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# High Human Herpesvirus 8 Seroprevalence in the Homosexual Population in Switzerland

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**The seroprevalence of human herpesvirus 8 (HHV-8) in the Swiss population was investigated. By enzyme-linked immunosorbent assay, sera reactive to the recombinant HHV-8 antigen orf 65.2 were found in 24% of human immunodeficiency virus (HIV)-positive patients without and in 92% of HIV-positive patients with Kaposi's sarcoma. Surprisingly, 20% of homosexual HIV-negative men, versus only 7% of heterosexual HIV-negative individuals and 5% of blood donors, had antibodies to HHV-8.**

In 1994, Chang et al. (7) identified DNA fragments of a novel herpesvirus in Kaposi's sarcoma (KS) tissue samples. This newly discovered virus was termed Kaposi's sarcoma-associated herpesvirus or human herpesvirus 8 (HHV-8). By PCR techniques, HHV-8 was found in more than 90% of human immunodeficiency virus (HIV)-associated KS lesions, classic KS, and posttransplant-associated KS (1, 5, 9, 13, 17, 20), as well as in two lymphoproliferative disorders, body cavity-based lymphoma and multicentric Castleman's disease (6, 23). Serological data obtained from immunoblotting and immunofluorescence studies link the distribution of HHV-8 with the risk of developing KS (11, 14, 15, 18, 22). In Western countries, a considerably higher seroprevalence of HHV-8 (ranging from 13 to 35%) was found in HIV-infected male homosexuals than in blood donors (0 to 8%). An increased seroprevalence was also reported from areas with endemic KS, such as central and eastern Africa and Mediterranean countries (12, 15, 21). Recently, three groups developed enzyme-linked immunosorbent assays (ELISAs) using selected HHV-8-encoded proteins with low sequence homologies to corresponding Epstein-Barr virus (EBV) proteins (2, 8, 21). However, the seroprevalence results obtained varied considerably. This may have been due to regional population differences, although different sensitivity levels of the tests or possible cross-reactivities to other herpesviruses, which have not been excluded, seem more likely. By the sensitive ELISA to the HHV-8 orf 65.2 protein (21), seroprevalence rates in various Swiss population groups were investigated.

A total of 571 sera from 113 HIV-positive and 458 HIV-negative individuals were analyzed. The HIV-infected group included 26 sera from patients with KS, 21 sera from asymptomatic (Centers for Disease Control and Prevention [CDC] stage A) subjects, and 66 sera from symptomatic (CDC stage B or C) patients. All patients were participants in the Swiss HIV Cohort Study. The HIV-negative group included 123 sera from individuals with various known herpesvirus infections, 35 sera from patients with lymphoproliferative diseases, 122 sera from individuals visiting an AIDS counseling center, of whom 54 were homosexual or bisexual men and 68 were heterosexual men or women, and 178 sera from blood donors. ELISAs were performed with, as antigen, recombinant orf 65.2 proteins expressed in M14 bacteria and purified by affinity chromatography on Ni-nitrilotriacetic acid resin (Qiagen, Basel, Switzerland), as described elsewhere (21). Sera were diluted 1:80 in phosphate-buffered saline containing 0.1% Tween 20 for cross-reactivity and 1:100 for seroprevalence studies. Cutoff values were calculated from blood donor sera as the mean plus 5 standard deviations. To adjust for interassay variability, the same five negative blood donor sera were used to determine the cutoff for each plate. Two reactive sera from patients with KS were included per plate as positive controls. All sera were blindly tested and reactive sera or sera with values close to the cut-off were retested at least once. For confirmation, indirect immunofluorescence assays (IFAs) for antibodies to latent HHV-8 antigens were done with, as target, the BC-3 cell line, as described elsewhere (3). A serum dilution of 1:40 was used and all slides were evaluated by two independent examiners. Immunoglobulin G antibodies to herpes simplex virus (HSV), cytomegalovirus (CMV), varicella-zoster virus (VZV), EBV, and HHV-6 were measured with commercial ELISAs and IFAs. Prevalence results among different patient groups were compared with the chi-square test.

Table 1 shows good concordance of antibody reactivity to orf 65.2, as measured by ELISA, and to latent antigen, as determined by IFA. For patients with KS, 92 and 88% of the sera were reactive in ELISA and IFA, respectively, with both assays together yielding 100% reactivity. For a control group of 35 patients with lymphoproliferative diseases, only two and one sera were reactive in ELISA and IFA, respectively. Our ELISA results are similar to those of Simpson et al. (21), who reported a seroprevalence of 84% among KS patients with the same assay. Other groups found prevalence rates ranging from 67 to 100% among patients with KS by using IFA or immunoblots (12, 14, 16, 22), while in ELISAs to HHV-8 orf 26 and orf 35, only 35 (2) and 60% (8), respectively, of the KS sera were reactive. Thus, the ELISA to orf 65.2 demonstrates good sensitivity, making the assay an excellent tool for seroepidemiological studies.

However, as pointed out by Rickinson (19), a major concern for seroepidemiological investigations with HHV-8 is possible antibody cross-reactivity between HHV-8 and antigens of other herpesviruses. Thus, 123 sera which had antibodies against at least one member of the herpesvirus group were tested for possible cross-reactivity with the HHV-8 orf 65.2 antigen. Thirteen sera (11%) were reactive to HHV-8 in the

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TABLE 1. Comparison of antibody reactivities to orf 65.2 protein (determined by ELISA) and to latent HHV-8 antigens (determined by IFA)

| ELISA result | No. (%) of sera with IFA result: | | | | | |
|---|---|---|---|---|---|---|
| | Patients with KS | | | Patients with lymphoproliferative diseases | | |
| | Positive | Negative | Total | Positive | Negative | Total |
| Positive | 21 | 3 | 24 (92) | 1 | 0 | 1 (3) |
| Negative | 2 | 0 | 2 (8) | 1 | 33 | 34 (97) |
| Total | 23 (88) | 3 (12) | 26 (100) | 2 (6) | 33 (94) | 35 (100) |

TABLE 3. Seroprevalence of antibodies against HHV-8 tends to increase with age[a]

| Age (yr) | No. of sera positive/no. tested (%) for HHV-8 antibodies | | | |
|---|---|---|---|---|
| | HIV-positive individuals without KS | HIV-negative individuals | | |
| | | Homosexuals | Heterosexuals | Blood donors |
| <30 | 2/7 (29) | 3/19 (16) | 2/35 (6) | 0/2 (0) |
| 30–40 | 5/33 (15) | 3/22 (14) | 1/24 (4) | 1/43 (2) |
| 40–50 | 8/35 (23) | 4/10 (40) | 1/5 (20) | 5/60 (8) |
| >50 | 6/12 (50) | 1/3 (33) | 1/4 (25) | 3/73 (4) |

[a] Mean age (range), in years, of groups studied: HIV-positive individuals without KS, 40 (22 to 70); HIV-negative homosexuals, 35 (20 to 79); HIV-negative heterosexuals, 31 (19 to 69); HIV-negative blood donors, 47 (29 to 77).

ELISA. The prevalence of antibodies to HHV-8 was similar in the HSV (5 of 31 [16%]), VZV (9 of 51 [18%]), CMV (3 of 19 [16%]), EBV (4 of 31 [13%]), and HHV-6 (3 of 18 [17%]) seropositive subgroups. In addition, three sera with very high antibody titers to other herpesviruses were negative for HHV-8 and three sera highly positive for HHV-8 were negative for all other herpesviruses studied. Thus, the orf 65.2 ELISA specifically detects antibodies to HHV-8, with no cross-reactivity to antibodies against the other members of the herpesvirus family.

HHV-8 seroprevalence was investigated in different HIV-positive and -negative groups. For HIV-positive patients with KS, 92% of the sera had antibodies to orf 65.2 versus 24% (21 of 87) in individuals without KS (Table 2), a prevalence rate comparable to published data ranging from 13 to 35% (8, 11, 12, 14, 16, 21, 22). There was neither a correlation of HHV-8 seropositivity with HIV disease stages—as patients with CDC stage A (5 of 21 [24%]) or stages B and C (16 of 66 [24%]) of HIV disease had the same seroprevalence—nor with CD4 counts (data not shown), as also found by two other studies (8, 21). Among the HIV-infected patients without KS, homosexuals had a higher prevalence of antibodies (30%) than did heterosexuals (25%) and intravenous drug users (0%), although the patient numbers tested were small in the two latter groups (Table 2). This is in agreement with various studies (8, 11, 12, 14, 16, 22) with the exception of that of Lennette et al. (15), who found HHV-8-reactive sera in nearly all American HIV-infected homosexual men tested.

Except in certain KS-endemic regions of southern Europe, the rate of infection with HHV-8 in the general population in Western countries is low (11, 12, 14, 21). In agreement with this observation, we found an HHV-8 prevalence of 7% in the heterosexual population (persons attending an AIDS counseling center) and of 5% in blood donors but a somewhat higher seroprevalence (13 to 18%) in individuals with known herpesvirus infections. This may reflect a higher rate of infection with HHV-8 in a population already infected with other herpesviruses and/or the presence of homosexuals in this population (see below). The seroprevalence in the HIV-negative population tended to increase with age (Table 3) but was almost identical between men and women (7 of 133 [5%] and 2 of 45 [4%], respectively, in blood donors and 3 of 33 [9%] and 2 of 35 [6%], respectively, in the heterosexual population). Of considerable interest, and in contrast to Simpson et al. (21), we found a significantly higher seroprevalence in HIV-negative homosexual men than in heterosexuals (20 versus 7% [Table 2]). The high rate of infection with HHV-8 in homosexuals and the fact that HIV-infected individuals who develop KS are mostly homosexuals strongly indicates that perhaps one important mode of transmission of HHV-8 is through homosexual practices. This view is supported by observations that the prevalence of sporadic cases of KS is increased in HIV-negative homosexuals (10) and that women who have sexual contacts with HIV-infected bisexual men are more likely to develop KS than women whose sexual partners are HIV-infected intravenous drug users (4).

TABLE 2. Seroprevalence of antibodies against HHV-8 orf 65.2 among HIV-infected and uninfected individuals[a]

| Group tested (*n*) | No. (%) of sera with HHV-8 antibodies |
|---|---|
| $HIV^+$ with KS (26) | 24 (92.3) |
| $HIV^+$ without KS (87) | 21 (24.1) |
| Homosexuals (63) | 19 (30.2) |
| Heterosexuals (8) | 2 (25.0) |
| Intravenous drug users (12) | 0 (0) |
| $HIV^-$ individuals (300) | 25 (8.3) |
| Homosexuals (54) | 11 (20.4) |
| Heterosexuals (68) | 5 (7.4) |
| Blood donors (178) | 9 (5.1) |

[a] $HIV^+$, HIV positive; $HIV^-$, HIV negative. Statistics (by the chi-square test): $HIV^-$ homosexuals versus heterosexuals, $P = 0.034$; $HIV^-$ homosexuals versus blood donors, $P = 0.0004$; $HIV^+$ individuals without KS versus $HIV^-$ individuals, $P < 0.0001$.

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