Increased Human Herpesvirus 8 Seroprevalence in Young Homosexual Men Who Have Multiple Sex Contacts with Different Partners

David J. Blackbourn, Dennis Osmond, Jay A. Levy, and Evelyne T. Lennette

Department of Medicine, University of California, San Francisco, and Department of Epidemiology and Biostatistics, San Francisco General Hospital and University of California, San Francisco; Virolab, Inc., Berkeley, California

The objective of this study was to evaluate the behavioral risks that are associated with human herpesvirus 8 (HHV-8) infection in a cohort of young homosexual men. Seventy-nine subjects (ages 22–33 years) who completed a questionnaire about their sexual and drug use behavior over the preceding year were recruited from the San Francisco Young Men’s Health Study. Plasma samples were tested for anti-HHV-8 antibodies using an indirect IFA. Thirty-eight subjects (48.1%) were infected with HHV-8. HHV-8 infection was significantly linked to an increasing number of male sex partners (P = .025, Mantel-Haenszel χ² test for trend), suggesting a strong association between HHV-8 infection and multiple homosexual contacts.

Epidemiologic evidence has suggested that the etiologic agent of Kaposi’s sarcoma (KS) is an infectious agent [1]. A sexual route of transmission for this agent has been proposed, including oral/anal contact [2]. Overwhelming data from molecular [3, 4] and seroepidemiologic [5–8] studies have implicated human herpesvirus 8 (HHV-8, also called KS-associated herpesvirus) as this etiologic agent, but the route of HHV-8 transmission has yet to be identified. As part of our ongoing longitudinal studies to evaluate both the clinical consequences of acute and chronic HHV-8 infection and the route of transmission, we studied plasma samples from a cohort of young homosexual men to determine if behavioral risk factors are associated with HHV-8 infection.

Methods

Subjects. Subjects were recruited from the San Francisco Young Men’s Health Study (SFYMHS), an ongoing longitudinal study of the risk of human immunodeficiency virus (HIV) infection in homosexual men between the ages of 18 and 29 years at baseline [9]. Over 600 young homosexual men are entering their fourth year of follow-up in this cohort. The subjects are evaluated annually for HIV infection and complete a questionnaire about their sexual and drug use behavior over the preceding year.

In the present study, we have analyzed sexual behavior reported during the 12-month period preceding the date that the plasma sample was taken for HHV-8 serology. Subjects were stratified for HIV status in order to determine whether there was an association between HHV-8 and HIV infection. Hence, we attempted to select 30 HIV-positive subjects and 50 HIV-negative individuals. Due to either missing or inadequate plasma samples, we studied 26 HHV-infected subjects and 53 HIV-negative individuals. Within the strata, the samples were chosen randomly. Approximately half of the subjects were at high risk for sexually transmitted infections, on the basis of reporting unsafe sex with >1 partners.

For statistical analyses, subjects were divided into 1 of 2 age groups: 22–27 years (25 subjects) or 28–33 years (54 subjects). The number of sex partners was categorized as 0, 1, 2–10, or >10. Data were available for the number of receptive anal intercourse (RAI) partners and receptive oral intercourse (ROI) partners.

HHV-8 IFA. Plasma samples were tested for the presence of anti–HHV-8 antibodies using the murine antibody–enhanced IFA (MIFA) [7]. The HHV-8 antigen reactivity in this assay is determined with the body cavity–based lymphoma cell line, BCBL-1. This cell line is infected with HHV-8 but not Epstein-Barr virus. To induce lytic (cytoplasmic) HHV-8 antigen expression for MIFA, BCBL-1 cells were treated for 4 days with phorbol ester (PMA, 20 ng/mL; Sigma, St. Louis) and recombinant interleukin-6 (200 U/mL; Collaborative Biomedical Products, Bedford, MA).

HIV serology. HIV serostatus was determined by ELISA (Organon Teknika, Durham, NC) on fingerstick samples collected in the home on filter paper. Samples positive by ELISA were confirmed by Western blot (Organon Teknika).

Statistical analyses. Statistical analyses were performed with either the χ² test for categorical variables or by the Mantel-Haenszel χ² test for trend for ordered variables.

Results

Cohort description. The cohort was predominantly Caucasian (61/79), so no association of HHV-8 infection with ethnicity could be identified reliably. Only 4 study participants were intravenous drug (IVD) users, precluding detecting an association between IVD use and parenteral HHV-8 infection.
**HHV-8 seroprevalence.** The HHV-8 MIFA assay detects antibodies to two types of HHV-8 antigen, which we call latent (nuclear) and lytic (cytoplasmic) antigens [7]. This procedure has been used by others to detect antibodies to HHV-8 [10]. Previous studies have suggested that antibodies to the latent antigens are found predominantly in subjects who either have KS or are predisposed to developing KS [7, 8, 11, 12]. In the present study, 38 subjects (48.1%) were infected by HHV-8, as determined by detection of antibodies to the lytic antigens. Of the infected individuals, 9 were also seropositive for the latent antigens (11.4% of subjects studied). A statistical comparison between HHV-8 infection and age category of the subjects revealed no association with lytic antigen seropositivity. However, in terms of latent antigen seropositivity, there was a trend toward increased frequency of HHV-8 infection with older age: 8 of 54 subjects in the group 28–33 years old but only 1 of 25 in the group 22–27 years old were positive for antibodies to the latent antigen. Nevertheless, this trend was not statistically significant.

**Association between HHV-8 infection and number of sex partners.** HHV-8 seroprevalence was significantly linked to the number of sex partners in the previous 12 months (P = .025) (table 1). In particular, in univariate analyses, HHV-8 seroprevalence was significantly associated with subjects reporting either ≥2 RAI intercourse partners (65% vs. 33%, P = .014) or ≥2 ROI partners (16.7% vs. 57.4%, P = .010). All HHV-8–infected subjects had had ROI. Nineteen subjects reported having had no RAI within the previous 12 months, but 13 of them had had ROI (table 1). Five (26.3%) of these individuals were HHV-8–seropositive, and they belonged to the cohort that had participated in oral sex. All 6 subjects who reported neither RAI nor ROI in the previous 12 months were HHV-8–seronegative (table 1).

**HIV seroprevalence.** Of the 26 individuals infected with HIV, 15 were HHV-8–seropositive. There was no statistically significant association between HIV and HHV-8 infection (P = .232).

**Discussion**

The objective of this study was to assess the behavioral risk factors that are associated with HHV-8 infection by evaluating a cohort of young homosexual men. Of the 79 subjects, 38 (48.1%) were infected with HHV-8; 9 of the 38 were seropositive for antibodies to HHV-8 latent antigens. Since no statistical association was found between HHV-8 lytic antigen seroreactivity and age, and the minimum age of study participants was 22 years, HHV-8 infection presumably occurred in these subjects earlier than this age. There was a trend toward older men being seropositive for HHV-8 latent antigens, consistent with a protracted incubation period between HHV-8 infection and development of KS [8, 11, 13]. However, this trend was not statistically significant. All 9 subjects who were seropositive for HHV-8 latent antigens reported having ≥2 sex partners in the preceding 12 months.

The prevalence of HHV-8 infection was associated with an increasing number of male sex partners, categorized as 0, 1, 2–10, or >10 in the previous 12 months (P = .025) (table 1). Although the 12-month period for which sexual behavior was reported does not necessarily represent the time during which HHV-8 infection was acquired, it is a reasonable surrogate for lifetime behavior. Temporal analysis of the sexual behavior of the SFYMHS participants over the 4-year period indicates that the number of sex partners of a subject has remained relatively constant for each year of the study. Therefore, HHV-8 infection is associated with multiple homosexual contacts.

**Table 1.** Association of HHV-8 seropositivity with the number and type of partners with whom receptive sex acts were performed in a cohort of young homosexual men in the preceding year.

<table>
<thead>
<tr>
<th>No. of partners, RAI</th>
<th>No. of subjects/ partner category (n = 79)</th>
<th>No. of subjects HHV-8-infected (n = 38)</th>
<th>% total HHV-8-positive subjects</th>
<th>No. of partners, ROI, within RAI category (n = 79)</th>
<th>No. of subjects HHV-8-infected (n = 38)</th>
<th>% total HHV-8-positive subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19</td>
<td>5</td>
<td>26.3</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>9</td>
<td>39.1</td>
<td>1</td>
<td>2</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2–10</td>
<td>9</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;10</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>2–10</td>
<td>29</td>
<td>20</td>
<td>69.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2–10</td>
<td>17</td>
<td>1.29</td>
</tr>
<tr>
<td>&gt;10</td>
<td>8</td>
<td>4</td>
<td>50.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2–10</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt;10</td>
<td>7</td>
<td>7.9</td>
</tr>
</tbody>
</table>

NOTE. HHV-8 infection is associated with increasing no. of male sex partners, categorized as 0, 1, 2–10, or >10 in preceding 12 months (P = .025). Specifically, HHV-8 seroprevalence was significantly associated with subjects reporting receptive anal intercourse (RAI) with ≥2 partners (65% vs. 33%, P = .014) or receptive oral intercourse (ROI) with ≥2 partners (16.7% vs. 57.4%, P = .010). Statistical analyses were performed by Mantel-Haenszel χ² test for trend.
A recent report described HHV-8 infection associated with homosexual activity in a cohort of older men from the San Francisco Men’s Health Study [8]. In the present study, a similar association of multiple sexual contacts with HHV-8 infection has been observed with young gay men (ages 22–33 years). Thus, homosexual exposure to seminal fluid via either the oral or anal route appears to be highly associated with the risk of HHV-8 infection. The low frequency of detection of HHV-8 in seminal fluid [14] could be one reason that more frequent sexual encounters, which increase the chance of exposure to virus-containing semen, are necessary before HHV-8 infection occurs. Other sources of infectious HHV-8 that may not be associated with sexual activity could include saliva and nasal fluids [15].

This study was designed to identify behavioral risks associated with HHV-8 infection but not to provide an estimate of the prevalence of HHV-8 in this population of young gay men. Indeed, the results of the present study support the association of HHV-8 infection with multiple homosexual contacts. However, since we stratified our subjects by HIV status, our estimated prevalence of HHV-8 may be higher than that of the cohort. Nevertheless, continued temporal evaluation of the uninfected subjects in this cohort may reveal both behavioral factors specifically associated with HHV-8 infection and clinical symptoms of acute HHV-8 infection.

Acknowledgment

We thank Ann Murai for help in preparing the manuscript.

References

1. Peterman TA, Jaffe HW, Beral V. Epidemiologic clues to the etiology of Kaposi’s sarcoma. AIDS 1993;7:605–11.