High Risk of Human Immunodeficiency Virus in Men Who Have Sex with Men with Herpes Simplex Virus Type 2 in the EXPLORE Study

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The relation between herpes simplex virus type 2 (HSV-2) and human immunodeficiency virus (HIV) acquisition was evaluated among 4,295 high-risk, HIV-negative men who have sex with men in an intensive behavioral intervention (colloquially referred to as “EXPLORE”) study in the United States from 1999 to 2003. Sexual behavior data were obtained by computer-assisted self-interview, and sera were collected semiannually for HIV and HSV-2 serology. HSV-2 infection was classified as “recent incident” (at the first HSV-2 seropositive visit), “remote incident” (within 24 months of the first positive visit), and “prevalent” (for visits >24 months after the first HSV-2 positive visit). Baseline HSV-2 prevalence was 20.3%. HSV-2 incidence was 1.9 (95% confidence interval (CI): 1.6, 2.2) per 100 person-years; significant risk factors were African-American race, unprotected receptive anal intercourse, an HIV-positive male sex partner, and six or more male partners in the prior 6 months. The behavioral intervention did not reduce HSV-2 acquisition (adjusted hazard ratio (HR) = 1.2, 95% CI: 0.9, 1.6). Overall HIV incidence was 1.9 (95% CI: 1.7, 2.2) per 100 person-years. HIV risk was elevated among men who have sex with men with recent incident HSV-2 (adjusted HR = 3.6, 95% CI: 1.7, 7.8), remote incident HSV-2 (adjusted HR = 1.7, 95% CI: 0.8, 3.3), and prevalent HSV-2 (adjusted HR = 1.5, 95% CI: 1.1, 2.1) infection compared with HSV-2 seronegative participants. HIV intervention strategies targeting HSV-2 prevention and suppression among men who have sex with men should be evaluated.

behavior therapy; herpes genitalis; herpesvirus 2, human; HIV; homosexuality, male

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; HR, hazard ratio; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2.

Genital herpes, most commonly caused by herpes simplex virus type 2 (HSV-2), is among the most prevalent sexually transmitted infections globally (1–3). Genital HSV-2 infection increases the risk of human immunodeficiency virus (HIV) type 1 acquisition (4–8), likely via mucosal or epithelial disruption, which provides a portal of entry for HIV.
and via recruitment of CD4-positive T lymphocytes during HSV-2 reactivation (9). Because the period immediately following genital HSV-2 acquisition is marked by increased frequency and duration of reactivations (10), people with early HSV-2 infection may have an even higher risk of HIV acquisition than those with established HSV-2 infection.

The EXPLORE study was a randomized, controlled, phase IIb efficacy trial of a 10-session behavioral intervention designed to reduce HIV acquisition among 4,295 high-risk, HIV-negative men who have sex with men. Although there was no significant reduction in HIV incidence (intervention effect = 15.7 percent, 95 percent confidence interval (CI): −8.4, 34.4), a statistically significant reduction of 20.5 percent (95 percent CI: 10.9, 29.0) in unprotected receptive anal intercourse with an HIV-positive or unknown serostatus partner was detected in the behavioral intervention arm (11).

We used blood samples and behavioral data collected during the EXPLORE study to assess risk factors for HSV-2 acquisition, to evaluate the association of prevalent and incident HSV-2 infection with HIV acquisition, and to determine the effect of the behavioral intervention on HSV-2 acquisition.

MATERIALS AND METHODS

The EXPLORE study was conducted from 1999 to 2003 in six US cities: New York, New York; San Francisco, California; Seattle, Washington; Boston, Massachusetts; Chicago, Illinois; and Denver, Colorado. Details of the eligibility criteria, study protocol, primary results, and baseline characteristics of the participants have been reported previously (www.explorestudy.org) (11–13). Institutional review boards at each study site approved the study. All participants provided written informed consent that included HIV and HSV-2 serologic testing.

Subjects

Participants were recruited between January 1999 and February 2001, as described previously (11, 13). Men were eligible if they were HIV seronegative, were aged 16 years or older, reported engaging in anal intercourse with at least one male partner in the past year, and were not in a mutually monogamous relationship with an HIV-negative partner lasting 2 or more years.

Study procedures

At enrollment, participants were randomized to either the intervention arm or the control arm. Those in the intervention arm received 10 individualized counseling sessions of 1-hour duration with specific themes delivered over 4–6 months, followed by quarterly maintenance sessions (12). Those in the control arm received semianual HIV testing with pre- and posttest counseling based on the Centers for Disease Control and Prevention’s Project RESPECT model (14). Sexual risk behavior data were collected semianually via audio computer-assisted self-interview to increase the accuracy of reporting of sensitive behaviors, such as unprotected sex (15–17). Blood samples were collected semianually for HIV testing, with samples stored for future batch testing for HSV-2 antibodies.

Laboratory methods

HSV-2 serologic testing was performed with the commercially available, type-specific HerpeSelect type 2 enzyme-linked immunosorbent assay (Focus Diagnostics, Cypress, California), with herpes simplex virus Western blot (18) confirmation of sera with index values between 0.9 and 3.5 because of the lower specificity of the enzyme-linked immunosorbent assay in the low positive (index values = 1.1–3.5) range (19, 20). HSV-2 testing was performed at the University of Washington after the study was completed. Final visit sera from consenting participants who had stored samples available were tested first, followed by testing of baseline sera of those identified as HSV-2 seropositive at the final visit. Those participants who appeared to seroconvert to HSV-2 during follow-up (based on a negative baseline result and a positive final visit result) had HSV-2 testing performed on all available interim sera, to determine the time of seroconversion. Results were provided to study sites and offered to participants.

Statistical methods

All behavioral and sexually transmitted infection data were reported at each visit for the 6-month period prior to that visit, including diagnosis or treatment for syphilis, gonorrhea, or chlamydia since the prior study visit. At each visit, participants were asked the following questions: “Since your last interview, have you had a recurrent episode or been newly diagnosed with penile or rectal herpetic ulcers?” and “Have you had sores on your penis or in or around your rectum since your last interview?” Participants answering “yes” to either question were categorized as having genital ulcer disease at that visit. The number of male sex partners since the prior study visit was dichotomized at the median (≤5 vs. >6 partners). Serodiscordant intercourse was defined as intercourse with an HIV-positive partner or a partner of unknown HIV status. The number of unprotected receptive anal sex acts was categorized in quartiles. Alcohol use and drug use were categorized as described previously (21), and depression was evaluated using a shortened seven-item version of the Center for Epidemiologic Studies Depression Scale (22), with a score of at least 13 of 28 considered as reflecting some depressive symptoms. Missing behavioral data were left as missing rather than being carried forward or imputed.

Participants who acquired HSV-2 during follow-up were classified as HSV-2 negative until the first visit at which they tested positive for HSV-2 antibodies. HSV-2 seroconverters were classified as having “recent incident” HSV-2 at the first HSV-2 seropositive visit, “remote incident” HSV-2 for all visits occurring within 24 months after the first positive visit, and “prevalent HSV-2” for all visits occurring more than 24 months after the first positive visit. Thus, a participant who acquired HSV-2 during follow-up could contribute person-time to multiple categories of HSV-2 status. Participants seropositive for HSV-2 at baseline were classified as having prevalent infection at all study visits. Ten participants
who acquired HSV-2 during follow-up but were missing inter- 

term sera reported genital ulcers during follow-up. For 

these 10 participants, the timing of HSV-2 acquisition was 

assigned as the first visit with missing serology when genital 

ulcers were reported. An additional 27 participants missing 

interim sera who seroconverted to HSV-2 did not report any 

genital ulcers. In analyses in which HSV-2 acquisition was 

the outcome, the time of HSV-2 acquisition for these 27 

participants was estimated as the midpoint between the last 

negative and the first positive HSV-2 test. In analyses in 

which HSV-2 was a predictor (e.g., time to HIV infection), 

we imputed the timing of HSV-2 acquisition, assuming an 

equal probability of HSV-2 acquisition at each study visit for 

these 27 participants. Given the less precise timing of HSV-2 

acquisition in these 37 participants, we conducted sensitivity 

analyses, assuming extreme values of HSV-2 acquisition 

time for these 37 participants.

HSV-2 acquisition rates were compared between the 

intervention and control groups by intention-to-treat analysis. 

A binomial regression model with log link \((23)\) was used to 

compute per-contact rates of HSV-2 acquisition for recep- 

tive, insertive, protected, and unprotected anal intercourse 

and by partner’s HIV status. The model assumes that the per- 

contact infectivity is independent of the number of sex acts 

of an individual, and so the per-contact rates should be the 

same for people who have a high number of sex acts versus 

low numbers of sex acts. To assess the validity of the model 

assumption, we repeated these calculations, successively 
excluding greater proportions of observations containing 

the highest numbers of reported anal sex acts to determine 

whether the calculated per-act infectivity remained constant 

regardless of the number of reported sex acts.

Cox proportional hazards regression models were used to 

assess the effect of the intervention on HSV-2 acquisition 

rates, the factors associated with HSV-2 acquisition, and the 
asociations of recent and remote incident and prevalent 

HSV-2 infection with HIV acquisition. Predictors of HSV-2 

acquisition were identified by use of univariate regression; 

those factors found to be significant in the univariate models 

were retained in the multivariate model, which also 

included study site, study arm, and age.

For the analysis of the associations of incident and prev- 

alent HSV-2 with HIV acquisition, all behavioral covariates 

found to be predictors of HIV infection in earlier analyses of 

this data set \((21)\) or thought to be important markers of 

sexual risk behavior were retained in the multivariate model, 
even though some of these did not alter the risk estimate 

appreciably. Stata, version 8.0, software (StataCorp LP, Col- 

lege Station, Texas) was used for all analyses.

RESULTS

A total of 4,295 HIV-negative men who have sex with 

men participated in the EXPLORE study. Of these men, 

3,909 (91 percent) had valid HSV-2 test results. The remain- 
der of the participants either did not have stored sera avail- 
able for HSV-2 testing \((n = 358)\) or had indeterminate 

HSV-2 test results even after the Western blot was per- 
formed \((n = 28)\), and they were excluded from the analyses. 

Of these 3,909 men, 1,980 were in the control arm, with a 

median follow-up time of 36.2 months \((range: 1.2–51.2 

months)\), and 1,929 were in the intervention arm, with a 

median follow-up of 36.1 months \((range: 2.1–52.7 months)\). 

The median age was 33 years \(\text{interquartile range: 27–39 

years})\); 27 percent were non-Caucasian \((14 \text{ percent} 

Hispanic, 7 percent African American, and 6 percent other)

and the median educational level was a college degree.

Incidence of HSV-2

Among the 3,909 participants with HSV-2 results, 793 

\((20.3 \text{ percent})\) were HSV-2 seropositive at enrollment, 2,949 

\((75.4 \text{ percent})\) remained seronegative throughout the study, 

and 167 \((4.3 \text{ percent})\) acquired HSV-2 during follow-up. 

There was no difference in HSV-2 incidence between the 

study arms \((figure 1)\). Of the participants who acquired 

HSV-2, 87 were in the 10-session behavioral intervention 

arm, and 80 were in the brief risk-reduction counseling 

control arm \(\text{adjusted hazard ratio} (HR) = 1.2, 95 \text{ percent} 

CI: 0.9, 1.6) \(\text{table 1})\).

The overall HSV-2 incidence rate was 1.9 \((95 \text{ percent CI:} 

1.6, 2.2) \text{ per 100 person-years}\). HSV-2 acquisition rates per 

10,000 sex acts with partners of unknown HSV-2 status 

ranged from 2.4 \((95 \text{ percent CI: 1.8, 3.1})\) for all anal 

intercourse with HIV-negative partners to 5.0 \((95 \text{ percent} 

CI: 2.7, 7.4)\) for all anal intercourse with HIV-positive partners. 

Rates were somewhat higher for all receptive acts \((3.9, 

95 \text{ percent CI: 2.8, 4.9})\) than for all insertive acts \((2.7, 

95 \text{ percent CI: 1.9, 3.6})\) and for protected acts \((4.2, 95 \text{ percent} 

CI: 3.2, 5.2)\) than for unprotected acts \((2.4, 95 \text{ percent} 

CI: 1.6, 3.1)\). In an analysis of the validity of the model assump- 
tions of independence of infectivity of individual sex acts, 
calculated per contact, HSV-2 acquisition rates varied with 

the number of sex acts reported, with exclusion of observ- 

ations with the highest reported numbers of sex acts causing 

the per-contact rates to increase; for example, if the obser- 

vations with the highest 50 percent of observations are ex- 

cluded, then the per-contact rates increase approximately 

threefold. No data on the HSV-2 serostatus of partners or the 
distribution of sex acts across partners were available.

Among HSV-2 seroconverters who had their timing of 

HSV-2 acquisition documented serologically, only 8.1 \text{ per- 

cent reported a first episode of genital ulcers in the 6-month 

interval just prior to the first positive HSV-2 test. Genital 

ulcers within the prior 6 months were reported at some point 
during the study by 31 percent of the participants who were 

HSV-2 seropositive at baseline, 32 percent of HSV-2 sero- 
converters, and 17 percent of participants who remained 

HSV-2 seronegative. A diagnosis of syphilis in the prior 

6 months was reported at 0.34 percent of all study visits 

and at 1.4 percent of visits at which participants reported 
genital ulcer symptoms but were HSV-2 seronegative.

Risk factors for HSV-2 acquisition

Factors associated with HSV-2 acquisition in the multivar- 

iate model included unprotected receptive anal intercourse 

(for \(\geq 5\) unprotected receptive anal intercourse acts vs. no
receptive anal intercourse within the prior 6 months: adjusted HR = 2.6, 95 percent CI: 1.6, 4.1), having at least one HIV-positive male partner within the past 6 months (adjusted HR = 1.6, 95 percent CI: 1.2, 2.3), having six or more male partners in the prior 6 months (adjusted HR = 1.5, 95 percent CI: 1.1, 2.1), and African-American compared with Caucasian race (adjusted HR = 1.9, 95 percent CI: 1.1, 3.2) (table 1). These findings were not sensitive to

![Kaplan-Meier curve of proportion of EXPLORE study participants in six US cities remaining herpes simplex virus type 2 (HSV-2) uninfected, by study arm, 1999–2003.](image)

### TABLE 1. Factors associated with herpes simplex virus type 2 acquisition among men who have sex with men in six US cities participating in the EXPLORE study, 1999–2003

<table>
<thead>
<tr>
<th></th>
<th>No. of events (n = 167)</th>
<th>Person-years at risk</th>
<th>Unadjusted hazard ratio</th>
<th>95% confidence interval</th>
<th>Adjusted hazard ratio*</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>119</td>
<td>6,545</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>21</td>
<td>1,201</td>
<td>1.0</td>
<td>0.6, 1.5</td>
<td>0.9</td>
<td>0.6, 1.5</td>
</tr>
<tr>
<td>African American</td>
<td>17</td>
<td>553</td>
<td>1.7</td>
<td>1.0, 2.8</td>
<td>1.9</td>
<td>1.1, 3.2</td>
</tr>
<tr>
<td>Other</td>
<td>10</td>
<td>522</td>
<td>1.1</td>
<td>0.6, 2.0</td>
<td>1.1</td>
<td>0.6, 2.1</td>
</tr>
<tr>
<td><strong>Study arm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>80</td>
<td>4,497</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behavioral intervention</td>
<td>87</td>
<td>4,325</td>
<td>1.1</td>
<td>0.8, 1.5</td>
<td>1.2</td>
<td>0.9, 1.6</td>
</tr>
<tr>
<td><strong>Unprotected receptive anal intercourse†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No receptive anal intercourse</td>
<td>32</td>
<td>2,988</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All receptive anal intercourse protected</td>
<td>44</td>
<td>2,172</td>
<td>1.9</td>
<td>1.2, 3.0</td>
<td>1.7</td>
<td>1.1, 2.7</td>
</tr>
<tr>
<td>1–4 times unprotected</td>
<td>35</td>
<td>1,768</td>
<td>1.9</td>
<td>1.2, 3.0</td>
<td>1.7</td>
<td>1.0, 2.7</td>
</tr>
<tr>
<td>≥5 times unprotected</td>
<td>47</td>
<td>1,635</td>
<td>2.7</td>
<td>1.7, 4.2</td>
<td>2.6</td>
<td>1.6, 4.1</td>
</tr>
<tr>
<td>Any male human immunodeficiency virus-positive partner†</td>
<td>52</td>
<td>1,871</td>
<td>1.8</td>
<td>1.3, 2.5</td>
<td>1.6</td>
<td>1.2, 2.3</td>
</tr>
<tr>
<td><strong>No. of male partners†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5</td>
<td>66</td>
<td>4,658</td>
<td>Referent</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥6</td>
<td>94</td>
<td>4,077</td>
<td>1.7</td>
<td>1.3, 2.4</td>
<td>1.5</td>
<td>1.1, 2.1</td>
</tr>
</tbody>
</table>

* Also adjusted for age and study site.
† In prior 6 months.
the timing assumptions made for the 37 participants with uncertain dates of HSV-2 acquisition, because the hazard ratios for these associations do not differ meaningfully as the timing of HSV-2 acquisition is varied from the beginning to the end of the follow-up period for these 37 participants (data not shown).

Association of HSV-2 with HIV acquisition

The overall HIV acquisition rate among this subset (91 percent) of the EXPLORE cohort was 1.9 (95 percent CI: 1.7, 2.2) per 100 person-years. Among the 20 participants who acquired both HSV-2 and HIV during follow-up, HSV-2 was detected before HIV for seven participants. The interval by which HSV-2 preceded HIV ranged from 21 days to 751 days (median: 478 days). For the other 13 participants, HSV-2 and HIV were first detected at the same study visit, and for eight of these 13, no interim sera were available for testing. HSV-2 acquisition was never detected after HIV acquisition, because participants exited the study when they acquired HIV.

HIV acquisition rates were elevated among participants with recent incident HSV-2 (6.9 per 100 person-years, 95 percent CI: 3.1, 15.4), remote incident HSV-2 (6.8 per 100 person-years, 95 percent CI: 3.9, 12.0), and prevalent HSV-2 (2.7 per 100 person-years, 95 percent CI: 2.2, 3.5) compared with the rate among participants who remained HSV-2 seronegative (1.5 per 100 person-years, 95 percent CI: 1.3, 1.8) (figure 2). The unadjusted hazard ratio of HIV acquisition was 5.9 (95 percent CI: 2.8, 12.5) for recent incident HSV-2, 3.1 (95 percent CI: 1.6, 6.1) for remote incident HSV-2, and 1.8 (95 percent CI: 1.3, 2.4) for prevalent HSV-2, compared with HSV-2 seronegative participants. When adjusted for the important markers of risk behavior as described in detail by Koblin et al. (21), the hazard ratio of HIV acquisition among those with recent incident HSV-2 was 3.6 (95 percent CI: 1.7, 7.8); that for remote incident HSV-2 was 1.7 (95 percent CI: 0.8, 3.3), and that for prevalent HSV-2 was 1.5 (95 percent CI: 1.1, 2.1) (table 2), compared with participants who were HSV-2 seronegative.

These findings are somewhat sensitive to assumptions about the timing of infection for the 37 participants with uncertain acquisition dates. The adjusted hazard ratios for recent incident and remote incident HSV-2 vary from 2.6 (95 percent CI: 1.1, 6.1) to 6.3 (95 percent CI: 3.7, 10.9) and from 1.6 (95 percent CI: 0.8, 3.2) to 0.7 (95 percent CI: 0.2, 2.1), respectively, as the time of HSV-2 infection is varied from the beginning to the end of the follow-up period for these 37 participants.

DISCUSSION

This multicenter trial is the largest prospective study of HSV-2 incidence and risk factors ever conducted among men who have sex with men, with 167 documented HSV-2 acquisition events among 218 participants. The overall HIV acquisition rate among EXPLORE participants was 1.9 per 100 person-years, and HIV acquisition rates were elevated among participants with recent incident HSV-2, remote incident HSV-2, and prevalent HSV-2 compared with those who remained HSV-2 seronegative. These findings support the hypothesis that HSV-2 is a risk factor for HIV acquisition, and they highlight the importance of interventions to reduce HSV-2 acquisition and transmission.

TABLE 2. Factors associated with human immunodeficiency virus acquisition among men who have sex with men in six US cities participating in the EXPLORE study, 1999–2003

<table>
<thead>
<tr>
<th>HSV-2 $^*$ status</th>
<th>No. of events (n = 218)$^+$</th>
<th>Person-years at risk</th>
<th>Unadjusted hazard ratio</th>
<th>95% confidence interval</th>
<th>Adjusted hazard ratio$^+$</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2 seronegative</td>
<td>133</td>
<td>8,724</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Recent incident HSV-2</td>
<td>6</td>
<td>87</td>
<td>5.9</td>
<td>2.8, 12.5</td>
<td>3.6</td>
<td>1.7, 7.8</td>
</tr>
<tr>
<td>Remote incident HSV-2</td>
<td>12</td>
<td>176</td>
<td>3.1</td>
<td>1.6, 6.1</td>
<td>1.7</td>
<td>0.8, 3.3</td>
</tr>
<tr>
<td>Prevalent HSV-2</td>
<td>67</td>
<td>2,451</td>
<td>1.8</td>
<td>1.3, 2.4</td>
<td>1.5</td>
<td>1.1, 2.1</td>
</tr>
</tbody>
</table>

$^*$ HSV-2, herpes simplex virus type 2.

$^+$ Because of missing interim HSV-2 serologies for 27 participants, the number of events and person-years at risk by HSV-2 status are approximations (refer to Materials and Methods).

$^+$ Adjusted for study arm, study site, age, race, unprotected receptive anal intercourse, any human immunodeficiency virus-positive male partner, number of male partners, heavy alcohol use, drug or alcohol use before or during sex, amphetamine use, popper use, depression, gonorrhea, and syphilis (all behaviors and sexually transmitted infections reported at each study visit, for the 6 months prior to visit).
seroconversions, and the first study to estimate HSV-2 acquisition rates on a per-contact basis among men who have sex with men, although these estimates are limited because the HSV-2 serostatus of their partners is unknown. It is also the only study to ascertain the effect of a behavioral intervention on HSV-2 acquisition among men who have sex with men. The intensive, 10-session risk-reduction behavioral intervention tested in the EXPLORE study did not reduce HIV incidence to a statistically significant degree and had no effect on HSV-2 incidence, despite the statistically significant reduction in reported unprotected receptive anal intercourse (11). Participants with HSV-2 infection in this cohort had elevated rates of HIV acquisition; furthermore, these data suggest that incident HSV-2 might confer a further increase in HIV risk compared with prevalent HSV-2 infection.

The elevated risk of HIV infection among HSV-2 seropositive participants in this cohort substantiates the findings of meta-analyses by Wald and Link (7) and Freeman et al. (8). In the latter meta-analysis, the relative risk of HIV acquisition among individuals with HSV-2 infection ranged from 1.7 (95 percent CI: 1.2, 2.4) for men who have sex with men to 3.1 (95 percent CI: 1.7, 5.6) for women. Additionally, the stronger association between HIV and recently acquired HSV-2, compared with prevalent HSV-2, observed in this cohort of men who have sex with men is consistent with the temporal relation between HSV-2 and HIV acquisition that has been suggested by several prior studies among heterosexual men and women in India and Africa (4, 6, 24, 25). For example, in a cohort of sexually transmitted disease clinic patients in India, the hazard ratio of HIV was over twice as high among participants with incident HSV-2 infection (HSV-2 acquired within 6 months or less) compared with participants who were HSV-2 seropositive at enrollment (HR = 3.81 vs. 1.67) (4). In a nested case-control study of HIV seroconversions in Mwanza, Tanzania, the incidence of HIV infection was higher (odds ratios = 5.6 for men and 4.8 for women) among persons who seroconverted to HSV-2 during follow-up than among those who were HSV-2 seropositive at enrollment (odds ratios = 3.7 for men and 2.9 for women) compared with HSV-2 seronegative subjects (25).

In these studies and the present study, many participants seroconverted to both HSV-2 and HIV during the same follow-up interval; thus, the temporal sequence of the two infections cannot be ascertained for all participants. The stronger association of incident HSV-2 with HIV infection is biologically plausible as the greater frequency, severity, and duration of reactivations of HSV-2 early after HSV-2 acquisition may increase the chance that HSV-2 is reactivating when a man is exposed to HIV. However, this association may also simply represent the simultaneous acquisition of HSV-2 and HIV from a dually infected partner. Given that up to 70 percent of HIV-positive men who have sex with men are also HSV-2 seropositive (26–28) and that rates of HSV-2 shedding are over three times higher in HIV-positive compared with HIV-negative individuals (29), exposure to both viruses during sexual contact with a high-risk partner is likely. Distinguishing between these two possibilities (a causal association vs. simultaneous acquisition) is very difficult and would require very frequent HSV-2 and HIV testing of study subjects. Another possibility is that some of the increased risk of HIV observed with incident HSV-2 infection may be due to residual confounding by factors that elevate risk of acquiring both HSV-2 and HIV. Although the hazard ratios for recent and remote incident HSV-2 were substantially attenuated by adjustment for sexual risk behaviors, there may be further confounding by inaccuracies in self-reported behavior or by unmeasured variables, such as genetic factors, that might influence risk of both HSV-2 and HIV acquisition.

The risk factors for HSV-2 acquisition in this cohort were unprotected receptive anal intercourse, a higher number of male sex partners, having an HIV-positive male partner, and African-American race. The increased risk of HSV-2 among African Americans in this cohort likely reflects the higher HSV-2 prevalence among African Americans (30, 31) combined with the tendency toward assortative mating (choosing a sexual partner of one’s own racial/ethnic group) (32, 33).

This is the first study to estimate per-contact HSV-2 acquisition rates among men who have sex with men. Because the HSV-2 serostatus of participants’ partners was unknown, we included all reported sexual acts in our per-contact analyses, recognizing that HSV-2 prevalence ranges from at least 20 percent in HIV-negative men who have sex with men to up to 70 percent among HIV-positive men who have sex with men (26, 28). The per-contact HSV-2 acquisition rates we have calculated are likely to be attenuated by the inclusion of not-at-risk acts (i.e., those with HSV-2 seronegative partners). If the prevalence of HSV-2 among the partners of participants is estimated to be 20 percent (the baseline prevalence in this cohort), then the true per-contact rates derived from at-risk acts might be expected to be approximately fivefold higher than the rates presented here. The increase in per-contact HSV-2 acquisition rates seen after exclusion of observations containing the highest numbers of sex acts indicates that the model assumptions of independence are not met, leading to further uncertainty in estimated rates. Our lack of information about the distribution of sex acts across partners and partner’s HSV-2 status, coupled with the evidence from sensitivity analyses that our results were quite sensitive to model assumptions, indicates that these estimates of per-contact rates could be inaccurate and should be interpreted as rough estimates. However, it would be difficult to obtain better estimates of per-contact rates without conducting a study among couples of HSV-2 discordant monogamous men who have sex with men. The higher per-contact HSV-2 acquisition rates observed for protected acts compared with unprotected acts may indicate that participants modified their behavior on the basis of their perception of their partner’s risk; participants may have been more likely to use a condom with a partner perceived to be high risk, as has been suggested by Warner et al. (34) and Casper et al. (35).

Strengths of this study include the high retention rate over up to 4 years of follow-up (87 percent in the control arm and 83 percent in the intervention arm) (11). The use of highly accurate serologic tests for HSV-2 and HIV allowed for precise outcome measurement. Collection of extensive behavioral data by use of audio computer-assisted self-interview allowed for a detailed assessment of HSV-2 risk factors and enabled adjustment for potential behavioral confounders of
the relation between HSV-2 infection and HIV acquisition. Although the intervention did not reduce HIV or HSV-2 acquisition rates to a statistically significant degree, the control arm received extensive counseling at semiannual visits based on the best available model (Project RESPECT (14)), which may have decreased the chance of detecting a difference between study arms. The absence of a decrease in HSV-2 acquisition, despite the significant reduction in self-reported unprotected receptive anal intercourse with an HIV-positive or unknown serostatus partner, may reflect the importance of unmeasured factors related to partner selection and is consistent with prior observations that the efficacy of behavioral interventions may be underestimated by self-reported behavior change (11, 36).

This study had several limitations. Limited demographic data were available on the partners of participants. No herpes simplex virus type 1 (HSV-1) testing was done in this study, because of issues of cost and the need for oral and genital cultures to determine the site of HSV-1 infection. Since HSV-1 is an increasingly common cause of genital herpes, particularly among men who have sex with men (37), this study may have underestimated the impact of genital herpes (due to either HSV-1 or HSV-2) on the risk of HIV acquisition in this population, although when assessed serologically, HSV-1 has not been found to be a risk factor for HIV acquisition among men who have sex with men (5). The finding that 17 percent of HSV-2 seronegative participants did report genital ulcer disease symptoms indicates that genital HSV-1 infections may have been relatively common in this cohort, especially given the low reported rates of syphilis. However, syphilis data were collected by self-report (of syphilis diagnosed since prior study visit) rather than by serologic screening of all participants; thus, syphilis may have been underdiagnosed in this cohort.

The timing of HSV-2 acquisition had to be imputed for 27 HSV-2 seroconverters who had no interim sera available for HSV-2 testing and was assigned on the basis of lesions for an additional 10 seroconverters. Sensitivity analyses revealed that these assumptions may have influenced the hazard ratios we obtained for the associations of recent and remote incident HSV-2 with HIV acquisition. All participants who were HSV-2 seropositive at enrollment were classified as having prevalent HSV-2 at all study visits, with potential misclassification as some of these participants likely acquired HSV-2 recently before enrollment into the EXPLORE study, given the annual 1.9 percent HSV-2 incidence in this population. However, the bias produced by this misclassification is expected to be conservative. Finally, for over half (13 of 20) of the participants who acquired both HSV-2 and HIV during follow-up, the temporal sequence of the two infections could not be ascertained, because both were detected at the same study visit. This has been a limitation of all prior studies of the association of incident and prevalent HSV-2 with HIV acquisition.

Since this intensive behavioral intervention did not reduce HSV-2 acquisition, it is important that other methods of HSV-2 prevention be evaluated among men who have sex with men, including education, counseling about HSV-2 based on type-specific serologic testing, HSV-2 suppressive therapy, and candidate HSV-2 vaccines. Serologic testing is needed to identify men who have sex with men with HSV-2 infection, as symptoms were neither sensitive nor specific for prevalent or incident HSV-2 infection. It is evident from these data that HSV-2 infection is an important risk factor for HIV acquisition among men who have sex with men, although it remains uncertain whether incident HSV-2 confers additional risk compared with prevalent HSV-2. Even considering only the prevalent HSV-2 infections, at least 33 percent of HIV acquisitions among HSV-2-infected participants in this cohort are attributable to HSV-2, on the basis of the adjusted hazard ratio of 1.5. Given these findings, HSV-2 infection appears to be an important target for HIV prevention interventions. Studies of HSV-2 suppression with acyclovir to prevent HIV acquisition and transmission are currently underway (38) and will help to further clarify the role of HSV-2 in the spread of HIV.

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