Herpes Simplex Virus Type 2 Infection Does Not Influence Viral Dynamics during Early HIV-1 Infection

Edward R. Cachay, Simon D. W. Frost, Douglas D. Richman, Davey M. Smith, and Susan J. Little

Objective. We sought to compare baseline and longitudinal plasma HIV-1 loads between herpes simplex virus type 2 (HSV-2)–seropositive and –seronegative individuals who are enrolled in a primary HIV-1 infection cohort in San Diego, California.

Design. The study was a retrospective cohort analysis.

Methods. We categorized antiretroviral-naive subjects on the basis of HSV-2 serostatus at baseline using an HSV-2 enzyme immunoassay. Low positive results (1.1–3.5) were confirmed by Western blotting. We compared baseline HIV-1 loads of the 2 groups using a linear model. To detect differences in HIV-1 dynamics, we analyzed longitudinal viral loads using a flexible semiparametric model, controlling for the time to antiretroviral therapy and stratifying by HIV-1 infection stage at entry.

Results. We studied 294 adult men. Ninety percent reported sex with men as their main HIV-1 risk factor. The seroprevalence of HSV-2 was 41.5%. The HSV-2–seropositive and –seronegative groups had similar baseline HIV-1 loads during acute infection (5.52 vs. 5.72 log10 copies/mL; P = .39) and early infection (4.57 vs. 4.67 log10 copies/mL; P = .5). Longitudinally, the difference in HIV-1 loads between HSV-2–seropositive and –seronegative men remained close to 0 during the first year of infection.

Conclusions. HSV-2 serostatus has minimal influence on the dynamics of HIV-1 during acute and early HIV-1 infection.

Herpes simplex virus type 2 (HSV-2) is the most frequent cause of genital ulcerative disease worldwide [1-4]. HSV-2 and HIV-1 infections are sexually transmitted, and both result in lifelong infection. HSV-2 is more frequent in HIV-1–seropositive than HIV-1–seronegative individuals. For example, in an Australian study, the reported HSV-2 seroprevalence in men who have sex with men was higher in HIV-1–seropositive than HIV-1–seronegative subjects (61% vs. 28%, respectively; P<.001) [5]. Epidemiological observations have suggested a strong bidirectional relationship between HSV-2 and HIV-1 infection, where each viral infection increases the acquisition and transmission of the other [6-11]. Furthermore, HSV-2 infection has been shown to exacerbate HIV-1 infection, with shedding of genital HSV-2 causing a concurrent increase in plasma HIV-1 loads that persist for up to 6 weeks [12]. The positive correlation between HSV-2 genital shedding and increases in blood HIV-1 loads was present whether or not genital lesions or symptoms were noted [14]. In a cross-sectional study of 217 Ugandans with primary HIV-1 infection, of whom 150 were coinfected with HSV-2, positive HSV-2 serostatus was associated with a plasma HIV-1 load 0.55 log10 copies/mL higher ~5 months after HIV-1 seroconversion (P = .004) [15]. This led investigators to suggest that HSV-2 coinfection might affect the course of HIV-1 progression by producing higher postseroconversion HIV-1 viremia [15, 16]. In another cross-sectional study of 339 African individuals with chronic HIV-1 infection, HSV-2 sero-
positivity was associated with a significantly higher plasma HIV-1 load (0.3 log_{10} copies/mL), compared with those in HSV-2–seronegative individuals [17]. Whether HSV-2 seropositivity reflected active (symptomatic or asymptomatic shedding) versus latent genital infection or recently acquired versus chronic HSV-2 infection was unclear. The exact mechanisms by which HSV-2 influences HIV-1 loads have not been delineated. Nevertheless, the results of these cross-sectional studies invite speculation that HSV-2 might be a modifiable cofactor that influences the establishment of a higher steady-state HIV-1 load (“set point”) during early HIV-1 infection. We therefore conducted a longitudinal study to investigate the effect of HSV-2 serostatus on the dynamics of HIV-1 in our well-characterized primary HIV-1 infection cohort at the University of California, San Diego (UCSD). We also sought to establish HSV-2 seroprevalence in individuals with HIV-1 infection of <1 year’s duration (recent HIV-1 infection).

PATIENTS, MATERIALS, AND METHODS

Patient population and data collection. Subjects in the study were recruited between June 1996 and June 2005 at the UCSD site of the Acute Infection and Early Disease Research Program (AIEDRP) network. Study participants signed an informed consent form approved by the Human Research Protection program at UCSD. Acute HIV-1 infection was defined by detectable HIV-1 RNA in the absence of HIV-1 antibody measured by EIA, with subsequently documented HIV-1 seroconversion. Early HIV-1 infection was defined by a positive HIV-1 EIA and a documented negative serologic test within the preceding 12 months or a negative result when a reduced-sensitivity (“detuned”) EIA was used [18]. All subjects were adult men and were antiretroviral naive at enrollment and during follow-up. Subjects were excluded if they were being treated with any type of HSV-2–specific antiviral therapy. Clinical assessments were conducted at baseline, biweekly for the first month, monthly for the next 5 months, and every 2 months thereafter.

The date of HIV-1 infection was estimated as reported elsewhere [19]. Briefly, (1) if HIV-1 EIA was negative and the HIV-1 load was >5000 copies/mL, infection was defined as having occurred 21 days previously; (2) if HIV-1 EIA was positive and Western blotting was indeterminate, infection was defined as having occurred 28 days previously; (3) if HIV-1 EIA was positive and Western blotting was positive but with ≤5 bands, infection was defined as having occurred 45 days previously; (4) if HIV-1 EIA was positive, Western blotting was positive (>5 bands), and a detuned EIA value was ≤0.75 (with a simultaneous CD4+ T cell percentage >14%), infection was defined as having occurred 85 days previously; (5) if none of the previous criteria were met, then infection was defined as the midpoint between the date of the first documented positive EIA result and the last historically documented negative EIA result, which must have been <365 days before enrollment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV-1 positive/HSV-2 negative (n = 172)</th>
<th>HIV-1 positive/HSV-2 positive (n = 122)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), years</td>
<td>35 (20–35)</td>
<td>40 (23–43)</td>
<td>&lt;.0001a</td>
</tr>
<tr>
<td>Race, no. (%) of subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>138 (80.2)</td>
<td>106 (86.9)</td>
<td>.20b</td>
</tr>
<tr>
<td>American Indian</td>
<td>11 (6.4)</td>
<td>9 (7.3)</td>
<td>.20</td>
</tr>
<tr>
<td>Black</td>
<td>12 (7.0)</td>
<td>3 (2.4)</td>
<td>.20</td>
</tr>
<tr>
<td>Asian</td>
<td>7 (4.1)</td>
<td>1 (0.8)</td>
<td>.20</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (2.3)</td>
<td>3 (2.4)</td>
<td>.20</td>
</tr>
<tr>
<td>Ethnicity, no. (%) of subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Hispanic</td>
<td>64 (37.2)</td>
<td>43 (35.0)</td>
<td>.91c</td>
</tr>
<tr>
<td>Hispanic</td>
<td>35 (20.3)</td>
<td>25 (20.3)</td>
<td>.91</td>
</tr>
<tr>
<td>Unknown</td>
<td>73 (42.4)</td>
<td>55 (44.7)</td>
<td>.91</td>
</tr>
<tr>
<td>HIV-1 stage, no. (%) of subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>46 (26.7)</td>
<td>39 (32.0)</td>
<td>.33c</td>
</tr>
<tr>
<td>Early</td>
<td>126 (73.3)</td>
<td>83 (68.0)</td>
<td>.33</td>
</tr>
<tr>
<td>CD4+ T cell count, median (range), cells/mm^3</td>
<td>498 (107–1380)</td>
<td>495.5 (154–1119)</td>
<td>.87d</td>
</tr>
<tr>
<td>HIV-1 load, median (range), log_{10} copies/mL</td>
<td>5.04 (1.70–7.24)</td>
<td>4.87 (1.70–7.76)</td>
<td>.57e</td>
</tr>
</tbody>
</table>

a Two-sample unpaired Student’s t test.

b Fisher’s exact test.

c χ^2 test.
Subjects in groups 1–3 were considered to have acute infection, and those in groups 4 and 5 were considered to have early infection (table 1). Risk factors for exposure to HIV-1, demographic characteristics, and HIV-1 treatment history were known for all subjects in the study.

**Study design.** A retrospective longitudinal cohort analysis of the influence of HSV-2 serostatus on the HIV-1 dynamics of coinfected individuals was performed. Subjects were categorized on the basis of HSV-2 serostatus. Comparison of viral loads between the 2 groups was conducted at baseline using a general linear model, controlling for age and stage of infection at study entry and longitudinally over the first 360 days of follow-up, to detect temporal differences in HIV-1 dynamics using a joint model of viral loads and time to therapy and, thus, to control for possible differences between groups in time to antiretroviral therapy. Similar models have been used to analyze CD4+ T cell counts [20] during antiretroviral therapy. Because viral loads are typically higher during acute infection, we used a simple semiparametric approach to capture nonlinearity in viral dynamics, as has been used elsewhere [21].

**Serologic testing.** Plasma and serum samples collected during study enrollment and subsequent follow-up visits were obtained and stored at −80°C. Baseline serum samples from all studied individuals were subjected to HSV-2–specific EIA (HerpeSelect; Focus Technologies) performed at ARUP Laboratories. Serum samples with ODs of 1.1–3.5 were confirmed as positive by HSV-2 Western blotting (University of Washington, Seattle). HIV-1 serologic testing and HIV-1 loads (Roche Amplicor) were available for analysis in the UCSD AIEDRP database.

**Statistical analysis.** Bivariate comparisons between baseline viral loads and CD4+ T cell counts between HSV-2–seropositive and –seronegative individuals were tested using linear models, unpaired Student’s t tests, and exact Wilcoxon tests (data not shown), with and without stratification by infection stage. For multivariate analysis, a linear model was fitted to test differences in log-transformed HIV-1 loads between the HSV-2–seropositive and –seronegative individuals at baseline, controlling for age and HIV-1 infection stage at entry. The model for the baseline log-transformed viral loads or square root–transformed CD4+ T cell count for individual i at time point \( t \), \( y_{ij} \), is as follows:

\[
\begin{align*}
y_{ij} & \sim \text{Normal}(\mu_{ij}, \sigma) , \\
\mu_{ij} & = \beta_{i1} + \beta_{i2} \times h_i + U_{ij} + \beta_{i3} \times (a_i - 37) + \beta_{i4} \times g_i .
\end{align*}
\]

In this model, \( h_i \in (0,1) \) is the HSV-2 serostatus, \( a_i \) is the age of the participant in years (which is then centered at 37 years), and \( g_i \in (0,1) \) is an indicator of the infection stage (acute or early). This model was fitted using the “glm” function in the statistical software R (version 2.2.1; available at: http://www.r-project.org) [22].

To model longitudinal viral loads while controlling for possible differences between groups in time to therapy, we fitted a joint model of viral loads and time to therapy. We adopted a Bayesian approach, as recommended by Guo and Carlin [20]. The model used for the longitudinal log-transformed viral loads for individual \( i \) at time point \( t \), \( y_{ij} \), is as follows:

\[
y_{ij} = \text{Normal}(\mu_{ij}, \sigma) , \\
\mu_{ij} = \beta_{i1} + \beta_{i2} \times h_i + U_{ij} + \beta_{i3} \times s_{ij} + \beta_{i4} \times s_{ij} + h_i + U_{ij} + \beta_{i5} \times s_{ij} + \beta_{i6} \times s_{ij} .
\]

The terms \( s_{ij} \) and \( s_{ij} \) are B spline bases for a natural cubic spline with 3 df, with knots placed at 30 and 120 days. The terms \( U_{ij} \), \( U_{ij} \), and \( U_{ij} \) are individual-level random effects, which are assumed to follow a multivariate normal distribution, Normal(0, Ω).

The model for the relative hazard of going on to therapy assumes that the time \( t \) is distributed as an exponential distribution, parameterized as a Weibull distribution with a fixed-scale parameter, as follows:

\[
t_i \sim \text{Weibull}(1, \nu_i) , \\
\log(\nu_i) = \beta_{i1} + \beta_{i2} \times h_i + r_i \times U_{ij} .
\]

Note that, in model (2b), variation in the random intercept \( U_{ij} \) of the viral load is assumed to correlate with time to therapy. The median time to therapy is calculated for the HSV-2–seronegative and –seropositive groups using the expressions \( \ln(2) \exp(-\beta_{i1}) \) and \( \ln(2) \exp(-\beta_{i2}+\beta_{i3}) \), respectively.

Models (2a) and (2b) were fitted using a Bayesian Markov Chain Monte Carlo approach in the WinBUGS program (version 1.4; available at: http://www.mrc-bsu.cam.ac.uk/bugs/) [23], using chains of 50,000 iterations with a 5000-iteration burn-in period. We assumed “vague” priors for the parameter values, either gamma distributions with shape and scale parameters of 0.1, univariate normal distributions with a mean of 0 and precision of 0.01, or multivariate normal distributions with variances of 100, and all covariances set to 0. Posterior distributions were summarized by the median and the 2.5% and 97.5% quantiles.
HSV-2 Coinfection and HIV-1 Dynamics

**RESULTS**

**Study population.** As of June 2005, 309 eligible subjects were enrolled in the UCSD AIEDRP. Twelve women (5 HSV-2 seropositive and 7 HSV-2 seronegative) were excluded from the final analysis because the female sample size was too small to adjust for possible confounding by sex. Three men who had low positive HSV-2 EIA titers and subsequent indeterminate HSV-2 Western blot results were also excluded from the study. Our final study cohort consisted of 294 men. More than 90% of subjects reported sex with men as their primary HIV-1 risk factor. A total of 122 subjects (41.5%) were coinfected with HSV-2 (table 1). HIV-1/HSV-2–coinfected subjects were significantly older than HIV-1–monoinfected individuals (mean age, 40.7 vs. 35.4 years, respectively; \( P < .0001 \), unpaired Student’s \( t \) test). Eighty-five subjects (28.9%) had estimated acute HIV-1 infection, and 209 (71.1%) had early HIV-1 infection. No difference was observed in the distribution of acute or early HIV-1 infection stage between the 2 study groups (\( \chi^2 \) test). There was also no significant difference in the initial CD4\(^+\) T cell count between the 2 groups (mean of square root–transformed CD4\(^+\) T cell count, 22.42 and 22.57 for HSV-2–seronegative and –seropositive individuals, respectively; \( P = .78 \), unpaired Student’s \( t \) test). CD4\(^+\) T cell counts were significantly lower among subjects with acute versus early HIV-1 infection (\( P = .037 \)), as reported elsewhere [24, 25] (figure 1).

**Serologic testing.** Serum samples subjected to HSV-2 EIA were obtained a median of 9 days (interquartile range [IQR], 5–15 days) from the date of study enrollment (baseline) and 93 days (IQR, 55–99 days) after the estimated date of infection (EDI). The median HSV-2 EIA optical density in the coinfected group was 6.23 (IQR, 3.1–9.3). Thirty-six serum samples had low HSV-2 EIA optical density results (IQR, 1.1–3.5) and were subjected to confirmatory HSV-2 Western blot analyses; 18 were positive, 15 were negative, and 3 were indeterminate. The latter 3 were excluded.

**Analysis of HIV-1 dynamics.** In bivariate analyses, baseline viral loads did not differ significantly between HSV-2–seropositive or –seronegative individuals, for those with either acute or early infection (figure 2). In a multivariate linear model controlling for age and disease stage at entry, HSV-2 status was associated with a 0.16 log\(_{10}\) lower baseline HIV-1 load, but this was also not statistically significant (\( P = .21 \)). As would be expected, HIV-1 loads were significantly higher (0.98 log\(_{10}\); \( P < .0001 \)) in subjects with acute infection than in subjects with early infection. Age was not significantly associated with baseline viral load (0.006 log\(_{10}\) per year; \( P = .36 \)).

The median period of follow-up was 188 and 217 days in the HSV-2–seropositive and –seronegative groups, respectively, after the EDI. We analyzed only the HIV-1 loads obtained while the individuals were antiretroviral naive or not receiving prophylactic HSV-2 therapy. Four patients required prophylactic HSV-2 therapy during the study period. Data were analyzed at 360 days of follow-up and were stratified by infection stage. A joint model of viral loads and time to therapy was used to...
control for possible differences in follow-up between the HSV-2–seropositive and –seronegative groups, as described elsewhere [20]. We adopted a Bayesian approach to fitting this model, because this has been shown to give improved parameter estimates, compared with standard maximum-likelihood approaches [20]; this approach generates full probability distributions of parameters of interest, which are described using 95% “credibility intervals,” the Bayesian analogue of confidence intervals. We used a semiparametric model of viral loads to capture nonlinear viral dynamics during early therapy and a simple parametric model of time to therapy in which individual-level variation in viral loads was linked to time to antiretroviral therapy.

For the 85 individuals who entered the study with acute infection, the median follow-up times were similar for HSV-2–seropositive (76 days [95% credibility interval, 51–117 days]) and HSV-2–seronegative (76 days [95% credibility interval, 53–112 days]) individuals. Individuals with higher HIV-1 loads initiated therapy sooner (parameter $\beta_{12}$ in model [2b], 0.40 [95% credibility interval, 0.15–0.70]). The estimated baseline viral load was slightly lower in HSV-2–seropositive individuals, but this was not significantly different between the 2 groups (parameter $\beta_{13}$ in model [2a], $-0.17 \log_{10}$ copies/mL [95% credibility intervals, $-0.73$ to $0.44 \log_{10}$ copies/mL]). Viral loads were higher during the beginning of the follow-up period (figure 3A and 3B), but the difference in mean viral loads between HSV-2–seropositive and –seronegative individuals remained close to 0 throughout the period of follow-up (figure 4A).

Longitudinal analyses of time to treatment and viral loads in the 209 individuals who entered the study with early infection were consistent with the analysis of acutely infected individuals (figure 3C and 3D). The estimated median time to antiretroviral therapy was 331 days (95% credibility interval, 222–520 days) in HSV-2–seropositive individuals and 208 days (95% credibility interval, 155–285) in HSV-2–seronegative individuals. The estimated baseline viral load was $0.12 \log_{10}$ copies/mL lower in HSV-2–seropositive subjects (95% credibility interval, $-0.45$ to $0.2 \log_{10}$ copies/mL), and this difference remained close to 0 throughout the period of follow-up (figure 4B).

**DISCUSSION**

The present large, retrospective, longitudinal study to assess the effect of HSV-2 serostatus on HIV-1 dynamics during early

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**Figure 3.** Scatter plots of viral loads according to herpes simplex virus type 2 (HSV-2) serostatus for individuals enrolled during acute infection (A and B) and early infection (C and D). Dots represent individual viral load values for each study subject. Solid lines represent the best-fit curve of the mean viral load over time. Interrupted lines represent the 95% credibility intervals.
HSV-2 Coinfection and HIV-1 Dynamics

Figure 4. Difference in mean viral load over time (solid lines) between herpes simplex virus type 2 (HSV-2)–seropositive and HSV-2–seronegative individuals with associated 95% credibility intervals for individuals enrolled with acute (A) and early (B) infection.

HIV-1 infection found no significant difference between either baseline viral load or longitudinal patterns of viremia between HSV-2–seropositive and –seronegative individuals. Both study groups were monitored for at least 6 months after their EDI. The strengths of our study are the number of individuals (n = 294) and the large number of viral load measurements (n = 2434), standardization of the acute and early stage of HIV-1 infection, and the use of sophisticated models to jointly model longitudinal viral loads and time to initiating antiretroviral therapy.

Our findings are in contrast to those of a previous study that included 219 subjects with primary HIV-1 infection from Rakai, Uganda [15]. In that study, 2 cross-sectional analyses were conducted. The first analysis occurred at 5 months and the second at ~15 months after the EDI. In the first analysis, there was a significantly higher mean ± SD HIV-1 load in HSV-2–seropositive subjects than in HSV-2–seronegative subjects (4.48 ± 0.10 vs. 3.93 ± 0.17 log_{10} copies/mL; P = .004). In the second analysis, which included only 58 patients from the same cohort, no differences were found in the plasma HIV-1 load among the HSV-2–seropositive and –seronegative subjects (mean ± SD, 4.53 ± 0.17 vs. 4.40 ± 0.17 log_{10} copies/mL; P = .6). There are important differences—such as sex, ethnicity, nutritional status, additional coinfections, and HIV-1 subtype—that distinguished our study from the Rakai cohort study. Our study population was composed primarily of healthy white men who have sex with men. In African countries, the coexistence of endemic diseases such as malaria and tuberculosis is relatively common and may have contributed to the observed higher HIV-1 loads [26, 27]. In the Rakai cohort, the prevalence of these coinfections was unknown, particularly because trimethoprim-sulfamethoxazole and isoniazid were not provided as prophylaxis [7]. It is also unknown to what extent differences in HIV-1 clades linked to dissimilar genetic background could have contributed to the differences observed between our study and the ones conducted in Africa [28, 29].

Our study had several limitations. First, we did not measure HSV-2 genital shedding, which could be more likely to influence HIV-1 dynamics than latent HSV-2 infection [12–14, 30] as assessed by HSV-2–positive serostatus. Longitudinal studies have shown that the frequency of HSV-2 genital shedding correlates inversely with the CD4+ T cell count in HIV-1–infected subjects [14, 31]. Indeed, the median percentage of the total rate of HSV-2 genital shedding in HIV-1–infected men with CD4+ T cell counts >500, 200–500, and <200 cells/mm³ was 6%, 15%, and 31%, respectively [14]. In our HSV-2–seropositive cohort, only 5 subjects (4.1%) had CD4+ T cell counts <200 cells/mm³. Nevertheless, we cannot exclude the possibility that these patients had a modest increase in their plasma HIV-1 RNA loads during periods of shedding. Second, we did not differentiate recently acquired (within 1 year of HSV-2 acquisition) from chronic HSV-2 serostatus. Recently acquired HSV-2 infection has been associated with a higher rate of HSV-2 shedding after the primary HSV-2 infection and a higher risk of HIV-1 acquisition [32, 33]. It is plausible that the more-severe inflammatory effects associated with recently acquired HSV-2 infection may have a more-pronounced effect on plasma HIV-1 load. However, if this situation were significant, it would have biased our results to show a difference between the 2 groups. Third, we only analyzed patients while they were antiretroviral naive, because it is not possible to evaluate the natural history in treated patients. In theory, HIV-1/HSV-2–coinfected subjects could have had higher plasma HIV-1 loads and,
thus, require an earlier initiation of antiretroviral therapy. However, HSV-2–seropositive individuals did not have significantly different HIV-1 loads at baseline than HSV-2–seronegative individuals, and our longitudinal model controlled for potential differences in times to therapy. Furthermore, there were no differences in the proportion of patients started on antiretroviral therapy between the HSV-2–seropositive and –seronegative individuals. Finally, although there is growing evidence about the increasing prevalence of HSV-1 as a cause of genital ulcerative disease [34], we did not assess HSV-1 seropositivity, because, to our knowledge, there are no reports that have indicated that HSV-1 influences plasma HIV-1 loads.

In summary, HSV-2 serostatus showed no influence on the dynamics of plasma HIV-1 loads during acute or early HIV-1 infection; therefore, it is unlikely that HSV-2 serostatus influences HIV-1 disease progression during primary HIV-1 infection in men. Prospective studies are needed to investigate whether (1) discordant results with prior studies are related to differences in HIV-1 clades or genetic background and (2) subjects who have concurrent HSV-2 genital shedding or recently acquired HSV-2 infection may experience a stronger influence on the initial set-point level of HIV load during primary infection.

Acknowledgments

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References


