are unrooted trees.

Fig. 5. Portion of the variable glycoprotein gene coding sequence (BHLF2) from laboratory strains (U1102, GS, AJ, Z29 and KF) and a Josephs et al. (1992) and PCR amplification products. The GS sequence is from HHV-6 strains. The dendrograms in Fig. 4, 5 and 6 are produced by sequences from the variable HHV-6 (BHLF2) glycoprotein gene in Fig. 4. Dendrogram showing relationships between nucleotide lengths are proportional to the similarity between nucleotide or amino acid sequences. These are unrooted trees.

Relationships between HHV-6 strain variants and with other herpesviruses

The relationships between the two groups of HHV-6 sequences can be summarized in dendrograms (Fig. 4 and 5). Clearly there are at least two groups of strain variants, group A or U1102-like and group B or Z29- or KF-like, in agreement with earlier findings on re-
striction enzyme polymorphisms and antigenic differentiations (Schirmer et al., 1991; Ablashi et al., 1991). These clusters contain very closely related strains showing nucleotide differences of only 4 to 6%. Each group shows at least 2 to 3% heterogeneity but at different genes. This is similar to or less than the degree of variation between variant and conserved genes compared for strains of HCMV or EBV (Chou & Dennison, 1991; Sample et al., 1990). EBV sequences with differences of 4% in the intergenic regions of groups of strains are referred to as 'homologous', in comparison to the more variable EBNA 2, 3A, 3B, 3C and EBER genes. Conserved EBV genes have not been analyzed to determine whether this intergenic sequence variation of 4% occurs throughout the genome. There are two groups of EBV strain variants called types 1 and 2, more recently designated variants A and B (Sample et al., 1990; Roizman et al., 1992). In contrast, HCMV strains have been identified by analysis of conserved genes gB and gH. Variation in gH distinguishes two groups, strain AD169-like and strain Towne-like, which differ by 50% (Pachl et al., 1989; Chou, 1992), and more heterogeneity between these groups has been shown for the gB sequence (Chou & Dennison, 1991).

For EBV strain groups (variants A or B) or HCMV strains, it is not clear whether there is consistent grouping by restriction fragment polymorphisms across the entire genome as has been shown for some HHV-6 isolates. If this is not the case, EBV and HCMV strains may vary more overall than HHV-6 strains. Another possibility, although not mutually exclusive, is that EBV and HCMV strains appear more variable in restriction enzyme analyses owing to genome structure differences such as internal repetitive sequences. Mutation rates are higher at these sites, presumably mediated by homologous recombination, and an example has been shown for the HHV-6 terminal repeats (Lindquester & Pellett, 1991). However, unlike EBV and HCMV HHV-6 does not contain extensive internal repeats (Martin et al., 1991).

PCR amplification products from conserved genes without repeats in HCMV strains or selected regions in EBV strains can be grouped by restriction enzyme polymorphisms (Chou & Dennison, 1991; Sample et al., 1990). Studies of virus isolated by cell culture may also distort views of overall variation. For example, the growth properties of the EBV strain groups differ in B lymphocytes and may favor group A isolations.

It has been proposed that in HCMV, gB and gH strain variation may be due to immune selection (Chou & Dennison, 1991; Chou, 1992), but there is only limited evidence to support this. A variant epitope in HCMV gH has been identified at the N terminus which distinguishes sera reacting with the AD169-like or Towne-like strain variants. This site elicits MAb and polyclonal antibodies that can neutralize virus infectivity (Urban et al., 1992). In contrast, neutralization epitopes in gB are conserved among strain variants (Meyer et al., 1990; Lehner et al., 1991). In HSV gH there is a single example: of 66 HSV-1 clinical isolates only one was resistant to neutralization by a MAb, and had the same nucleotide and resulting amino acid substitution (although overall 0-4% variance existed between four laboratory strains) as an antibody-selected variant isolated in the laboratory (Gompels et al., 1991). Thus, it is possible that immune selection may operate on HHV-6 gH, giving rise to variants. But if it does so, it is relatively infrequent and variation is at a low level. For example, the variation observed is far less than has been recorded for HIV gp120 (to 39%), a proposed target for immune selection (Myers, 1990). Furthermore, HHV-6 undergoes sequence drift clearly independent of immune selection. The dendrogram in Fig. 5 shows relationships determined from nucleotide sequences, but the same result is obtained for the intergenic sequence as well as the encoded amino acid sequence. In addition, the variant BamHI site, a genetic polymorphism which appears to be maintained in isolates of the Z29-like group, is a non-coding change. These aspects of nucleotide sequence drift have not been examined for HCMV. Moreover, further study is required to determine whether the 2 to 3% heterogeneity observed within HHV-6 strain groups is also distributed throughout the genome.

How do these HHV-6 variant groups relate to the other herpesvirus species? In Fig. 6, relationships between the amino acid sequences of the gH protein family are shown for different herpesvirus species, prototypes for the alpha-, beta- and gammaherpesvirus subfamilies. Nucleotide sequences could not be compared because they are not conserved between herpesvirus species. The 5% difference between HHV-6 strain groups represented by strains U1102 (HHV-6A) and L9 (HHV-6B) is identical to the variation observed between HCMV (HHV-5) strains AD169 and Towne (Pachl et al.,...
Fig. 6. Dendrogram showing relationships between herpesvirus species and strains in the gH protein family. Details as for Fig. 4. The relationships between gH amino acid sequences were examined for prototypes of herpesvirus species. Alphaherpesviruses are represented and strains in the gH protein family. Details as for Fig. 4. The relationships between gH amino acid sequences were examined for HSV-1 (or HHV-1; alpha-l, GC-rich base composition) and VZV (or HHV-3; alpha-2, AT-rich); betaherpesviruses by HCMV (or HHV-5; beta-1, GC-rich) and HHV-6 (beta-2, AT-rich); gammaherpesviruses by EBV (or HHV-4; gamma-1, GC-rich) and herpesvirus saimiri (HVS or SHV-2; gamma-2, AT-rich). Strains of HCMV AD169 (HCMV-A) and Towne (HCMV-T) are shown with strains of HHV-6 variant A (U1102) and variant B (L9).

1989) but clearly different from the diversity of 75 to 85% between other herpesvirus species prototypes (Fig. 1 and 6). Among the most closely related distinct herpesvirus species are the alphaherpesviruses equine herpesvirus 1 and 4 (EHV-1 and EHV-4) and HHV-1 and -2 (HSV-1 and HSV-2) (Roizman et al., 1992). HHV-1 and HHV-2 are 16 to 33% variant in sequences examined across their genomes (McGeoch et al., 1987). The HHV-2 gH sequence has not been determined, but the gH sequences from EHV-1 and EHV-4 are available for comparison; the amino acid sequences are 15% variant and of different length, 848 and 855 residues, respectively (Nicolson et al., 1990). Again this is distinct from variation between strains of HCMV and HHV-6. Thus, in conclusion, on the basis of sequence analysis of selected genes and intergenic regions, HHV-6 strains form at least two groups which appear to be strain variants.

A consensus has been agreed (XVII International Herpesvirus Workshop, Edinburgh, August 1992) that the HHV-6 strain variant groups be termed 'variant A' for U1102-like viruses and 'variant B' for Z29-like viruses, similar to a precedent for naming EBV strain variant groups (Sample et al., 1990; Roizman et al., 1992). The International Committee on Taxonomy of Viruses (ICTV) herpesvirus study group recommends designation of separate species if the viruses differ across their entire genome and have distinct biological and epidemiological characteristics (Roizman et al., 1992). The naming involves the host and serial arabic numbers; for example if HHV-6 strain groups should be regarded as separate species the next available nomenclature is human herpesvirus 8 or HHV-8 (Roizman et al., 1992).

However, as summarized in the previous sections, no difference in epidemiology has been identified between the HHV-6 strain groups. Furthermore, the level of nucleotide sequence variation found here between the groups was similar to that found between current strains of other herpesvirus species; the groups are more closely related to each other than any other pairs of designated herpesvirus species. Within the ICTV guidelines for herpesviruses (Roizman et al., 1992) there exists a recommendation for naming groups of strains of HHV-4 (EBV). These are designated variants A and B (previously termed types 1 and 2; Sample et al., 1990). The term 'groups' cannot be used as this denotes genome structures with respect to their repetitive and unique sequence organization (groups A to F; Roizman et al., 1992). Therefore the decision has been made to designate HHV-6 strain groups variants A and B, and the data presented here would support this nomenclature.

After the completion of these studies, sequence analyses of PCR products were presented for 10 isolates (including laboratory strains GS, U1102 and Z29) of two other regions of the genome, the tegument gene and major capsid gene (Aubin et al., 1993). The variation observed between strain groups is 50% and in agreement with the studies we have presented here. Preliminary PCR and sequence analyses of the gH gene and intergenic region of the isolates of Aubin et al. (1993) agree with their grouping by studies of the tegument and major capsid genes. We found similar variation between HHV-6 variant groups (50%) and heterogeneity (20%) in group B variants in the N-terminal gH sequence. Furthermore, Chou & Marousek (1992) have recently shown that sequences for the HHV-6 glycoprotein B homologues have little variation, of only 40%, between strains GS (variant A) and Z29 (variant B) as compared to the greater variation, 110%, observed between gB sequences of HCMV (Chou & Dennison, 1991). In studies of restriction enzyme polymorphisms of geographically distinct HSV-1 strain groups, 30% sequence variation has been calculated (Sakaoka et al., 1987). The geographical distributions of HCMV and HHV-6 strain variants have not been determined. Continued sequencing studies on representative laboratory strains and analysis of further isolates or direct examination by PCR of HHV-6 sequences from tissue samples may determine the distribution and possible overlap or additional distinctions between HHV-6 strains. Further study is required to determine how these distinctions relate to biological properties or epidemiology.

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References


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