High Prevalence of Multiple Human Herpesviruses in Saliva from Human Immunodeficiency Virus-Infected Persons in the Era of Highly Active Antiretroviral Therapy

Craig S. Miller,1,2* Joseph R. Berger,3 Yunanan Mootoor,3 Sergei A. Avdiushko,2 Hua Zhu,4 and Richard J. Kryscio4

Department of Microbiology, Immunology & Molecular Genetics, 1 Department of Oral Health Practice,2 Departments of Neurology and Internal Medicine, 3 and Department of Statistics,4 University of Kentucky College of Medicine and College of Dentistry, Lexington, Kentucky 40536-0297

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Human immunodeficiency virus (HIV) infection is associated with an increased risk for human herpesviruses (HHVs) and their related diseases. Methods for limiting the transmission of HHVs require a better understanding of the prevalence and infectiousness of oral HHVs in HIV-infected patients. We performed quantitative PCR to investigate the prevalence, quantity, risk, and correlates of salivary HHVs from 58 HIV-seropositive individuals in a case control study. HHVs were significantly more prevalent in the salivas of HIV-seropositive persons than in those of the controls (odds ratios [ORs], 4.2 to 26.2; P ≤ 0.008). In HIV-infected patients, Epstein-Barr virus (EBV), human herpesvirus 8 (HHV-8), cytomegalovirus (CMV), and herpes simplex virus type 1 (HSV-1) were detected in 90%, 57%, 31% and 16% of samples, respectively, compared with 48%, 24%, 2%, and 2%, respectively, of samples from controls. Multiple HHVs were observed in 71% of HIV-seropositive persons and only 16% of controls (OR, 13.0; 95% confidence interval, 5.29 to 32.56). HIV-positive patients had significantly higher EBV loads than HIV-negative persons (P < 0.0001). HIV-infected patients with CD4 counts above 200 cells/μl had increased probability for having HHV-8 in saliva (P = 0.009) compared with patients whose counts were less than 200. In contrast, HSV-1, EBV, and CMV were detected more often when CD4 counts were low. High salivary HHV loads were detected for those (n = 7) with oral lesions. These findings suggest that saliva is a potential risk factor for the acquisition of multiple HHVs, and several host factors may function to accelerate HHV reactivation or replication in patients with HIV infection.

Human herpesviruses (HHVs) are widely distributed pathogens that cause benign and malignant disease. Serological evidence of infection with HHVs is found in the majority of the world’s populations (23, 28, 32, 40, 45, 60, 64, 65, 74). Spread of HHVs is by contact with infected secretions, usually early in life. However, modes of transmission of select HHVs are not fully understood (2). Of the bodily secretions and fluids documented to harbor HHVs, saliva appears important for transmission of several HHVs (11, 16, 17, 33, 52, 53, 55, 56, 69). The amount and frequency of HHVs appearing in saliva are likely determinants of risk for transmission (48).

Herpes simplex virus 1 (HSV-1), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus 8 (HHV-8) (also called Kaposi’s sarcoma [KS]-associated herpesvirus), are members of the Herpesviridae family that have common associations with orofacial diseases in humans. They are shed in saliva asymptptomatically and simultaneously at various rates and levels (48). The rates and viral loads detected are influenced by the biological properties of the virus, method of detection, frequency of sampling, oral health, and social behaviors and immunological status of the patient (46, 49). By sensitive PCR assay, the mean detection rates for EBV (30, 31, 48, 63), CMV (15, 44), HSV-1 (15, 27, 48, 49, 67), and HHV-8 (7, 14, 36, 38, 44, 48) nucleic acids in the oral cavity of healthy adults at a single visit are approximately 31%, 9%, 6%, and <1%, respectively. The prevalence of HHVs in the saliva of human immunodeficiency virus (HIV)-seropositive patients has been less often reported, with reports generally focusing on individual prevalence, not the simultaneous presence of HHVs. In these reports, the prevalence of HHVs appears higher in HIV-seropositive patients (3, 4, 6, 19, 38, 44, 46, 68). However, in one study that examined multiple HHVs, rates of detection in saliva of HIV-seropositive patients were similar to that of the general population, except for CMV (20).

Almost 45 million people worldwide have been infected by HIV, and prior to highly active antiretroviral therapy (HAART), more than 75% of all HIV-infected persons developed HHV-related diseases (39, 54, 61, 66, 70). The advent of HAART has decreased the incidence of opportunistic HHV diseases and improved survival for those fortunate to receive therapy (1, 9, 20, 39, 50, 59). Whether HAART has altered the rate of HHV reemergence from latency or the ability of HHVs to produce clinical manifestations is not well known. Clearly, HHV-related malignancies remain a significant problem for the HIV infected (5, 73), and laboratory assessments of HHVs in bodily fluids that could be predictive of the development of HHV-related diseases would be of great benefit to this population.
Materials and Methods

Study population and procedures. Fifty-eight HIV-seropositive subjects were recruited from the Bluegrass Care Clinic of the University of Kentucky, College of Medicine, in Lexington, Kentucky. Fifty-eight age- and sex-matched control subjects were recruited from campus volunteers and from patients in the Neurology Clinic at the University of Kentucky. Enzyme-linked immunosorbent assays for HIV were performed on all controls to insure that they were HIV seronegative. All subjects were 18 years or older. All controls were in good general health and did not have histories of liver or kidney dysfunction. All subjects were free of symptoms of acute illness (i.e., fever, sore throat, body aches, and diarrhea) and malignancies at the time of enrollment. Pregnancy, use of immunosuppressant medications, and antiretroviral therapy 1 week before the study enrollment date were additional exclusion criteria. The study was approved by the University Institutional Review Board, and all patients provided written informed consent as part of the study protocol.

Oral examinations were performed by a study physician. Subjects were asked specifically about previous tonsil surgery and inspected for gum bleeding, mouth ulcers, mouth pain, dry mouth, presence of tonsillar tissue, oral mucosal lesions, and cervical lymphadenopathy. Unstimulated whole saliva (5 ml) was collected at a single visit by the method described by Navazesh (51). All samples were maintained on ice, divided into 1-ml aliquots, placed into bar code-labeled cryotubes, and frozen at -85°C until use. Venous blood was collected, processed, and analyzed for HIV serostatus using ELISA. Lymphocyte subsets were assayed via flow cytometry.

PCR primers and probes. The primers and probes used were designed from published sources, as we have previously reported (48). Briefly, HSV-1 primers and probes were designed for glycoprotein G as described by Rynarz et al. (58). Primers and probes for EBV were directed to the BALF5 gene encoding viral DNA polymerase according to the method of Kimura et al. (55). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72). Primers and probes for CMV were designed for glycoprotein B as described in the work of Li et al. (41). HHV-8 primers and probes were designed for HHV-8 minor capsid protein as described by White and Campbell (72).

Real-time quantitative PCR. Real-time quantitative PCR was used for the detection and quantification of HHVs in saliva. The DNA from 1 ml of each saliva specimen was centrifuged, and the DNA was isolated from the cell pellet using the QIAamp DNA Mini kit (QIAGEN, Valencia, CA). Herpesviruses are more readily detected in the cell pellet than in the supernatant (48). Each 50-µl PCR mixture contained 10 µl purified DNA template in a final volume consisting of 1× TaqMan Universal PCR master mix (PE Applied Biosystems), 900 nM primers, and 250 nM TaqMan probe. Real-time PCR was performed on an ABI Prism 7700 sequence detection system (PE Applied Biosystems). Cycling parameters were 50°C for 2 min, 95°C for 10 min, and 40 cycles at 95°C for 15 s and 60°C for 1 min. Each PCR run contained negative controls, including reaction mixtures without DNA template as well as several specimens that were known to contain no HHV DNA, a positive amplicon control, and a 10-fold dilution series (1 × 10<sup>3</sup> to 1 × 10<sup>9</sup> genome equivalents per reaction) of either genomic HHV DNA or cloned HHV sequences. The positive control standards were HHV-1 genomic DNA; plasmid pGEM-BALF5 containing the EBV BALF5 gene, kindly provided by H. Kimura, Nagoya University, Nagoya, Japan; plasmid pCR2.1 with a cloned 254-bp fragment of CMV glycoprotein B, kindly provided by Y.-W. Tang, Vanderbilt University, Nashville, Tennessee; and plasmid pMCP, containing nucleotides 47259 to 47554 of the HHV-8 genome, kindly provided by T. B. Campbell of the University of Colorado, Denver. Each specimen was analyzed in duplicate. Results were scored positive if both reactions yielded a threshold cycle value (C<sub>t</sub>) above the limit of detection for the standards. Reactions that yielded one positive and one negative result were repeated in duplicate. Samples were scored positive only when both repeat reactions yielded positive results. Viral genome copy number results are reported as the means of the two runs.

Statistical analysis. Odds ratios (and 95% confidence intervals) to measure the association between demographics (age and gender), or risk factors (CD4+ counts, use of HAART, etc.) and the presence of a virus were based on 2×2 contingency tables. P values associated with these odds ratios were based on chi-square statistics or Fisher's exact test. A stepwise logistic regression model with a significance level to enter of 0.20 and a significance level to stay of 0.05 was used in the multivariate analysis. Mean titer levels were compared between controls and HIV patients using two sample t tests after log transforming the viral DNA copy numbers. Statistical significance was determined at the 0.05 level. All data were analyzed with use of the SAS statistical analysis software (SAS Institute).

Results

Demographic and clinical findings. The 58 HIV-infected individuals were demographically similar to the 58 controls (Table 1). The majority of subjects in both groups were Caucasian men. None of the participants in either group had clinical KS, and 47/58 (81%) of the HIV-seropositive patients were taking HAART. The majority of HIV-positive patients were adequately controlled as determined by HIV loads and CD4 counts. Oral lesions were noted in seven individuals; all were HIV seropositive.

Prevalence of salivary HHVs. HHVs were more prevalent in salivas from HIV-seropositive persons than healthy controls (Fig. 1). Ninety-seven percent of HIV-positive patients had detectable salivary HHV DNAs. In contrast, only 34 of 58 (58.6%) of the healthy controls had HHV DNAs in saliva (P ≤ 0.008).

![FIG. 1. Prevalence of HHVs in saliva as determined by quantitative PCR. The chi-square statistic demonstrated that the rate of detecting each virus in the HIV-seropositive group was significantly greater than the rate for the controls (P ≤ 0.008).](http://jcm.asm.org/onlinelibrary/dl/2015/53/11/2410.Miller.EtAl_2410_Fig1.jpg)
In the HIV-seropositive group, the most prevalent viral DNA was EBV (90%), followed by HHV-8 (57%), CMV (31%), and HSV-1 (16%). Detection rates in the HIV-seronegative group were as follows: EBV, 48%; HHV-8, 24%; CMV, 2%; and HSV-1, 2%.

**Viral loads.** Concentrations of HHV DNA in the expressors ranged greatly from a few copies to millions and varied by HIV serostatus and HHV type (Table 2). CMV and HHV-8 were detected, in general, at lower genome copy numbers than EBV seropositive persons was 13 times the odds of having multiple HHVs in the controls (odds ratio [OR], 13.13; 95% confidence interval [CI], 5.29 to 32.56). Viruses most likely to be simultaneously present in the saliva of HIV-seropositive persons were HHV-8 and CMV (P = 0.0004), HHV-8 and EBV (P < 0.0001), CMV and EBV (P < 0.0001), EBV and HSV-1 (P = 0.001), and HHV-8, CMV, and EBV (P = 0.0004) (data not shown).

**Relationships with immunological characteristics and HIV disease severity.** The presence of select HHVs correlated with the level of cellular immunosuppression (Fig. 2). CMV was more likely to be present when CD4 counts were less than 350 cells/mm³ than above this level (P = 0.047) and when HIV viral loads were between 400 and 5,000 copies/ml than when they were above 5,000 (P = 0.048). HHV-8 was more likely to be present in saliva when CD4 counts were greater than 200 cells/mm³ than when they were less than 200 (P = 0.009). Salivary EBV and HSV-1 loads increased with increasing HIV loads (Spearman’s correlation, 0.29 [P = 0.028] and 0.40 [P = 0.002], respectively), and HHV-8 loads correlated with the CD8 count (Pearson’s correlation, 0.46; P = 0.0003).

**Immunological status and multiple salivary HHVs.** Table 3 shows that the CD4 count was significantly associated with the simultaneous presence of CMV and EBV (P = 0.002) and CMV, EBV, and HHV-8 (P = 0.003) in HIV-positive patients. When the CD4 count was less than 200 cells/mm³, CMV and EBV were 18 times more likely to be present in saliva than when the CD4 count was greater than 400 cells/mm³ (OR, 18.12; 95% CI, 1.84 to 178.57; P = 0.007). Also, HIV-positive

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**TABLE 2. HHV genome copy numbers in saliva**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Subject group (n*)</th>
<th>Median (range)</th>
<th>Geometric mean</th>
<th>Standard error</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>HIV – (1)</td>
<td>20,516</td>
<td>20,517</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>HIV + (9)</td>
<td>14,948 (1,254–82,806)</td>
<td>12,777</td>
<td>6,228</td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td>HIV – (28)</td>
<td>1,759 (18–114,614)</td>
<td>1,775</td>
<td>926</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>HIV + (52)</td>
<td>308,875 (16–53,247,808)</td>
<td>128,028</td>
<td>78,058</td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>HIV – (1)</td>
<td>762</td>
<td>762</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>HIV + (17)</td>
<td>1,501 (89–9,584)</td>
<td>1,180</td>
<td>355</td>
<td></td>
</tr>
<tr>
<td>HHV-8</td>
<td>HIV – (14)</td>
<td>172 (5–2,224)</td>
<td>167</td>
<td>92</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>HIV + (33)</td>
<td>217 (5–1,087,543)</td>
<td>311</td>
<td>138</td>
<td></td>
</tr>
</tbody>
</table>

a n, no. of subjects.

b Logarithm was taken of viral loads for each virus, and a two-sample t test was performed to determine if the mean viral loads were different between the groups. All values reported are per ml of saliva.

c NA, not available due to low frequencies.

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**FIG. 2.** Relationship of CD4 count and presence of HHVs in saliva from HIV-seropositive patients. Fisher’s exact test was performed and revealed the following P values: *, P = 0.058 for comparing CD4 counts of <200 cells/mm³ versus CD4 counts of ≥400 cells/mm³; ***, P = 0.002 for comparing CD4 counts of 201 to 399 cells/mm³ versus CD4 counts of ≥400 cells/mm³; ***, P = 0.002 for comparing CD4 counts of <200 cells/mm³ versus counts of >400 cells/mm³.

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**TABLE 3. Relationship of CD4 count and presence of multiple salivary HHVs in HIV-positive patients**

<table>
<thead>
<tr>
<th>CD4 count (n*)</th>
<th>CMV and EBV</th>
<th>EBV and HHV-8</th>
<th>CMV, EBV and HHV-8</th>
<th>CMV, EBV and HHV-8</th>
<th>HHV-1, CMV, EBV and HHV-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200 (13)</td>
<td>5*</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>200–399 (15)</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>7*</td>
<td>1</td>
</tr>
<tr>
<td>&gt;400 (30)</td>
<td>1</td>
<td>0</td>
<td>12</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

a n, no. of patients.

b *, P = 0.002.
patients with a CD4 count between 200 and 399 cells/mm³ were 12 times more likely to have the simultaneous presence of CMV, EBV, and HHV-8 in saliva than patients with a CD4 count greater than 400 cells/mm³ (OR, 12.25; 95% CI, 2.11 to 70.92; *P = 0.003).

### Risk factors for HHVs

Several factors (i.e., age, race, gender, CD4 and CD8 cell count, CD4/CD8 ratios, HIV viral loads, HAART use, presence of tonsils, bleeding gums, oral lesions, and abnormalities of the mouth and/or neck) were assessed for their association with the detection of HHV DNAs in saliva, first in univariate analysis and then in multivariate models. In the univariate analyses, HIV-positive patients were significantly more likely to shed HHVs than the controls (OR, 4.15 [95% CI, 1.87 to 9.19] for HHV-8, 9.29 [95% CI, 3.45 to 24.98] for EBV, 10.47 [95% CI, 1.28 to 85.53] for HSV-1, and 26.19 [95% CI, 3.34 to 205.22] for CMV). No other demographic (age, race, gender) or clinical risk factors were significantly related to the odds of shedding these viruses. To investigate why HHV shedding was related to HIV disease, a number of clinical and immunological related factors were examined using the HIV-positive patients only. A summary of multivariate analyses follows.

Patients with HIV loads over 5,000 copies/ml were significantly more likely to shed HSV-1 than patients with HIV loads under 5,000 (adjusted OR, 14.3; 95% CI, 2.7 to 100). Intermediate HIV loads (in the range of 400 to 5,000 copies/ml) had a significantly larger chance of shedding CMV than patients with loads below 400 (adjusted OR, 7.08; 95% CI, 1.21 to 41.46). In contrast, patients whose HIV loads were below 400 copies/ml were more likely to express HHV-8 and EBV than those with HIV loads over 400 (adjusted OR, 4.08; 95% CI, 1.23 to 13.59) or between 400 and 5,000 copies/ml, respectively (adjusted OR, 0.11; 95% CI, 0.01 to 0.97).

CD4 count was predictive of one HHV in saliva. An increasing CD4 count significantly protected patients from expressing EBV in saliva (adjusted OR, 0.998; 95% CI, 0.997 to 1.00).

### Oral lesions

Seven HIV-positive patients had oral lesions (Table 4). Two had hairy leukoplakia of the tongue, one had an aphthous-like ulcer on the labial mucosa, and the remainder had erythematous patches of the tongue and palate consistent with erythematous candidiasis. All salivas from these patients were positive for EBV, and none were positive for HSV-1. CMV was detected in highest copy number for the patient having an erythematous lesion of the palate. EBV was detected in high copy number for both patients with hairy leukoplakia. HHV-8 was detected in highest copy number for the patient having an ulcer of the labial mucosa and in low copies for two patients with nonulcerative oral lesions. The presence of oral lesions was not associated with immunological parameters or HIV loads (data not shown).

### DISCUSSION

This study represents the first report of the prevalence, loads, risks, and correlates of multiple salivary HHVs in HIV-seropositive patients who lacked clinical evidence of KS during the HAART era. Our analyses demonstrate that HSV-1, EBV, CMV, and HHV-8 were more prevalent and EBV loads were significantly higher in the saliva of HIV-positive patients than in that of matched healthy controls. The risk for salivary HHV shedding related to immune status but not demographic findings. Immunosuppression increased the likelihood for the individual presence of HSV-1 and CMV (CD4 count less than 400 cells/mm³) and the simultaneous presence of CMV with EBV (CD4 count less than 200 cells/mm³). In contrast, CD4 counts greater than 200 cells/mm³ were significantly associated with the detection of HHV-8 (*P ≤ 0.009*).

HSV-1, EBV, CMV, and HHV-8 were more prevalent in HIV-infected patients than in the age- and sex-matched controls. A 4- to 26-fold-increased odds of HHV shedding in the saliva of HIV seropositive patients was observed. The frequencies of detection in the HIV-positive and HIV-negative groups are consistent with those previously reported, indicating that HHVs are frequently shed asymptomatically in the saliva of HIV-infected patients who take HAART (3, 4, 6, 19, 37, 38, 44, 68). The higher prevalence in the HIV-positive group may reflect altered health status or differences in herpesvirus seropositivity between the groups, particularly for CMV.

In prior studies of HIV-infected patients without KS or AIDS, the detection rates in saliva for HSV-1/HSV-2 were 24% (4, 20), EBV, 71% to 93% (6, 18, 24, 42, 43), CMV, 17% to 77% (19, 20, 25, 44), and HHV-8, 0% to 57% (3, 4, 11, 13, 36–38, 56, 68, 71). Most prior studies examined for individual HHVs in saliva, not their simultaneous presence, and oral lesions may have been present or not reported (44). In this cohort, 88% of the HIV-positive patients were free of demonstrable oral lesions. Thus, these findings help clarify the prevalence of HHV shedding in the saliva of HIV-positive patients who are asymptomatic, not taking antiretroviral medications, and in large part lacking oral mucosal lesions. Interestingly, all patients who had oral lesions had EBV DNA in their saliva.

Salivary EBV loads were high for the two HIV-positive pa-

<table>
<thead>
<tr>
<th>Oral lesion</th>
<th>Clinical diagnosis</th>
<th>HSV-1</th>
<th>EBV</th>
<th>CMV</th>
<th>HHV-8</th>
<th>CD4 count</th>
<th>CD4/CD8 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer, mandibular labial mucosa</td>
<td>Aphthous</td>
<td>0</td>
<td>24,042</td>
<td>144</td>
<td>1,087,543*</td>
<td>332</td>
<td>0.1</td>
</tr>
<tr>
<td>Erosion, lateral palatal vault</td>
<td>Erythematous candidiasis</td>
<td>0</td>
<td>6,588,386</td>
<td>9,584*</td>
<td>0</td>
<td>42</td>
<td>0.05</td>
</tr>
<tr>
<td>Erythema, midline soft palate</td>
<td>Erythematous candidiasis</td>
<td>0</td>
<td>121</td>
<td>751</td>
<td>0</td>
<td>24</td>
<td>0.03</td>
</tr>
<tr>
<td>Erythema, dorsum of tongue</td>
<td>Median rhomboid glossitis</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>171</td>
<td>0.2</td>
</tr>
<tr>
<td>Erythema, dorsum of tongue</td>
<td>Median rhomboid glossitis</td>
<td>0</td>
<td>3,041,076</td>
<td>0</td>
<td>27</td>
<td>344</td>
<td>0.5</td>
</tr>
<tr>
<td>White patches, bilateral, tongue</td>
<td>Hairy leukoplakia</td>
<td>0</td>
<td>1,127,921</td>
<td>0</td>
<td>677</td>
<td>365</td>
<td>0.2</td>
</tr>
<tr>
<td>White patch, dorso lateral, tongue</td>
<td>Hairy leukoplakia</td>
<td>0</td>
<td>41,486,288**</td>
<td>0</td>
<td>0</td>
<td>161</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* = highest copy number detected in entire study population; ** = second-highest copy number detected in entire study population.
tients who had hairy leukoplaikia, and the highest observed viral copy numbers of CMV and HHV-8 were from patients with oral lesions. These findings suggest that interactions with HHVs may contribute to the persistent nature of certain mucosal lesions.

To date, few studies have reported HHV loads in the saliva of HIV-infected patients. We found that salivary HHV loads varied greatly within the population, and HSV-1 loads (i.e., geometric mean) tended to be at least 10-fold higher than CMV and HHV-8 loads for both HIV-seropositive and HIV-negative patients. EBV loads were highest of the four HHVs examined in saliva of HIV-positive persons (i.e., about 2 logs higher than loads for healthy controls). This is consistent with data in a previous report (6) and suggests that EBV not only is more prevalent but replicates more freely in the oral cavity of HIV-positive patients. Although EBV replicates in oropharyngeal tissue, we did not observe a correlation with the presence of pharyngeal tonsils. This may be due to the presence of additional replication sites, such as palatine and lingual tonsillar tissue in the oral cavity, the recruitment of EBV-infected lymphocytes to sites of chronic periodontal inflammation, or other undetermined factors. Unlike the findings of others, we did not observe a correlation of EBV load (or any HHV load) with the number of CD4 T cells in the peripheral blood (43). However, EBV and HSV-1 loads increased with increasing HIV loads, and the HHV-8 load correlated with CD8 count. HHV-8 has been detected in CD8 T cells (26, 62), which raises the possibility that the saliva of HIV-infected patients contains HHV-8-infected T cells or the cytokines they secrete may enhance HHV-8 replication.

HAART is known to diminish HHV-related diseases (29), but its effects on asymptomatic HHV shedding have not been fully explored. Gandhi et al. reported a trend toward increased HHV-8 salivary shedding with HAART use (21), but few other reports exist. In our study, 81% of HIV-positive patients were taking HAART, yet 97% of HIV-positive individuals had at least one HHV DNA detected in saliva and 71% had at least two HHVs in saliva. HHV loads and prevalence profiles were similar whether patients were taking HAART or not (data not shown). These data, combined with our previous findings (48), suggest that HAART, with its targeted specificity, does not fully explore HHV-related malignancies.

Immune function helps regulate the clinical appearance of HHVs and the development of HHV-related diseases. In this study, HSV-1, EBV, and CMV were detected individually more often in saliva at lower CD4 counts (<400 cells/mm³), and CMV and EBV were detected simultaneously significantly more often when CD4 counts were <200 cells/mm³. In contrast, HHV-8 was more likely to be present alone or together with CMV and EBV when CD4 counts were above 200 cells/mm³ (P = 0.003). Higher prevalence of HSV-1, EBV, and CMV in saliva at low CD4 counts has been previously observed (19, 25). Likewise, the finding that HHV-8 is more likely to be present in saliva at higher CD4 counts is consistent with the recent findings of Gandhi et al. (21). Together, these findings suggest that CD4 cells can contribute to the control of select oral HHVs (i.e., HSV-1, EBV, and CMV), possibly within germinai centers of oropharyngeal tissues, where CD4 cells form cognate interactions with B cells (22, 34). In contrast, the presence of CD4 and CD8 cells may enhance HHV-8 replication in other cell types as suggested by Gandhi et al. (21). This could be biologically significant in that HHV-8 may rely on cytokines produced by immune cells or the presence and interactions of CD4 and CD8 cells for efficient replication. These interactions could be in contrast to the regulation of HSV-1, CMV, and EBV replication provided by cytokines (i.e., interferon) and the immune response at oropharyngeal and more distant latent sites (8, 12, 47).

In summary, our observations suggest that EBV and HHV-8 expression in saliva is a frequent event in HIV-infected individuals in the presence of HAART therapy. The frequency and viral loads detected are suggestive that HHVs in persons with adequately controlled HIV infection could be transmitted by contact with saliva, and risk for transmission may be greater during certain stages of HIV infection. These data increase our understanding of the correlates between asymptomatic HHV shedding and health status and provide a basis for future studies that can investigate if salivary levels of HHVs are useful for monitoring disease states and risk for progression of HHV-related malignancies.

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