SEXUAL TRANSMISSION AND THE NATURAL HISTORY OF HUMAN HERPESVIRUS 8 INFECTION

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ABSTRACT

Background Although human herpesvirus 8 (HHV-8) has been suspected to be the etiologic agent of Kaposi's sarcoma, little is known about its seroprevalence in the population, its modes of transmission, and its natural history.

Methods The San Francisco Men's Health Study, begun in 1984, is a study of a population-based sample of men in an area with a high incidence of human immunodeficiency virus (HIV) infection. We studied all 400 men infected at base line with HIV and a sample of 400 uninfected men. Base-line serum samples were assayed for antibodies to HHV-8 latency–associated nuclear antigen (anti-LANA). In addition to the seroprevalence and risk factors for anti-LANA seropositivity, we analyzed the time to the development of Kaposi's sarcoma.

Results Anti-LANA antibodies were found in 223 of 593 men (37.6 percent) who reported any homosexual activity in the previous five years and in none of 195 exclusively heterosexual men. Anti-LANA seropositivity correlated with a history of sexually transmitted diseases and had a linear association with the number of male sexual-intercourse partners. Among the men who were infected with both HIV and HHV-8 at base line, the 10-year probability of Kaposi's sarcoma was 49.6 percent. Base-line anti-LANA seropositivity preceded and was independently associated with subsequent Kaposi's sarcoma, even after adjustment for CD4 cell counts and the number of homosexual partners.

Conclusions The prevalence of HHV-8 infection is high among homosexual men, correlates with the number of homosexual partners, and is temporally and independently associated with Kaposi's sarcoma. These observations are further evidence that HHV-8 has an etiologic role in Kaposi's sarcoma and is sexually transmitted among men. (N Engl J Med 1998;338:948-54.)

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A MONG persons infected with the human immunodeficiency virus (HIV), the disproportionate incidence of Kaposi's sarcoma among homosexual or bisexual men has long suggested that a sexually transmitted cofactor, in addition to background HIV infection, is responsible for the development of Kaposi's sarcoma.[1] A novel herpesvirus, termed human herpesvirus 8 (HHV-8) or Kaposi's sarcoma–associated herpesvirus, became a candidate for the etiologic cofactor when its DNA sequences were discovered in tissue specimens of Kaposi's sarcoma.[2] Subsequently, the detection of HHV-8 DNA sequences in all forms of Kaposi's sarcoma[3-7] has strengthened the biologic argument. Cross-sectional epidemiologic studies have determined that HHV-8 seropositivity in various populations is strongly correlated with the population's risk of Kaposi's sarcoma,[8-12] and a few longitudinal studies have shown that HHV-8 infection precedes the onset of Kaposi's sarcoma.[13-15] Longitudinal studies are critical in the evaluation of causality, because they exclude the possibility that HHV-8 infection is the result rather than the cause of Kaposi's sarcoma. Definitive epidemiologic evidence, however, requires longitudinal observation for Kaposi's sarcoma with careful control for potential confounding factors such as the patient's degree of sexual exposure, degree of immunocompromise, and geographic area of residence and changing temporal trends in HHV-8 seroprevalence.

If HHV-8 is assumed to be the cause of Kaposi's sarcoma, it becomes imperative to understand its distribution within populations, its modes of transmission, and its natural history. The high prevalence of infection among homosexual or bisexual men in the United States has suggested that HHV-8 may be sexually transmitted.[8,9,11] The available studies, however, offer only indirect evidence of sexual transmission, because they have not correlated individual sexual behavior with HHV-8 infection.

To address several unresolved aspects of HHV-8 infection, we used data from the San Francisco Men's Health Study. This study enrolled a population-based sample of men residing in an area of high HIV incidence for whom detailed behavioral histories and 10-year follow-up for the development of Kaposi's sarcoma are available. We present population-based estimates of the seroprevalence of HHV-8 and further data that support an independent association of HHV-8 infection with Kaposi's sarcoma.

METHODS

Population and Sampling

The San Francisco Men's Health Study was initiated to study the natural history of HIV infection.[16] The target population was unmarried men in San Francisco at high risk for HIV infection. Study sampling has been described in detail elsewhere.[17] In brief, there was stratified two-stage cluster sampling of all households in the 19 census tracts in San Francisco with the highest incidence of the acquired immunodeficiency syndrome (AIDS) through 1983. First, a sample of blocks was chosen from each tract with probability proportionate to the estimated number of households on each block. Second, a sample of households was randomly selected from each block. In each household, all unmarried men 25 to 54 years of age were invited to enroll. At base line, from June 1984 through January 1985, 1034 men were interviewed and had biologic specimens collected. Eight hundred twenty-two men reported themselves to be homosexual or bisexual, and 212 reported that they were exclusively heterosexual. At base line, 400 men were HIV-seropositive, 624 were HIV-seronegative, and 10 refused testing. No exclusively heterosexual man was HIV-seropositive. Subsequently, the men were followed at six-month intervals through 1994, by which time AIDS had developed in 63 percent of the men who were infected with HIV at base line. The current study of HHV-8 infection included all 400 men who were infected with HIV at base line, a random sample of 200 of the 422 homosexual or bisexual men who were not infected with HIV at base line, and 200 of the 212 exclusively heterosexual men.

Epidemiologic, Behavioral, and Immunologic Variables

The base-line questionnaires requested information on demographics, self-reported medical history (including sexually transmitted diseases and transfusions), and illicit-drug use. On the basis of their self-reported sexual practices during the previous five years, the men were classified as exclusively homosexual or exclusively heterosexual or were placed into one of three intermediate bisexual categories (Table 1). They were also asked how many male and female sexual-intercourse partners they had had during the previous two years, and when they started to have intercourse regularly. Intercourse was defined as any insertive or receptive act in which a man's penis was placed into another person's mouth, vagina, or anus. Analysis of lymphocyte subpopulations was performed with standard techniques.[18]

Antibodies to HHV-8

Base-line serum samples that had been stored at −70°C were tested in a blinded fashion for evidence of HHV-8 infection by a previously described indirect immunofluorescence assay that measures antibodies to a latency-associated nuclear antigen (anti-LANA).[8] This assay uses as its substrate isolated nuclei from a cell line, BCBL-1, that is latently infected with HHV-8 and not infected with the Epstein–Barr virus. After incubation with 1:40 diluted test serum, unbound antibody is washed away, and a secondary antibody, Texas red–conjugated goat antihuman IgG, is added. Samples are considered anti-LANA–positive if they produce a punctate nuclear pattern in all BCBL-1 nuclei visualized but not in parallel testing with an HHV-8–uninfected Ramos cell line. All samples that tested anti-LANA–positive were blindly retested, along with a random sample of those that initially tested anti-LANA–negative. Samples that produced disparate results (less than 1 percent of the subjects) were tested a third time, and the majority outcome was taken as final.

Diagnosis of Kaposi's Sarcoma

At each follow-up visit, the subjects were asked whether Kaposi's sarcoma had been diagnosed by their personal physicians in the preceding interval. A physician's diagnosis, based on prevailing clinical practices, was considered to be evidence of Kaposi's sarcoma in this study. The subjects also underwent a detailed der-

TABLE 1. POPULATION-BASED ESTIMATES OF THE PREVALENCE OF HHV-8 ANTI-LANA ANTIBODIES ACCORDING TO SOCIODEMOGRAPHIC CHARACTERISTICS, SEXUAL ORIENTATION, AND HIV SEROLOGIC STATUS.

CHARACTERISTIC	STUDY SAMPLE		TARGET POPULATION*
	NO. TESTED†	PERCENT ANTI-LANA– POSITIVE	PERCENT ANTI-LANA– POSITIVE (95% CI)
Age (yr)‡			
25–29	188	25.0	23.9 (16.7–31.1)
30–34	244	29.9	28.3 (23.0–33.6)
35–39	186	26.3	23.8 (17.5–30.0)
40–44	98	32.6	29.0 (19.4–38.6)
45–49	44	31.8	26.7 (13.6–39.7)
50–54	28	25.0	18.9 (5.3–32.6)
Race or ethnic group‡			
White	672	27.8	25.3 (21.8–29.0)
Hispanic	42	38.1	33.6 (17.6–49.6)
African American	43	23.3	22.5 (8.7–36.4)
Other	26	30.8	29.2 (10.3–48.1)
Education‡			
High school or less	84	33.3	31.2 (21.0–41.4)
1 yr college	192	29.2	26.0 (20.1–32.0)
Associate's degree	59	30.5	28.9 (16.6–41.2)
Bachelor's degree	288	27.1	24.9 (19.3–30.3)
Postgraduate degree	146	24.7	22.5 (14.8–30.3)
Sexual orientation§¶			
Exclusively homosexual	475	39.6	34.9 (30.6–39.2)
Primarily homosexual	94	35.1	31.6 (22.2–41.0)
Mostly homosexual	8	12.5	12.2 (0.0–35.0)
Equally or primarily heterosexual	16	6.3	3.5 (0.0–10.4)
Exclusively heterosexual	195	0	0 (0.0–1.90)
HIV infection¶			
Uninfected	393	8.7	11.5 (7.8–15.3)
Infected	396	47.7	48.6 (44.0–53.2)

*Estimates are weighted for original sampling fraction and differences in participation rates between census tracts and sampling of serum samples for this study. CI denotes confidence interval.

†Data were not available for all subjects in every category.

‡P>0.20 by overall chi-square test of independence.

§The category "primarily homosexual" included subjects who had a small degree of heterosexual activity, "mostly homosexual" included subjects who had a substantial degree of heterosexual activity, and "equally or primarily heterosexual" included subjects more than half of whose sexual activity was heterosexual.

¶P<0.001 for the comparison between subgroups.

matologic examination; those with suggestive lesions that had not already been diagnosed as Kaposi's sarcoma were referred to their personal physicians or to dermatologists. Finally, all subjects were cross-referenced to a data base maintained by the San Francisco Department of Public Health that actively pursued both AIDS-defining diagnoses and secondary AIDS-defining diagnoses in all cases of AIDS in San Francisco.

Statistical Analysis

In addition to estimates of HHV-8 seroprevalence in the study sample, population-based seroprevalence was estimated by assigning probability weights to each subject that took into account the subject's original probability of selection according to census tract, the original response rate per census tract, and his probability of being selected for analysis in this substudy of HHV-8 infec-

tion. Variances were estimated by taking into account these probability weights and the original block-cluster sampling scheme.[19,20]

To determine the risk factors for HHV-8 infection, univariate analyses of the associations between categorical variables and HHV-8 seropositivity were first evaluated with a chi-square test of independence. Multivariable logistic regression was used to determine independent associations. Goodness of fit was assessed by the Hosmer–Lemeshow test.[21]

Kaplan–Meier techniques were used to estimate the distribution of times from entry into the cohort to the diagnosis of Kaposi's sarcoma according to base-line anti-LANA serologic status.[22] The distributions were compared by the log-rank test. After adjustment for potential confounders, the independent association between HHV-8 and Kaposi's sarcoma was determined with multivariable proportional-hazards regression.[23,24] All analyses were performed with STATA (version 5.0)[25] and SAS (version 6.11)[26] software.

RESULTS

Stored base-line serum was available for 397 of the 400 HIV-infected subjects in the San Francisco Men's Health Study. Of the 200 subjects in the random sample of exclusively heterosexual men, none of whom were HIV-infected at base line, base-line serum was available for 195. Of 200 homosexual or bisexual men who were not HIV-infected at base line, base-line serum was available for 198. One subject had an indeterminate result on the anti-LANA assay on three separate readings and hence was eliminated from subsequent analyses; this resulted in 789 subjects available for analysis.

Population-Based HHV-8 Seroprevalence

The target population of the San Francisco Men's Health Study was men at high risk for HIV infection early in the AIDS epidemic in San Francisco. Of the 789 men sampled, 223 (28.3 percent) had antibodies to HHV-8 LANA. To the extent that the cohort was representative of the target population, the prevalence of HHV-8 anti-LANA seropositivity can be extrapolated from the study sample to the target population (Table 1). In this population, the overall estimate of HHV-8 anti-LANA seroprevalence was 25.8 percent (95 percent confidence interval, 22.6 to 29.1). HHV-8 infection was not associated with age, race or ethnic group, or education but was associated in a graded fashion with the subjects' self-reported degree of homosexual activity in the previous five years. None of the 195 exclusively heterosexual men were anti-LANA–seropositive, as compared, for example, with 12.5 percent of the men who reported some heterosexual but mostly homosexual activity, and 39.6 percent of the men who were exclusively homosexual (P<0.001 by chi-square test for trend). HHV-8 seropositivity was also strongly correlated with base-line HIV infection (P<0.001). Among the homosexual or bisexual men, 189 of 396 HIV-infected men (47.7 percent) were anti-LANA–seropositive, as compared with 34 of 197 HIV-uninfected men (17.3 percent) (P<0.001). The seroprevalences

TABLE 2. ASSOCIATION OF HHV-8 ANTI-LANA–POSITIVITY WITH SELF-REPORTED HISTORY OF SEXUALLY TRANSMITTED DISEASES AND OTHER MEDICAL CONDITIONS.

PRIOR CONDITION	NO. TESTED*	NO. ANTI-LANA– POSITIVE (%)	RELATIVE PREVALENCE OF HHV-8 (95% CI)†
Urethral or rectal gonorrhea			
No	316	33 (10)	
Yes	471	189 (40)	3.84 (2.73–5.40)
Syphilis			
No	585	118 (20)	
Yes	201	105 (52)	2.59 (2.10–3.19)
Nonspecific urethritis			
No	477	109 (23)	
Yes	309	113 (37)	1.60 (1.28–2.00)
Genital or anal– rectal herpes			
No	565	86 (15)	
Yes	223	48 (22)	1.41 (1.03–1.94)
Giardiasis			
No	721	186 (26)	
Yes	63	36 (57)	2.21 (1.73–2.84)
Salmonella or shigella			
No	722	187 (26)	
Yes	64	35 (55)	2.11 (1.64–2.72)
Diabetes			
No	777	220 (28)	
Yes	10	2 (20)	0.70 (0.20–2.45)
Infectious mononucleosis			
No	691	193 (28)	
Yes	96	28 (29)	1.04 (0.75–1.46)
Asthma			
No	722	206 (29)	
Yes	67	17 (25)	0.89 (0.58–1.36)
Hay fever			
No	624	170 (27)	
Yes	165	53 (32)	1.18 (0.91–1.52)

*Data were not available for all subjects in every category.

†CI denotes confidence interval.

in the study sample were in general equivalent to the population-based estimates. Therefore, for simplicity of presentation, the results for all subsequent analyses are shown for the study sample alone.

HHV-8 as a Sexually Transmitted Agent

Although HHV-8 infection was strongly associated with sexual orientation, more direct analyses were undertaken to test the hypothesis that HHV-8 is sexually transmitted. First, anti-LANA seropositivity was associated with a history of several sexually transmitted diseases (Table 2). It was not associated with several diseases that are not believed to be transmitted by intimate sexual contact, such as infectious mononucleosis, asthma, and hay fever. Of particular interest was the association with specific intestinal infections, which in this largely homosexual or bisexual population are probably spread by fecal–oral exposure associated with sexual activity. Second, there was a linear association between the prevalence of anti-LANA antibodies and the number of male

intercourse partners in the previous two years (Fig. 1). Third, men with more than five years of regular homosexual intercourse (defined as at least once monthly) had a greater prevalence of HHV-8 infection (213 of 535 men, 39.8 percent) than men with between one and five years of regular homosexual intercourse (7 of 44 men, 15.9 percent), who in turn had a greater prevalence than men who either had no history of homosexual intercourse or denied a regular pattern of activity (2 of 197 men, 1.0 percent) (P<0.001 by chi-square test for trend).

Associations of Potential Risk Factors with HHV-8 Infection

Modes of transmission other than sexual were also examined (Table 3). The univariate association of HHV-8 infection with a history of transfusion was of borderline statistical significance (P=0.06). There was a significant univariate association with sharing of needles for recreational-drug use in the previous
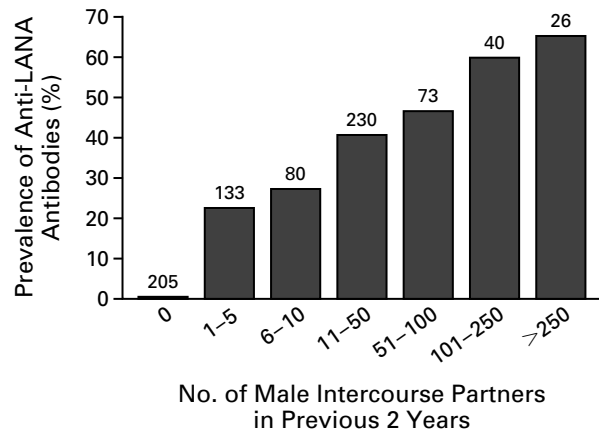

Figure 1. Prevalence of HHV-8 Anti-LANA Antibodies According to the Number of Male Intercourse Partners in the Previous Two Years.

The number of subjects in each group is shown above the bars. P<0.001 by chi-square test for linear trend.

TABLE 3. UNIVARIATE AND MULTIVARIABLE LOGISTIC-REGRESSION ANALYSES OF THE ASSOCIATION BETWEEN THE PREVALENCE OF HHV-8 ANTI-LANA ANTIBODIES AND SEXUAL PRACTICES, INJECTION-DRUG USE, HISTORY OF TRANSFUSION, CD4 LYMPHOCYTE COUNT, AND HIV SEROLOGIC STATUS.

VARIABLE	NO. TESTED*	NO. ANTI-LANA– POSITIVE (%)	UNIVARIATE ODDS RATIO	MULTIVARIABLE ODDS RATIO (95% CI)†	P VALUE
No. of male intercourse partners in previous 2 yr					
0‡	205	1 (0.5)			
1–5	133	30 (23)	59.4	29.8 (3.91–226.5)	0.001
6–10	80	22 (28)	77.4	29.0 (3.68–228.2)	0.001
11–50	230	94 (41)	141.0	46.5 (6.16–351.6)	<0.001
51–100	73	34 (47)	177.8	59.5 (7.57–467.2)	<0.001
101–250	40	24 (60)	306.0	96.6 (11.4–820.4)	<0.001
>250	26	17 (65)	385.3	105.6 (12.5–895.5)	<0.001
Shared needles in previous 5 yr					
No‡	716	189 (26)			
Yes	72	33 (46)	2.36	1.34 (0.74–2.42)	0.34
History of transfusion					
No‡	758	209 (28)			
Yes	19	9 (47)	2.36	2.30 (0.80–6.63)	0.12
CD4 count (cells/mm³)					
>1000‡	243	36 (15)			
751–1000	190	48 (25)	1.94	0.90 (0.45–1.76)	0.75
501–750	205	72 (35)	3.11	0.76 (0.41–1.42)	0.39
≤500	112	53 (47)	5.17	0.86 (0.46–1.60)	0.64
HIV infection					
No‡	393	34 (9)			
Yes	396	189 (48)	9.64	4.00 (2.28–7.00)	<0.001

*Data were not available for all subjects in every category.

†Each variable was adjusted for all other variables in the table. The Hosmer–Lemeshow goodness-of-fit statistic=4.63 with 7 df (P=0.70). CI denotes confidence interval.

‡Men with this factor served as the reference category.

five years (P<0.001). Because HIV-related immunocompromise could conceivably be a risk factor for the acquisition of HHV-8, both HIV infection itself and the absolute CD4 lymphocyte count were examined, and both were associated with HHV-8 seropositivity (P<0.001 for both).

Because all these factors might be confounded by their association with a subject's sexual practices, a multivariable logistic-regression model was applied in which each of the factors was adjusted for the number of male intercourse partners in the previous two years, as well as for the other factors in question (Table 3). The association between the number of male intercourse partners and anti-LANA seropositivity remained very strong. After adjustment for the number of sexual partners, the associations with sharing of needles and the CD4 lymphocyte count were no longer significant. The association with HIV infection, however, remained significant. For a history of transfusion, the adjusted odds ratio was not markedly changed from the unadjusted estimate, but it was not statistically significant (odds ratio, 2.30; 95 percent confidence interval, 0.80 to 6.63; P=0.12).

Temporal Associations of HHV-8 Infection with Kaposi's Sarcoma

Ten-year follow-up of the 391 subjects who were HIV-infected but free of Kaposi's sarcoma revealed 113 incident cases of Kaposi's sarcoma. At 10 years, 18 of these men (4.6 percent) had been lost to follow-up for ascertainment of Kaposi's sarcoma. No Kaposi's sarcoma was observed in the HIV-uninfected men (including those who were infected with HHV-8), and hence they were not included in subsequent analyses. Figure 2 shows the Kaplan–Meier estimates of time from enrollment in the cohort to the development of Kaposi's sarcoma according to the initial anti-LANA serologic status. Base-line anti-LANA seropositivity was significantly associated with the subsequent development of Kaposi's sarcoma (relative hazard, 2.40; 95 percent confidence interval, 1.61 to 3.58; P<0.001).

In the subjects with HHV-8 and HIV infection at base line, the median time to the development of Kaposi's sarcoma was not quite reached, although the probability of Kaposi's sarcoma at 10 years was 49.6 percent (95 percent confidence interval, 41.3 to 58.7 percent). After adjustment in a proportional-hazards regression model for the degree of homosexual activity, as measured by the number of male intercourse partners in the previous two years, as well as for propensity for HIV-related opportunistic disease, as measured by CD4 count and age, the relative hazard associated with being anti-LANA–seropositive at base line was 2.51 (95 percent confidence interval, 1.67 to 3.77; P<0.001), not a substantially altered value. Thus, HHV-8 infection preceded and
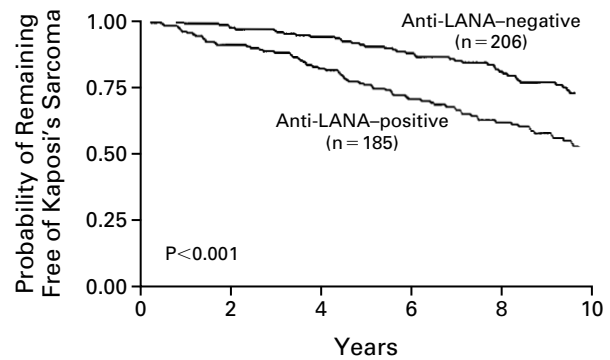

Figure 2. Kaplan–Meier Estimates of Time from Study Enrollment to the Development of Kaposi's Sarcoma According to Base-Line HHV-8 Anti-LANA–Antibody Status among 391 HIV-Infected Men.

was associated with Kaposi's sarcoma independently of the degree of sexual activity and HIV-related immunocompromise.

DISCUSSION

In assessing potential causative agents of Kaposi's sarcoma,[27-29] one must ask whether their epidemiology matches that predicted by the epidemiology of Kaposi's sarcoma. That is, the epidemiology of the candidate agent should probably be that of a sexually transmitted agent. We found that HHV-8 seroprevalence was more common among homosexual or bisexual men, as did previous studies.[8-11] A correlation with sexual orientation, however, is not direct proof of sexual transmission. We directly addressed the hypothesis of sexual transmission with information on individual sexual activity. In addition to being correlated with a history of sexually transmitted disease and the number of years of regular intercourse with men, HHV-8 infection had a striking independent correlation with the number of male intercourse partners. This is the most suggestive evidence to date that HHV-8 is sexually transmitted.

Sexual exposure was by far the strongest risk factor for HHV-8 infection, but surprisingly, the association between HHV-8 infection and HIV infection remained significant even after adjustment for CD4 count. This is most likely explained by some specific sexual practice, not fully captured by the number of sexual partners, that was associated with the acquisition of both HIV and HHV-8. Alternatively, the association may be explained by clustering of HIV and HHV-8 in those who transmitted the infections to these men or by some aspect of HIV infection apart from the decline in the CD4 cell count that confers host susceptibility to HHV-8. In the multivariable model, although the odds ratio for

a history of transfusion was not meaningfully altered from its univariate estimate, history of transfusion was not significantly associated with HHV-8 infection. However, transfusion cannot be excluded as a risk factor, because the confidence interval still includes meaningful measures of association. Hence, although the lack of an association with needle sharing might argue against parenteral transmission, our study cannot draw firm conclusions about the role of transfusion.

Base-line anti-LANA reactivity, which indicates HHV-8 infection, preceded and was strongly associated with the subsequent development of Kaposi's sarcoma. Although Kaposi's sarcoma did arise in men whose base-line anti-LANA test was negative, in most of these subjects HHV-8 infection almost certainly supervened during follow-up. This can be inferred because virtually all patients with Kaposi's sarcoma are infected with HHV-8 (as judged by polymerase-chain-reaction [PCR] assay of viral DNA),[2,6] and at least 80 percent are anti-LANA–seropositive at or after diagnosis.[8-10] Seroconversion analyses among those who were initially anti-LANA–negative are under way. In addition, because of incomplete sensitivity of the anti-LANA assay, some base-line seronegative results may have been false negatives. Thus, the observed association between base-line HHV-8 infection, as measured by anti-LANA results, and Kaposi's sarcoma probably substantially underestimates the true association between HHV-8 and the tumor.

Adjustment for the number of male sexual partners as well as the degree of HIV-related immunocompromise did not alter the association between HHV-8 and Kaposi's sarcoma. Adjustment for sexual exposure is particularly important, because it excludes the possibility that HHV-8 seropositivity might be a marker for the still undiscovered sexually transmitted agent of Kaposi's sarcoma.[30] Finally, because the study was conducted in a single geographic area during a specific period, potential confounding by regional or temporal differences in HHV-8 prevalence was eliminated. In the absence of an animal model or an unethical experimental exposure of humans to HHV-8, this type of well-controlled longitudinal analysis is likely to yield the strongest evidence of a causal role of HHV-8 in Kaposi's sarcoma.

The median time from cohort entry to the development of Kaposi's sarcoma in our subjects coinfected with HIV and HHV-8 was approximately 10 years. This is substantially longer than the 3.5 years estimated by Whitby et al.,[13] who measured HHV-8 infection by PCR in peripheral-blood mononuclear cells. PCR is less sensitive than serologic tests for the detection of HHV-8 infection[10,15] and may be more likely to detect infection in subjects with a higher viral burden or at a more advanced stage of HHV-8 infection, who may be at risk for more rapid progres-

sion to Kaposi's sarcoma. In addition, the sample size in the study by Whitby et al. (11 subjects) may have contributed to the discrepancy.

Gao et al. also analyzed the median time to the development of Kaposi's sarcoma.[9,15] They estimated that patients in whom Kaposi's sarcoma developed had a median of 3.8 years of preceding HHV-8 infection. This measure is fundamentally different from ours; we estimated the probability that Kaposi's sarcoma would develop in men who were coinfected with HIV and HHV-8 at entry into the study. These differences in design doubtless contribute in important ways to the different estimates. Certainly, shorter times to the development of Kaposi's sarcoma do occur and will probably be evident in some initially anti-LANA–seronegative men in our study in whom HHV-8 infection and Kaposi's sarcoma developed during follow-up. Our study, however, clearly shows that many persons coinfected with HIV and HHV-8 will have much longer intervals from HHV-8 infection to the development of Kaposi's sarcoma. Finally, estimates of time to Kaposi's sarcoma made in HIV-infected populations such as ours will probably be quite different from those in groups without HIV infection.

Because our study intentionally included a large proportion of homosexual and bisexual men, our population-based overall seroprevalence estimate should not be considered representative of all urban men. Instead, the value of population-based sampling is in the estimates of subpopulation seroprevalences it allows. The seroprevalence of HHV-8 was 37.6 percent in men reporting any homosexual activity and 0 percent in exclusively heterosexual men. These population-based estimates of HHV-8 seroprevalence avoid the selection biases associated with samples from clinics or blood banks or other convenience samples.

Previous work with the anti-LANA assay in a clinic-based population found an HHV-8 seroprevalence of 27 percent in homosexual or bisexual men.[8] Similar estimates have been obtained with other serologic assays that measure antibodies to latent-phase viral antigens.[9,15] However, because 15 to 20 percent of patients with Kaposi's sarcoma who have PCR evidence of HHV-8 in their lesions are seronegative according to these assays, their sensitivity is incomplete. Lennette et al., using an assay designed to detect antibodies to lytic HHV-8 antigens, reported that more than 90 percent of homosexual or bisexual men were infected with HHV-8.[11] However, subsequent work with lytic antigens in enzyme immunoassays[10,31,32] and indirect immunofluorescent assays[33] has failed to confirm such a high seroprevalence in homosexual or bisexual men. Regardless of which prevalence estimate is correct, characterizing the epidemiology of the anti-LANA–seropositive state is important. Because we have shown that anti-

LANA reactivity is a risk factor for Kaposi's sarcoma, anti-LANA positivity must mark a special subpopulation of infected persons who are at higher risk for Kaposi's sarcoma, even if HHV-8 is ubiquitous in homosexual and bisexual men.

Understanding the epidemiology of HHV-8 is a critical first step in designing interventions to decrease the transmission of this agent. Because many of the risk factors responsible for HIV infection probably also increase the risk of HHV-8 infection, many of these prevention efforts have already begun. The potential transmission of HHV-8 through unprotected sexual practices provides yet another impetus to reinforce safe sexual behavior.

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