Prospective Study of Human Herpesvirus 6, Human Herpesvirus 7, and Cytomegalovirus Infections in Human Immunodeficiency Virus-Positive Patients

GIULIANA FABIO,[1,2] SOPHIA N. KNIGHT,[1] I. MICHAEL KIDD,[1] SHANITA M. NOIBI,[1]
MARGARET A. JOHNSON,[3] VINCENT C. EMERY,[1] PAUL D. GRIFFITHS,[1]
AND DUNCAN A. CLARK[1]*

*Departments of Virology[1] and Thoracic Medicine,[3] Royal Free Hospital School of Medicine,
Hampstead, London NW3 2PF, United Kingdom, and Department of
Biomedical Sciences, University of Modena, Modena, Italy[2]*

Blood samples from human immunodeficiency virus (HIV)-positive patients were monitored for cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), and HHV-7 by PCR. We detected CMV in 17% of the patients, HHV-6 in 6%, and HHV-7 in 3%. The viral loads of CMV were significantly higher than those of HHV-6 ($P = 0.007$) or HHV-7 ($P = 0.01$). Detection of CMV and HHV-6 was associated with low and high CD4 counts, respectively.

Human herpesvirus 6 (HHV-6) and HHV-7 are ubiquitous, lymphotropic herpesviruses that are classified along with cytomegalovirus (CMV) in the *Betaherpesvirinae* subfamily. Both viruses persist in the host following primary infection and have the potential to cause disease upon reactivation, particularly in the immunocompromised host.

The role of HHV-6 either as a cofactor in human immunodeficiency virus (HIV) disease progression or as an opportunistic infection in AIDS is unknown, although a number of mechanisms have been proposed (13, 20). It has been reported that HHV-6 is widely disseminated at death in both AIDS patients and controls (2, 4). Knox and Carrigan (16, 17, 18) reported active HHV-6 infection in a variety of AIDS postmortem tissues and, more recently, in lymph node biopsies taken relatively early in the course of HIV disease.

In cross-sectional studies, the prevalence of HHV-6 in peripheral blood mononuclear cells (PBMC) from HIV patients has been reported to be either greater than (1) or similar to (11) that of healthy controls. Reduced HHV-6 prevalence in PBMC from HIV-positive patients with lower CD4 cell counts has also been reported (7, 8, 11), although not consistently (1). This is in contrast to the other human herpesviruses, including CMV, which are found to reactivate more frequently in the context of severe HIV-induced immunosuppression (10, 21).

Little is known about the interactions of HHV-7 and HIV in vivo. Both viruses use CD4 as a cellular receptor, and HHV-7 has been shown to interfere with HIV infection of T cells and macrophages (5, 19). To date, only the frequency of HHV-7 detection in the saliva and urine of HIV patients has been reported (6, 12).

We prospectively monitored HIV-positive patients for betaherpesvirus infections by PCR, including quantitative-competitive PCR to determine the viral load. DNA was extracted from heparinized blood by using silica-matrix columns (QIAamp Blood Kit; Qiagen), and 30 ng of DNA was tested by qualitative PCR assays for CMV, HHV-6, and HHV-7 as described previously (14, 15, 24). The viral loads in positive samples were determined by quantitative-competitive PCR assays developed in our laboratory (2, 9, 14).

One thousand and one blood samples from 247 patients were tested, with a median of 3 samples per patient (range, 1 to 14). The qualitative PCR results are shown in Fig. 1. We detected CMV in the blood of 41 (17%) of 247 patients, HHV-6 in 15 (6%) of 247 patients, and HHV-7 in 7 (3%) of 247 patients (Fig. 1A). One or more viruses were detected in 54 (22%) of 247 patients (Fig. 1A) and 86 (9%) of 1,001 samples (Fig. 1B). Only 5 (0.5%) of the 1,001 samples were PCR positive for more than one virus. These findings suggest that most patients in whom at least two betaherpesviruses were detected had sequential infections.

PCR-positive samples that were subsequently below the threshold of quantification (<10 copies for each assay) were given an arbitrary viral load of one genome copy per PCR, which is equivalent to 400 genomes/ml of blood. Four samples that were PCR positive for CMV only were unavailable for quantitation. The median maximum viral loads were 10,300 genome copies/ml of blood for CMV (range, 400 to 49,700,000), 400 genomes/ml of blood for HHV-6 (range, 400 to 7,180,000), and 400 genomes/ml of blood for HHV-7 (range, 400 to 2,400). The CMV viral loads were significantly higher than those of HHV-6 (Mann-Whitney U test; $P = 0.007$) and HHV-7 ($P = 0.01$).

The samples were divided into quartiles according to CD4 cell count per microliter of blood (Table 1). Cell counts were determined as close to the virological surveillance sampling time as possible (median, 0 days; range, 0 to 200 days). There was a significant difference in CMV detection among the four CD4 groups (χ^2 test; $P = 0.001$), with the lowest CD4 group (range, 0 to 40) containing the highest number of positive samples. Likewise, there was a statistically significant difference in the occurrence of HHV-6 by CD4 groups (Fisher's exact test; $P = 0.006$). However, the most samples found positive were in the group containing the highest CD4 cell counts (range, 361 to 1,660). There was no significant difference in the number of HHV-7-positive samples among the quartiles.

In this prospective study, HHV-6 and HHV-7 were not detected frequently in the peripheral blood of HIV-positive pa-

* Corresponding author. Mailing address: Department of Virology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, United Kingdom. Phone: 00441717940500, ext. 3746. Fax: 00441718302854.
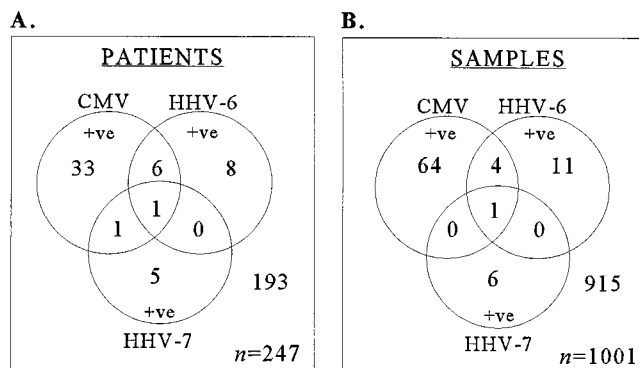
A.

B.

FIG. 1. Betaherpesvirus infections of HIV-positive patients on a per-patient basis (A) and a per-sample basis (B). Numbers within the circles indicate PCR-positive (+ve) patients or samples, while the number beside each diagram indicates the number of PCR-negative patients or samples. n refers to the total number studied.

tients ranging from early to late stages of disease, despite the fact that a number of HHV-6 isolates have been obtained from individual AIDS patients (22). Although the PCR assays do not directly detect active infection, we tested quantities of PBMC DNA below a threshold at which normal persistence is detected in healthy controls (14). Therefore, the DNAemia is likely to represent an increased viral burden in blood as a result of virus replication. Secchiero et al. (23) used the detection of HHV-6 DNA in serum by PCR as a direct marker for active infection and found 4 of 20 samples from HIV patients to contain HHV-6. However, we have previously reported that HHV-6 or HHV-7 DNA can be detected in serum by PCR in only 50% of children with primary HHV-6 or HHV-7 infections, indicating the possible insensitivity of such an approach (3). Our finding that HHV-6 infection was detected less frequently in persons with low CD4 cell counts is in agreement with cross-sectional studies (7, 8, 11). However, the situation in blood may potentially underestimate the pathogenic relevance of HHV-6 in late-stage HIV disease since we found higher median viral loads in AIDS autopsy tissues than in controls (2). The paucity of HHV-7 infections, at least in peripheral blood, does not indicate an obvious role for HHV-7 in HIV disease pathogenesis. Based on in vitro findings, it is possible that HIV outcompetes HHV-7 for infection of CD4$^+$ T cells (19).

In summary, infections of HIV-positive patients with the betaherpesviruses are temporally and numerically distinct. CMV was the most commonly detected virus and predominated in persons with low CD4 cell counts, presumably as the virus reactivated in the context of severe immunosuppression. In contrast, HHV-6 and HHV-7 infections were infrequent at any stage of disease. It remains possible that both of these

viruses, in particular, HHV-6, are important pathogens in HIV infection, but monitoring of blood for infection may underestimate this.

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TABLE 1. Detection of CMV, HHV-6, and HHV-7 in 1,001 blood samples by CD4 cell count

Virus	No. of samples (%) PCR positive in quartile groups with CD4 cell counts				P value
	0–40 ($n = 283$)	41–170 ($n = 232$)	171–360 ($n = 247$)	361–1,660 ($n = 239$)	
CMV	41 (14.5)	7 (3.0)	4 (1.6)	17 (7.1)	0.001[a]
HHV-6	1 (0.4)	1 (0.4)	5 (2.0)	9 (3.8)	0.006[b]
HHV-7	2 (0.7)	1 (0.4)	1 (0.4)	3 (1.3)	0.73[b]

[a] χ^2 test.
[b] Fisher's exact test.

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