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Definition and Distribution Analysis of Glycoprotein B Gene Alleles of Human Herpesvirus 7

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As for other herpesviruses, glycoprotein B (gB) of human herpesvirus 7 (HHV-7) is believed to play a major role in virus infection and as a target of the host immunogenic response. Using nested PCR, we amplified the whole HHV-7 gB gene from 108 human peripheral blood mononuclear cell samples and studied its variability. By means of restriction fragment length polymorphism (RFLP) analysis, three distinct patterns, designated I, II, and III, were defined and detected at frequencies of 93, 5, and 2%, respectively. Determination of the nucleotide sequence allowed us to recognize five critical positions in the gB gene with six specific combinations of point changes at these positions. These combinations were gB alleles A, B, C, D, E, and F. Alleles D and E corresponded to RFLP patterns II and III, respectively, while the other four alleles corresponded to RFLP pattern I. Identical gB alleles were detected in serial samples as well as in paired samples of blood and saliva from the same individuals, except for one case. In contrast, the distribution of gB alleles differed according to the geographical origin of the human samples: C was the most frequent allele in both African and Caribbean samples, whereas F was the most frequent allele in European ones. Although none of the allele-specific nucleotide changes induced any modification at the protein level, the definition of gB alleles provided convenient viral markers for the study of both HHV-7 infections and human population genetics.

Human herpesvirus 7 (HHV-7) was first isolated from purified stimulated $CD4^+$ T cells from a healthy individual (15). Later on, HHV-7 was isolated from stimulated peripheral blood mononuclear cells (PBMCs) from a patient with chronic fatigue syndrome and an infant with febrile syndrome (3, 25). Epidemiological studies have demonstrated that HHV-7 is widely spread in the human population, with a prevalence exceeding 90%, and that primary infection occurs early in life (11, 31, 33). Saliva contains infectious viruses and is suspected to be the source of human transmission (5, 32). HHV-7 is a member of the *Betaherpesvirinae* subfamily and is closely related to human herpesvirus 6 (HHV-6) (4, 13, 22, 23). As yet, no clear association of HHV-7 with a human disease has been convincingly reported. Interestingly, the tropism of HHV-7 is restricted to $CD4^+$ lymphocytes (6, 15), and the CD4 protein is involved in the cell receptor for this herpesvirus, resulting in a competitive interaction between human immunodeficiency virus and HHV-7, at least in vitro (21).

The genetic variability of herpesviruses is of major interest. For Epstein-Barr virus, human cytomegalovirus (HCMV), and HHV-6 strains, this variability correlates with phenotypic differences, such as B-cell immortalization capacity (27), immunogenicity (8), and cell tropism (26), respectively. In addition, it has provided useful markers for epidemiological studies (2, 9, 10, 12, 14). So far, the variability of HHV-7 strains has not been extensively studied, but preliminary data have indicated a very high degree of homology between different HHV-7 strains (13, 22, 23). For instance, a comparison of the sequences of HHV-7 strains JI and IM over a 1,062-bp-long genomic region, including parts of open reading frames (ORF) U10 and U11 and their intergenic region, showed strict identity between these two strains (24). One study revealed a polymorphism in the number of telomere-like repeated sequences located at the left end of each terminal repeat of the HHV-7 genome (30). This genetic polymorphism was shown to be stable over time in an infected individual and after virus passages in vitro but seemed to be strain specific. In addition, it has been hypothesized that the number of *Dra*I internal repeats in the R2 region may be a strain-specific genetic marker (23).

Recently, we focused on an analysis of the glycoprotein B (gB) gene of HHV-7. gB is considered well conserved among herpesviruses and plays an important role in the early events of virus-cell interactions (8, 19, 28). It is also known to be the target of neutralizing antibodies and other immune effectors. In addition, studies of the interstrain variability of HCMV and HHV-6 in the gB gene revealed a genetic polymorphism that was related to either group-specific or variant-specific phenotypic differences (8, 19).

Regarding HHV-7 gB, our initial strategy was to investigate a large number of HHV-7 isolates. However, HHV-7 isolation from human samples was difficult due to the restricted permissiveness of cell cultures and the low viral load in specimens. We therefore amplified the whole gB gene from numerous samples by means of nested PCR and characterized the amplified DNA products. Here, we present the results of an analysis of 108 PBMC samples which allowed us both to confirm the high level of conservation of the HHV-7 gB gene and to define specific alleles of this gene.

MATERIALS AND METHODS

Samples. PBMC samples were obtained from 228 unrelated individuals for whom a virological diagnosis procedure had been performed in our laboratory: 45 subjects were at risk of retrovirus infection, 90 subjects suffered from chronic fatigue syndrome and had been tested for HHV-6 infection, 47 subjects had cardiac transplants, and 46 healthy subjects from various geographical origins were studied as controls. The PCR HHV-7-positive subjects were classified according to their birthplaces into four groups: Europeans ($n = 69$), the majority of whom were French; Asians ($n = 5$), from China ($n = 1$), Vietnam ($n = 1$),

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TABLE 1. Oligonucleotides used for PCR and sequencing of the HHV-7 gB gene

Primer	Sequence (5′→3′)[a]	Sense	Location[b]	Use
gBN1	GCAAGTTTCCCATATACCGGCTCC	+	59896–56918	PCR
gBN2	CATTAAGCCGGGTGCTCCATCGTG	−	54279–54303	PCR
gB1bam	acacggatccATGAAAATTCTATTCCTTGAGTG	+	56849–56871	PCR, sequencing
gB2sal	acacgtcgacTCACAGTTCTTCTGTTGA	−	54401–54418	PCR
gBMF1	CCATCATCAAACGTTTTCAGC	−	56109–56130	Sequencing
gBMF4	TAACGGAATCAAATCCAACG	−	56390–56409	Sequencing
gBMF5	TTACCGTACACTGCTGAAATG	−	55486–55506	Sequencing

[a] Lowercase letters correspond to additional sequences not present in the HHV-7 genome.
[b] Based on the JI nucleotide sequence.

India (n = 1), and Sri Lanka (n = 2); Africans (n = 16), from Congo (n = 1), Mali (n = 5), Zaire (n = 2), Senegal (n = 3), Guinea (n = 1), and Ivory Coast (n = 4); and Caribbeans (n = 18). For some of these subjects, saliva samples collected simultaneously with PBMC samples and/or serial PBMC samples collected monthly were also available for study. All of the samples were coded and further tested in a blind manner.

Preparation of DNA samples. PBMCs were Ficoll purified from heparinized blood specimens. Cells were lysed in TE (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) buffer containing 0.5% sodium dodecyl sulfate and 0.2 mg of proteinase K per ml for 18 h at 37°C. Nucleic acids were extracted by phenol-chloroform treatment and ethanol precipitated. Nucleic acids from 1 ml of whole saliva samples were purified as described previously (17).

DNA amplification. Samples corresponding to 1 μg of PBMC DNA or 100 μl of saliva were subjected to nested PCR by use of a hot-start procedure with AmpliWax (Perkin-Elmer, Norwalk, Conn.). The first round of PCR, with outer primers gBN1 and gBN2 (Table 1), included 30 cycles, each cycle consisting of a denaturation step at 92°C for 1 min, a primer annealing step at 55°C for 1 min, and an elongation step at 72°C for 4 min. In the first cycle, the denaturation step was increased to 5 min. The second amplification round, with inner primers gB1bam and gB2sal (Table 1), included 40 cycles with step characteristics identical to those given above. Reaction volumes were 50 and 100 μl for the first and second PCRs, respectively. Five microliters of the first-round amplification mixture was secondarily amplified. In order to increase the fidelity of polymerase activity, we used a mixture of *Taq* polymerase (Perkin-Elmer) and *Pfu* DNA polymerase (Stratagene, La Jolla, Calif.) at a respective unit ratio of 29:1. The use of biotinylated primers during the second round of nested PCR allowed us to obtain a single-stranded DNA matrix for nucleotide sequencing experiments (see below). PCR products were electrophoresed on 1.5% agarose gels, visualized after ethidium bromide staining, and compared to a 500-bp DNA molecular weight marker ladder (Gibco-BRL, Paisley, United Kingdom). The steps of sample DNA extraction, mixture preparation, the two rounds of nested PCR, and PCR product analysis were done in four different laboratory areas to prevent PCR carryover. Negative controls and blank reactions were systematically included in each PCR.

Characterization of amplified products. Amplification products were extracted from agarose gels by the Jetsorb procedure (Genomed, Montreuil, France). Purified DNA fragments corresponding to the whole HHV-7 gB gene were digested with the restriction endonucleases *Hin*fI, *Bst*EII, *Dra*I, *Apa*I, *Hin*dIII, and *Sfa*NI in accordance with the manufacturer's instructions (Boehringer, Mannheim, Germany). Positive digestion controls were added in each experiment when the loss of a unique restriction site was suspected. Digestion products were electrophoresed on 1.5 to 2% agarose gels, visualized after ethidium bromide staining, and compared to a 123-bp DNA molecular weight marker ladder (Gibco-BRL).

Nucleotide sequence determination. Sequence data for 2,470 bp from the gB gene of samples IM and 7660 were obtained with PCR sequencing procedures and an automated sequencer (ABI, Foster City, Calif.). A portion of the purified amplification products was submitted to alkaline denaturation and single-stranded DNA separation with streptavidin-coated magnetic beads (Dynal, Oslo, Norway) before nucleotide sequencing by the general dideoxynucleotide chain termination method. Sequencing was performed by use of a T7 polymerase sequencing kit (Pharmacia, Uppsala, Sweden) with [α-^{35}S]dATP and oligonucleotides gB1bam, gBMF1, gBMF4, and gBMF5 (Table 1). Partial nucleotide sequencing was performed twice with distinct DNA templates obtained from separate PCRs in order to assess the reproducibility of results.

Statistical analysis. Qualitative analysis of allele distribution was performed by means of a chi-square test or Fisher's exact test as appropriate with the software StatView 4.5 (Abacus Concepts, Berkeley, Calif.).

RESULTS

Analysis of HHV-7 gB-specific PCR products by means of restriction fragment length polymorphism (RFLP). Of the 212 PBMC and 15 saliva samples tested, 108 (51%) and 8 (53%), respectively, provided HHV-7 gB-specific PCR products and were considered for further molecular analysis.

The 108 distinct PCR products obtained from PBMC samples were submitted to digestion with five different restriction endonucleases, the cleavage sites of which were determined to be present in the HHV-7 gB gene on the basis of the published sequence of reference strain JI (23).

As shown in Table 2, no variation in digestion pattern was observed with *Hin*fI, *Dra*I, and *Taq*I with regard to both the number of fragments and their lengths. Only seven cleavage sites of the eight expected from the nucleotide sequence of JI were found with *Dra*I for all of the samples tested. In contrast, variability was observed with regard to the *Apa*I and *Bst*EII digestion patterns, these two enzymes exhibiting nonoverlapping unique cleavage sites, as determined on the basis of the JI sequence. This finding allowed us to define three RFLP patterns, designated I, II, and III. Pattern I, corresponding to the presence of both *Apa*I and *Bst*EII cleavage sites, was the most frequent (101 of 108; 93%). Pattern II, corresponding to the absence of both sites, was found in five cases (5%), and pattern III, corresponding to the presence of the *Bst*EII site alone, was found in two cases (2%). A fourth possible theoretical pattern, corresponding to the presence of the *Apa*I site alone, was not found in our study. Patterns II and III were found only in European samples (Table 2). However, the absence of detection of these two patterns in African, Caribbean, and Asian samples might have been related to the low overall frequencies of these patterns and the low number of subjects in these groups compared to the European group. These preliminary results prompted us to perform a more accurate analysis of gB gene polymorphisms.

TABLE 2. Restriction analysis of HHV-7 gB-specific PCR products

Samples		No. of cleavage sites per enzyme					RFLP pattern
Geographical origin	No.	*Hin*fI	*Dra*I	*Taq*I	*Apa*I	*Bst*EII	
European	62	8	7	4	1	1	I
	5	8	7	4	0	0	II
	2	8	7	4	0	1	III
African	16	8	7	4	1	1	I
Caribbean	18	8	7	4	1	1	I
Asian	5	8	7	4	1	1	I
All	101	8	7	4	1	1	I
	5	8	7	4	0	0	II
	2	8	7	4	0	1	III

TABLE 3. Nucleotide sequence analysis of the HHV-7 gB gene

Sample	Nucleotide sequence at crucial codons of the gB gene[a]					No. (%) showing sequence[b]	RFLP pattern
	119	137	185	220	406		
Prototype							
IM	GTG	TCA	TTT	GGG	ATT	NA	I
7660	**GTA**	**TCG**	**TTC**	**GGC**	**ATC**	NA	II
gB allele							
A	GTG	**TCG**	**TTC**	GGG	ATT	1 (2)	I
B	GTG	**TCG**	TTT	GGG	ATT	3 (6)	I
C	GTG	**TCG**	**TTC**	GGG	**ATC**	20 (41)	I
D	**GTA**	**TCG**	**TTC**	**GGC**	**ATC**	5 (10)	II
E	**GTA**	TCA	TTT	GGG	ATT	1 (2)	III
F	GTG	TCA	TTT	GGG	ATT	19 (39)	I

[a] Based on the codon numbers of the published JI sequence (23); the codon sequences of sample 7660 are indicated in bold.
[b] Number of samples out of the 49 tested exhibiting the indicated genetic profile. NA, not applicable.

Nucleotide sequence analysis of the HHV-7 gB gene. In a first step, the complete nucleotide sequence of the gB gene was obtained from two different HHV-7-positive samples, IM and 7660, which exhibited RFLP patterns I and II, respectively. The IM sample was the source of the HHV-7 IM isolate, which was previously characterized (24) and which exhibited pattern I after serial propagation in cell cultures (data not shown). Sequencing data for IM and 7660 were compared. Among the 2,700 bp studied (nucleotide sequence positions 54401 to 57101, including a portion of the 3′ end of the upstream U40 ORF), only five positions were shown to exhibit genetic polymorphisms, indicating a very high degree of nucleotide sequence conservation (99.8% homology). Moreover, the genetic variations depicted in Table 3 altered the third base of each codon and did not result in any change in the predicted amino acid sequence. This finding implied the perfect conservation of the gB protein sequence for the two distinct samples IM and 7660. As expected, two of the five crucial positions fit the polymorphisms of restriction patterns observed previously: the G-to-A substitution at codon 119 induced the disappearance of the unique *Apa*I site, and the G-to-C substitution at codon 220 induced the disappearance of the unique *Bst*EII site. The other three positions did not correspond to nucleotide sequences accessible to restriction analysis. A comparison of the IM and 7660 sequences with the JI sequence (GenBank Data Library accession no. U43400) revealed an additional change at codon 436: an A-to-G substitution in JI resulted in the appearance of one additional theoretical *Dra*I site which was not present in any of the 108 HHV-7-positive samples that we tested. This finding led to the predicted change of an aspartic acid residue (for IM and 7660) into an asparagine residue (for JI). This additional change did not affect significantly the general conclusion that the gB gene of HHV-7 was highly conserved, with 99.8% nucleotide sequence homology as established from the comparison of three distinct viruses.

Definition of HHV-7 gB gene alleles. The finding of three RFLP patterns with the *Apa*I and *Bst*EII enzymes as well as the recognition of five critical positions in the nucleotide sequence raised the question of the interpretation of gB gene polymorphisms. In order to obtain a more complete view of this phenomenon, the genetic study was expanded to 49 different HHV-7-positive samples, including IM and 7660, from among the 108 previously characterized by means of restriction analysis. These samples were selected in order to be representative of diverse restriction patterns and geographical origins. Given the high degree of conservation observed for IM, 7660, and JI, determination of the nucleotide sequence was restricted to the regions containing the five critical positions defined above. Partial sequencing was performed on 783 bp (261 codons) for each of the 49 samples studied. No other genetic variation besides the five already known was detected during this analysis. Surprisingly, among the 32 theoretical possible associations between the sequences of the five critical positions, only 6 were detected with different frequencies (Table 3). This finding strongly suggested that these genetic point alterations did not occur at random and were apparently associated in a stable manner. These diverse combinations might be considered allelic forms of the gB gene. The six specific combinations were arbitrarily designated by letters A to F: alleles A, B, C, and F corresponded to RFLP pattern I, allele D corresponded to pattern II, and allele E corresponded to pattern III. Alleles C and F were the most frequently detected, corresponding to 41 and 39% of all the samples tested, respectively.

Distribution of gB gene alleles according to geographical origin, infection follow-up, and body compartment. The definition of gB alleles as specific stable combinations of silent genetic point alterations might be questioned if the distribution of such alleles were shown to occur at random in the general population. As reported in Table 3, the frequency of gB alleles ranged from 2% (E) to 41% (C), strongly suggesting a nonrandom distribution. When the group of 43 samples exhibiting RFLP pattern I was studied, the distribution of corresponding alleles was again heterogeneous, despite the fact that the definition of alleles had not been used for the selection of samples: 1 sample (2%) was A, 3 samples (7%) were B, 20 samples (46%) were C, and 19 samples (44%) were F. A marked imbalance was also evident when the distribution of gB alleles was studied according to the geographical origin of samples (Table 4): the C allele was the most frequent allele in African and Caribbean samples, while the F allele was the most frequent allele in European samples, the difference in distribution being highly significant ($P = 0.01$, chi-square test). Interestingly, no significant distribution difference was found when African samples were compared with Caribbean ones.

Conversely, if gB alleles truly were stable combinations of specific genetic alterations, one should expect that the same gB allele would be found in serial samples from the same individual. PBMC samples obtained monthly from three individuals were analyzed, and the same gB allele was repeatedly detected in each (Table 5). In two subjects (7660 and 3562), the gB allele detected was D, an allele which was not frequently found among the samples that we tested. This repeated detection of an uncommon allele in serial samples from the same individual provided additional circumstantial evidence that gB alleles were not artifacts but were stable genetic entities. Similarly, we characterized the gB alleles of paired PBMC and saliva samples collected at the same time and found concomitantly

TABLE 4. Geographical distribution of HHV-7 gB alleles

Samples		No. of samples with the following gB allele[a]					
Geographical origin	No.	A	B	C	D	E	F
European	19	0	2	2	5	1	9
African	7	1	1	3	0	0	2
Caribbean	18	0	0	13	0	0	5
Asian	5	0	0	2	0	0	3
All	49	1	3	20	5	1	19

[a] See Table 3 for the sequences of gB alleles.

TABLE 5. Detection of HHV-7 gB alleles in serial blood specimens obtained from the same subject

Subject	gB allele[a] found in blood sample at mo:			
	0	1	2	3
7660	D[b]	D	D	D
3562	D	D	NT[c]	NT
1962	F	F	F	NT

[a] See Table 3 for the sequences of gB alleles.
[b] The entire nucleotide sequence of the gB gene has been determined for this sample (see text and Table 3).
[c] NT, not tested.

HHV-7 positive (Table 6). For six subjects, the gB allele was identical in the PBMC and saliva samples, whether this allele was very frequent (C and F) or uncommon (B). This finding confirmed the hypothesis that gB alleles were stable genetic combinations independent of the specimen tested. However, in subject 39, there was a discrepancy between PBMCs and saliva: the gB allele was F in PBMCs and D in saliva. As discussed below, the most likely explanation for this discrepancy was the existence of a mixed infection of the same individual with two different strains of HHV-7.

DISCUSSION

Among the 108 PBMC samples that we studied, the gB gene of HHV-7 appeared to be highly conserved in terms of amino acid sequence, with 99.8% homology at the nucleotide sequence level. Our sequence findings were in very good agreement with those previously published for the gB gene (20, 22, 23). However, a change at codon 436 inducing the appearance of an additional *Dra*I restriction site in the nucleotide sequence of HHV-7 strain JI (23) was not found in any of our HHV-7-positive samples. Similarly, a change at codon 502 inducing the appearance of an extra *Sfa*NI restriction site in the nucleotide sequence of the Japanese HHV-7 strain KHR (20) was not found in our samples (data not shown). Whether these changes correspond to an adaptation of HHV-7 strains to cell cultures or additional sites of genetic polymorphism in HHV-7 isolates infecting humans remains to be determined. Nevertheless, these two particular changes did not conflict with the high homology observed between the viruses present in our samples and the few previously characterized strains of HHV-7. This high homology may be considered surprising. Like other members of the *Betaherpesvirinae* subfamily, HHV-7 gB, a constituent of the virus envelope, is believed to be involved in interactions with a cell receptor(s), in particular, virus binding to cell surface proteoglycans (28), and to behave as a major target for the immunologic response. Therefore, this gene might be expected to be under high selection pressure resulting from the action of immune effectors as well as patterns of cell protein expression; this selection pressure would be the source of genetic diversity. On the other hand, HHV-7 is ubiquitous, and preliminary studies indicate that it is not a major pathogen. The high level of conservation of gB might then reflect the high degree of adaptation of the virus to its natural host and the absence of high evolutionary pressure following the divergence of HHV-7 strains. This finding is important for the development of novel tools dedicated to the study of HHV-7 infection in basic science as well as in clinical diagnosis. The gB protein and the gB gene now appear to be convenient targets for generating HHV-7-specific universal reagents, such as monoclonal antibodies, recombinant proteins, primers, and probes.

Despite its high level of conservation, the HHV-7 gB gene was found to exhibit a certain degree of polymorphism, albeit to a lesser extent than the gB genes of other herpesviruses, such as HCMV and HHV-6 (7–10). For HCMV and HHV-6, the variability of the gB gene was found to induce amino acid changes which resulted in distinct virus phenotypes. A 4% divergence at the amino acid level was reported between HHV-6 variants A and B (10), and these two variants could be distinguished by means of gB epitopes recognized by monoclonal antibodies (7). HCMV gB genotypes, which might differ with geographical origin (34), like HHV-7 gB genotypes, were reported to induce various degrees of severity of disease expression (16, 29). Clearly, the level of HHV-7 gB gene variability was not so high and did not support the classification of HHV-7 strains into variants, types, or subtypes, as for the two other members of the *Betaherpesvirinae* subfamily.

We identified five critical positions for gB gene polymorphisms; the stable combination of specific changes at these positions allowed us to define six allelic forms of the gene. It is possible that we incorrectly interpreted simple PCR artifacts, but this hypothesis could be easily ruled out. *Taq* DNA polymerase copying errors were not expected to occur in such a reproducible manner in so many independent assays. Similarly, cross-contamination of numerous samples by a limited panel of PCR products mimicking gB alleles did not correlate with the procedures that we used and the results that we obtained; strict measures were undertaken to avoid intersample carryover, negative controls and blank reactions provided consistently negative PCR results throughout the entire study, and independent assays performed in a blind manner with coded samples provided the same results. In addition, one might wonder whether the gB gene polymorphisms discovered were not simply the result of mutations occurring at random in each infected individual. Indeed, our strategy of analysis (nested PCR) was not designed for the detection of minor genetic forms in quasispecies mixtures and therefore might have underestimated gB gene polymorphisms. If this were the case, it would be surprising that only the same five critical positions and the same six specific combinations of markers at these positions would have emerged from our analysis, given the high number of other possibilities (2,470 nucleotide positions; 384 combinations for a silent mutation at the third base of the five codons mentioned). The most likely hypothesis was that the polymorphic traits that we observed corresponded to stable associations of genetic markers which were serially propagated within human populations through HHV-7 transmission. In our opinion, this consideration justifies the use of the term gB allele to designate these stable genetic associations. The use of a genetic polymorphism which does not induce any obvious phenotypic difference as a basis for genetic classification might be questioned. However, gB alleles, which in theory all induce the

TABLE 6. Detection of HHV-7 gB alleles in paired saliva and PBMC samples

Subject	gB allele[a] found in:	
	PBMCs	Saliva
39	F	D
47	B	B
136	B	B
167	C	C
171	F	F
178	F	F
206	F	F

[a] See Table 3 for the sequences of gB alleles.

same gB phenotype, might be tightly associated with some specific alleles of other genes inducing marked phenotypic changes. Of interest is the fact that the C and F gB alleles were each significantly linked with one of the two allelic forms of the protein p100 gene (ORF U11) corresponding to two different amino acid sequences (unpublished data). A less likely hypothesis was that the changes in the gB gene, albeit silent at the protein level, had an effect on either the replication or the transcription of the HHV-7 genome by means of conformational differences or preferential nucleotide usage. Finally, the absence of any selection pressure on the diverse gB alleles might also explain the stability of these associations.

In agreement with our hypothesis of stable propagation in human populations, the distribution of gB alleles according to the geographical origin of samples was not found to occur at random, since major differences in detection frequency were observed. Interestingly, samples from African and Caribbean subjects, who are known to be originally related, were not significantly different, but they differed greatly from European samples with regard to the distribution of the C and F alleles. Also in agreement with our hypothesis is the similarity of gB alleles found in serial samples or in different body compartments of the same individual. One exception was subject 39, who had different alleles in PBMCs and saliva. This result was repeatedly found in different assays combining independent PCR runs and nucleotide sequence determinations. The most likely explanation is that this subject was infected with two distinct strains of HHV-7 as a consequence of either coinfection or superinfection. Mixed infections with two strains of the same viral species have been reported for many members of the *Herpesviridae* family, such as HCMV (8), Epstein-Barr virus (14), and HHV-6 (12). Our results suggest that this is also the case for HHV-7 strains and raise the question of possible genetic intraspecies recombination, a question that should be addressed in the future.

A major consequence of the differential distribution of HHV-gB alleles among different geographical groups is the possibility of using these alleles as markers for studying worldwide population movements and genetics. An ideal viral marker for that purpose should be ubiquitous, specific for humans, acquired early in life, and nonpathogenic or poorly pathogenic. So far, HHV-7 infection fulfills all of these criteria and may be an even better candidate than other viruses with a high level of genomic stability, such as human T-cell leukemia virus type 1 (18) or JC virus (1). As shown above, the RFLP strategy that we initially developed for the gB gene is not discriminating enough. Conversely, the sequencing approach provides unambiguous complete information but is time-consuming and not adaptable to very large studies. We are presently developing novel alternative strategies for the allele-specific detection of the HHV-7 genome which will allow us to test our hypotheses on a larger scale.

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