ANTIBODIES AGAINST HUMAN HERPESVIRUS 8 IN BLACK SOUTH AFRICAN PATIENTS WITH CANCER

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ABSTRACT

Background Infection with human herpesvirus 8 (HHV-8) has been consistently linked to Kaposi's sarcoma, but its mode of transmission, association with other cancers, and interaction with the human immunodeficiency virus type 1 (HIV-1) are largely unknown.

Methods Between January 1992 and December 1997, we interviewed 3591 black patients with cancer in Johannesburg and Soweto, South Africa. Blood was tested for antibodies against HIV-1 and HHV-8 in 3344 of the patients. Antibodies against HHV-8 were detected with an indirect immunofluorescence assay. The intensity of the fluorescent signal correlated well with the titers of antibodies ($P<0.001$). The relations among the presence of anti–HHV-8 antibodies, sociodemographic and behavioral factors, type of cancer, and the presence or absence of coexistent HIV-1 infection were examined with the use of unconditional logistic-regression models.

Results Among the 3293 subjects with cancers other than Kaposi's sarcoma, the standardized seroprevalence of antibodies against HHV-8 was 32 percent, which did not differ significantly from the standardized seroprevalence among black blood donors. Among these 3293 patients, the prevalence of antibodies against HHV-8 increased with increasing age ($P<0.001$) and an increasing number of sexual partners ($P=0.05$) and decreased with increasing years of education ($P=0.007$); it was not strongly associated with HIV-1 infection. Anti–HHV-8 antibodies were more frequent among black than white blood donors ($P<0.001$). Among the 51 patients with Kaposi's sarcoma, the standardized seroprevalence of antibodies against HHV-8 was 83 percent, significantly higher than the prevalence among those without Kaposi's sarcoma ($P<0.001$). For 16 other specific types of cancer, including multiple myeloma (108 cases) and prostate cancer (202 cases), the variation in the standardized seroprevalence of antibodies against HHV-8 was not remarkable. At a given intensity of fluorescence of anti–HHV-8 antibodies, Kaposi's sarcoma was more frequent among HIV-1–positive patients than among those who were HIV-1–negative ($P<0.001$).

Conclusions Among black patients with cancer in South Africa, the seroprevalence of anti–HHV-8 antibodies is high and is specifically associated with Kaposi's sarcoma, particularly at high titers. (N Engl J Med 1999;340:1863-71.)

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HUMAN herpesvirus 8 (HHV-8, also known as Kaposi's sarcoma–associated herpesvirus) has been consistently linked to Kaposi's sarcoma.[1-3] HHV-8 is detectable in the lesions of nearly all patients with Kaposi's sarcoma,[4,5] and its presence in the blood predicts the development of Kaposi's sarcoma.[6,7] This neoplasm occurs frequently in patients with the acquired immunodeficiency syndrome (AIDS),[8] but it was common in central Africa even before the advent of AIDS.[9] With the AIDS epidemic now spreading through central and southern Africa, Kaposi's sarcoma has become the most commonly diagnosed cancer in some countries in those regions.[10,11] The incidence of Kaposi's sarcoma is increasing rapidly in South Africa.[12] In the United States and most of Europe Kaposi's sarcoma, which was rare before the advent of AIDS, now occurs mainly among homosexual men with AIDS.[8]

Little is known about the transmission of HHV-8, its association with other cancers, or its interaction with the human immunodeficiency virus type 1 (HIV-1). The few studies investigating the spread of HHV-8 infection have tended to be small, but they suggest that the routes include sexual transmission among homosexual men in the United States[13] and mother-to-child transmission in South Africa.[14]

HHV-8 has also been found in association with primary-effusion lymphoma in patients with AIDS,[15-17] and some investigators have suggested, largely on the basis of molecular studies, that multiple myeloma[18] and prostate cancer[5] are also associated with HHV-8 infection. These reports, however, have not been confirmed.[19-22] We studied the relations among the presence of antibodies against HHV-8, the risk of cancer, and other factors, including the presence or absence

of HIV-1 infection, in over 3000 black patients from Johannesburg and Soweto, South Africa, in whom cancer was diagnosed between 1992 and 1997.

METHODS

Study Population

Data from two large epidemiologic studies conducted by researchers from the South African National Cancer Registry and the Department of Medicine of the University of the Witwatersrand, in collaboration with investigators in the United Kingdom, are included in this report. In the first study, performed between January 1992 and December 1994, information was collected on the age, sex, and birthplace of 1015 black inpatients with cancer at the Chris Hani–Baragwanath, Hillbrow, and Johannesburg hospitals, which are the main teaching hospitals in Johannesburg and Soweto.[12] In the second study, performed between January 1994 and October 1997, trained nurses interviewed 2576 black inpatients with cancer at the same three hospitals using a standard questionnaire in the language of the patient (usually Zulu or Sotho). Questions were asked about sociodemographic and behavioral characteristics, including age, sex, birthplace, residence, years of education, and reproductive and lifetime sexual history. Diagnoses of cancer were established, where appropriate, by biopsy, examination of blood or bone marrow, or cytologic techniques, or by a combination of these methods. Both studies were approved by the ethics committee of the University of the Witwatersrand, and informed consent was obtained. For comparative purposes, serum samples were obtained consecutively from 85 black and 224 white blood donors at the Johannesburg blood-transfusion service (unpublished data). Samples were collected in each study and stored at −30°C.

Serologic Tests for HHV-8 and HIV-1

The serum samples were shipped by air on dry ice to the Institute of Cancer Research in London for HHV-8 testing. All assays were performed by a single observer who was unaware of each patient's personal characteristics and diagnosis. A body-cavity–related B-cell lymphoma (primary effusion lymphoma) cell line, BCP-1, which is positive for HHV-8 and negative for the Epstein–Barr virus (EBV), was used for an indirect immunofluorescence assay to detect IgG antibodies against HHV-8 antigen.[23,24] Latently infected BCP-1 cells were fixed in 4 percent paraformaldehyde and were made permeable with 0.2 percent Triton X-100. Cells were resuspended in phosphate-buffered saline and fixed on glass slides. The samples were diluted 1:100 in phosphate-buffered saline with 3 percent fetal-calf serum. The diluted serum was added to the fixed BCP-1 cells and incubated at 22°C for 45 minutes.

After the slides were washed in phosphate-buffered saline with 3 percent fetal-calf serum, rabbit antihuman IgG labeled with fluorescein isothiocyanate (Dako, High Wycombe, United Kingdom), diluted 1:40 in phosphate-buffered saline with 3 percent fetal-calf serum, was added, and the slides were incubated at 22°C for 20 minutes. The slides were then washed in phosphate-buffered saline without fetal-calf serum and screened by ultraviolet microscopy for the nuclear stippling pattern characteristic of antibodies against the latent nuclear antigen of HHV-8 encoded by orf73.[23,25-29] The HHV-8–negative cell lines Raji and Daudi were used as controls.

Serum samples that were positive for antibodies against HHV-8 by the immunofluorescence assay were then scored as low, medium, or high according to the intensity of the fluorescent signal. These scores correlated well with the intensity of fluorescence as measured by fluorescence-activated cell-sorter (FACS) analysis. Fig. 1A shows the results of FACS analysis for six subjects, two of whose samples had been scored as low, two as medium, and two as high in terms of the fluorescent-signal intensity. The reproducibility of these results was examined whenever there was sufficient serum for retesting from the patients with

Kaposi's sarcoma (47 patients) and from 94 matched controls without Kaposi's sarcoma (for each retested sample from a patient with Kaposi's sarcoma, we selected serum samples from 2 control subjects without Kaposi's sarcoma who had the same HIV-1 status and whose age was as close as possible to that of the patient).

The serum samples from these 141 subjects were retested in a blinded fashion with the immunofluorescence assay, and the intensity of the fluorescent signal was scored again (absent, low, medium, or high). The results of the repeated test were in complete agreement with those of the first test for the patients with Kaposi's sarcoma (40 were positive according to the immunofluorescence assay, and 7 were negative), and in 94 percent agreement for those who did not have Kaposi's sarcoma (about one third were positive according to the immunofluorescence assay). The scores for signal intensity also agreed well: exact agreement was obtained for 85 percent of those with Kaposi's sarcoma and for 90 percent of those without Kaposi's sarcoma.

To study the relation between the intensity of fluorescence and the titer of antibodies to HHV-8, the 141 serum samples selected for retesting were also examined in a blinded fashion at doubling dilutions, starting at 1:100 for antibodies against HHV-8.[30] The score for the fluorescent-signal intensity was strongly related to antibody titer — the median titers were 1:200 for low signal intensity, 1:51,200 for medium signal intensity, and 1:204,800 for high signal intensity (P<0.001) (Fig. 1B).

Testing for HIV-1 was conducted at the Serology Department of the South African Institute for Medical Research in Johannesburg. After presumptive positive results by enzyme-linked immunosorbent assay, the presence of HIV-1 infection was confirmed in the first study either by Western blot analysis or by three third-generation tests.[12] In the second study, one test was performed for each patient and those with an equivocal result were considered to be HIV-1–negative.

Statistical Analysis

We recruited 3591 patients between 1992 and 1997. Serum samples from 3344 of them (93 percent) were tested for antibodies to HHV-8, and these results are the basis for the analyses presented in this report. Since the factors influencing HHV-8 infection in the general population are not known, we initially examined the relation between antibodies against HHV-8 and age, sex, education, place of birth, parity, and number of lifetime sexual partners in the patients without Kaposi's sarcoma. Odds ratios were calculated by unconditional logistic regression and, because information on factors other than age and sex were not collected in the first study, analyses with respect to the other four factors were performed for the second study only. The results were examined at two levels of fluorescence intensity by immunofluorescence assay (i.e., according to whether there was any positive result, which corresponded to an antibody titer of at least 1:100, and according to whether there were positive results at a high signal intensity, which corresponded to a median antibody titer of 1:204,800).

To examine the relation between antibodies against HHV-8 and type of cancer, an unconditional logistic-regression model was used to calculate the log of the odds of HHV-8 seropositivity for each type of cancer for which there were 50 or more patients and for the blood donors, with adjustment for age (<35, 35 to 44, 45 to 54, 55 to 64, or ≥65 years), sex, years of education (unknown, none, one to five, or six or more), and number of sexual partners (unknown, zero to two, three or four, or five or more). Since adjustment for years of education and number of sexual partners had no material effect on the outcome of the analyses according to cancer type, the results for both studies were combined, with the patients from the first study assigned to the unknown categories for years of education and number of sexual partners. To present these results in a directly interpretable form, estimates of the adjusted log (odds) of infection for each type of cancer were transformed into estimates of standard-
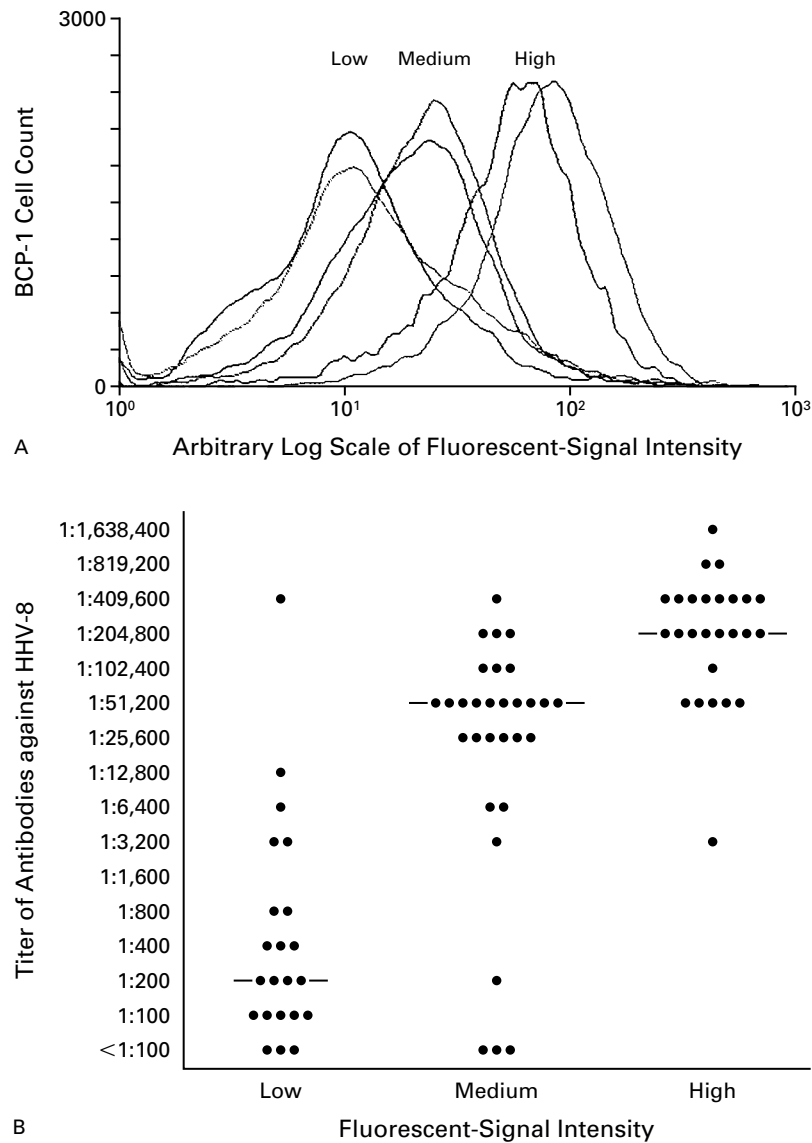
Figure 1. Fluorescent-Signal Intensity and Distribution of Titers of Antibodies against Human Herpesvirus 8 (HHV-8).

Panel A shows the results of a fluorescence-activated cell-sorter analysis of six patients. The intensity of the fluorescent signal, as determined by immunofluorescence assay, was low in two patients, medium in two, and high in two. Panel B shows the distribution of titers of anti–HHV-8 antibodies among those with low-intensity signals, those with medium-intensity signals, and those with high-intensity signals (those with no signals were excluded). The median titer in each group is indicated by a horizontal line. BCP-1 is a body-cavity–related B-cell lymphoma (primary effusion lymphoma) cell line, which is positive for HHV-8 and negative for the Epstein–Barr virus.

ized rates of prevalence with corresponding 95 percent confidence intervals. The resulting estimates represent prevalence rates for each cancer type standardized to a population containing men and women in equal proportion between the ages of 35 and 44 years, with one to five years of education and three or four sexual partners.

To study the relation between Kaposi's sarcoma and fluorescent-signal intensity for anti–HHV-8 antibodies separately in HIV-1–positive and HIV-1–negative patients, floating absolute risks and their corresponding floated confidence intervals were calculated,[31] with the patients with other cancers as the comparison group. Presentation of the results in this way permitted us to make valid comparisons between any two groups, regardless of signal intensity or HIV-1 status, after taking into account the variation in each floating absolute risk. These risks were calculated with the use of unconditional logistic-regression models, with adjustment for age, sex, and, if known, years of education and number of sexual partners.
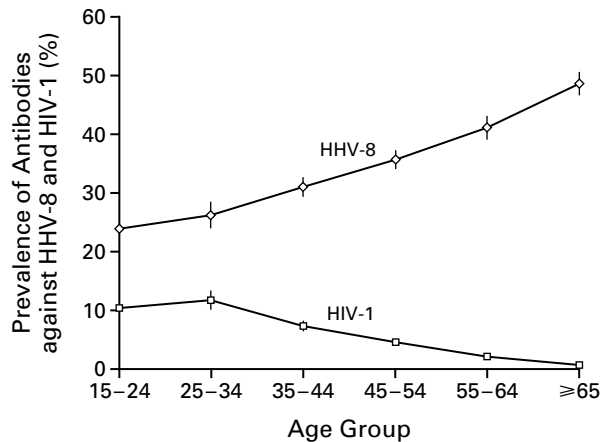
Figure 2. Age-Specific Seroprevalence of Antibodies against Human Herpesvirus 8 (HHV-8) and Human Immunodeficiency Virus Type 1 (HIV-1) in the Patients without Kaposi's Sarcoma. Vertical bars indicate standard errors.

RESULTS

Antibodies against HHV-8 in Relation to Demographic and Behavioral Factors

The serum samples of 1196 of the 3293 patients who did not have Kaposi's sarcoma were positive for antibodies against HHV-8 according to the immunofluorescence assay. Of the 1196 positive samples, 58 percent (698 of 1196) had a low fluorescent-signal intensity, 30 percent (359 of 1196) had a medium intensity, and 12 percent (139 of 1196) had a high intensity. The seroprevalence of antibodies against HHV-8 increased steadily with age — from 24 percent for those 15 to 24 years of age to 49 percent for those 65 or more years of age (P<0.001 by test for trend) (Fig. 2). The corresponding age-specific seroprevalence rates for patients with a high signal intensity were 1.0 percent and 7.9 percent, respectively (P for trend, <0.001).

Table 1 shows the age- and sex-adjusted odds ratios for the presence of antibodies against HHV-8 according to sex, years of education, place of birth (urban or rural area), parity, and number of lifetime sexual partners. The presence of these antibodies was not significantly related to sex, place of birth, or parity. However, the prevalence of seropositivity decreased with increasing years of education (P for trend=0.007) and increased with the number of sexual partners (P for trend=0.05). Broadly similar relations were seen when the analyses were restricted to the patients who were seronegative for HIV-1 (Table 1). Adjustment for years of education and number of sexual partners with respect to the other factors shown in Table 1 did not alter the results materially.

TABLE 1. ODDS RATIOS FOR HUMAN HERPESVIRUS 8 SEROPOSITIVITY ACCORDING TO CHARACTERISTICS OF PATIENTS WITHOUT KAPOSI'S SARCOMA.

CHARACTERISTIC	ALL PATIENTS/ HIV-1–NEGA-TIVE PATIENTS*	ODDS RATIO (95% CI)†	
		ALL PATIENTS (N=3293)	HIV-1–NEGATIVE PATIENTS (N=3117)
Sex			
Male‡	1285/1223	1.0	1.0
Female	2004/1894	1.0 (0.9–1.2)	1.0 (0.9–1.2)
Test for heterogeneity§		χ²=0.2; P=0.7	χ²=0.0; P=0.9
Education			
None‡	603/587	1.0	1.0
1–5 yr	928/883	0.8 (0.6–1.0)	0.8 (0.6–1.0)
≥6 yr	779/729	0.7 (0.6–0.9)	0.7 (0.6–0.9)
Test for trend§		χ²=7.3; P=0.007	χ²=7.2; P=0.007
Place of birth			
Urban area‡	937/878	1.0	1.0
Rural area	1298/1254	1.1 (0.9–1.4)	1.1 (0.9–1.3)
Test for heterogeneity§		χ²=1.7; P=0.2	χ²=0.6; P=0.4
Parity			
0–2‡	433/403	1.0	1.0
3–4	468/440	1.1 (0.9–1.5)	1.1 (0.8–1.5)
≥5	412/402	1.2 (0.9–1.6)	1.2 (0.9–1.6)
Test for trend§		χ²=1.7; P=0.2	χ²=1.6; P=0.2
No. of lifetime sexual partners			
0–2‡	459/447	1.0	1.0
3–4	1043/1008	1.2 (1.0–1.5)	1.2 (1.0–1.5)
≥5	623/576	1.3 (1.0–1.7)	1.3 (1.0–1.8)
Test for trend§		χ²=3.8; P=0.05	χ²=4.4; P=0.04

*Data were not available for all patients for all variables.

†The odds ratios have been adjusted for age and, where appropriate, sex. CI denotes confidence interval.

‡These patients served as the reference group.

§All values were calculated with the χ² test with 1 df.

Antibodies against HHV-8 in Relation to Cancer

The presence of antibodies against HHV-8 among patients with any of 17 types of cancer and among the black blood donors is shown in Figure 3 at two levels of fluorescent-signal intensity. Prevalence rates were standardized according to age, sex, years of education, and number of sexual partners. At each level of intensity, the standardized prevalence of antibodies against HHV-8 among the patients with Kaposi's sarcoma differed significantly from the rates found for other cancers (for any positive signal, P<0.001; for a high-intensity signal, P<0.001). The seroprevalence rates among those with the other 16 types of cancer were similar, and there was little evidence of variation in the rates according to type of cancer (for any positive signal, P=0.05; for a high-intensity signal, P=0.3).

The standardized prevalence of antibodies against HHV-8 among the 51 patients with Kaposi's sarcoma was 83 percent (95 percent confidence interval, 70 to 91 percent) on the basis of the presence of any
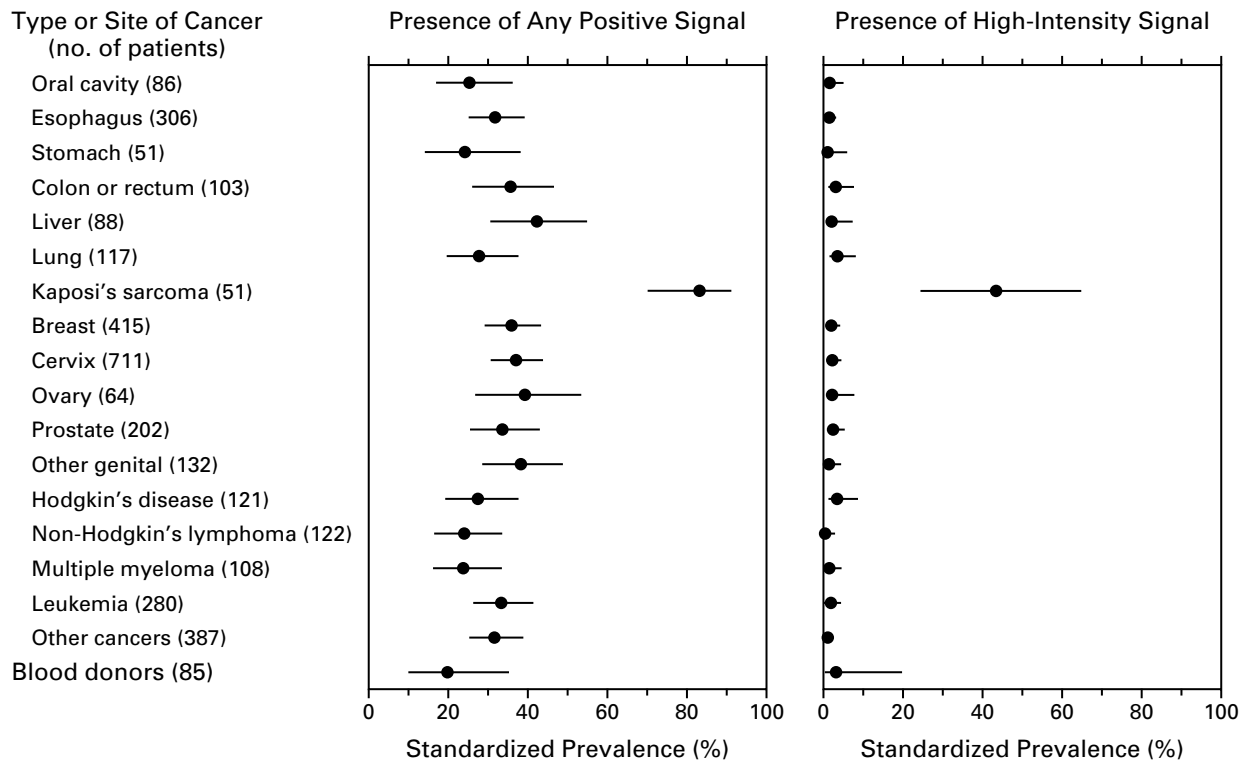
Figure 3. Standardized Prevalence of Antibodies against Human Herpesvirus 8 (HHV-8) at Two Levels of Fluorescent-Signal Intensity among 3344 Black South African Patients with Cancer and 85 Black Blood Donors.

The results are given separately for each type or location of cancer that was identified in 50 or more patients. Seroprevalence rates are standardized according to age, sex, years of education, and number of lifetime sexual partners. Horizontal lines denote 95 percent confidence intervals.

positive signal and 45 percent (95 percent confidence interval, 26 to 66 percent) on the basis of the presence of a high-intensity signal. The corresponding figures for the patients with all other cancers combined were 32 percent (95 percent confidence interval, 28 to 38 percent) and 2.0 percent (95 percent confidence interval, 1.1 to 3.6 percent), respectively, which do not differ significantly from the values for the black blood donors (20 percent [95 percent confidence interval, 10 to 35 percent] and 3 percent [95 percent confidence interval, 0.4 to 20 percent]). For the patients with multiple myeloma, the corresponding figures were 24 percent (95 percent confidence interval, 16 to 33 percent) and 1.5 percent (95 percent confidence interval, 0.5 to 5 percent), and for those with prostate cancer the figures were 33 percent (95 percent confidence interval, 25 to 43 percent) and 2.4 percent (95 percent confidence interval, 1 to 5 percent). None of the rates among the patients with myeloma or prostate cancer differed significantly from those among the patients with cancer other than Kaposi's sarcoma, nor were there any significant trends with increasing signal intensity for the patients with any of the other cancers.

Antibodies against HIV-1 in Relation to Antibodies against HHV-8 and the Presence of Kaposi's Sarcoma

The standardized seroprevalence of anti–HIV-1 antibodies was 75 percent among the patients with Kaposi's sarcoma and 5 percent among those with other cancers. As expected, the highest prevalence rates for anti–HIV-1 antibodies were found in young adults (Fig. 2), and the prevalence of such antibodies among the patients without Kaposi's sarcoma increased with the number of sexual partners. The odds ratio for the presence of HIV-1 antibodies among the patients with three or four sexual partners, as compared with those with zero to two partners, was 1.3 (95 percent confidence interval, 0.7 to 2.6), and the odds ratio among the patients with five or more sexual partners, as compared with those with zero to two partners, was 3.1 (95 percent confidence interval, 1.6 to 6.0).

There was little difference in the standardized prevalence of antibodies against HHV-8 among the
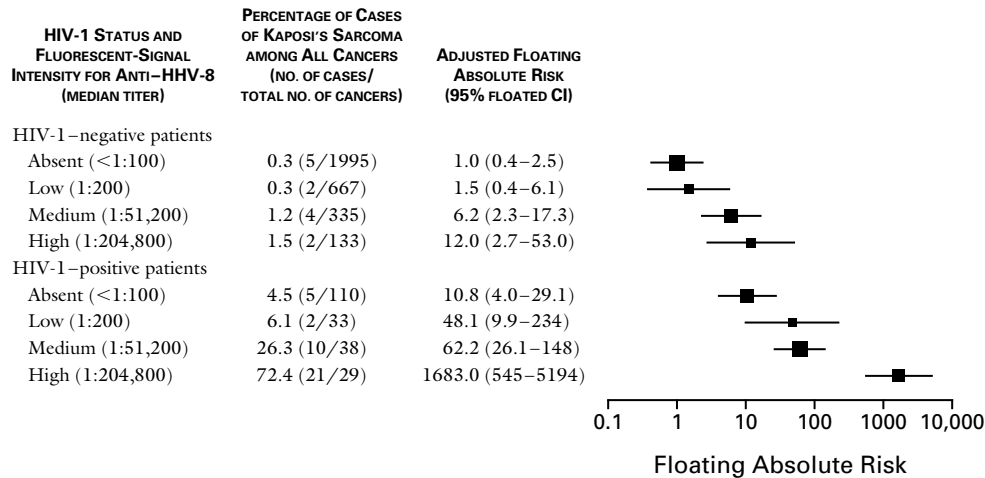
HIV-1 STATUS AND FLUORESCENT-SIGNAL INTENSITY FOR ANTI–HHV-8 (MEDIAN TITER)	PERCENTAGE OF CASES OF KAPOSI'S SARCOMA AMONG ALL CANCERS (NO. OF CASES/ TOTAL NO. OF CANCERS)	ADJUSTED FLOATING ABSOLUTE RISK (95% FLOATED CI)
HIV-1–negative patients		
Absent (<1:100)	0.3 (5/1995)	1.0 (0.4–2.5)
Low (1:200)	0.3 (2/667)	1.5 (0.4–6.1)
Medium (1:51,200)	1.2 (4/335)	6.2 (2.3–17.3)
High (1:204,800)	1.5 (2/133)	12.0 (2.7–53.0)
HIV-1–positive patients		
Absent (<1:100)	4.5 (5/110)	10.8 (4.0–29.1)
Low (1:200)	6.1 (2/33)	48.1 (9.9–234)
Medium (1:51,200)	26.3 (10/38)	62.2 (26.1–148)
High (1:204,800)	72.4 (21/29)	1683.0 (545–5194)

Floating Absolute Risk

Figure 4. Risk of Kaposi's Sarcoma among Patients with Cancer, According to Fluorescent-Signal Intensity and HIV-1 Status.

The floating absolute risks and corresponding 95 percent floated confidence intervals for Kaposi's sarcoma have been adjusted for age, sex, years of education, and number of lifetime sexual partners. For the HIV-1–negative patients, P<0.001 for trend; for the HIV-1–positive patients, P<0.001 for trend. The size of the boxes is inversely related to the influence of chance. CI denotes confidence interval, and HHV-8 human herpesvirus 8.

patients who were seropositive for HIV-1 and those who were seronegative (30 percent vs. 33 percent). Only a weak relation was found between the fluorescent-signal intensity for anti–HHV-8 antibodies and HIV-1 infection, with the patients who were seropositive for HIV-1 about twice as likely as the seronegative patients to have medium-intensity signals (odds ratio, 2.0; 95 percent confidence interval, 1.3 to 3.2) or high-intensity signals (odds ratio, 2.0; 95 percent confidence interval, 0.9 to 4.4) for anti–HHV-8 antibodies.

Both among patients who were seronegative for HIV-1 and those who were seropositive, Kaposi's sarcoma was more frequent among those with high-intensity fluorescent signals for antibodies against HHV-8 (P<0.001 for trend with increasing signal intensity) (Fig. 4). However, at each level of HHV-8 signal intensity, Kaposi's sarcoma was more likely among the HIV-1–positive patients than among those who were HIV-1–negative (P<0.001). Since signal intensity is an indirect measure of antibody titer, we repeated the analyses using the results from the 141 patients whose titers of antibody against HHV-8 had been measured directly. Associations similar to those shown in Figure 4 were found.

Figure 5 shows the distribution of anti–HHV-8 antibody titers for 47 patients with Kaposi's sarcoma and controls matched for age and HIV-1 status. Eighty-five percent of the serum samples from these patients had anti–HHV-8 titers ≥1:100, and 68 percent had titers ≥1:51,000. The corresponding figures were 35 percent and 13 percent, respectively,

for the matched controls. Figure 5 also shows that HIV-1 infection did not have an appreciable effect on the titer of anti–HHV-8 antibodies.

DISCUSSION

We found that about one third of the serum samples from the black South African patients with cancer whom we studied had antibodies against HHV-8. In this population, the prevalence of antibodies against HHV-8 increased with increasing age and a higher number of sexual partners; it was higher among those with fewer years of education, but it was not strongly related to HIV-1 infection. The seroprevalence of HHV-8 was highest among the patients with Kaposi's sarcoma, as compared with the patients with any of the other 16 types of cancer studied, including multiple myeloma and prostate cancer. Kaposi's sarcoma was more common than other tumors among patients with high titers of antibody against HHV-8, and, at a given anti–HHV-8 titer, Kaposi's sarcoma was more frequent in HIV-1–positive patients than in those who were HIV-1–negative.

The black patients with cancers other than Kaposi's sarcoma appeared to be typical of other black populations in South Africa with respect to HHV-8 infection in that the prevalence of anti–HHV-8 antibodies among them was similar to that among the black blood donors from the same area (Fig. 3). The standardized prevalence of antibodies against HHV-8 was substantially greater among black blood donors than among white blood donors from the same area (20 percent vs. 5 percent) (unpublished data). With
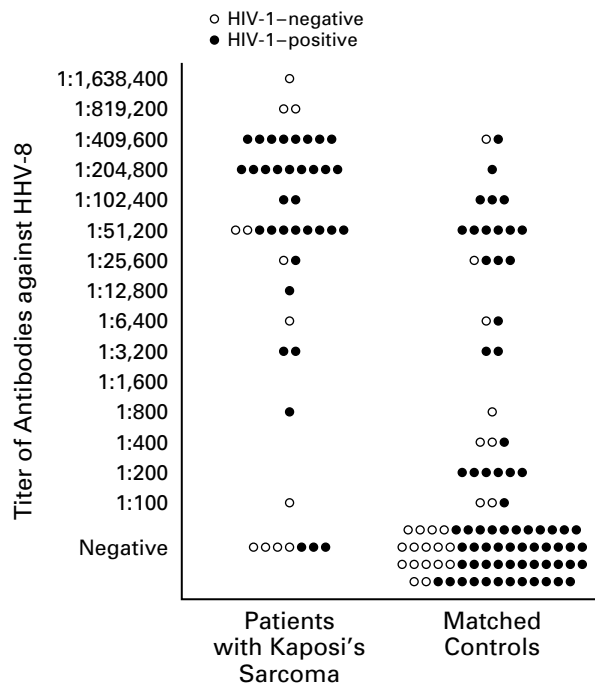
Figure 5. Distribution of Titers of Anti–Human Herpesvirus 8 (HHV-8) Antibodies in 47 Patients with Kaposi's Sarcoma and 94 Matched Controls.

For each patient with Kaposi's sarcoma, two controls without Kaposi's sarcoma were selected, matched for HIV-1 status and age.

the use of similar assays, less than 5 percent of blood donors from the United States or the United Kingdom have been shown to have antibodies against HHV-8.[23,25,30]

The immunofluorescence assay we used detects antibodies against the latent nuclear antigen of HHV-8 encoded by orf73[27-29]; it has been used to study the seroprevalence of this virus.[13,14,21,23,25,26,30] Like other serologic assays, the immunofluorescence assay is not sensitive enough to identify every person infected with HHV-8, but it does detect antibodies against HHV-8 in over 80 percent of patients with Kaposi's sarcoma. Sensitive polymerase-chain-reaction methods can demonstrate the presence of HHV-8 in the tumor tissue of virtually all patients with Kaposi's sarcoma. On the other hand, the immunofluorescence assay is highly specific and reveals antibodies against particular HHV-8 antigens.[27-29] An immunofluorescence assay against lytically induced HHV-8–positive cells has been reported to detect infection in a higher proportion of North American blood donors than the assay for antibodies against latent HHV-8 antigens used in our study.[32] However, that assay is likely to be less specific than the one we used, because it detects antibodies against undefined HHV-8

proteins. In blinded comparative studies the immunofluorescence assay we used was shown to be the most sensitive and specific assay for HHV-8.[33] From an epidemiologic perspective, the immunofluorescence assay and the fluorescent-signal-intensity scoring system we used are highly reproducible and correlate well with directly measured titers of antibody against HHV-8.

Comparatively little is known about factors associated with HHV-8 infection in Africa or, indeed, elsewhere in the world. Studies of homosexual men in Western countries suggest that the prevalence of antibodies against HHV-8 increases when sexual partners are numerous,[13,26] findings parallel with our results. We have shown that among black South African patients with cancer other than Kaposi's sarcoma, there is a trend toward increasing prevalence of antibodies against HHV-8 with increasing numbers of sexual partners. Our results also suggest that, in this population, sexual transmission of HHV-8 might not be as efficient as sexual transmission of HIV-1, since the odds ratio associated with having had five or more sexual partners, as compared with zero to two partners, was only 1.3 (95 percent confidence interval, 1.0 to 1.7) for antibodies against HHV-8 but 3.1 (95 percent confidence interval, 1.5 to 6.0) for antibodies against HIV-1.

Furthermore, transmission from mother to child appears to be common in Johannesburg, with about one third of the mothers infected with HHV-8 transmitting the virus to their children.[14] Therefore, sexual transmission is clearly not the only mode of spreading HHV-8 in South Africa, and there may be other, as yet unknown, routes. The fact that HHV-8 seroprevalence increases with age suggests that the virus may have been prevalent in this population for some time — a recently introduced virus that is transmitted in adults mainly by a sexual route would be found predominantly among young adults, as is the case for HIV-1 in this population (Fig. 2) and throughout Africa. The lower prevalence of anti–HHV-8 antibodies among whites than among blacks and the observation that among blacks the prevalence declines with increasing education suggest that factors associated with poverty may contribute to the transmission of the virus.

Kaposi's sarcoma was the only cancer among the 17 types we studied that was associated with a high seroprevalence of antibodies against HHV-8. These results indicate that HHV-8 has little, if any, specific association with the other cancers we studied, including multiple myeloma and prostate cancer. Although some molecular studies have suggested an association between HHV-8 and multiple myeloma or prostate cancer,[5,18] various other serologic and molecular studies have not been able to confirm these associations.[19-21]

Kaposi's sarcoma was especially frequent among

those with high signal intensities in the fluorescent test and high titers of antibody against HHV-8 (Fig. 4 and 5). This association is similar to the relation between EBV in African Burkitt's lymphoma[34] and nasopharyngeal cancer,[35] in which high antibody titers correlate with the risk of disease. Our results were measured in serum samples collected at the time that Kaposi's sarcoma was diagnosed, and no data are available on how signal intensity or antibody titer vary over time in the same patient before diagnosis. It seems unlikely that these associations are a consequence of the disease, since the detection of HHV-8 by the polymerase chain reaction in peripheral-blood mononuclear cells, which is also an indicator of a high viral load, has been shown to predict the subsequent development of Kaposi's sarcoma in HIV-1–positive patients.[6,7] In Italy, a study of titers of antibodies against HHV-8 in blood donors found the highest titers among populations at highest risk for HIV-1–negative, or classic, Kaposi's sarcoma.[30]

HIV-1 infection does not appear to have a substantial effect on the presence or titer of anti–HHV-8 antibodies (Fig. 5) — further evidence that the association between the titer of anti–HHV-8 antibodies and Kaposi's sarcoma is not a consequence of the disease. Instead, it appears that infection with HIV-1 has a separate effect with respect to Kaposi's sarcoma, which is independent of the anti–HHV-8 antibody titer (Fig. 4). The HHV-8 viral load may differ between persons infected with HIV-1 and those who are uninfected who have similar antibody titers, but relevant data are not yet available. Alternatively, HIV-1 infection may affect the development of Kaposi's sarcoma in a way that is unrelated to the HHV-8 viral load, perhaps through HIV-1–related immune dysregulation or its transactivation (Tat) protein.[36] Currently, however, there is no obvious explanation for our finding of striking differences in the likelihood of HHV-8–associated Kaposi's sarcoma between HIV-1–positive patients and those who were HIV-1–negative.

REFERENCES

1. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994; 266:1865-9.
2. Moore PS, Chang Y. Kaposi's sarcoma (KS), KS-associated herpesvirus, and the criteria for causality in the age of molecular biology. Am J Epidemiol 1998;147:217-21.
3. Boshoff C, Weiss RA. Kaposi's sarcoma-associated herpesvirus. In: von de Woude G, Klein G, eds. Advances in cancer research. Vol. 75. San Diego, Calif.: Academic Press, 1998:56-86.
4. Boshoff C, Schultz TF, Kennedy MM, et al. Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. Nat Med 1995;1:1274-8.
5. Staskus KA, Zhong W, Gebhard K, et al. Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells. J Virol 1997;71:715-9.
6. Whitby D, Howard MR, Tenant-Flowers M, et al. Detection of Kaposi's sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. Lancet 1995;346:799-802.
7. Moore PS, Kingsley LA, Holmberg SD, et al. Kaposi's sarcoma-associated herpesvirus infection prior to onset of Kaposi's sarcoma. AIDS 1996; 10:175-80.
8. Beral V, Peterman TA, Berkelman RL, Jaffe HW. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? Lancet 1990; 335:123-8.
9. Cook-Mozaffari P, Newton R, Beral V, Burkitt DP. The geographical distribution of Kaposi's sarcoma and of lymphomas in Africa before the AIDS epidemic. Br J Cancer 1998;78:1521-8.
10. Wabinga HR, Parkin DM, Wabwire-Mangen F, Mugerwa JW. Cancer in Kampala, Uganda, in 1989-91: changes in incidence in the era of AIDS. Int J Cancer 1993;54:23-36.
11. Bassett MT, Chokunoga E, Mauchaza B, Levy L, Ferlay J, Parkin DM. Cancer in the African population of Harare, Zimbabwe, 1990-1992. Int J Cancer 1995;63:29-36. [Erratum, Int J Cancer 1995;63:76.]
12. Sitas F, Bezwoda WR, Levin V, et al. Association between human immunodeficiency virus type 1 infection and cancer in the black population of Johannesburg and Soweto, South Africa. Br J Cancer 1997;75:1704-7.
13. Martin JN, Ganem DE, Osmond DH, Page-Shafer KA, Macrae D, Kedes DH. Sexual transmission and the natural history of human herpesvirus 8 infection. N Engl J Med 1998;338:948-54.
14. Bourboulia D, Whitby D, Boshoff C, et al. Serologic evidence for mother-to-child transmission of Kaposi sarcoma-associated herpesvirus infection. JAMA 1998;280:31-2.
15. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma–associated herpesvirus-like DNA sequences in AIDS-related body-cavity–based lymphomas. N Engl J Med 1995;332:1186-91.
16. Ansari MQ, Dawson DB, Nador R, et al. Primary body cavity-based AIDS-related lymphomas. Am J Clin Pathol 1996;105:221-9.
17. Gaidano G, Pastore C, Gloghini A, et al. Distribution of human herpesvirus-8 sequences throughout the spectrum of AIDS-related neoplasia. AIDS 1996;10:941-9.
18. Rettig MB, Ma HJ, Vescio RA, et al. Kaposi's sarcoma-associated herpesvirus infection of bone marrow dendritic cells from multiple myeloma patients. Science 1997;276:1851-4.
19. Parravicini C, Lauri E, Baldini L, et al. Kaposi's sarcoma–associated herpesvirus infection and multiple myeloma. Science 1997;278:1969-70.
20. Masood R, Zheng T, Tupule A, et al. Kaposi's sarcoma–associated herpesvirus infection and multiple myeloma. Science 1997;278:1970-1.
21. Whitby D, Boshoff C, Luppi M, Torelli G. Kaposi's sarcoma–associated herpesvirus infection and multiple myeloma. Science 1997;278:1971-2.
22. Munker R, Tasaka T, Park D, Miller CW, Koeffler HP. HHV-8 (KSHV) does not establish latency in prostate cancer cell lines. Prostate 1997;33:286-8.
23. Gao SJ, Kingsley L, Li M, et al. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Nat Med 1996; 2:925-8.
24. Boshoff C, Gao SJ, Healy LE, et al. Establishing a KSHV+ cell line (BCP-1) from peripheral blood and characterizing its growth in Nod/SCID mice. Blood 1998;91:1671-9.
25. Simpson GR, Schulz TF, Whitby D, et al. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. Lancet 1996; 348:1133-8.
26. Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. Nat Med 1996;2:918-24. [Erratum, Nat Med 1996;2: 1041.]

27. Rainbow L, Platt GM, Simpson GR, et al. The 222- to 234-kilodalton latent nuclear protein (LNA) of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) is encoded by orf73 and is a component of the latency-associated nuclear antigen. J Virol 1997;71:5915-21.

28. Kellam P, Boshoff C, Whitby D, Matthews S, Weiss RA, Talbot SJ. Identification of a major latent nuclear antigen, LNA-1, in the human herpesvirus 8 genome. J Hum Virol 1997;1:19-29.

29. Kedes DH, Lagunoff M, Renne R, Ganem D. Identification of the gene encoding the major latency-associated nuclear antigen of the Kaposi's sarcoma-associated herpesvirus. J Clin Invest 1997;100:2606-10.

30. Whitby D, Luppi M, Barozzi P, Boshoff C, Weiss RA, Torelli G. Human herpesvirus 8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. J Natl Cancer Inst 1998;90:395-7.

31. Easton DF, Peto J, Babiker AGAG. Floating absolute risk: an alternative to relative risk in survival and case-control analysis avoiding an arbitrary reference group. Stat Med 1991;10:1025-35.

32. Lennette ET, Blackbourn DJ, Levy JA. Antibodies to human herpesvirus type 8 in the general population and in Kaposi's sarcoma patients. Lancet 1996;348:858-61.

33. Rabkin CS, Schulz TF, Whitby D, et al. Interassay correlation of human herpesvirus serologic tests. J Infect Dis 1998;178:304-9.

34. de-Thé G, Geser A, Day NE, et al. Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma from Ugandan prospective study. Nature 1978;274:756-61.

35. de-Thé G, Lavoue MF, Muenz L. Differences in EBV antibody titres of patients with nasopharyngeal carcinoma originating from high, intermediate and low incidence areas. In: de-Thé, Ito Y, eds. Nasopharyngeal carcinoma: etiology and control. Lyons, France: International Agency for Research on Cancer, 1978. (IARC scientific publications no. 20.)

36. Ensoli B, Barillari G, Salahuddin SZ, Gallo RC, Wong-Staal F. Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. Nature 1990;345:84-6.