High prevalence of Epstein-Barr virus type 2 among homosexual men is caused by sexual transmission


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High Prevalence of Epstein-Barr Virus Type 2 among Homosexual Men Is Caused by Sexual Transmission

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To investigate whether Epstein-Barr virus (EBV) type 2 infection is highly prevalent among homosexual men, the prevalence of EBV type 2 was studied among homosexual and heterosexual white men who were at high and low risk for sexually transmitted diseases; these data were correlated with sexual behavior. The prevalence of EBV type 2 among homosexual men was significantly higher than it was among heterosexual men (39% vs. 6%). Among high-risk heterosexual men, prevalence was significantly higher than it was among low-risk heterosexual men (15% vs. 0). In univariate analyses, EBV type 2 infection in homosexual men was significantly associated with human immunodeficiency virus (HIV) seropositivity, increased numbers of intercourse partners, non-Dutch nationality, and human herpesvirus 8 seropositivity. In multivariate analyses, an independent association with EBV type 2 was observed only for HIV seropositivity and number of sex partners. These data support the conclusion that EBV type 2 infection is more prevalent among white homosexual men and is caused by sexual transmission.

Epstein-Barr virus (EBV), a γ-herpesvirus, causes widespread, persistent infection of the human population [1]. Two EBV types exist, 1 and 2, which differ in the genes encoding transformation-associated EBV nuclear antigen (EBNA)-2 [2, 3] and EBNA-3A, -3B, and -3C [4] and, therefore, display distinct biologic differences. Both virus types can be transmitted orally and infect B lymphocytes in vivo. In addition, EBV can be transmitted via a hematogenous route, through sexual contact.

In healthy persons, it has been shown that only a single EBV strain is present in peripheral blood B lymphocytes [5]. Type 1 strains are more prevalent in white and Asian populations, whereas both types are common in Africa and New Guinea. However, within the white population, human immunodeficiency virus (HIV) type 1–infected persons are exceptional with regard to their EBV status; they frequently are infected with EBV type 2, and a high percentage of AIDS patients is superinfected with types 1 and 2 [6]. This has been ascribed to immunodeficiency.

Interestingly, among HIV-infected patients with hemophilia, the prevalence of EBV type 2 (super)infection was found to be much lower than that among HIV-infected homosexual men [7], which suggests that HIV-induced immunosuppression is not the principal explanation for EBV superinfection. Recently, we used a type-specific polymerase chain reaction (PCR) directly on peripheral blood mononuclear cells (PBMC) to study EBV type prevalence in different groups of HIV-1–infected persons [8], including those progressing to AIDS who developed non-Hodgkin’s lymphoma, opportunistic infections, or Kaposi’s sarcoma (AIDS-KS), as well as long-term asymptomatic persons. We found high prevalence of EBV type 2 in all groups (50%–62%). However, infection with EBV type 2 was not related to the degree of immunodeficiency and frequently was already found early in HIV-1 infection. Therefore, these results might be a reflection of high EBV type 2 prevalence among homosexual men. In addition, our finding that patients with AIDS-KS, in particular, had a high prevalence of EBV type 2 infection suggested that type 2 infections might be associated with specific sexual behavior, because the Kaposi’s sarcoma–inducing human herpesvirus 8 (HHV-8) has been shown to be sexually transmitted, probably by orogenital contact [9, 10]. To further test this hypothesis, we extended the EBV type analysis to groups of HIV-1–seronegative homosexual and heterosexual
Materials and Methods

Study population. We analyzed blood samples from 85 HIV-1–
seropositive homosexual men and 113 HIV-1–seronegative ho-
mosexual men who all were participants of the Amsterdam Cohort
studies on AIDS and HIV-1 infection. These persons at risk for
HIV-1 infection visited the Municipal Health Services every 3
months, when a medical history and physical examination were
carried out, blood samples were collected for HIV-1 serologic
and immunologic studies, and PBMC were cryopreserved. At entry and
every 6 months, participants completed a standardized behavioral
questionnaire.

Furthermore, we studied blood samples from 108 healthy blood
bank donors, 54 men and 54 women (“low-risk heterosexuals”), as
well as 60 randomly selected heterosexual men who visited the
Amsterdam outpatient clinic for sexually transmitted diseases
(“high-risk heterosexuals”). The median age was 39 years (range,
21–66 years) and did not differ between groups.

Lymphocyte isolation and B cell lines. PBMC were isolated
from heparinized blood by ficoll-hypaque density centrifugation.
B cells were purified by positive selection by use of CD19 micro-
beads and MiniMacs (Milteny Biotec, Bergisch Gladbach, Ger-
many) according to the protocol described by the manufacturer.

The B95.8 and Ag876 cell lines were used as sources of EBV
type 1 and EBV type 2, respectively. The EBV-negative B cell line
BJAB was used as a negative control.

DNA extraction and PCR for EBV typing. B cells were lysed
by addition of L6 lysis buffer. Genomic DNA was extracted by
precipitation with isopropanol, and 1–2 μg was amplified in a
nested PCR in 50-μL reactions containing 5 μL of 10× PCR buffer,
1.5 mM MgCl₂, 10 mM dNTPs, and 1 U of DNA Taq polymerase,
as described elsewhere [8], with the use of non–type-discriminating
EBNA-2 primers in the first reaction. The region within the EBNA-
2 gene that discriminates EBV type 1 from EBV type 2 was am-
plified with type-specific nested primers. After PCR, the identity
of the amplified EBV fragment was confirmed by Southern blot
analysis with γ³P(dATP)-labeled type-specific probes.

The assay was shown to be type specific and could detect as few
as 5 EBV copies/10⁴ EBV-negative human diploid genomes or ge-
nomes containing the other EBV type.

HHV-8 serology. Serum samples were tested for antibodies to
recombinant HHV-8 lytic-phase capsid (open-reading frame [ORF]
65) antigen and latency-associated nuclear (ORF73) antigen by
EIA, as described elsewhere [11].

Statistical analysis. For comparison of EBV type 2 prevalence
between homosexual and high- and low-risk heterosexual men, χ²
tests were used. Among homosexual men, we assessed risk factors
for EBV type 2 infection. Several demographic and sexually related
variables were examined. Because the number of sex partners is
the number during the 5 years that preceded study entry through
the date of EBV typing and, thus, depends on the time the partici-
patant was in follow-up, we represented this variable as the number
of sex partners corrected for time since follow-up. Logistic regres-
sion was used to obtain univariate and multivariate odds ratios
and 95% confidence intervals, to quantify variation in estimates.

Multivariate analyses were done with a stepwise forward procedure
(SPSS, version 7.5, for Windows; SPSS, Chicago).

Results

EBV types in homosexual and heterosexual men. EBV type
was analyzed among 85 HIV-1–infected homosexual men, 113
HIV-1–seronegative homosexual men, and 114 HIV-1–seroneg-
Table 1. Prevalence of Epstein-Barr virus (EBV) type 2 infection among 171 homosexual men, by sexual behavior and other characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. with EBV type 2 infection/total no. (%)</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤30</td>
<td>10/16 (62.5)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>31–35</td>
<td>15/33 (45.5)</td>
<td>0.50</td>
<td>0.15–1.70</td>
</tr>
<tr>
<td>36–40</td>
<td>22/47 (46.8)</td>
<td>0.53</td>
<td>0.17–1.69</td>
</tr>
<tr>
<td>41–45</td>
<td>19/36 (52.8)</td>
<td>0.67</td>
<td>0.20–2.24</td>
</tr>
<tr>
<td>&gt;45</td>
<td>22/39 (56.4)</td>
<td>0.78</td>
<td>0.24–2.56</td>
</tr>
<tr>
<td>Nationality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Dutch</td>
<td>13/17 (76.5)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Dutch</td>
<td>75/154 (48.7)</td>
<td>0.29</td>
<td>0.09–0.94</td>
</tr>
<tr>
<td>HHV-8 infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>47/106 (44.3)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Yes</td>
<td>41/65 (63.1)</td>
<td>2.14</td>
<td>1.14–4.04</td>
</tr>
<tr>
<td>HIV infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>34/90 (37.8)</td>
<td>3.29</td>
<td>1.76–6.18</td>
</tr>
<tr>
<td>Yes</td>
<td>54/81 (66.7)</td>
<td>1.47</td>
<td>0.55–3.94</td>
</tr>
<tr>
<td>Sex partners during preceding 5 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤100</td>
<td>15/39 (38.5)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>101–200</td>
<td>17/36 (47.2)</td>
<td>1.47</td>
<td>0.55–3.94</td>
</tr>
<tr>
<td>201–300</td>
<td>15/30 (50.0)</td>
<td>1.68</td>
<td>0.60–4.69</td>
</tr>
<tr>
<td>301–400</td>
<td>7/19 (36.8)</td>
<td>1.07</td>
<td>0.32–3.60</td>
</tr>
<tr>
<td>401–500</td>
<td>11/18 (61.1)</td>
<td>3.06</td>
<td>0.90–10.47</td>
</tr>
<tr>
<td>&gt;500</td>
<td>19/24 (79.2)</td>
<td>7.05</td>
<td>2.05–24.24</td>
</tr>
<tr>
<td>Oroanal partners in 6 months preceding EBV typing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>19/52 (36.5)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>9/24 (37.5)</td>
<td>1.04</td>
<td>0.38–2.83</td>
</tr>
<tr>
<td>2–5</td>
<td>10/18 (55.6)</td>
<td>2.17</td>
<td>0.73–6.44</td>
</tr>
<tr>
<td>&gt;5</td>
<td>17/11 (63.6)</td>
<td>3.04</td>
<td>0.79–11.74</td>
</tr>
<tr>
<td>Unknown</td>
<td>53/66 (65.2)</td>
<td>1.04</td>
<td>0.41–2.63</td>
</tr>
<tr>
<td>Orogenital partners in 6 months preceding EBV typing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>19/33 (57.6)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>19/40 (47.5)</td>
<td>0.67</td>
<td>0.26–1.69</td>
</tr>
<tr>
<td>2–5</td>
<td>19/44 (43.2)</td>
<td>0.56</td>
<td>0.22–1.39</td>
</tr>
<tr>
<td>&gt;5</td>
<td>24/41 (58.5)</td>
<td>1.04</td>
<td>0.41–2.63</td>
</tr>
<tr>
<td>Anogenital partners in 6 months preceding EBV typing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>37/63 (58.7)</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>18/37 (48.6)</td>
<td>0.67</td>
<td>0.29–1.51</td>
</tr>
<tr>
<td>2–5</td>
<td>15/37 (40.5)</td>
<td>0.48</td>
<td>0.21–1.09</td>
</tr>
<tr>
<td>&gt;5</td>
<td>11/21 (52.4)</td>
<td>0.77</td>
<td>0.29–2.09</td>
</tr>
</tbody>
</table>

NOTE. Logistic regression was used to obtain univariate and multivariate odds ratios (ORs) and 95% confidence intervals (CIs), to quantify variation in estimates. HHV-8, human herpesvirus 8; HIV, human immunodeficiency virus.

Estimated risk for no. of sex partners is corrected for time since follow-up (categorical variable), because no. of sex partners is reported as no. during 5 years preceding study entry through date of EBV testing. Trend toward higher EBV type 2 prevalence with increasing nos. of partners, .004.

Receptive and/or active.

Trend toward higher EBV type 2 prevalence with increasing nos. of oroanal sex partners, .055.

As shown in figure 1B, none of the heterosexual men who were at low risk for sexually transmitted diseases (n = 53) were infected with EBV type 2. The same result was obtained for 54 HIV-negative homosexual women who were at low risk (data not shown). In contrast, within the group of heterosexual men who were at high risk for sexually transmitted diseases (n = 40), 10% harbored EBV type 2 only and an additional 5% had a dual infection. The EBV type 2 prevalence among high-risk heterosexual men (15%) was significantly higher than that among low-risk heterosexual men (0%; P < .001).

EBV-2 infection in homosexual men in relation to sexual behavior. Data on sexual behavior and HHV-8 serologic studies...
were available for the 171 homosexual men (table 1). Subjects who harbored an EBV type 2 strain ($n = 88$) were compared with those without EBV type 2 infection ($n = 83$). Univariate analysis showed that EBV type 2 prevalence increased with the number of sex partners. Also, HIV-seropositive men, HHV-8–seropositive men, and men with non-Dutch nationality had a higher prevalence of EBV type 2 infection. No association was found with specific sexual techniques, although there was a borderline significant trend toward higher EBV type 2 prevalence with higher numbers of partners engaging in oroanal sex (active, receptive, or both).

In multivariate analysis, EBV type 2 infection was statistically significantly associated with HIV infection and the number of sex partners. Homosexual men with >500 sex partners had a nearly 10 times greater risk of being infected with EBV type 2 than did men with <100 sex partners. HIV-infected homosexual men had an almost 4 times greater risk of having an EBV type 2 infection (table 1).

Discussion

Until now, analysis of EBV strains in B lymphoblastoid cell lines has shown that 1%–3% of healthy white EBV carriers in Europe harbor a type 2 strain [6]. To our knowledge, this is the first study in which EBV type prevalence was investigated among white homosexual men and was compared with that among heterosexual individuals by use of direct EBV type analysis of peripheral blood B lymphocytes. We observed a high prevalence of EBV type 2 infection among homosexual men, compared with that among heterosexual men, and obtained evidence that EBV type 2 is sexually transmitted, at least among homosexual men.

Our study provides evidence that, in western Europe, EBV type 2 is transmitted by sexual contact. First, among homosexual men, we found a significant association between EBV type 2 infection and a higher number of partners. This high EBV type 2 prevalence could not be attributed to a specific sexual technique, although there was a trend in the number of partners with whom oroanal sex was practiced. Second, HIV-positive homosexual men have higher EBV type 2 prevalence than do HIV-negative homosexual men. This was not due to the degree of immunodeficiency, as shown in our previous study [8]. A more likely explanation may be that HIV-positive persons tend to have sex with HIV-seroconcordant persons, leading to more EBV type 2 exposure for those who are HIV-positive. This also has been suggested as an explanation for HHV-8 prevalence [10].

Third, EBV type 2 prevalence among high-risk heterosexual men was significantly higher than that among low-risk heterosexual men, suggesting that EBV type 2 infection also is related to sexual behavior in heterosexual contacts.

Interestingly, EBV type 2–infected homosexual men had a higher prevalence of HHV-8 infection than did EBV-1–infected homosexual men, in the univariate analysis. Because HHV-8 prevalence also increases with an increase in the number of sex partners, this suggests that EBV type 2 may resemble HHV-8 in its mode of sexual transmission [9, 10].

The fact that EBV type 2 prevalence among HIV-negative homosexual men is higher than that among HIV-negative heterosexual men probably was not caused by differences in EBV load (recent data in our laboratory show no difference in EBV load between persons infected with type 1 or type 2) and supports the recently proposed idea that EBV type 2 infection is endemic among white homosexual men [7]. This could be explained by the fact that EBV type 2 has been introduced into this community by sexual contacts with persons from areas where EBV type 2 is endemic and is further transmitted by multiple sexual contacts.

Sexual transmission of EBV is quite likely, because EBV is shed from many mucosal sites, including both female and male genital tracts [12]. One group reported that, among homosexual men, a higher percentage shed virus from the genital tract (50%) than from the oropharynx (25%) [13]. In addition, EBV has been detected in the anal region of sexually active homosexual men [14]. The fact that EBV type 2 is difficult to contract, despite the high prevalence among homosexual men, argues against kissing as the main route of transmission, and sexual transmission is likely in this study group. In parts of Africa, where EBV type 2 prevalence is high among the adult population, as well as among children with Burkitt’s lymphoma, EBV type 2 probably is acquired by oral transmission during childhood.

The fact that homosexual men with >500 partners had a nearly 10-fold–greater risk of EBV type 2 infection, whereas this infection was only moderately increased in homosexual men with 100–400 sex partners, indicates that EBV type 2 is not easily transmitted and, therefore, requires high exposure. Although the mechanism of this relatively poor efficiency of transmission of EBV type 2 remains to be established, it may explain why EBV type 2 infection is highly prevalent among persons with multiple sex partners and virtually is not found among the general white population.

Acknowledgments

This study was part of the Amsterdam Cohort Studies on AIDS and human immunodeficiency virus (HIV) type 1 infection, a collaboration of the Municipal Health Service, the Academic Medical Center, and the CLB. We thank H. Fennema for collecting blood samples from HIV-seronegative heterosexual white men and I. Spijkerman for initial analyses.

References


