JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1991, p. 367–372
0095-1137/91/020367-06$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 29, No. 2

# Several Groups among Human Herpesvirus 6 Strains Can Be Distinguished by Southern Blotting and Polymerase Chain Reaction

JEAN-THIERRY AUBIN,[1] HELENE COLLANDRE,[2] DANIEL CANDOTTI,[1] DIDIER INGRAND,[1] CHRISTINE ROUZIOUX,[3] MARIANNE BURGARD,[3] STEPHANE RICHARD,[4] JEAN-MARIE HURAUX,[1] AND HENRI AGUT[1]*

*Laboratoire de Bactériologie-Virologie, C.E.R.V.I.,[1] and Laboratoire de Neurohistologie,[4] Hôpital de la Pitié-Salpêtrière, 75013 Paris, and Unité d'Oncologie Virale, Institut Pasteur,[2] and Laboratoire de Bactériologie-Virologie, Hôpital Necker,[3] 75015 Paris, France*

Received 2 July 1990/Accepted 23 October 1990

**Eight human herpesvirus 6 (HHV-6) strains were studied by Southern blot and polymerase chain reaction. DNA from infected cells was digested by a panel of restriction enzymes and hybridized with cloned *Bam*HI fragments corresponding to about 30% of the HHV-6 strain SIE genome. In parallel, this DNA was amplified by polymerase chain reaction using pairs of primers derived from the strain SIE nucleotide sequence. Subsequently, amplification products were analyzed by hybridization, digestion with restriction endonucleases, and partial nucleotide sequencing. Overall results indicated that all strains were closely related to one another. However, concordant differences in restriction patterns allowed at least two groups to be distinguished, typified by strains SIE and HST, respectively. Differences between the two groups were found to reflect a limited number of punctual changes in nucleotide sequences. These results strengthen the idea of a unique HHV-6 species with genetic polymorphism. In addition, this study provides useful markers for the diagnosis and molecular epidemiology of HHV-6 infections.**

Human herpesvirus 6 (HHV-6) was originally isolated from patients with lymphoproliferative disorders by using mononuclear blood cell culture (21). By the same procedure, distinct HHV-6 isolates were subsequently obtained (3–5, 10, 18, 25). Despite partial homology with cytomegalovirus (11, 17), all of the isolates were found to be distinct from other known human herpesviruses. They all hybridized with pZVH14, a probe derived from the original human B-lymphotropic virus (HBLV) strain (15). However, digestion with restriction endonucleases showed different patterns for HHV-6 strains (13) which, in some cases, suggest the existence of new T-lymphotropic human herpesviruses related to HHV-6 (5). Recently, a new lymphotropic herpesvirus isolate which exhibited only partial hybridization with HHV-6 probes led to the definition of HHV-7 (12).

Taken together, these results address at least two issues: (i) the distinction between HHV-6 and other putative herpesvirus species and (ii) the distinction between different strains belonging to the same HHV-6 species. These issues are not only a semantic problem. HHV-6 has been found to be the causative agent of exanthem subitum (24, 26), but it may be responsible for other pathologies. The mechanism of its transmission remains basically unknown and so does the site of its maintenance in a latent state. It would be difficult to draw any conclusion pertaining to these issues without being sure that the results concern the same infectious agent or, in some situations, the same strain.

The present report describes the study of eight HHV-6 strains from different origins by both Southern blot (23) and polymerase chain reaction (PCR). The results indicate very close homology between these strains, despite a marked genetic polymorphism, which could provide useful epidemiological markers.

* Corresponding author.

## MATERIALS AND METHODS

**Viruses and cells.** Eight HHV-6 strains were studied. Strain SIE was isolated from an Ivory Coast patient (3), and strain TAN was from a Congolese patient (4). In both cases, the presence of HHV-6 was associated with retroviral infections. Strains BOU, MAR, BLE, and MBE were isolated from children born to human immunodeficiency virus-seropositive mothers during attempts to culture human immunodeficiency virus from peripheral blood mononuclear cells. No evidence of human immunodeficiency virus infection was found among these children, whose ages exceeded 18 months at the time of peripheral blood mononuclear cell culture. Isolation of strain HBLV, kindly provided by S. Z. Salahuddin, and that of strain HST, kindly provided by K. Yamanishi, have been previously reported (21, 26).

Viruses were grown in phytohemagglutinin-stimulated peripheral blood mononuclear cells from a single healthy donor as previously described (2), except for strain HBLV, which was propagated in the HSB-2 cell line. When the cytopathic effect was at its peak, infected cells were harvested and treated for either constitution of infectious stocks or total DNA extraction.

**Preparation of DNA from infected cells.** Cells were lysed overnight in TE (10 mM Tris hydrochloride [pH 7.5], 1 mM EDTA) buffer containing 0.5% sodium dodecyl sulfate (SDS) and 100 μg of proteinase K per ml at 37°C. Nucleic acids were extracted by phenol-chloroform treatment and ethanol precipitated (19). Treatment with 100 μg of RNase A per ml was performed for 1 h, after which DNA was phenol extracted and ethanol precipitated again. Samples were kept at −20°C. DNA from control uninfected cells was prepared by the same procedure.

**DNA probes.** *Bam*HI fragments of purified DNA from strain SIE have been cloned by one of us (6). Cosmids cHC15 and cHC17, derived from cosmid pWE15 (Clontech), contain 30- and 20-kb fragments, respectively, with no overlapping region between the two inserts. Plasmid pHC5,

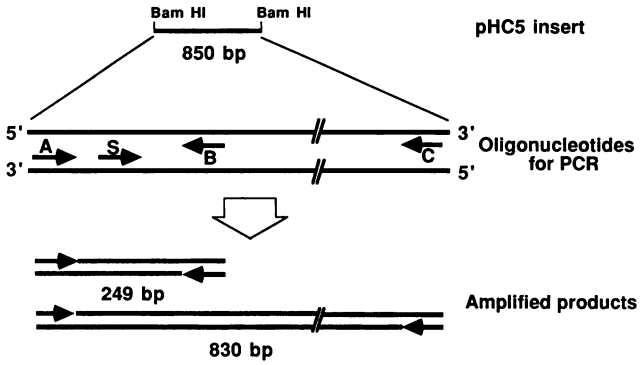


FIG. 1. Location of primers and probe for HHV-6 PCR.

derived from the pTZ19R vector, contains an 850-bp *Bam*HI fragment homologous to that in the pZVH14 insert (15). For hybridization, plasmids and cosmids were labeled with [$\alpha$-$^{32}$P]dATP by using the Multiprime DNA labeling system (Amersham).

**Southern blot hybridization.** DNAs from infected and uninfected cells were digested with restriction enzymes (Boehringer) in accordance with the instructions of the manufacturer and electrophoresed in 0.8% agarose gels with the following molecular weight markers: bacteriophage $\lambda$ digested by *Hin*dIII and the phage $\Phi$X174 replicative form digested by *Hae*III. Gels were stained with ethidium bromide, and DNA was blotted onto nylon membranes (Hybond N+; Amersham) with a vacuum blotting system. The membranes were prehybridized for 1 h at 42°C in hybridization buffer containing 5× SSC (1× SSC is 0.15 M NaCl–0.015 M sodium citrate, pH 7), 5× Denhardt solution (9), 0.5% SDS, 50% formamide, and 100 $\mu$g of denatured salmon sperm DNA per ml. Hybridization was performed overnight at the same temperature in the same buffer containing $10^6$ cpm of denatured labeled probe per ml. Subsequently, the membranes were washed under stringent conditions (0.2× SSC, 65°C) before autoradiography.

**DNA amplification.** DNA was amplified essentially as described elsewhere (7, 20). Briefly, a 1-$\mu$g sample of DNA was amplified during 40 cycles, each cycle consisting of denaturation at 92°C for 1 min, primer annealing at 55°C for 1 min, and chain elongation with *Taq* polymerase (Cetus) at 72°C for 1.25 min. In the first cycle, samples were denatured at 92°C for 7 min, and in the last cycle, the extension step was increased to 7 min.

PCR products were electrophoresed in 1.2% agarose gels and visualized after ethidium bromide staining. In this case, molecular weight markers were $\Phi$X174 digested by *Hae*III or a 123-bp DNA ladder (Gibco BRL). To confirm its specificity, DNA was blotted onto a nylon membrane and hybridized to a 5′-end-labeled oligonucleotide probe.

For PCR, the oligonucleotides used as primers or probes were designated A (5′-GATCCGACGCCTACAAACAC-3′), B (5′-TACCGACATCCTTGACATATTAC-3′), C (5′-CGG TGTCACACAGCATGAACTCTC-3′), and S (5′-GGCTG ATTAGGATTAATAGGAGA-3′). Amplification using primers A and B led to a 249-bp fragment, whereas the primer pair A-C allowed an 830-bp fragment to be amplified (Fig. 1). In both cases, the amplified fragment hybridized to the S probe.

**Characterization of amplified products.** The amplified 830-bp DNA product was extracted from agarose gels by the Geneclean procedure (Bio 101 Inc.). The PCR product was then digested with appropriate restriction endonucleases and analyzed on agarose gels, the concentrations of which ranged from 1.2 to 2%. After ethidium bromide staining, DNA was blotted onto nylon membranes and hybridized with the labeled S probe or plasmid pHCS. Alternatively, the purified DNA fragment was submitted to nucleotide sequencing by the general dideoxynucleotide-chain termination method (22). Sequencing reactions were performed with the T7 Polymerase Sequencing Kit (Pharmacia) with [$\alpha$-$^{35}$S]dATP and oligonucleotide B as the primer.

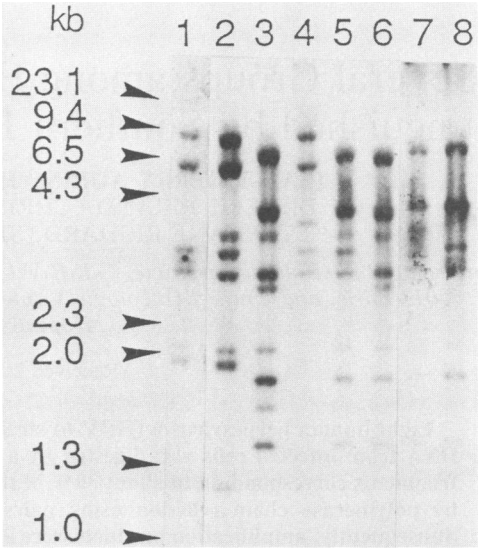


FIG. 2. Hybridization of cHC15 to infected cell DNAs digested with *Bgl*II. Each lane corresponds to infection with an HHV-6 strain. Lanes: 1, SIE; 2, TAN; 3, HST; 4, HBLV; 5, BOU; 6, MAR; 7, BLE; 8, MBE. The arrowheads indicate the positions of molecular size markers.

**Nucleotide sequence accession numbers.** The nucleotide sequence data reported here have been submitted to GenBank and assigned accession numbers M58512 and M58513.

## RESULTS

**Restriction mapping by Southern blotting.** DNA extracted from infected peripheral blood mononuclear cells was digested by various restriction enzymes and hybridized with cloned DNA fragments of HHV-6 strain SIE. Digestion with *Bgl*II and hybridization with cosmid cHC15, which contains a 30-kb *Bam*HI insert of HHV-6 DNA, revealed different profiles, depending on the viral strains tested (Fig. 2; Table 1). However, the cumulative molecular sizes of hybridized fragments were very similar for all strains, ranging from 31 to 35 kb, which was close to the size of the fragment used as the probe. The difference between the profiles of each possible pairwise combination of strains was scored. The comparison of scores allowed the following two groups of strains to be distinguished: SIE, TAN, and HBLV (Fig. 2, lanes 1, 2, and 4) on the one hand and HST, BOU, MAR, BLE, and MBE (Fig. 2, lanes 3, 5, 6, 7, and 8) on the other. These were designated groups I and II, respectively. The number of differences between two strains of the same group was much smaller than the number of differences between two strains from distinct groups (Table 1). Only three strains, HST, MAR, and BLE, exhibited identical profiles with *Bgl*II

TABLE 1. *Bgl*II DNA fragments visualized by hybridization with cHC15

| Sizes[a] (kb) of *Bgl*II DNA fragments of strain: | | | | | | | |
|---|---|---|---|---|---|---|---|
| SIE | TAN | HST | HBLV | BOU | MAR | BLE | MBE |
| 9 | 9 | —[b] | 9 | — | — | — | — |
| — | — | 7.4 | — | 7.4 | 7.4 | 7.4 | 7.4 |
| 6.5 | 6.5 | — | 6.5 | — | — | — | — |
| — | — | 4.6 | — | 4.6 | 4.6 | 4.6 | 4.6 |
| — | — | | 4 | — | — | — | — |
| — | 3.7 | 3.7 | — | 3.7 | 3.7 | 3.7 | — |
| 3.5 | 3.5 | — | 3.5 | 3.5 | — | — | 3.5 |
| 3.3 | — | — | 3.3 | 3.3 | — | — | 3.3 |
| 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| — | — | 2.7 | — | — | 2.7 | 2.7 | — |
| 2.1 | 2.1 | 2.1 | — | 2.1 | 2.1 | 2.1 | 2.1 |
| — | — | — | 2.0 | — | — | — | — |
| 1.9 | 1.9 | — | — | — | — | — | — |
| — | — | 1.8 | — | 1.8 | 1.8 | 1.8 | 1.8 |
| — | — | 1.6 | — | 1.6 | 1.6 | 1.6 | 1.6 |
| — | — | 1.4 | — | 1.4 | 1.4 | 1.4 | 1.4 |
| 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |
| 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| — | — | 0.5 | — | 0.5 | 0.5 | 0.5 | 0.5 |

[a] The size of each fragment was estimated by using molecular weight markers as described in Materials and Methods.
[b] —, Absence of the corresponding DNA fragment detected by hybridization.

digestion and cHC15 hybridization. When cHC15 hybridization was performed with DNA digested by *Hin*dIII, *Pvu*II, and *Sal*I, the differences between restriction patterns allowed the same two groups to be defined (Table 2). In this case, the restriction patterns were strictly identical within each group. DNA fragments obtained from *Bgl*II and *Hin*dIII digestion were also probed with cHC17. This cosmid contains a 20-kb *Bam*HI insert of SIE DNA which exhibits no sequence that overlaps with the cHC15 insert. No *Hin*dIII restriction site was evident in this region of HHV-6 DNA for any strain. On the other hand, cHC17 hybridized with several *Bgl*II fragments, the aggregate molecular sizes of which were comparable for all strains. Differences in restriction patterns allowed the same two groups, I and II, to be distinguished (data not shown).

TABLE 2. DNA fragments visualized by cHC15 hybridization after digestion with *Pvu*II, *Hin*dIII, and *Sal*I

| Sizes[a] (kb) of fragments obtained with: | | | | | |
|---|---|---|---|---|---|
| *Pvu*II | | *Hin*dIII | | *Sal*I | |
| I[b] | II[c] | I | II | I | II |
| 17 | —[d] | 23 | 23 | 27 | 27 |
| 16 | 16 | 15 | 15 | 20 | 20 |
| 7.4 | — | — | 6 | — | 9 |
| 5.6 | 5.6 | 5.9 | — | 6.2 | 6.2 |
| — | 5.4 | 5.4 | — | 5.4 | — |
| — | 4.6 | — | 5.1 | 3.7 | — |
| 3.5 | 3.5 | 3.1 | 3.1 | | |
| 1.5 | 1.5 | 2.5 | — | | |
| | | — | 2 | | |
| | | — | 0.7 | | |
| | | 0.6 | 0.6 | | |

[a] See Table 1, footnote *a*.
[b] I, Group I HHV-6 strains.
[c] II, Group II HHV-6 strains.
[d] See Table 1, footnote *b*.

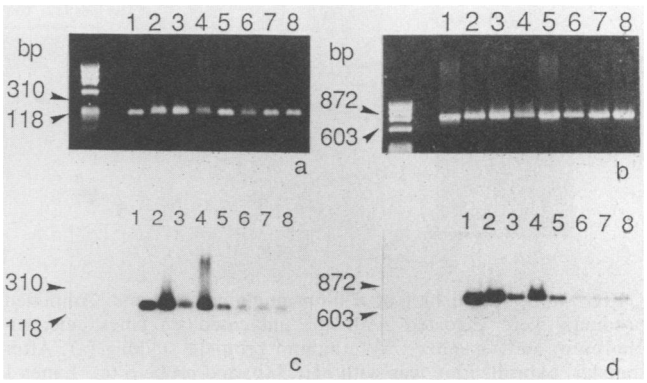


FIG. 3. Amplification of infected cell DNAs. Lanes 1 to 8 correspond to the eight HHV-6 strains, as in Fig. 1. Amplified products were visualized by ethidium bromide staining (a and b) and hybridization with labeled probe S (c and d). Amplification was performed with primer pair A-B (a and c) or A-C (b and d). The arrowheads indicate the positions of molecular size markers.

In addition, plasmid pHC5, containing an 850-bp *Bam*HI fragment of strain SIE DNA, was used for hybridization. All strains provided a single band after *Bam*HI digestion, whereas an internal *Hin*dIII restriction site was present in group II strains only. An internal restriction site for *Bgl*II was present in strains SIE, TAN, HST, HBLV, and MAR and absent from strains BOU, BLE, and MBE (data not shown).

All restriction mapping results were reproducible in at least three independent experiments using different passages of each strain. Furthermore, no positive signal was ever detected when DNA from uninfected cells was hybridized with probes cHC15, cHC17, and pHC5.

**Amplification by PCR.** To investigate the genetic polymorphism of HHV-6 strains by another approach, DNA from infected cells was submitted to specific amplification by PCR in the region corresponding to the pHC5 insert as described in Materials and Methods and illustrated in Fig. 1. The expected amplification products were obtained for all strains, both with 249-bp amplification and with 830-bp amplification (Fig. 3a and b, respectively). Hybridization with specific oligonucleotide S gave a positive signal in all cases (Fig. 3c and d). However, although comparable amounts of amplified DNA were present, as visualized by ethidium bromide staining, the hybridization signals were much stronger for group I than for group II strains (compare, for instance, HST and HBLV in Fig. 3a to d, lanes 3 and 4), suggesting lower affinity of the probe for amplified DNAs from group II strains. This was confirmed by increasing the stringency of washing: when membranes were washed at 55 instead of 50°C, positive hybridization signals were found only with group I strains (data not shown).

**Restriction enzyme patterns of amplified products.** The fragments obtained by the 830-bp amplification reaction were purified and analyzed by restriction enzyme digestion. Restriction fragments were visualized with ethidium bromide staining, hybridization with pHC5, and hybridization with probe S (Fig. 4a to c, respectively). As expected, probe S hybridized with a single fragment and gave a weaker signal for group II than for group I strains. On the other hand, pHC5 gave a positive signal with several fragments. This plasmid did not hybridize strongly with the smallest fragments because of the highly stringent conditions used. The combination of these three detection methods in multiple

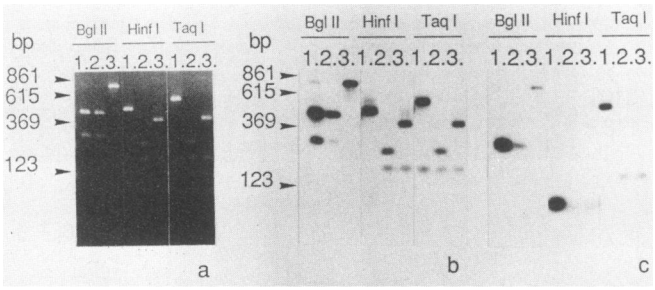


FIG. 4. Southern blot of 830-bp amplified products. Amplified products were digested with the indicated enzymes, electrophoresed, and visualized by ethidium bromide staining (a). After transfer, hybridization was with pHC5 (b) and probe S (c). Lanes 1 to 3 correspond to strains SIE, HST, and MBE, respectively. The arrowheads indicate the locations of molecular size markers.


FIG. 5. Partial nucleotide sequences of amplified DNA fragments from strains SIE and HST. The sequence of strain HST (lines II) is compared with that of strain SIE (lines I). Nucleotide changes are indicated by boxed letters, and new restriction sites resulting from the modifications in the HST sequence are shown. Each number refers to the first nucleotide of the sequenced region. The bold horizontal line defines the location of probe S.

experiments was needed to determine the complete restriction profile for four enzymes (Table 3). Digestion with *Hin*dIII permitted us to distinguish group I strains, which have no internal restriction site, from group II strains, which do exhibit one. This confirms the results of Southern blotting with total DNA. All group I strains exhibited identical patterns after digestion with *Bgl*II, *Hin*fI, or *Taq*I (Fig. 4, lanes 1), whereas by using these enzymes, two restriction profiles could be defined among group II strains (Fig. 4, lanes 2 and 3). The first one was exhibited by strains HST and MAR, and the second one was exhibited by strains BOU, BLE, and MBE. The difference between these two profiles could be attributed to the modification of a single restriction site for each enzyme. Hence, despite this additional subdivision among group II strains, the genetic polymorphism of the amplified 830-bp fragment correlated with the variability observed in the Southern blot analysis of infected-cell DNA when large probes were used.

**Partial nucleotide sequencing of the amplified fragment.** To investigate the basis of the HHV-6 variability more accurately, partial nucleotide sequencing of the amplified 830-bp fragment was performed for two group I strains, SIE and TAN, and two group II strains, HST and MBE (Fig. 5). In the 163-bp region analyzed, no difference in nucleotide

TABLE 3. Restriction enzyme patterns of the 830-bp fragment obtained by PCR

| Restriction enzyme | Sizes[a] (bp) of fragments obtained with strain: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SIE | TAN | HST | HBLV | BOU | MAR | BLE | MBE |
| *Hin*dIII | 830 | 830 | — | 830 | — | — | — | — |
| | —[b] | — | 610 | — | 610 | 610 | 610 | 610 |
| | — | — | 220 | — | 220 | 220 | 220 | 220 |
| *Hin*fI | 530 | 530 | — | 530 | — | — | — | — |
| | — | — | — | — | 450 | — | 450 | 450 |
| | — | — | 300 | — | — | 300 | — | — |
| | — | — | 200 | — | 200 | 200 | 200 | 200 |
| | — | — | 150 | — | — | 150 | — | — |
| | 110 | 110 | — | 110 | — | — | — | — |
| | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 90 | 90 | 90 | 90 | 90 | 90 | 90 | 90 |
| *Taq*I | 630 | 630 | — | 630 | — | — | — | — |
| | — | — | — | — | 450 | — | 450 | 450 |
| | — | — | 270 | — | — | 270 | — | — |
| | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| | — | — | 180 | — | 180 | 180 | 180 | 180 |
| | — | — | 160 | — | — | 160 | — | — |
| *Bgl*II | — | — | — | — | 830 | — | 830 | 830 |
| | 530 | 530 | 530 | 530 | — | 530 | — | — |
| | 300 | 300 | 300 | 300 | — | 300 | — | — |

[a] The size of each fragment was estimated by using molecular weight markers as described in Materials and Methods.
[b] —, Absence of the corresponding DNA fragment.

sequence was found either between SIE and TAN or between HST and MBE. When the HST sequence was compared with that of SIE, only six point differences were detected, which defined 96% homology between the two strains. Two modifications were located at positions 43 and 46, in the 23-bp region corresponding to probe S. The sequence of S is derived from strain SIE, and this could explain the lower affinity of this probe to products amplified from group II strains. The A-to-G change at position 151 induces the appearance of two additional restriction sites, one *Hin*dIII site and one *Taq*I site. The changes at positions 43 and 160 create two new *Hin*fI sites. These findings were consistent with the results of restriction enzyme analysis. In addition, a unique and identical open reading frame was found in the region sequenced for both strains. When HST was compared with SIE, the nucleotide changes observed led to two substitutions in the predicted amino acid sequence: the modification at position 43 induced an Ile-to-Met change, and the two modifications at positions 79 and 81 induced a modification from Asn to Gln. None of the six nucleotide changes introduced a nonsense codon in the open reading frame of the HST DNA sequence.

## DISCUSSION

We studied eight different HHV-6 strains by Southern blotting and PCR. Both methods evidenced genetic variations which, taken together, allowed one strain to be distinguished from another. Moreover, genetic polymorphism permitted two distinct subgroups, I and II, to be recognized among the eight strains studied. Although Southern blotting was applied to regions of DNA of sizes and locations different from those explored by PCR, both methods led to the same primary classification.

Although marked differences in restriction profiles between the two groups were observed, many arguments are consistent with the idea that all of these strains belong to the same virus species. (i) Our study with Southern blotting explored an aggregate DNA length of at least 50 kb, which corresponds to about 30% of the HHV-6 DNA, and for all strains the cumulative molecular sizes of hybridized fragments were similar and closely related to the complexity of the fragment used as the probe. This situation was clearly distinct from that reported recently in the isolation of a new virus designated HHV-7 (12). (ii) PCR allowed amplification of the expected 830-bp fragment from the DNAs of all eight strains. It might be argued that this region was so highly conserved among human herpesviruses that it was not HHV-6 specific. However, the amplification was not possible with DNAs from other human herpesviruses, such as herpes simplex virus type 1, varicella-zoster virus, cytomegalovirus, and Epstein-Barr virus (8). Moreover, the amplified region exhibited genetic polymorphism analogous to that evidenced in larger parts of the HHV-6 genome. (iii) The partial nucleotide sequencing of four strains indicated 96% homology between the two groups of HHV-6 strains. (iv) In addition, the results of specific immunofluorescence staining with a reference HHV-6-positive human serum, as well as growth kinetics and susceptibilities to antiviral drugs, were similar for all strains (unpublished data; HBLV was not tested for antiviral susceptibility).

The genetic polymorphism of HHV-6 has already been described (14). In particular, Jarrett and coworkers and Kikuta and coworkers reported differences in *Hin*dIII patterns (13, 16) which were in agreement with the present findings. It is unlikely that differences observed in restriction profiles were due to genomic inversions or other putative forms of genome reorganization, since most of these differences were highly reproducible and did not concern fragments present in submolar ratios. Sequencing data indicated that the modification of restriction sites was mainly the consequence of point mutations, some of which were assumed to be silent mutations. As shown with PCR, such genetic polymorphism could also alter the detection of HHV-6 sequences with specific oligonucleotide probes, and this must be kept in mind for diagnosis.

The classification founded on polymorphism provides a basis for the characterization of new HHV-6 isolates. We acknowledge that the classification into two groups is arbitrary and limiting, since most strains can also be distinguished from one another inside the same group. Furthermore, preliminary observations indicate that the search for molecular markers of pathogenicity using restriction enzyme patterns is hazardous. The HST strain was isolated from a child with exanthem subitum, and it could not be distinguished from the MAR strain from an infant with no symptom of the disease. Among the eight strains studied, group I strains were isolated from immunocompromised adults whereas group II strains were obtained from children. This discrimination might be coincidental and needs further investigations, including a much larger number of isolates. As far as the geographic origin of the strains is concerned, our approach to the genetic polymorphism of HHV-6 provided no specific marker: for instance, strain HST from Japan could not be distinguished from strain MAR from France, although no obvious epidemiological link was found between them.

Nevertheless, genetic polymorphism could be a useful tool for other epidemiological studies. Indeed, the distinction between two strains of the same viral species is of value for studying virus transmission. Analysis of DNA restriction patterns would allow investigation of blood-borne transmission or mother-to-child infection, for example. Similarly, these procedures would allow endogenous reactivation of latent HHV-6 to be differentiated from a new exogenous infection. Molecular investigations have proved extremely helpful in addressing these matters for other human herpesviruses, such as cytomegalovirus (1). Hence, the ability of PCR to provide fragments for restriction mapping or nucleotide sequencing can be considered a promising contribution to molecular epidemiology studies. The efficiency of enzymatic amplification allows this type of analysis with human samples, even in the absence of virus culture. It can therefore be expected that analysis of HHV-6 polymorphism by PCR will provide significant insights into the pathogenicity and biological properties of this virus.

## ACKNOWLEDGMENTS

We thank S. Z. Salahuddin for the gift of strain HBLV, K. Yamanishi for the gift of strain HST, O. Fichot for helpful advice on nucleotide sequence determination, and K. M. Kean for critical review of the manuscript.

This work was supported by the Caisse Regionale d'Assurance Maladie d'Ile de France and the Agence Nationale de Recherches sur le SIDA.

## REFERENCES

1. **Adler, S. P.** 1985. The molecular epidemiology of cytomegalovirus transmission among children attending a day care center. J. Infect. Dis. **152**:760–768.
2. **Agut, H., H. Collandre, J. T. Aubin, D. Guetard, V. Favier, D. Ingrand, L. Montagnier, and J. M. Huraux.** 1989. In vitro

sensitivity of human herpesvirus-6 to antiviral drugs. Res. Virol. **140**:219–228.
3. **Agut, H., D. Guetard, H. Collandre, C. Dauguet, L. Montagnier, J. M. Miclea, H. Baurmann, and A. Gessain.** 1988. Concomitant infection by human herpesvirus 6, HTLV-1 and HIV-2. Lancet **i**:712.
4. **Agut, H., D. Kerouedan, P. M'Pele, H. Collandre, C. Samba-Lefevre, M. Rosenheim, D. Ingrand, A. Itoua-Ngaporo, M. Gentilini, L. Montagnier, and J. M. Huraux.** 1989. Cocultivation of human herpesvirus 6 and HIV-1 from blood lymphocytes. Res. Virol. **140**:23–26.
5. **Becker, W. B., S. Engelbrecht, M. L. B. Becker, C. Piek, B. A. Robson, L. Wood, and P. Jacobs.** 1989. New T-lymphotropic human herpesvirus. Lancet **i**:41.
6. **Collandre, H.** Unpublished data.
7. **Collandre, H., J. T. Aubin, H. Agut, J. M. Béchet, and L. Montagnier.** J. Virol. Methods, in press.
8. **Collandre, H., et al.** Submitted for publication.
9. **Denhardt, D. T.** 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–652.
10. **Downing, R. G., N. Sewankambo, D. Serwadda, R. Honess, D. Crawford, R. Jarrett, and B. E. Griffin.** 1987. Isolation of human lymphotropic herpesviruses from Uganda. Lancet **ii**:390.
11. **Efstathiou, S., U. A. Gompels, M. A. Craxton, R. W. Honess, and K. Ward.** 1988. DNA homology between a novel human herpesvirus (HHV-6) and human cytomegalovirus. Lancet **i**:63–64.
12. **Frenkel, N., E. C. Schirmer, L. S. Wyatt, G. Katsafanas, E. Roffman, R. M. Danovich, and C. H. June.** 1990. Isolation of a new herpesvirus from human CD4+ T cells. Proc. Natl. Acad. Sci. USA **87**:748–752.
13. **Jarrett, R. F., A. Gallagher, S. Gledhill, M. D. Jones, I. Teo, and B. E. Griffin.** 1989. Variation in restriction map of HHV-6 genome. Lancet **i**:448–449.
14. **Josephs, S. F., D. V. Ablashi, S. Z. Salahuddin, B. Kramarsky, B. R. Franza, P. Pellett, A. Buchbinder, S. Memon, F. Wong-Staal, and R. C. Gallo.** 1988. Molecular studies of HHV-6. J. Virol. Methods **21**:179–190.
15. **Josephs, S. F., S. Z. Salahuddin, D. V. Ablashi, F. Schachter, F. Wong-Staal, and R. C. Gallo.** 1986. Genomic analysis of the human B-lymphotropic virus (HBLV). Science **234**:601–603.
16. **Kikuta, H., H. Lu, S. Matsumoto, S. F. Josephs, and R. C. Gallo.** 1989. Polymorphism of human herpesvirus 6 DNA from five Japanese patients with exanthem subitum. J. Infect. Dis. **160**: 550–551.
17. **Lawrence, G. L., M. Chee, M. A. Craxton, U. A. Gompels, R. W. Honess, and B. G. Barrell.** 1990. Human herpesvirus 6 is closely related to human cytomegalovirus. J. Virol. **64**:287–299.
18. **Lopez, C., P. Pellett, J. Stewart, C. Goldsmith, K. Sanderlin, J. Black, D. Warfield, and P. Feorino.** 1988. Characteristics of human herpesvirus 6. J. Infect. Dis. **157**:1271–1273.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim.** 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230**:1350–1354.
21. **Salahuddin, S. Z., D. V. Ablashi, P. D. Markham, S. F. Josephs, S. Sturzenegger, M. Kaplan, G. Halligan, P. Biberfeld, F. Wong-Staal, B. Kramarsky, and R. C. Gallo.** 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science **234**:596–601.
22. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
23. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
24. **Takahashi, K., S. Sonoda, K. Kawakami, K. Miyata, T. Oki, T. Nagata, T. Okuno, and K. Yamanishi.** 1989. Human herpesvirus 6 and exanthem subitum. Lancet **i**:1463.
25. **Tedder, R. S., M. Briggs, C. H. Cameron, R. W. Honess, D. Robertson, and H. Whittle.** 1987. A novel lymphotropic herpesvirus. Lancet **ii**:390–392.
26. **Yamanishi, K., T. Okuno, K. Shiraki, M. Takahashi, T. Kondo, Y. Asano, and T. Kurata.** 1988. Identification of human herpesvirus 6 as causal agent for exanthem subitum. Lancet **i**:1065–1067.