Detection of DNA of Lymphotropic Herpesviruses in Plasma of Human Immunodeficiency Virus-Infected Patients: Frequency and Clinical Significance

Francesco Broccolo,* Simona Bossolasco, Anna M. Careddu,† Giuseppe Tambussi,
Adriano Lazzarin, and Paola Cinque

Division of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy

The frequency and clinical significance of detection of DNA of cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), HHV-7, and HHV-8 in plasma were investigated by PCR. The plasma was obtained from 120 selected human immunodeficiency virus (HIV)-infected patients, of whom 75 had AIDS-related manifestations, 32 had primary HIV infection (PHI), and 13 had asymptomatic infections. Nested PCR analysis revealed that none of the lymphotropic herpesviruses tested were found in patients with PHI, in asymptomatic HIV-positive individuals, or in HIV-negative controls. By contrast, DNA of one or more of the viruses was found in 42 (56%) of 75 patients with AIDS-related manifestations, including CMV disease (CMV-D) or AIDS-related tumors. The presence of CMV DNA in plasma was significantly associated with CMV-D ($P < 0.001$). By contrast, EBV detection was not significantly associated with AIDS-related lymphomas ($P = 0.31$). Interestingly, the presence of HHV-8 DNA in plasma was significantly associated with Kaposi's sarcoma (KS) disease ($P < 0.001$) and with the clinical status of KS patients ($P < 0.001$). CMV (primarily), EBV, and HHV-8 were the viruses most commonly reactivated in the context of severe immunosuppression ($P < 0.05$). In contrast, HHV-6 and HHV-7 infections were infrequent at any stage of disease. In conclusion, plasma PCR was confirmed to be useful in the diagnosis of CMV-D but not in that of tumors or other conditions possibly associated with EBV, HHV-6, and HHV-7. Our findings support the hypothesis of a direct involvement of HHV-8 replication in KS pathogenesis, thus emphasizing the usefulness of sensitive and specific diagnostic tests to monitor HHV-8 infection.

Human lymphotropic herpesviruses (cytomegalovirus [CMV], Epstein-Barr virus [EBV], human herpesvirus 6 [HHV-6] variants A and B, HHV-7, and HHV-8) are ubiquitous viruses that infect the majority of the human population (approximately 50 to 70% for CMV and 90% for EBV, HHV-6, and HHV-7), except for HHV-8, whose seropositivity rate in the normal adult population does not exceed 35% (17, 26, 42). Primary infections with these viruses in the immunocompetent host generally cause benign illnesses. Following primary infection, herpesviruses persist in the host in a latent form and have the potential to cause disease upon reactivation, particularly in the immunocompromised host, such as with human immunodeficiency virus (HIV) infection (3, 35). In contrast, reactivation of these viruses in nonimmunocompromised hosts is rare.

Some of these viruses, such as EBV, CMV, and HHV-6, are often found by PCR in peripheral blood mononuclear cells (PBMCs) or other cell types, without being necessarily associated with clinical manifestations (12, 18). In contrast, a number of reports have demonstrated that detection of CMV DNA in plasma identifies persons with active CMV disease (CMV-D) (7, 37) or at high risk for the subsequent development of CMV-D (14, 36, 38). However, little is known about the detection rate of other lymphotropic herpesviruses in plasma and its clinical significance. In particular, EBV, which has been linked pathogenetically to the development of AIDS-related non-Hodgkin's lymphoma (NHL), has only rarely been found in the plasma of patients with such disease (10). HHV-6 has been found in tissues from patients with AIDS-related tumors, including NHL, Hodgkin's disease, or Kaposi's sarcoma (KS), but there is no clear demonstration that this virus is etiologically linked to the development of these conditions (5, 12). Furthermore, HHV-6 DNA has been found in the plasma of HIV-infected patients without clear correlation with disease (35). HHV-8 has been associated with all forms of KS, body cavity lymphomas (BCL), and multicentric Castleman's disease (MCD) (1, 9), and it has been found sporadically in plasma of HIV-infected patients with KS or other disorders (22, 24). However, the clinical relevance of HHV-8 plasma viremia remains to be established (8).

The aim of the present study was to evaluate the frequency of detection of lymphotropic herpesvirus DNA in the plasma of HIV-infected patients with several AIDS-related disorders and its diagnostic value. Additionally, we extended our examination to asymptomatic HIV-positive patients and to those with primary HIV infection (PHI) in order to verify whether the reactivation of these herpesviruses was related to the degree of immunodeficiency.

MATERIALS AND METHODS

Patients and controls. A total of 120 HIV-infected patients (80 men, 40 women; median age, 38 years [range, 24 to 56]) observed at the Division of

* Corresponding author. Mailing address: Unit of Human Virology, DIBIT, San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy. Phone: (39) 02-26434904. Fax: (39) 02-26434905. E-mail: broccolo.francesco@hsr.it.

† Present address: Unit of Human Virology, DIBIT, San Raffaele Scientific Institute, 20132 Milan, Italy.

Infectious Diseases of San Raffaele Hospital, Milan, Italy, from October 1993 to October 2000 were studied retrospectively. Seventy-five patients were selected for having one or more herpesvirus-associated pathological conditions, including CMV-D alone (20 patients), systemic lymphoma (20 patients, 18 with NHLs), primary central nervous system lymphoma (P-CNSL) (10 patients), or KS disease with different clinical statuses (25 patients); 14 patients also presented with CMV-D in combination with these tumors. Thirty-two patients with PHI, as defined by documented antibody seroconversion during the 3 months preceding sampling, and 13 asymptomatic HIV-infected patients (showing no clinical signs or symptoms of HIV disease) were also studied. The median $CD4^+$ cell count was $15/\mu l$ (range, 2 to $96/\mu l$) in patients with CMV-D alone, $42/\mu l$ (range, 6 to $966/\mu l$) in systemic lymphoma patients, $12/\mu l$ (range, 2 to $46/\mu l$) in P-CNSL patients, $135/\mu l$ (range, 4 to $1,149/\mu l$) in individuals with KS, $654/\mu l$ (range, 204 to $1,231/\mu l$) in patients with PHI, and $361/\mu l$ (range, 87 to $663/\mu l$) in asymptomatic HIV-seropositive patients. As controls, 50 healthy HIV-negative blood donors (28 men and 22 women; median age, 37 years [range, 21 to 60]) were also examined. The median $CD4^+$ cell count was $810/\mu l$ (range, 520 to 1,222), and serology assays for HIV-1 were negative. All of the 13 asymptomatic HIV-infected patients, but not all of the other patients, were receiving highly active antiretroviral treatment at the time of sampling.

Disease definitions. Patients were defined as having CMV-D if they had one or more of the following: (i) retinitis consistent with CMV etiology, as confirmed by an experienced ophthalmologist, (ii) gastrointestinal disease (including gastroenteritis, esophagitis, or colitis) as confirmed by endoscopy with biopsy, (iii) encephalitis with clinicoradiological picture suggestive of a CMV etiology, in the presence of CMV DNA in the cerebrospinal fluid (CSF) as detected by PCR, or (iv) ≥ 20 pp65 antigenemia-positive polymorphonuclear (PMN) cells per 200,000 (29). All patients with CMV-D alone included in the study had $CD4^+$ lymphocyte counts lower than $100/\mu l$, and at the time of sampling none of them had received anti-CMV therapy during the preceding 2 months. To monitor ganciclovir (GCV) treatment, blood specimens before (day 0) and after (21 days) induction therapy were drawn from 20 patients with CMV-D alone. GCV was administered intravenously at the standard dose of 5 mg/kg of body weight every 12 h for 3 weeks.

Twenty-five HIV-infected patients with histologically confirmed KS were included in the study. The KS stage was heterogeneous among the patient population. Fourteen individuals had cutaneous involvement, while 11 had visceral and/or lymph node involvement. Most patients were receiving therapy for KS at the time of sampling. The patients were stratified into three groups according to clinical status: patients with progressive disease (PD) (10 patients), complete remission (CR) (10 patients), or partial remission (PR) (5 patients). PD was considered to have occurred when either new lesions or new sites of disease developed, previously existing lesions became at least 25% larger, or lesion-associated edema appeared or worsened. CR was defined as the absence of detectable residual disease for at least 4 weeks or, if pigmented lesions persisted, at least one representative lesion was free of malignant cells upon biopsy. PR was defined as either a flattening of at least 50% of nodular lesions or a 50% decrease, lasting more than 4 weeks, in the number and/or size of the previous lesions, with no new lesions appearing and no worsening of tumor-associated edema.

Histopathological examination. NHL, P-CNSL, and KS were all diagnosed by histopathology on tissue obtained by biopsy. Tissues were formalin fixed for 3 to 5 h and examined after hematoxylin and eosin staining. Immunochemical techniques were used for diagnosis of lymphomas as previously described (11). The presence of small EBV-encoded nuclear RNA transcripts (EBERs) was sought by in situ hybridization using a mixture of fluoresceinylated synthetic oligonucleotides specific for EBER (Dakopatts) according to the procedure suggested by the manufacturer (9). Tissues of 17 patients with lymphoma (10 with P-CNSL, 5 with NHLs, and 2 with HL) were examined for the presence of EBERs.

Sample preparation and nucleic acid extraction. Plasma samples were precleared by centrifuging twice (first at $900 \times g$ for 15 min at 4°C and then at $1,500 \times g$ for 15 min at 4°C). Plasma aliquots of 200 μl were used for nucleic acid extraction by means of the QIAamp blood kit (Qiagen, Inc., Chatsworth, Calif.). The DNA was eluted into 100 μl of water and stored at -80°C until testing by nested PCR.

Evaluation of $CD4^+$ cell counts and plasma HIV type 1 (HIV-1) load. $CD4^+$ T-cell counts were performed by fluorescence-activated cell sorting (FACS) analysis for all individuals included in this study. The HIV RNA load in plasma samples of patients with KS was measured by the branched DNA assay (version 3.0; Chiron Corp., Emeryville, Calif.).

PCR protocols. Nested PCRs for CMV, EBV, HHV-6, HHV-7, and HHV-8 DNA detection were carried out as previously described (6). The positions of the primers used in this study are shown in Table 1. Samples which gave positive

TABLE 1. Primers used in this study[a]

Virus DNA assayed	Upstream primer (5'→3')	Downstream primer (5'→3')	GenBank accession no.	Amplicon length (bp)	Target gene	Sensitivity (no. of genomes)
EBV	EB-3: [109332]AGGAGGGGTGGTTTGGAAAG[109351]	EB-4: [109609]AGACAATGGACTCCCTTAGC[109628]	V01555	297	EBNA1	4
	EB-1: [109353]ATCGTGGTCAAGGAGGTTCC[109372]	EB-2: [109542]ACTCAATGGTGTAAGACGAC[109561]		209		
CMV	A: [120655]CGCTGATCTTGGTATCGCAGTACAC[120679]	B: [120853]ACGTTGATGCTGTGGGGATGTTCAGCA[120877]	X17403	222	HCMVUL83	10
	C: [120725]TGGGGGCAGATGCTTCGGGCCC[120744]	D: [120827]CGACGCCATGCCACCGCG[120844]		119		
HHV-6	P0: [4520]CCGCAATCCAACTCAACCTAGCGG[4543]	P4: [4931]GTGAGAACGGAATTCGAACAGTGCTG[4956]	S57540	435	ORF RF5	10
	P1: [4688]CCCATTTACGATTTCCTGCACCACCTCTCTGC[4719]	P3: [4843]TTCAGGGACCGTGTCATTGAGCATGTC[4872]		186		
HHV-7	H7-1: [88173]AGTTCCAGCACTGCAATCG[88192]	H7-2: [88562]CACAAAAGCGTCGCTATCCGCATACACCAA[88580]	AF037218	408	U57	10
	H7-3: [88228]CGCATACAACCAACCCTACTG[88247]	H7-4: [88472]GACTCATTATGGGGATCGAC[88491]		264		
HHV-8	KS-1: [47287]AGCCGAAAAGGATTCCACCAT[47307]	KS-2: [47500]TCCGTGTTGTCTACGTCCAG[47520]	U75698	233	ORF26	4
	NS-1: [47321]ACGGATTTGACCCCGTGTTC[47341]	NS-2: [47461]AATGACACATTGGTGGTATA[47481]		160		

[a] For each assay, the first and second lines refer to the first and second PCR rounds, respectively.

TABLE 2. Number and percentage of plasma PCR-positive lymphotropic herpesvirus cases among 120 patients with HIV infection

Diagnosis	No. of patients	No. (%) positive for:				
		EBV	CMV	HHV-6	HHV-7	HHV-8
CMV-D	20	2 (10)	17 (85)	0	0	0
SL[a]	13	3	3	0	0	0
SL + CMV-D	7	0	5	0	0	1
Total	20[b]	3 (15)	8 (40)	0	0	1 (5)
P-CNSL	6	0	0	0	0	0
P-CNSL + CMV-D	4	0	4	0	0	0
Total	10	0	4 (40)	0	0	0
KS	21	0	3	0	0	8
KS + BCL	1[c]	1	1	0	0	1
KS + CMV-D	3	1	3	2	0	1
Total	25	2 (10)	7 (33)	2 (10)	0	10 (40)
PHI	32	0	0	0	0	0
HIV—asymptomatic	13	0	0	0	0	0
Total no. (%) of plasma PCR-positive patients	120	7 (6)	36 (30)	2 (2)	0	11 (9)

[a] SL, systemic lymphoma.
[b] Including 18 patients with AIDS-related NHL and 2 patients with Hodgkin's lymphoma.
[c] This patient was also affected by NHL.

findings for HHV-6 were also tested by a second PCR assay that allows differentiation between HHV-6 A and B variants (subtype PCR) (11). After PCR, 10 μl of amplified product was electrophoresed on a 2% agarose gel stained with ethidium bromide. Meticulous laboratory technique and adherence to standard PCR anticontamination procedures were the norm (23). In each PCR assay, every plasma sample was run in duplicate together with a DNA-free water control. A sample was considered either positive or negative in the case of concordant results. In the case of discordant results, both DNA extraction and PCR were repeated, and the sample was considered positive if at least two concordant results were obtained out of four examinations. To avoid possible contamination of PCR mixtures, all reactions were performed under controlled conditions. The analytic sensitivity of each virus-specific PCR is given in Table 1 in terms of the minimum number of genome copies required for positive detection.

Statistical analysis. Between-group characteristics were compared by using a χ^2 test for categorical variables and a Kruskal-Wallis test (>2 groups) or a Mann-Whitney U test (2 groups) for continuous variables. A P value of <0.05 was considered significant.

RESULTS

Frequency of detection of lymphotropic herpesvirus DNA in plasma of HIV-infected patients. The presence of DNA of one or more herpesviruses was demonstrated in the plasma of 42 of 75 (56%) patients with herpesvirus-associated pathological disorders (Table 2). All of the plasma samples from patients with PHI, individuals with asymptomatic HIV-infection, and HIV-seronegative controls were PCR negative for all viruses (Table 2). More than one virus was detected in 7 of 42 patients (17%); in all these cases CMV DNA was present together with EBV, HHV-6, or HHV-8 (Table 3). We found that CMV was the primary virus detected and that CMV, EBV, and HHV-8 were the viruses most commonly detected and those most commonly

TABLE 3. Plasma PCR results for seven patients for whom two or more lymphotropic herpesviruses were detected

Patient no. (days)[a]	Diagnosis	CD4 count/ mm³	Anti-CMV treatment	Plasma PCR result[b] for:			
				EBV	CMV	HHV-6 (variant)	HHV-8
1	CMV-D+KS	0	None	−	+	+ (A)	+
2a	CMV-D+KS	4	None	+	+	+ (A)	−
2b (+21)			GCV	+	+	+ (A)	−
2c (+30)			GCV	+	−	+ (A)	−
3a	CMV-D	25	GCV	+	+	−	−
3b (+21)			GCV	+	+	−	−
3c (+60)			GCV	−	+	−	−
4a	CMV-D	23	GCV	+	+	−	−
4b (+21)			GCV	+	+	−	−
5	KS	11	None	−	+	−	+
6a	NHL	130	None	−	+	−	−
6b (+90)				−	+	−	+
7	BCL+KS	24	None	+	+	−	+

[a] In patient designations, a, b, and c stand for first, second, and third samples, respectively. The number of days after the first sample is given in parentheses.
[b] +, positive; −, negative. No sample was HHV-7 DNA positive.

reactivated in the context of severe immunosuppression ($P < 0.05$ by the χ^2 test). In contrast, HHV-6 and HHV-7 infections were infrequent at any stage of disease.

Relationship between presence of viral DNA in plasma and disease. PCR for EBV DNA was positive in the plasma of seven patients. These included 5 of 55 patients with AIDS-related tumors (3 with NHLs alone, 1 with KS alone, and 1 with KS, NHL, and BCL) and 2 of 20 patients with CMV-D alone but no malignancies (Table 2). Therefore, the presence of EBV DNA was not associated with AIDS-related lymphomas ($P = 0.31$ by the χ^2 test). All the lymphomas tested by in-situ hybridization (17 of 17 tested) expressed EBERs. Only 2 of these 17 patients, both with NHLs, were PCR positive for EBV.

CMV was the most commonly detected virus. CMV PCR was positive for 36 patients, including 29 patients with CMV-D and 7 with other AIDS-associated conditions. CMV-D was demonstrated in 34 of 120 patients including 14 with retinitis, 10 with encephalitis, 3 with gastroenteritis, 3 with pulmonary infection, and 4 with positive pp65 antigenemia of ≥20 per 200,000 PMN cells. Overall, the clinical sensitivity and specificity of plasma CMV PCR for diagnosis of CMV-D were 85 and 92%, respectively. The presence of CMV DNA in plasma was significantly associated with CMV-D ($P < 0.001$ by the χ^2 test). At the beginning of therapy with GCV, CMV DNA was found in the plasma of 17 (85%) of 20 patients with CMV-D alone. At the end of induction therapy, 3 (15%) of 20 patients were still CMV DNA positive (patients 2, 3, and 4 [Table 3]). The presence of CMV DNA in patient 3 was related to a GCV-resistant mutant strain (data not shown). For all three patients who were still PCR positive after therapy, no clinical improvement was observed; by contrast, for all 17 patients with CMV-D which showed clinical improvement, the result of PCR was negative after treatment.

HHV-6, variant A, was detected in two patients patients 1 and 2 [Table 3]) both with KS and CMV-D. For both patients CMV DNA was found concomitantly in the plasma, whereas one patient was also EBV DNA positive. Three serial plasma

samples, drawn from the latter patient before and after GCV treatment, were examined (Table 3); whereas HHV-6 and EBV were found in all three specimens examined, CMV DNA was detected only in the first two specimens. Semiquantitative PCR revealed the presence of HHV-6 DNA after 10^{-2} dilution, whereas CMV and EBV DNA were found in undiluted samples only.

HHV-7 DNA was detected in none of the 120 patients examined.

Plasma HHV-8 DNA was detected in 10 (40%) of 25 KS patients and in 1 patient with NHL. However, for the latter patient, the presence of HHV-8 DNA was found 20 months after a clinical diagnosis of gastric KS which was not confirmed by histological examination. Therefore, the presence of HHV-8 DNA in plasma was significantly associated with KS disease ($P < 0.001$ by the χ^2 test).

Relationship between presence of HHV-8 DNA in plasma and clinical status of KS patients. It was remarkable that HHV-8 DNA was detected in 9 of 10 patients with PD and that only 1 (a patient with PR) of 15 patients in clinical remission tested positive for HHV-8 by PCR. Indeed, when patients were stratified according to clinical status into three groups (patients with PD, CR, and PR), the presence of HHV-8 DNA in plasma was shown to be associated with the clinical status of KS patients ($P < 0.001$ by the χ^2 test).

Relationship between detection of HHV-8 and CD4$^+$ T-cell count, and between HHV-8 detection and HIV load, in patients with KS. Overall, the median plasma HIV RNA level was 38,000 copies/ml (range, 195 to 1,100,000 copies/ml). Among subjects with KS, the presence of HHV-8 DNA in plasma was significantly associated with plasma HIV-1 RNA levels ($P = 0.03$ by the Mann-Whitney U test) but not with CD4 lymphocyte counts ($P = 0.4$ by the Mann-Whitney U test).

Relationship between clinical status of KS patients and CD4$^+$ T-cell count, and between clinical status and HIV viral load. Finally, no significant differences in either the CD4 lymphocyte count ($P = 0.48$ by the Kruskal-Wallis test) or the plasma HIV-1 viral load ($P = 0.15$) were observed among KS patients with different clinical statuses.

DISCUSSION

One of the major goals in the study of the pathological events related to infections with lymphotropic herpesviruses has been the search for reliable tests to detect their replication in vivo. Most of the techniques currently used, such as antibody detection (27) or PCR on DNA extracted from tissue or blood cells (18), are often unable to discriminate between latent (clinically silent) and active (often clinically relevant) infection. Plasma viremia, however, could be useful in the diagnosis of active viral infection, especially for those viruses, such as herpesviruses, which establish a lifelong persistent infection with an alternating pattern of latency and reactivation. In recent years, numerous investigators (7, 14, 36, 38) have demonstrated that the presence of CMV DNA in plasma is a reliable marker of active CMV infections. However, little is known about the detection and clinical significance of other lymphotropic herpesviruses in plasma. We investigated these issues in HIV-infected patients with and without herpesvirus-related manifestations.

Our results indicate the presence of one or more viruses in plasma in 56% of AIDS-related disorders but not in PHI patients, asymptomatic HIV-seropositive patients, or healthy individuals, suggesting that latent viruses might be reactivated in patients with AIDS-associated immunosuppression. Moreover, the absence of herpesvirus DNA from the plasma of all asymptomatic HIV patients, as well as from that of patients with PHI, suggests that in these patients, there is only a modest immune dysregulation and, as is observed for HIV-negative patients, herpesvirus replication is tightly controlled.

EBV genomes have been detected by in situ hybridization in about one-half of systemic AIDS-related lymphomas and in virtually all cases of P-CNSL (28). Detection of EBV in body fluid has been considered a possible tumor marker for diagnosis of these disorders, and detection of EBV DNA in the CSF has been shown to be highly reliable for diagnosis of P-CNSL (2, 6, 10, 11). In plasma, EBV DNA has been found in 68% of patients with acute infectious mononucleosis and in 73% of HIV-infected patients with NHL (25). In contrast, in our study, EBV DNA was found in plasma from only 15% of patients with NHLs and none of the patients with P-CNSL. These discrepancies might be explained by different parameters used in patient selection (i.e., duration of disease and time point of sampling, antiviral therapy, CD4$^+$ cell number) and/or different sensitivity limits of the assays used in the two studies. In this regard, the decreased sensitivity of our PCR assay could be a possible explanation of the incongruent results, but the ability of our assay to detect 4 genome equivalents is comparable to the 2 EBV genome equivalents reported in the previous study (25). Moreover, with our PCR assay we detected EBV DNA in plasma of 20 of 40 patients (50%) with infectious mononucleosis (data not shown), a finding which is consistent with most of the published data. More-sensitive PCR, more-efficient methods of extracting viral DNA from plasma (i.e., phenol-chloroform extraction), and better methods of determining virion concentration deserve consideration for possible application in the diagnosis of AIDS-associated lymphoproliferative disorders. Although our finding does not support the use of plasma PCR for diagnostic testing of EBV, nevertheless the presence of EBV DNA in the plasma of three patients with NHLs suggests that this virus may reactivate in concomitance with this pathology.

However, the exact character of EBV DNA detectable in plasma is still unknown. Necrosis of circulating EBV-infected B lymphocytes or cells originating from any other tissue could be another possible explanation for the sporadic presence of EBV DNA in the plasma of patients with AIDS-related NHLs. Also it is likely that the outcome of plasma PCR varies strongly with the DNA preparation method used. Therefore, additional investigation is needed to precisely characterize the EBV DNA detectable in plasma samples.

In this study, the relatively high frequency of lymphotropic herpesvirus detection in the plasma of patients with AIDS-related disorders underlines the possibility that these viruses, CMV in particular, can reactivate under conditions of profound immunosuppression. A good clinical outcome after GCV treatment was associated with clearance of CMV DNA from plasma in all but 3 (patients 2, 3, and 4 [Table 3]) of the 20 patients. Correspondingly, a lack of clinical improvement, associated with persistence of CMV DNA in the plasma, con-

firmed that PCR results correlate with clinical outcome. These findings confirm that the presence of CMV DNA in plasma is a specific marker of active infection. This is of particular importance for AIDS patients, where similar clinical pictures might be sustained by different opportunistic agents. Additionally, we found CMV DNA in 33% of plasma specimens of patients with KS. Herpesviruses, especially CMV, have been discussed as possible "KS cofactors," because homosexual men, who are the main risk group for KS, show a high incidence of infections with these viruses (15). Several reports have raised the question of whether the presence of CMV in AIDS-associated KS lesions reflects a disseminated infection with this virus or whether CMV can be considered a causative agent for the development of KS (6, 21).

HHV-6 DNA was detected only in the plasma of two AIDS patients, both of whom had both KS and CMV-D. Bovenzi et al. detected HHV-6 DNA in 35% of KS biopsy specimens (5). HHV-6 and CMV may contribute to the pathogenesis of KS by inducing the release of cytokines such as tumor necrosis factor alpha, interleukin-1, interleukin-6, and gamma interferon (IFN-γ). These cytokines promote the activation and growth of endothelial cells and the expression of adhesion molecules and integrins. The production and release of angiogenic molecules by these endothelial cells remains an interesting possibility to be investigated in order to determine the possible contribution of HHV-6 and CMV to the pathogenesis of KS. In our study, HHV-6 was found concomitantly with CMV in the plasma of two patients, suggesting that both viruses should be investigated together to evaluate an association between these two herpesviruses and clinical syndrome. Simultaneous infection with HHV-6 has also been found in a variety of postmortem tissues from AIDS patients (M. Corbellino, P. Lusso, R. C. Gallo, C. Parravicini, M. Galli, and M. Moroni, Letter, Lancet **342:**1242, 1993), suggesting that in terminally ill AIDS patients HHV-6 infection is widely disseminated and therefore likely to be active, but its association with clinical disease is unclear (4, 40). In agreement with the above study, we found a higher rate of HHV-6 detection in autopsied CSF specimens than in samples obtained before death (unpublished data). HHV-6 infection is common after transplantation, and it has been indicated that HHV-6 seroconversion is a marker for CMV disease after transplantation (13). With regard to a possible association between HHV-6 and human tumors, studies have shown the presence of HHV-6 genomes in lymphoproliferative lesions, including NHLs and HD cases, but no association with the tumor was convincingly demonstrated (12). In this regard, we recently found HHV-6 DNA in the CSF of patients with neurological disorders, but again, no clear association of this virus with induction of neurological disease could be revealed (4).

Like HHV-6, HHV-7 has been found in peripheral blood of 83% of healthy HIV-seronegative subjects but only in 3% of HIV-positive patients (16). To our knowledge, no association between HHV-7 and HIV infection has been demonstrated in vivo, and the absence of the HHV-7 genome from the plasma of all of our HIV-infected patients, irrespective of clinical conditions, does not support a role for this virus in HIV infection.

HHV-8 has been etiologically linked with different forms of KS occurring in both HIV-infected and non-HIV-infected patients (1, 9, 32). PCR-based assays have been used to detect viral DNA in a variety of tissues and body fluids (9, 22, 24) including PBMCs, saliva, and plasma. The findings of HHV-8 DNA in the plasma of people with AIDS-KS has been described by others (8, 22, 24, 39), but the clinical relevance of HHV-8 plasma viremia remains to be established (8). HHV-8 DNA in plasma has been detected only sporadically (7 to 28%) for these patients (22, 24). Conversely, Tedeschi and coworkers have documented the presence of persistent viremia in a considerable fraction (71%) of European AIDS-KS patients (39). However, the different prevalences of HHV-8 DNA in the plasma of patients with KS could be due to the low HHV-8 copy number (F. Broccolo, G. Locatelli, L. Sarmati, S. Piergiovanni, F. Veglia, M. Andreoni, S. Butto', B. Ensoli, P. Lusso, and M. S. Malnati, submitted for publication) or to the fluctuation in HHV-8 viremia (intermittent viremic phase) as described by others (19). In our study, HHV-8 DNA was detected in 10 (40%) of 25 patients with KS disease with different clinical statuses. Interestingly, for the patient with a clinical diagnosis of gastric KS that was not confirmed by the histological examination, HHV-8 DNA was found only in the second sample (sample 6b [Table 3]) of plasma, obtained 90 days after the end of therapy with IFN-α. The absence of HHV-8 DNA in the first sample from one KS patient treated with IFN-α could indicate that IFN-α causes regression and therefore HHV-8 was no longer detected; that finding could also mean that IFN-α induces KS regression by clearing the HHV-8 and/or inhibiting its reactivation (31). This study provides the first demonstration that a significant association exists between the presence of HHV-8 DNA in plasma and the clinical status of persons with KS. Indeed, when patients were stratified according to clinical status, the presence of HHV-8 DNA in plasma was clearly shown to be associated with the activity of the clinical manifestation, thus indicating that detection of HHV-8 DNA might prove to be a marker of KS activity. By contrast, it should be noted that among KS patients with different clinical statuses, no significant differences in either CD4 lymphocyte count or plasma HIV-1 load were observed. As has been suggested by others, HHV-8 replication may be immunologically controlled (41), but from our own and others' data, detection rates of HHV-8 do not necessarily inversely correlate with CD4 cell counts. Finally, we also found that the presence of HHV-8 DNA in plasma has an association with HIV-1 load in HIV-infected KS patients, suggesting a synergistic interaction between HHV-8 and HIV, as also reported in another study (30). Elevated HIV RNA levels may play a role in inducing HHV-8 reactivation through either increased immunosuppression or direct activation of HHV-8 itself. Alternatively, increased levels of HHV-8 activation could stimulate HIV replication. Since other herpesviruses have been shown to *trans*-activate HIV and probably to contribute to progression of HIV infection (33, 34), it is conceivable that HHV-8 may have a similar effect.

A significant number of coinfections were observed in the plasma of our patients, and interestingly, CMV was always present with other herpesviruses. In particular, the CMV genome was detected in all of the seven patients whose plasma was also positive for either EBV, HHV-6, or HHV-8 (Table 3). This association may be casual, given the high prevalence of CMV infection in HIV-infected patients. An interaction between CMV and other herpesviruses has been shown in a

number of reports. In particular, CMV and HHV-6 coinfection has previously been observed in immunocompetent children and organ transplant recipients (20), as well as in the CSF of AIDS patients with neurological disease (4, 11). As for the possible association between HHV-8 and CMV, initial studies on KS had revealed the presence of CMV in 27% of AIDS-associated KS lesions. Whether CMV may act as a cofactor in the pathogenesis of KS remains an interesting possibility to be demonstrated.

In conclusion, this study confirms that PCR analysis of plasma is a valid tool for diagnosis of CMV-D in AIDS patients. By contrast, our findings do not support the use of plasma PCR for diagnosis of AIDS-associated lymphoproliferative disorders. Furthermore, the significant association between the presence of HHV-8 DNA in plasma and the activity of the clinical manifestation of KS supports the hypothesis of a direct involvement of HHV-8 active replication in KS pathogenesis and lesion dissemination. Finally, we observed that CMV (primarily), EBV, and HHV-8 were the most commonly detected viruses, presumably due to reactivation in the context of severe immunosuppression, while no significant reactivation of HHV-6 or HHV-7 was demonstrated at any stage. However, the presence of herpesvirus sequences in a remarkable proportion of patients with AIDS-related neoplasms, as well as the frequent concomitance of two or more herpesviruses, may have important implications for the pathogenesis and treatment of HIV-related opportunistic conditions.

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