

## SEROCONVERSION TO ANTIBODIES AGAINST KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS-RELATED LATENT NUCLEAR ANTIGENS BEFORE THE DEVELOPMENT OF KAPOSI'S SARCOMA

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### ABSTRACT

**Background** If Kaposi's sarcoma-associated herpesvirus (KSHV) is the cause of Kaposi's sarcoma, serologic evidence of infection should be present in patients before the disease develops.

**Methods** Using an immunoblot assay for two latent nuclear antigens of KSHV, we tested serum samples from homosexual male patients with the acquired immunodeficiency syndrome (AIDS) with and without Kaposi's sarcoma, HIV-infected men with hemophilia, HIV-seronegative blood donors, and HIV-seronegative patients with high titers of antibodies against Epstein-Barr virus (EBV). Serial serum samples obtained from patients with Kaposi's sarcoma before the diagnosis of the disease were tested for evidence of seroconversion.

**Results** Of 40 patients with Kaposi's sarcoma, 32 (80 percent) were positive for antibodies against KSHV antigens by the immunoblot assay, as compared with only 7 of 40 homosexual men (18 percent) without Kaposi's sarcoma immediately before the onset of AIDS. Of 122 blood donors, 22 EBV-infected patients, and 20 HIV-infected men with hemophilia, none were seropositive. When studied by the immunoblot assay over a period of 13 to 103 months, 21 of the 40 patients with Kaposi's sarcoma (52 percent) seroconverted 6 to 75 months before the clinical appearance of Kaposi's sarcoma. The median duration of antibody seropositivity for KSHV-related latent nuclear antigens before the diagnosis of Kaposi's sarcoma was 33 months.

**Conclusions** In most patients with Kaposi's sarcoma and AIDS, seroconversion to positivity for antibodies against KSHV-related nuclear antigens occurs before the clinical appearance of Kaposi's sarcoma. This supports the hypothesis that Kaposi's sarcoma results from infection with KSHV. (N Engl J Med 1996;335:233-41.)

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**K**APOSI'S sarcoma-associated herpesvirus (KSHV; also known as human herpesvirus 8)<sup>1,2</sup> is found in over 95 percent of Kaposi's sarcomas of all types, but rarely in solid tissue from control patients with neither the acquired immunodeficiency syndrome (AIDS) nor Kaposi's sarcoma.<sup>1,3-7</sup> KSHV is closely related to herpesvirus saimiri and Epstein-Barr virus (EBV),<sup>2</sup> which

are both tumorigenic gammaherpesviruses.<sup>8,9</sup> Infection with other human herpesviruses usually takes place during childhood, and nearly all adults are infected before adolescence. If KSHV is also ubiquitous, then people with and those without Kaposi's sarcoma would be equally likely to be infected, and this virus would not be likely to cause Kaposi's sarcoma.<sup>10,11</sup> However, if KSHV infection is rare and is primarily found in adults at risk for Kaposi's sarcoma, then it is likely that the virus has a causal role in the sarcoma.<sup>12</sup>

A rare B-cell body-cavity-based lymphoma is also associated with KSHV.<sup>13</sup> KSHV was first cultured in vitro with BC-1, a naturally infected B-cell line also infected with EBV that was derived from an AIDS-related body-cavity-based lymphoma.<sup>14</sup> Immunofluorescence assays using this cell line show that patients with Kaposi's sarcoma have a specific immune response to KSHV-related antigens.<sup>2</sup> We describe a new immunoblot assay based on KSHV-related latent nuclear antigens. Using this assay, we show KSHV-specific antibody seroconversion among homosexual men infected with the human immunodeficiency virus (HIV) in whom Kaposi's sarcoma subsequently developed.

### METHODS

#### Study Design and Patients

The serum samples studied were from 40 HIV-seropositive homosexual men enrolled in the Multicenter AIDS Cohort Study in whom AIDS-associated Kaposi's sarcoma developed.<sup>15</sup> Serum samples were collected at regular intervals from the time of entry into the study until the visit immediately before the onset of Ka-

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posi's sarcoma. The first of four control groups included 40 high-risk HIV-seropositive homosexual men enrolled in the study who died from AIDS but did not have Kaposi's sarcoma; these men were frequency-matched to the case patients according to the CD4+ cell count at the study visit preceding the diagnosis of AIDS. A second control group of 20 low-risk patients with hemophilia who had HIV infection but no Kaposi's sarcoma was also studied. Two other control groups, not infected with HIV, included 122 blood donors (seronegative for HIV, human T-cell lymphotropic virus type 1, hepatitis B virus, hepatitis C virus, and *Treponema pallidum*), recruited from various sites in the United States, and 22 patients with high antibody titers to EBV viral capsid antigen, recruited from clinical sites at Columbia University–Presbyterian Hospital. Case and control serum samples were evaluated in a blinded, randomized manner to determine the sensitivity and specificity of the assay. After unblinding, samples with discordant results (i.e., a case sample that tested negative or a control sample that tested positive) were tested again.

Samples from two HIV-seronegative patients in complete remission from Kaposi's sarcoma were also examined. The first patient was a homosexual man with six serum samples obtained over a 5-year period; he had remained in complete remission from Kaposi's sarcoma for 3½ years. The second patient had a single serum sample drawn seven years after he had a complete remission from Kaposi's sarcoma after surgical excision; in this patient, a disseminated body-cavity-based lymphoma containing KSHV also developed.

#### Cell Lines and Cultures

BC-1 is infected with both KSHV and EBV.<sup>14</sup> The form of EBV found in BC-1 is capable of both lytic replication and immortalization of lymphocytes.<sup>2</sup> Resting cells express EBV nuclear antigen 1 (EBNA-1) but not latent membrane protein 1 (LMP-1), EBNA-2, or lytic-phase antigens. CB33 (a gift of Dr. Riccardo Dalla Favera), an EBV-infected lymphoblastoid cell line; P3HR-1; and Raji (American Type Culture Collection, Rockville, Md.) containing various forms of EBV possess the major forms of EBV lytic-phase and latent-phase antigen expression. All the cell lines were cultured with RPMI-1640 medium, supplemented with 10 percent fetal-calf serum.

To induce expression of viral lytic-phase antigens, the cells were treated with 20 ng of phorbol ester (12-*O*-tetradecanoylphorbol-13-acetate, TPA, Sigma Chemical, St. Louis) per milliliter or 3 mM sodium *n*-butyrate (Sigma) for 48 hours. To inhibit expression of viral lytic-phase antigens, the cells were treated with 150 µg of phosphonoacetic acid (Sigma) per milliliter for 48 hours.

#### Preparation of Cytoplasmic and Nuclear Fractions

The BC-1 cells were solubilized for 10 minutes with three volumes of hypotonic buffer containing 10 mM TRIS–hydrochloric acid (pH 7.9), 1.5 mM magnesium chloride, 10 mM potassium chloride, and 1 percent Nonidet P-40, supplemented with 100 µM phenylmethylsulfonyl fluoride (Sigma) and 27 µM *N*α-p-tosyl-L-lysine chloromethyl ketone (Sigma) to prevent proteolytic degradation. The samples were then centrifuged at 2000×*g* for 10 minutes, and the supernatant and the pellet containing the cytoplasmic and nuclear fractions, respectively, were separated.

#### Immunoblot Assay

The KSHV-related latent nuclear antigens (p226 and p234) were found in uninduced BC-1 cell lysates by testing serum samples from an unblinded convenience sample of 21 patients with AIDS-associated Kaposi's sarcoma and 5 control patients with AIDS but no Kaposi's sarcoma. These serum samples were different from those used subsequently in the blinded evaluation of the assay. Initial evaluation identified a doublet of 226-kd and 234-kd proteins that reacted with 20 of the 21 case serum samples but none of the controls. The proteins used in the immunoassay were prepared from lysed cells or nuclear fractions with 100 µg of pro-

tein per lane in loading buffer, which were separated on a 10 percent sodium dodecyl sulfate–polyacrylamide gel<sup>16</sup> and transferred electrophoretically to a nitrocellulose membrane.<sup>17</sup> After saturation with 5 percent skim milk, the membrane was incubated with the serum samples from the patients at a dilution of 1:100 in 1 percent skim milk. Antibody binding was detected with goat antihuman IgG, IgM, and IgA alkaline phosphatase conjugate (Sigma) at a dilution of 1:5000 and was developed with 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt and nitroblue tetrazolium chloride as substrates (GIBCO BRL, Grand Island, N.Y.). Only serum samples that showed reactivity to both p226 and p234 were scored as positive. Preparations of BC-1 nuclear fractions were used in the immunoblot assays of serum samples from patients.

#### Polymerase-Chain-Reaction Amplification

Peripheral-blood mononuclear cells from the patients with Kaposi's sarcoma, the control homosexual patients with AIDS, and the HIV-infected control patients with hemophilia were examined by nested polymerase chain reaction (PCR) as described elsewhere.<sup>18</sup> To control for contamination of the PCR, only samples that tested consistently positive with two nonoverlapping sets of primers were considered positive; all samples were tested in a random, blinded fashion; water control samples were processed and amplified alternately with the specimens from patients; and the samples were extracted in laboratories separate from those where the PCR amplification was performed. The results of PCR amplification for 10 patients with Kaposi's sarcoma, 23 control homosexual patients with AIDS, and 19 control HIV-infected patients with hemophilia have been reported elsewhere.<sup>18</sup>

#### Statistical Analysis

The statistical analyses were performed with Epi Info, version 6 (USD, Stone Mountain, Ga.), with the chi-square test used to compare dichotomous variables and the Kruskal–Wallis test used for continuous variables. Data obtained by the immunoblot and PCR assays were compared by the McNemar test for paired data. Least-squares curve fitting was used to estimate the trend in seroprevalence over time for the patients with Kaposi's sarcoma. Seroreversion was assumed to have occurred halfway between the date of a patient's last seronegative examination and the date of the first seropositive examination.

## RESULTS

#### Identification of p226 and p234

The doublet of KSHV-related latent nuclear antigens comprises a 226-kd protein and a 234-kd protein that react with serum samples from patients with Kaposi's sarcoma (Fig. 1A and 1D). Representative immunoblots with BC-1 and three EBV-infected cell lines are shown in Figure 1 for serum samples from a patient with AIDS-associated Kaposi's sarcoma (Fig. 1A); a patient with AIDS but no Kaposi's sarcoma (Fig. 1B); a patient with nasopharyngeal carcinoma, a condition associated with high titers of antibody against EBV antigens<sup>19</sup> (Fig. 1C); and an HIV-seronegative patient with Kaposi's sarcoma (Fig. 1D). The BC-1 antigen doublet was detectable with serum samples from most patients with Kaposi's sarcoma (Fig. 1A and 1D) but not with most control serum samples (Fig. 1B and 1C). The doublet was also not detected in lysates of three resting B-cell lines that were not infected with KSHV

(Fig. 1) or after the induction of lytic-phase antigens with phorbol ester or butyrate (data not shown). Since BC-1 is infected with EBV, antibodies to EBNA-1 can also be detected with the BC-1 lysate (Fig. 1).

#### Characterization of p226 and p234 as Latent Nuclear Proteins

To characterize p226 and p234 further, BC-1 cells were treated with phorbol ester, butyrate, and phosphonoacetic acid. Both phorbol ester and butyrate can induce EBV into lytic replication,<sup>20,21</sup> and butyrate induces the expression of a KSHV-related 40-kd lytic-phase antigen.<sup>22</sup> Treatment with phosphonoacetic acid inhibits the lytic replication of EBV and can be used to distinguish lytic viral proteins from latent viral proteins.<sup>23</sup> Treating BC-1 cells with inducing agents did not increase the synthesis of the p226-p234 doublet, and treatment with phosphonoacetic acid did not inhibit expression of the antigen doublet (data not shown). Similar results were found for EBNA-1. When BC-1 cells were fractionated into nuclear and cytoplasmic fractions and tested by the immunoblot assay with a reactive serum, most of the antigen doublet was present in the nuclear fraction (Fig. 2). These results indicate that the p226 and p234 proteins are latent nuclear antigens.

#### Sensitivity and Specificity of the Latent Nuclear Antigens for KSHV

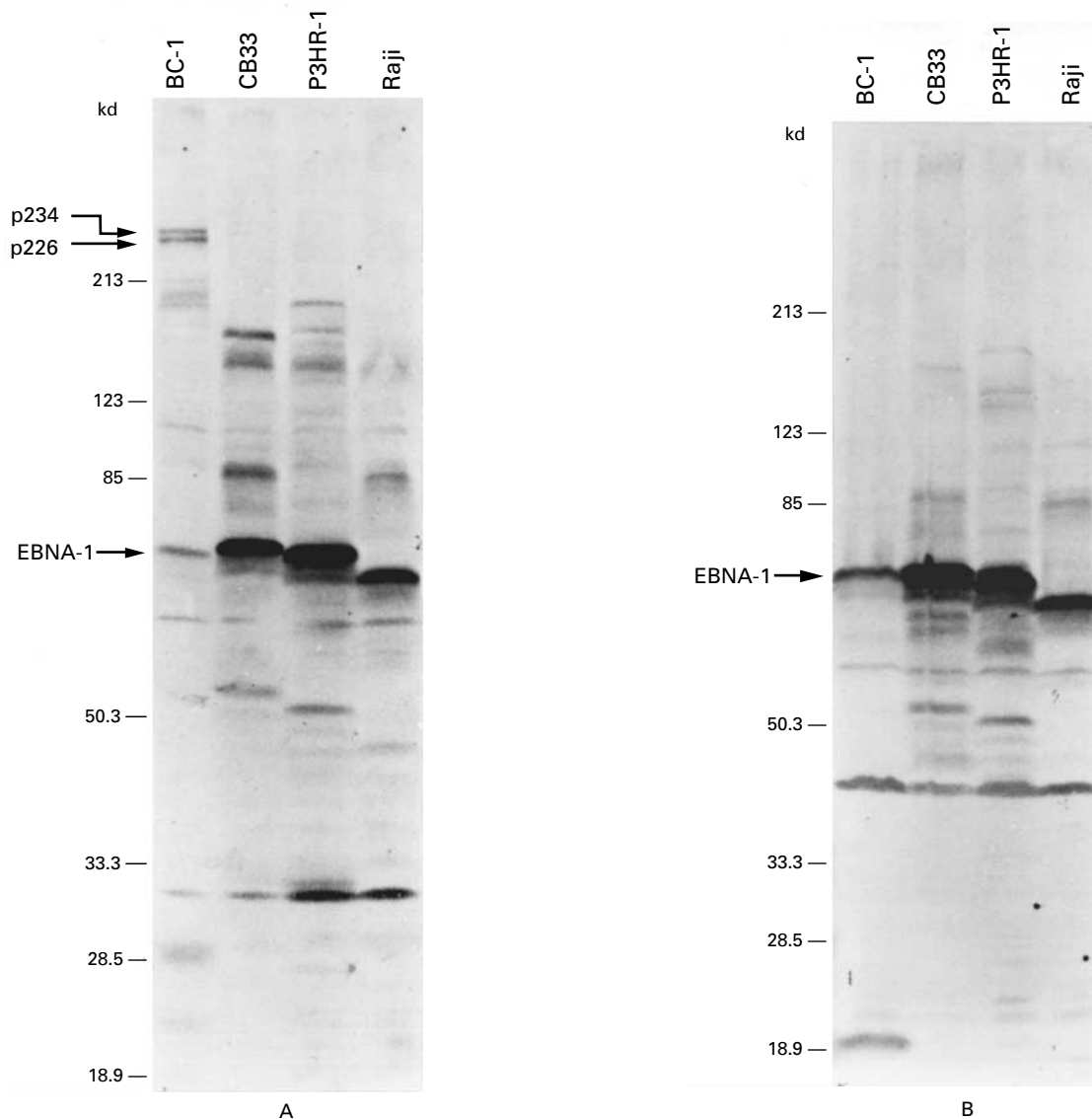
At the study visit immediately before the onset of AIDS, serum samples from 32 of the 40 patients with AIDS-associated Kaposi's sarcoma (80 percent) reacted with the KSHV-related latent nuclear antigens, whereas only 7 of the 40 homosexual control patients with AIDS (18 percent) were seropositive (odds ratio, 18.9; 95 percent confidence interval, 5.4 to 69.8) (Table 1). The two groups did not differ significantly in age, date of entry into the cohort, CD4+ cell count at entry, CD4+ cell count before the diagnosis of AIDS, or use of antiretroviral medication (data not shown). Two of the control serum samples and two serum samples from the patients with Kaposi's sarcoma produced weak bands that were not identified during the blinded evaluation but were clearly detectable after unblinding.

Although there was no significant difference in CD4+ cell count between the patients with Kaposi's sarcoma and the control patients with AIDS, the 8 seronegative patients with Kaposi's sarcoma had a higher median CD4+ cell count at the study visit before the onset of disease than the remaining 32 seropositive patients with Kaposi's sarcoma (396 vs. 229 cells per cubic millimeter,  $P=0.039$ ). Kaposi's sarcoma also developed more rapidly after enrollment in these seronegative patients with Kaposi's

sarcoma than in the seropositive patients (25 vs. 54 months,  $P=0.005$ ). Serum samples from the 20 HIV-infected patients with hemophilia, the 122 blood donors, and the 22 patients who were seropositive for EBV viral capsid antigen were all unreactive to the antigen doublet. The specificity of the serologic assay in predicting Kaposi's sarcoma ranged from 82 percent (in the control group composed of homosexual patients with AIDS) to 100 percent (in the control groups composed of HIV-infected patients with hemophilia, blood donors, and patients with high titers of antibodies against EBV) (Table 1). In contrast, antibodies against EBNA-1 were present in all the serum samples from the homosexual patients with AIDS and the EBV-infected patients, 90 percent of the serum samples from the HIV-infected patients with hemophilia, and 89 percent of the blood donors, as is consistent with the ubiquity of EBV infection.

To examine the persistence of antibodies against KSHV-related antigens after remission of Kaposi's sarcoma, serum samples from two patients were analyzed. All the serum samples collected serially over a five-year period from an HIV-seronegative homosexual man with Kaposi's sarcoma were positive for antibodies directed against KSHV-related nuclear antigens. The patient had had recurrent Kaposi's sarcoma for 1½ years but remained in remission for 3½ years after the excision of the tumor and local irradiation. Similarly, serum from an HIV-seronegative heterosexual man with a KSHV-infected body-cavity-based lymphoma was seropositive on immunoblotting seven years after complete remission of Kaposi's sarcoma.

The serologic assay was more sensitive in detecting KSHV infection than nested-PCR amplification of KSHV DNA from peripheral-blood mononuclear cells (Tables 1 and 2). If one assumes that all patients with Kaposi's sarcoma were infected at the time of their last visit before the diagnosis of Kaposi's sarcoma, the sensitivity of the immunoblot assay in detecting KSHV infection was 80 percent, as compared with 52 percent for PCR (two-tailed  $P=0.02$ ). Among the control patients with AIDS, the specificity was similar, with 18 percent found to be positive by the immunoblot assay as compared with 8 percent by PCR (two-tailed  $P=0.22$ ) (Table 2). Two of the 19 samples from patients with hemophilia and 1 sample from a homosexual control patient with AIDS that were previously reported to be positive for KSHV DNA by PCR<sup>18</sup> were not positive by PCR when they were tested again in this study. Both the patients with hemophilia were found to be seronegative, but the homosexual control patient with AIDS was found to be seropositive (Patient 38, Fig. 3) by the immunoblot assay. It is likely that the samples had earlier tested positive by PCR because of contamination.



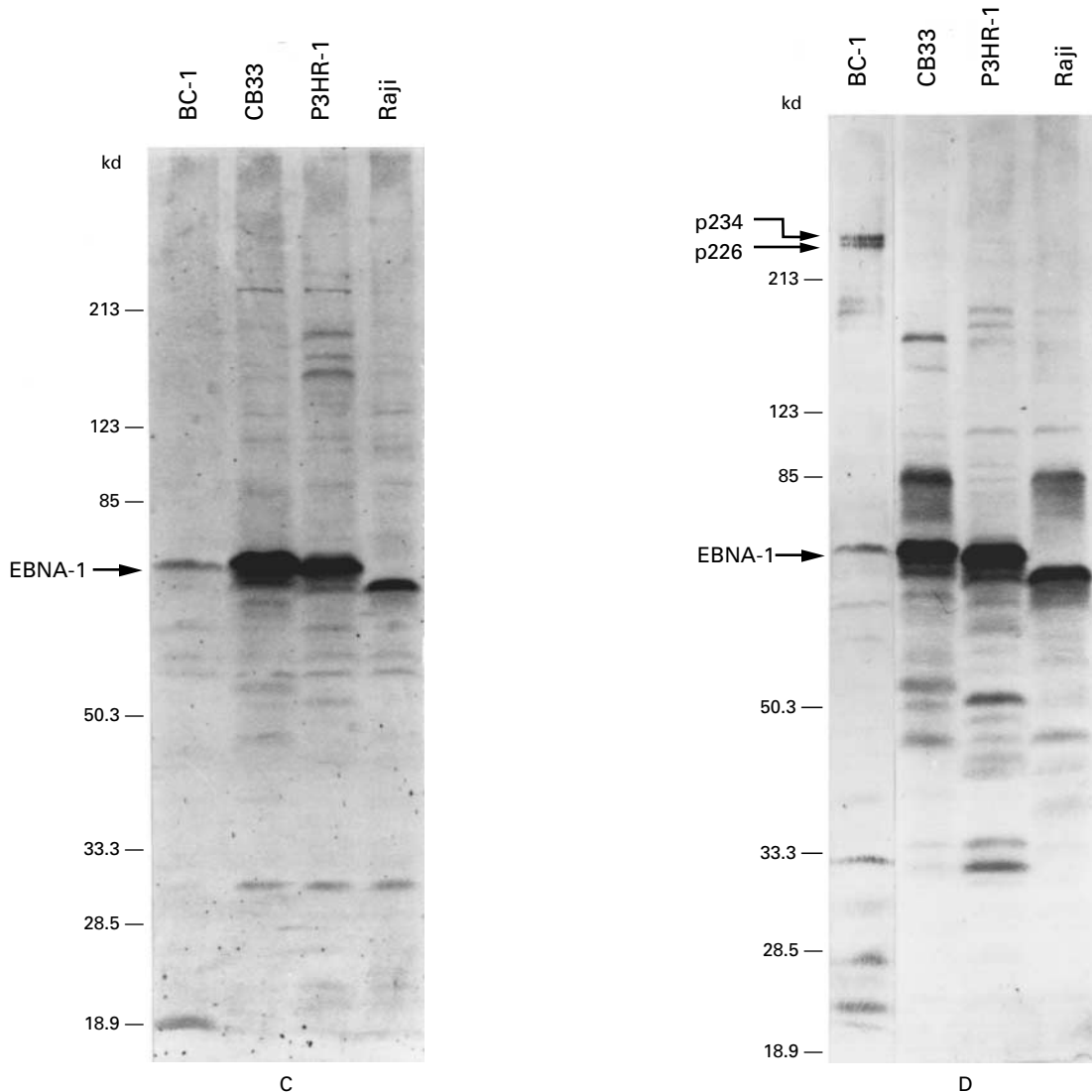
**Figure 1.** Representative Immunoblots of Serum Samples from a Patient with AIDS-Associated Kaposi's Sarcoma (Panel A), a Patient with AIDS but No Kaposi's Sarcoma (Panel B), a Patient with Nasopharyngeal Carcinoma (Panel C), and an HIV-Seronegative Patient with Kaposi's Sarcoma (Panel D).

The doublet of 226-kd and 234-kd latent nuclear antigens was detected with serum samples from the patients with Kaposi's sarcoma in lysates of the BC-1 cell line, which is infected with KSHV and EBV, but not in the other three cell lines, which are infected with EBV (Panels A and D). The latent nuclear antigens were not detected with serum samples from either of the patients without Kaposi's sarcoma (Panels B and C). The 65-to-76-kd EBNA-1 was detected in all four cell lines with all four serum samples.

**Longitudinal Examination of Patients with Kaposi's Sarcoma**

We studied serum samples from the 40 patients with AIDS-associated Kaposi's sarcoma that were obtained serially from the time of entry into the study until the last study visit before the development of Kaposi's sarcoma. Nearly all the patients had one of three immunoblot patterns (Fig. 3). Eleven patients (28 percent; Patients 30 through 40)

were seropositive at all visits, 13 to 103 months before the diagnosis of their disease. These patients appear to have been infected before entering the study. Twenty-one patients (52 percent; Patients 9 through 29) seroconverted from negative to positive for antibodies against KSHV latent nuclear antigens by the immunoblot assay after entering the study but before Kaposi's sarcoma was diagnosed. Seroconversion occurred 6 to 75 months before Ka-

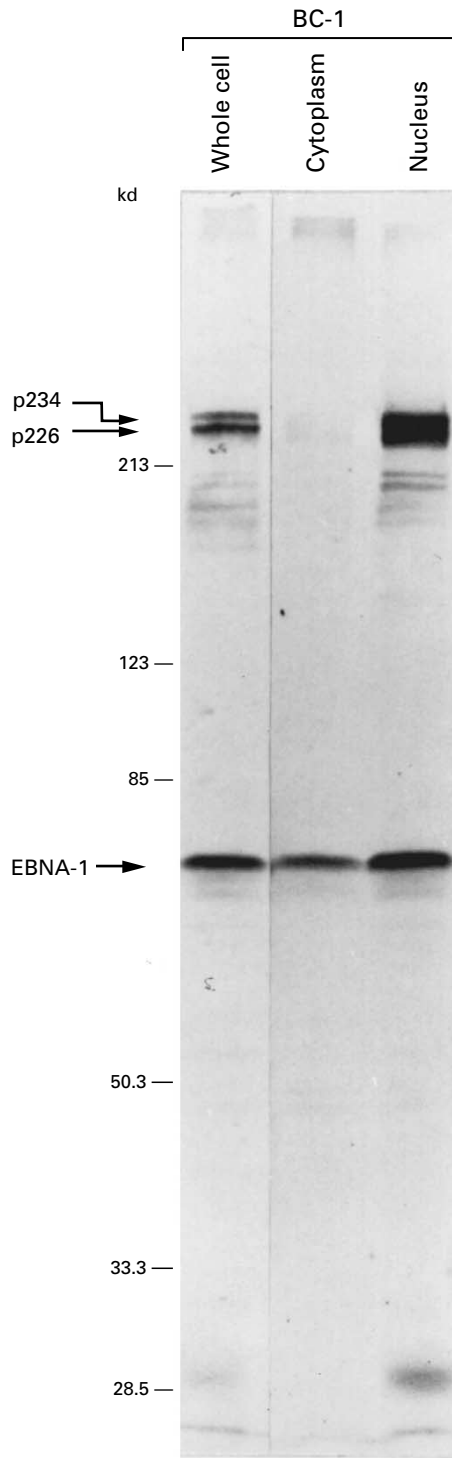


posi's sarcoma was diagnosed. Six patients (15 percent; Patients 1 through 6) were seronegative every time they were studied. Only two patients (5 percent; Patients 7 and 8) changed from seropositive to seronegative ("seroreverted") during the course of the study.

The results of nested-PCR examination of peripheral-blood mononuclear cells obtained at entry into the study and at the last visit before the diagnosis of Kaposi's sarcoma were consistent with the data on seroconversion obtained by the immunoblot assay (Fig. 3), although PCR was less sensitive than immunoblotting. Five samples of peripheral blood obtained at study entry were positive by PCR, as compared with 21 samples obtained immediately before

the diagnosis of Kaposi's sarcoma. Paired samples from two patients with Kaposi's sarcoma (Patients 4 and 5) were positive by PCR at both visits, but neither had detectable antibodies against KSHV latent nuclear antigens.

To examine the rate of seroconversion to antibodies against latent nuclear antigens while accounting for the duration of follow-up, we plotted the prevalence of seropositivity by the immunoblot assay shown in Figure 3 over time (Fig. 4). The seropositivity rate increased linearly with time during the 80 months before the diagnosis of Kaposi's sarcoma (chi-square for linear trend=30.3;  $P<0.001$ ;  $r^2=0.97$ ; 95 percent confidence interval, 0.92 to 1.00). Fifty percent of the patients with Kaposi's sarcoma were seropositive 33



**Figure 2.** Immunoblots with Serum from a Patient with AIDS-Associated Kaposi's Sarcoma with Whole BC-1 Cells and Cytoplasmic and Nuclear Fractions of BC-1. Most of the 226-kd and 234-kd latent nuclear antigens are in the nuclear fraction.

**TABLE 1.** DETECTION OF ANTIBODIES AGAINST KSHV LATENT NUCLEAR ANTIGENS AND EBV EBNA-1.

GROUP	NO. OF PATIENTS	KSHV LATENT NUCLEAR ANTIGENS	KSHV PCR	EBV EBNA-1
Homosexual patients with AIDS				
Kaposi's sarcoma	40	32 (80)*	21 (52)	40 (100)
No Kaposi's sarcoma	40	7 (18)*	3 (8)	40 (100)
Patients with hemophilia	20	0	0	18 (90)
Blood donors	122	0	Not done	108 (89)
Serum samples with high EBV titers†	22	0	Not done	22 (100)

\*This group includes two patients whose serum samples produced weak bands that were found to be seropositive after unblinding.

†The geometric mean titer of antibodies against EBV viral capsid antigen by the indirect immunofluorescence antibody assay was 1229 (range, 320 to 2560).

**TABLE 2.** COMPARISON OF THE IMMUNOBLOT ASSAY FOR LATENT NUCLEAR ANTIGENS WITH NESTED-PCR AMPLIFICATION TO DETECT KSHV INFECTION AMONG HOMOSEXUAL MEN WITH AIDS.

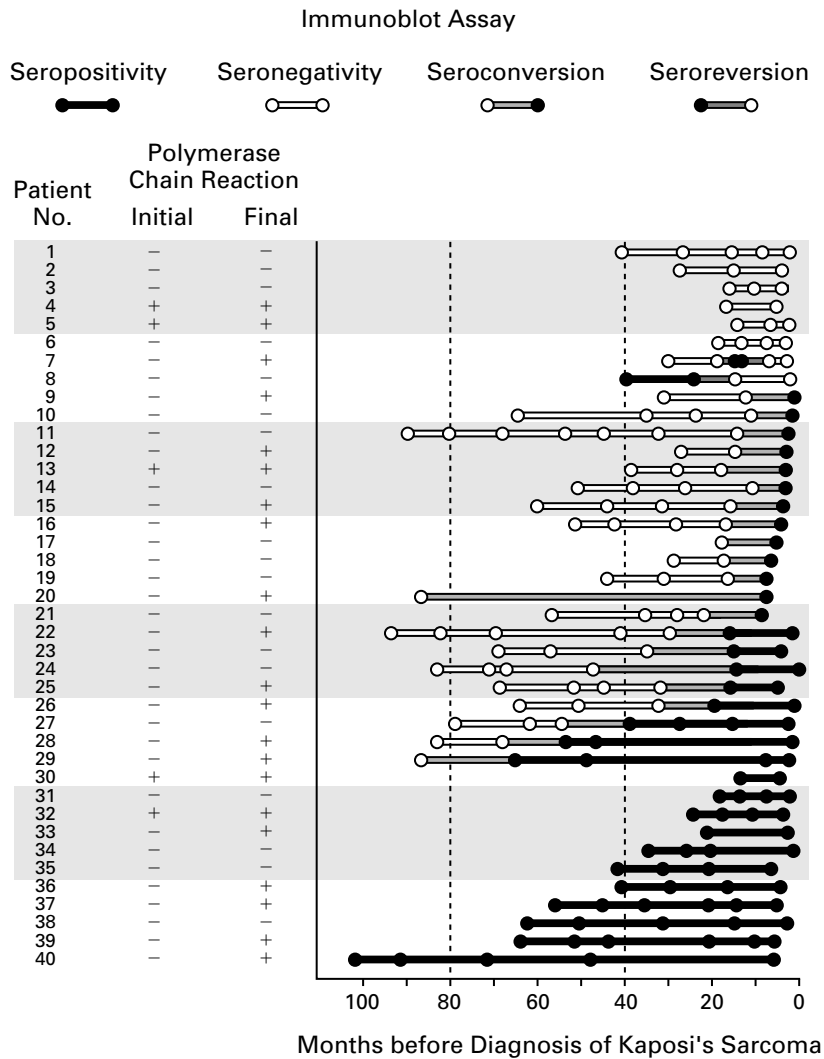
GROUP AND RESULTS OF IMMUNOBLOT ASSAY	RESULTS OF PCR*		
	POSITIVE	NEGATIVE	TOTAL
	no. (%)		
<b>Patients with Kaposi's sarcoma</b>			
Seropositive	18 (45)	14 (35)	32 (80)
Seronegative	3 (8)	5 (12)	8 (20)
Total	21 (52)	19 (48)	40 (100)
<b>Patients without Kaposi's sarcoma</b>			
Seropositive	2 (5)	5 (12)	7 (18)
Seronegative	1 (2)	32 (80)	33 (82)
Total	3 (8)	37 (92)	40 (100)

\*Because of rounding, not all percentages total 100.

months before their disease was diagnosed (Fig. 4), and 10 patients seroconverted during the year before their diagnoses of Kaposi's sarcoma (Fig. 3).

### DISCUSSION

The immunoblot assay detects antibodies against two nuclear antigens found in KSHV-infected cells. These antigens have not been conclusively shown to be KSHV proteins, but they are likely to be encoded by viral genes expressed during latency. The antigen doublet is not found in EBV-infected B-cell lines, but it is present in a KSHV-infected body-cavity-based lymphoma cell line uninfected with EBV (unpublished data). The establishment of a similar cell line has recently been reported.<sup>24</sup>

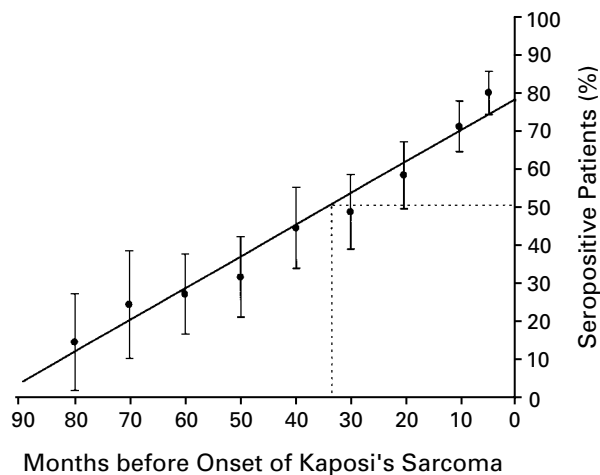


**Figure 3.** Longitudinal Data Obtained by PCR and the Immunoblot Assay on the 40 Study Patients with AIDS-Associated Kaposi's Sarcoma.

Eleven patients (Patients 30 through 40) were seropositive for antibodies against latent nuclear antigens by the immunoblot assay when they entered the study, and 21 (Patients 9 through 29) seroconverted before the onset of disease. Six patients (Patients 1 through 6) were seronegative every time they were studied, and two (Patients 7 and 8) had inconsistent patterns of seropositivity ("seroreversion"). Results of the nested-PCR amplification of KSHV DNA from peripheral-blood mononuclear cells at entry into the study ("initial PCR") and immediately before the onset of disease ("final PCR") are shown; plus signs denote positive, and minus signs negative.

Antibodies against these latent nuclear antigens can be detected years before the onset of Kaposi's sarcoma, and most patients (though not all) appear to remain seropositive after their initial seroconversions. This is similar to the antibody response to EBNA-1. Antibodies against EBNA-1 are detectable throughout a patient's life after EBV infection,<sup>25</sup> and nearly all the patients we studied were immunoreactive to EBNA-1, as is consistent with the results of other studies of EBV prevalence.<sup>26-28</sup>

If antibodies against latent nuclear antigens are reliable indicators of infection with KSHV, then KSHV infection is uncommon among persons at low risk for Kaposi's sarcoma, including HIV-infected patients with hemophilia. Seropositivity rates among homosexual control patients with AIDS but not Kaposi's sarcoma were higher than those in other control groups, as is consistent with the higher incidence of Kaposi's sarcoma among homosexual men than among other HIV risk groups.<sup>29,30</sup> Over



**Figure 4.** Estimated Prevalence of Seropositivity for Antibodies against KSHV-Associated Latent Nuclear Antigens among 40 Patients with AIDS-Associated Kaposi's Sarcoma, According to the Number of Months before the Diagnosis of Kaposi's Sarcoma.

The longitudinal data from Figure 3 have been adapted here, with the seroconversion of each patient considered to have occurred halfway between that patient's last negative serologic test and the first positive test. Fifty percent of the patients were seropositive 33 months before the onset of Kaposi's sarcoma (dashed lines). Means ( $\pm$ SE) calculated from a binomial distribution are shown.

half the patients with Kaposi's sarcoma seroconverted to positivity for antibodies against KSHV-related latent nuclear antigens, and one fourth seroconverted during the year before the onset of disease. These findings should not be extrapolated to persons who have Kaposi's sarcoma that is unrelated to AIDS, in whom the latency period could be considerably longer because of an intact immune system.

Although the immunoblot assay was 80 percent sensitive in our study, one fifth of the patients with Kaposi's sarcoma were not seropositive. Most of these seronegative patients, including two who were positive by PCR, tested negative at multiple visits. The seronegative patients had significantly higher CD4+ cell counts than the seropositive patients, and Kaposi's sarcoma developed more rapidly among the seronegative patients after enrollment in the study. It is possible that about 20 percent of infected patients do not generate antibodies against KSHV latent nuclear antigens and that these patients go on to have Kaposi's sarcoma earlier in their AIDS illness because of a lack of protective antibodies. Other possible explanations for the seronegativity include insensitivity of the test, variation in the strain of KSHV, loss of humoral immunity with the worsening of AIDS,<sup>31</sup> misdiagnosis of Kaposi's sarcoma,<sup>32</sup> or the occurrence of infection after the last study visit but before the onset of Kaposi's sarcoma. Non-

reactivity<sup>31,33,34</sup> and delayed seroconversion<sup>35</sup> have been reported with other assays in patients with AIDS. The immunoblot assay we used may also be sensitive to variations in the handling of specimens. We have found low seropositivity rates (44 percent) for serum samples from patients with Kaposi's sarcoma from one site in a separate study; the low rates are probably due to repeated cycles of freezing and thawing (unpublished data).

The reactivation of the virus cannot entirely explain our results. Homosexual patients with Kaposi's sarcoma and AIDS were significantly more likely to be seropositive for antibodies against KSHV latent nuclear antigens than homosexual control patients with AIDS, even though the two groups had similar CD4+ cell counts at their diagnoses of AIDS. All but two of the patients with Kaposi's sarcoma were positive for antibodies against latent nuclear antigens at all study visits after seroconversion. Furthermore, antibodies against KSHV-related latent nuclear antigens are found in nonimmunocompromised patients with Kaposi's sarcoma. Nonetheless, viral reactivation may be responsible for some of the serologic responses seen in this study, but not for all.

Blinded and controlled evaluations of samples of peripheral-blood mononuclear cells by PCR<sup>18,36</sup> also suggest that KSHV infection is uncommon among persons at low risk for Kaposi's sarcoma. The results of recent PCR-based studies suggesting that KSHV is common among adults<sup>37-39</sup> have not been reproduced by others.<sup>40-42</sup> Our experience and that of others<sup>41</sup> indicate that the detection of KSHV DNA by PCR inevitably leads to overestimates of the rate of infection because of laboratory contamination, even under stringent control conditions. Although improvements in test sensitivity will undoubtedly increase the present estimates of infection rates in the control groups we examined, our results are consistent with those of serologic studies using a KSHV-related lytic-phase antigen,<sup>22</sup> and we find no evidence that persons at low risk are infected with KSHV at the same rate as patients with Kaposi's sarcoma. This strongly suggests that KSHV infection is not ubiquitous among adults from North America and that KSHV infection has an etiologic role in Kaposi's sarcoma.

EBV proteins expressed during latent infection have important roles in virus-induced cell immortalization and transformation.<sup>43</sup> The KSHV nuclear antigens are defined as latent because their synthesis is neither enhanced by phorbol esters nor inhibited by antiviral drugs that inhibit the viral DNA polymerase. The transcription of other presumed lytic-phase KSHV genes, such as the major capsid-protein gene, is induced and inhibited under these conditions (unpublished data). If, like EBV, KSHV is a transforming virus, latently expressed KSHV proteins may play an important part in KSHV-related tumorigenesis.

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