Comparison of the Prevalence of Antibodies to Human Herpesvirus 8 (Kaposi’s Sarcoma–Associated Herpesvirus) in Brazil and Colorado

Xing-quan Zhang, Lisa Fitzpatrick, Thomas B. Campbell, Roberto Badaro, Mauro Schechter, Maria de Fatima Melo, Carlos Brites, Diana P. Sampaio, and Robert T. Schooley

Division of Infectious Disease, Department of Medicine, University of Colorado Health Sciences Center, Denver; Federal University of Bahia, Salvador, and Federal University of Rio de Janeiro, Brazil

The prevalence of human herpesvirus 8 (HHV-8; Kaposi’s sarcoma [KS] herpesvirus) infection was determined by IFA in 297 persons living in Brazil and Colorado. The prevalence of antibody to HHV-8 in human immunodeficiency virus (HIV) type 1–seropositive gay men with and without KS was similar in Brazil and Colorado. In Brazil, the prevalence of HHV-8 antibody was significantly greater in HIV-1–seronegative gay men than in HIV-1–seronegative male intravenous drug users. HHV-8–seropositive Brazilian gay men who had a clinical diagnosis of KS or who were infected with HIV-1 had significantly higher titers of HHV-8 antibody than did HHV-8–seropositive, HIV-1–seronegative Brazilian gay men. These findings provide further support for the association between HHV-8 infection and KS and suggest that, as in the United States, HHV-8 infection is transmitted sexually in Brazil.

Kaposi’s sarcoma (KS) has long been a common neoplasm in elderly men in eastern Europe and in parts of Africa, but prior to the onset of the AIDS epidemic, KS occurred only sporadically in North America and western Europe. The pattern of KS occurrence in patients with human immunodeficiency virus (HIV) type 1 infection led to the hypothesis that KS is a sexually transmitted disease. Most recently, KS has been linked to infection with human herpesvirus (HHV) 8 [1]. Infection by HHV-8 has been detected by molecular techniques in nearly all persons with either AIDS-related or non-AIDS–related KS, while HHV-8 infection appears to be uncommon in persons not at high risk for KS [2–4]. These observations strongly suggest that HHV-8 infection plays a causal role in the pathogenesis of KS. Since little is known about the epidemiology of HHV-8 infection in South America, the present study was performed to determine the prevalence of HHV-8 infection in populations in Brazil and to compare the patterns of HHV-8 infection in Brazil with US patterns of HHV-8 infection.

Materials and Methods

Study population. Serum samples from 297 persons from Brazil and Colorado were collected. The Brazilian cohort consisted of 267 persons from Rio de Janeiro and Salvador: 14 homosexual men with HIV-1 infection and clinical KS (median age 34 years; range, 24–56), 80 homosexual men with HIV-1 infection but no history of KS (median age 33 years; range, 18–55), 78 homosexual men who were HIV-1–seronegative and had no history of KS (median age 26 years; range, 18–42), 24 male hemodialysis patients who were HIV-1–seronegative and had no history of KS (median age 37 years; range, 19–68), 26 female hemodialysis patients who were HIV-1–seronegative and had no history of KS (median age 37 years; range, 20–63), and 45 heterosexual male intravenous drug users who were HIV-1–seronegative and had no history of KS (median age 24 years; range, 15–50). For all Brazilian subjects, the presence or absence of antibodies to HIV-1 was determined by ELISA, and reactive samples were confirmed by Western blot. The Colorado cohort consisted of 30 persons from the Denver area: 14 HIV-1–infected homosexual men with a history of KS (median age 32 years; range, 28–51), 10 HIV-1–infected homosexual men with no history of KS (median age 35 years; range, 26–44), and 6 HIV-1–seronegative, Epstein-Barr virus (EBV)–seropositive heterosexual laboratory workers who had no history of KS (4 men, 2 women; median age 34 years; range, 25–51). For the US cohort, HIV-1 seropositivity was confirmed by review of medical records, and HIV-1 seronegativity was confirmed by a nonreactive antibody test within 6 months of study entry. For all subjects with KS in both cohorts, the diagnosis of KS was established by biopsy.

Cell lines. The HHV-8– and EBV-infected cell line HBL-6 [5], was the gift of P. Moore (Columbia University, New York). The HHV-8–infected, EBV-negative cell line BC-3 was provided by E. Cesnarm (Cornell Medical Center, New York). Both the HBL-6 and BC-3 cell lines were maintained in RPMI 1640 medium that contained 10% and 20% fetal calf serum, respectively.
Preparation of cell nuclei. About 1.5 x 10^7 HBL-6 cells were centrifuged at 1800 g for 10 min. All subsequent steps were done at 4°C. Cells were washed once with ice-cold PBS and resuspended in 1 mL of wash buffer (10 mM HEPES, pH 7.8, 15 mM KCl, 1 mM MgCl₂, 1 mM DTT, 0.1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride) and centrifuged at 7000 g for 5 min. Cell pellets were resuspended in 1 mL of buffer wash buffer with 0.2% NP-40. Nuclei were isolated by centrifugation at 12,000 g for 10 min. Pelleted nuclei were resuspended in 0.1 mL of PBS and quantitated with a hemocytometer.

Detection of HHV-8 antibody by IFA. HBL-6 or BC-3 cells or extracted nuclei were counted and resuspended in PBS at 62 with a hemocytometer. Pelleted nuclei were resuspended in 0.1 mL of PBS and quantitated for HHV-8 antibody. DNA from HBL-6 cells served as a positive control, and HHV-8 DNA was not detected in PBMC from 2 of 2 IFA-negative HIV-1-infected patients (1 with KS). HHV-8 DNA was not detected in 2 HHV-8 IFA-negative subjects who did not have HIV-1 risk factors. HHV-8 DNA was not detected in the serum of any of these 9 subjects.

Preparation of cell nuclei. DNA from peripheral blood mononuclear cells (PBMC) and serum from patients with or without KS was extracted by use of commercial kits (QIAamp blood and QIAamp tissue; Qiagen, Chatsworth CA). PCR was performed with primers KS1 and KS2 as previously described [2]. The PCR reactions contained 2 μg of sample DNA, 1.5 mM MgCl₂, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.2 mM dNTPs, and 1 U of Taq polymerase in a final volume of 100 μL. The PCR mixture was prepared in a thermal cycler (model 9600; Perkin-Elmer Cetus, Norwalk, CT) for 35 cycles: 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min. Amplification products were visualized on an ethidium-stained 2% agarose gel, and samples were scored for the presence of the expected 233-bp product. DNA from HBL-6 cells served as a positive control, and DNA from H9 cells served as a negative control.

Results

Detection of HHV-8 antibodies by IFA. To determine the sensitivity of the IFA, a 1:10 dilution of serum samples from 14 Brazilian gay men with HIV-1 infection and KS (HIV-/KS⁺) was screened. The prevalence of HHV-8 antibody detected by nuclear staining of HBL-6 cells was 64% (9/14). In contrast, only 1 of 50 HIV-1-seronegative Brazilian hemodialysis patients (a woman) had detectable nuclear staining at a 1:10 dilution of serum (P = .005, Fisher’s exact test). The use of TPA-treated HBL-6 cells or the EBV-negative BC-3 cell line in the IFA also gave nuclear staining in the same 9 of 14 serum samples from the Brazilian HIV-1-/KS⁺ group. Similarly, an IFA that used isolated HBL-6 cell nuclei detected antibody in 10 (71%) of 14 of these patients. On the basis of these results, nuclear staining of uninduced HBL-6 cells at serum dilutions of 1:10 or greater was considered to be indicative of HHV-8 antibody in all subsequent IFAs.

Comparison of HHV-8 antibody detection by IFA and PCR amplification of HHV-8 DNA from PBMC. In a small subset of the Colorado cohort for which PBMC were available, the HHV-8 IFA was compared with PCR amplification of HHV-8 DNA from PBMC. Five of 5 HIV-1-infected patients with a positive IFA (4 with KS) had detectable HHV-8 DNA, while HHV-8 DNA was not detected in PBMC from 2 of 2 IFA-negative HIV-1-infected patients (1 with KS). HHV-8 DNA was not detected in 2 HHV-8 IFA-negative patients who did not have HIV-1 risk factors. HHV-8 DNA was not detected in the serum of any of these 9 subjects.

Prevalence of HHV-8 antibody in Brazilian and US subjects. The prevalence of HHV-8 antibody in subject groups with or without HIV-1 infection and with or without KS in Brazil and Colorado (figure 1) was compared by Fisher’s exact test. For HIV-1-infected patients with and without KS, the prevalence of antibody to HHV-8 was not significantly different in the Brazilian groups compared with the corresponding Colorado groups (P > .1 for each comparison).

Within the Brazilian cohort, the prevalence of HHV-8 antibody was highest in HIV-1-infected gay men with KS (64%, P ≤ .001 compared with all other Brazilian groups) but was not significantly different in Brazilian gay men who were or were not HIV-1-infected (18% vs. 12%, P = .4). The prevalences of HHV-8 antibody in male Brazilian hemodialysis patients and male intravenous drug users were not significantly different (0 for each group, P > .99). However, the prevalence of HHV-8 antibody among Brazilian men without HIV-1 infection was
HHV-8 antibody titer is higher in persons with KS. Within the combined Brazilian and Colorado cohorts, the distribution of end-point titers and the geometric mean titer of HHV-8 antibody varied by subject group (figure 2). For this analysis, the groups were compared by one-tailed Mann-Whitney test. The distribution of end-point titers among HHV-8–seropositive persons was significantly greater in HIV-1–infected gay men with KS than in HIV-1–infected gay men without KS ($P = .02$) or in HIV-1–seronegative gay men without KS ($P = .004$). HHV-8 antibody end-point titers were also significantly greater in HIV-1–infected gay men without KS than in HIV-1–seronegative gay men without KS ($P = .04$).

Discussion

In the present study, the HBL-6 cell line [5] that is dually infected with HHV-8 and EBV was used in an IFA to detect HHV-8 antibody. The HBL-6 and closely related BC-1 cell lines have been used previously to determine the seroprevalence of HHV-8 [6–8]. Although HBL-6 and BC-1 cells show both cytoplasmic and nuclear fluorescence by IFA, the nuclear fluorescence is specific for antibody to latent HHV-8 nuclear antigens [7, 8]. In the present study, only nuclear fluorescence of HBL-6 cells was used to detect HHV-8 antibody by IFA, and the ability to detect HHV-8 antibody in patients with KS was unchanged when the EBV-negative BC-3 cells or isolated HBL-6 cell nuclei were used in the IFA. Even though the expression of EBV antigens is induced by treatment with phorbol esters, the use of TPA-treated HBL-6 cells did affect the IFA results. In a small subset of subjects in the present study, there was complete agreement between the IFA and PCR amplification of HHV-8 DNA from PBMC, and only 1 of 6 EBV-seropositive laboratory workers was reactive by the HBL-6 IFA. Taken together, these results strongly suggest that the IFA used in the present study detected antibodies specific for latent nuclear HHV-8 antigens.

HHV-8 nucleic acids are detected in 93%–100% of KS tumors from AIDS and non-AIDS patients [1, 4, 9, 10]. Our finding that only 64%–71% of AIDS patients with KS are HHV-8–seropositive is consistent with previous studies that have reported a 65%–88% sensitivity for the HBL-6 or BC-1 cell IFA [6, 7] and indicates that this IFA underestimates the prevalence of HHV-8 infection in persons with KS. Antibody to latent nuclear antigen is detected in only 52%–88% of persons with AIDS-related KS by IFAs that use other cell lines (BCP-1 and BCBL-1) [3, 11, 12]. Although the detection of HHV-8 antibody by IFA underestimates the true prevalence of HHV-8 infection, these assays are more sensitive indicators of HHV-8 infection than is PCR amplification of HHV-8 DNA from PBMC [7]. Since titers of HHV-8 antibody are lower in HHV-8–seropositive persons without KS, it is possible that the sensitivity of the IFA is further reduced in these groups. Thus, in our study the prevalence of HHV-8 antibody by IFA is a minimum estimate of the prevalence of HHV-8 infection in the non-KS Brazilian groups, and the true prevalence of HHV-8 infection in these groups is not known.

Our finding that among HHV-8–seropositive patients, the HBL-6 cell IFA titer is greater in persons with KS is consistent with results of previous studies [3, 8, 12]. Our finding that among gay men without KS, coinfection with HHV-8 and HIV-1 results in a greater HHV-8 antibody titer has not been previously reported. These findings provide further support for the hypothesis that HIV-1 infection augments HHV-8 replication either directly by activation of HHV-8 gene expression [13] or indirectly by chemokine production or immunosuppression [3]. Unfortunately, CD4 lymphocyte counts and HIV-1 virus load measurements are not available for many of the subjects in our Brazilian cohort. Therefore, we do not know the relationship between HHV-8 antibody titers and the stage of HIV-1 disease in those subjects. Further studies are needed to define the effects of HIV-1 replication and immunopathogenesis on HHV-8 virus burden in dually infected persons.

The high prevalence of HHV-8 antibody in homosexual men in the United States compared with other HIV-1 risk groups suggests that HHV-8 infection is primarily sexually transmitted [11–14]. The finding that infection with HIV-1 and other sexually transmitted diseases are risk factors for HHV-8 infection for men in San Francisco provides further evidence for sexual transmission of HHV-8 [14]. In the present study, the prevalence of HHV-8 antibody was not significantly different in HIV-1–
infected or ±seronegative Brazilian gay men. Our inability to
detect a difference in HHV-8 prevalence in Brazilian gay men
with or without HIV-1 infection is likely due to the relatively
few subjects in each Brazilian subgroup and should not be
interpreted as evidence against sexual transmission of HHV-8.
Rather, antibody to HHV-8 was rarely detected in Brazilian
intravenous drug injectors or hemodialysis patients, and among
Brazilian men without HIV-1 infection, the prevalence of HHV-
8 antibodies was significantly greater in homosexuals than in
heterosexual intravenous drug users. Thus, the prevalence of
antibody to HHV-8 in Brazilian men was not affected by ex-
posure to blood but was affected by sexual practices. These
findings provide evidence that HHV-8 infection is sexually
transmitted in Brazil and that the epidemiology of HHV-8
infection is similar in Brazil and in the United States.

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