KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma

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A major controversy regarding Kaposi's sarcoma-associated herpesvirus (KSHV or HHV8)[1,2] is whether or not it is a ubiquitous infection of humans[3,4]. Immunoassays based on KSHV- and Epstein-Barr virus (EBV)-coinfected cell lines show that most US AIDS-KS patients have specific antibodies to KSHV-related antigens[2,5,6]. We have developed a sensitive indirect immunofluorescence assay (IFA) based on an EBV-negative, KSHV-infected cell line, BCP-1. When we used this IFA assay, KSHV-related antibodies were found in 71–88% of serum samples from US, Italian and Ugandan AIDS-KS patients, as well as all serum samples examined from HIV-seronegative KS patients. Although none of the US blood donors examined were KSHV seropositive by IFA, intermediate and high seroprevalence rates were found in Italian and Ugandan control populations. Antibody kinetics showed that more than half of the AIDS-KS patients who were examined IgG-seroconverted before KS development, and antibody levels did not decline after seroconversion. For these patients, seropositivity rates increased linearly with time, suggesting that the rate of infection was constant and that the risk of developing KS once infected with KSHV is not highly dependent on the duration of infection. These data strongly suggest that KSHV is not ubiquitous in most populations and that the virus may be under strict immunologic control in healthy KSHV-infected persons.

Kaposi's sarcoma-associated herpesvirus was first cultured using cell lines established from body cavity-based lymphomas (BCBL) coinfected with EBV (ref. 7, 8). BCP-1 was derived from the peripheral blood of an HIV-seronegative BCBL patient (unpublished data) and is similar to a previously described KSHV-infected, EBV-negative cell line[9]. Two types of BCP-1 immunofluorescence staining occur in AIDS-KS patient serum samples at low dilutions (<1:160): a homogeneous cytoplasmic staining and a specular nuclear staining (Fig. 1). At 1:160 dilution or higher, the cytoplasmic staining disappears; however, the specular nuclear staining pattern is retained.

To determine the specificity of the nuclear versus cytoplasmic IFA staining patterns, five pairs of serum samples from AIDS-KS and control patients were adsorbed using the EBV-infected, KSHV-negative cell line CB33 (Table 1). Cytoplasmic staining of BCP-1 cells disappeared from CB33-adsorbed case and control serum samples, whereas the nuclear staining remained largely unchanged, suggesting that cytoplasmic staining is nonspecific but that nuclear staining may be specific for KSHV-related antigens.

To determine IFA end-point titers and to evaluate the assay, we examined serum dilutions from 40 homosexual AIDS-KS patients and various HIV-seropositive and HIV-seronegative control patients from the United States (Fig. 2). At dilutions lower than 1:160, all groups had cytoplasmic staining. However, at 1:160 or greater dilution, only 12 (30%) of the serum samples from the homosexual control AIDS patients reacted to the BCP-1 cells compared with 35 (88%) from the AIDS-KS patients. None of the 122 serum samples from blood donors, 69 patients with elevated antibody titers to EBV, or 20 HIV-seropositive hemophiliacs reacted at dilutions of 1:160 or greater.

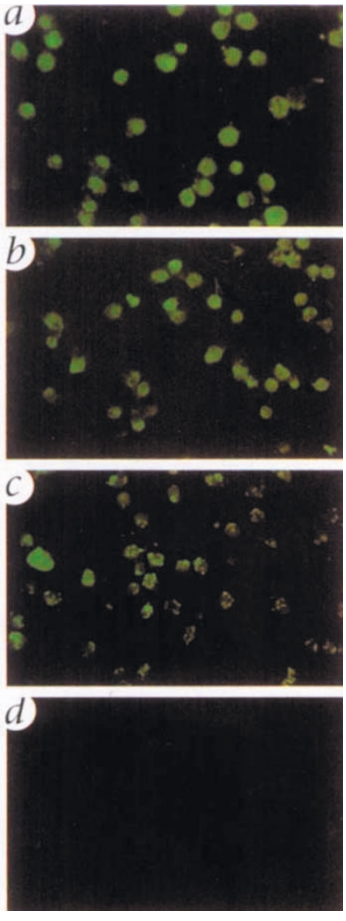

Fig. 1 Immunofluorescence assay staining of BCP-1 cells with serum samples from an AIDS-KS patient (*a* and *c*) and an AIDS patient without KS (*b* and *d*) examined at dilution of 1:20 (*a* and *b*) and 1:160 (*c* and *d*). Serum samples from both the AIDS-KS patient and the AIDS patient without KS have diffuse cytoplasmic staining at 1:20 dilution but not 1:160. Only serum from the AIDS-KS patient has specular nuclear staining at dilutions of both 1:20 and 1:160.

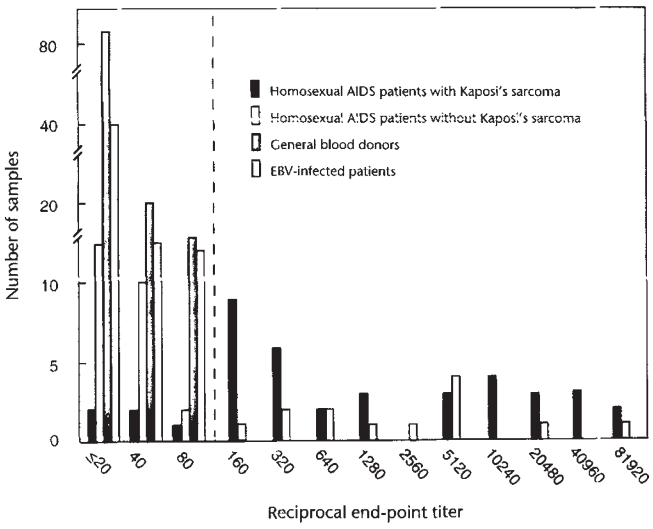

Fig. 2 Immunofluorescence assay antibody end-point titers for serum samples from AIDS patients with or without KS, patients with high EBV antibody titers and general blood donors from the United States. At dilutions lower than 1:160, the majority of serum samples from all the groups examined had diffuse cytoplasmic staining. Only AIDS-KS patients (88%) and homosexual control AIDS patients (30%) had nuclear staining and end-point titers greater than 1:160.

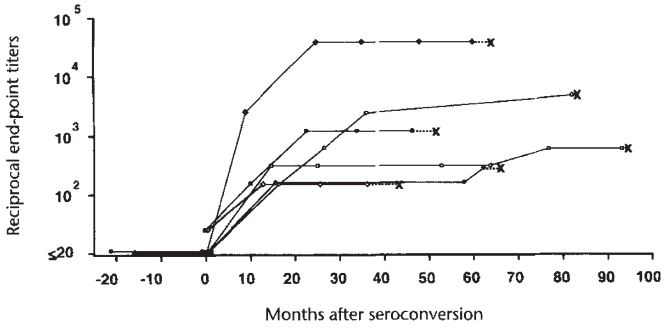

Fig. 3 Immunoglobulin-γ (IgG) end-point titers for six AIDS-KS patients with three or more samples drawn after seroconversion (IFA titer greater than 1:160 and a fourfold or greater rise in end-point titer). IgG end-point titers remained elevated for 36–93 months after seroconversion until KS onset (X) consistent with a prolonged antibody response after primary infection.

Comparison with previously reported latent nuclear antigen (LNA) immunoblot assay[6] demonstrates that the IFA is at least as sensitive as the LNA-based immunoassay (Table 2). It is unknown whether these assays measure antibodies to the same antigen. All but one of the 32 AIDS-KS patients found to be KSHV seropositive by LNA immunoassay were also positive by IFA, and 4 AIDS-KS patients were positive by IFA alone. Among homosexual AIDS control patients, the IFA identified all 7 (18%) of the patients recognized as KSHV seropositive by LNA immunoassay, and an additional 5 (13%) patients that were seronegative by LNA immunoassay, as KSHV seropositive by IFA (Table 2).

Compared with the US incidence, the incidence of non-AIDS-KS is higher in Italy[10], and especially high in Uganda, which may have one of the world's highest rates of disease[11]. Sera from 10 of 14 (71%) Italian and 14 of 18 (78%) Ugandan AIDS-KS patients were seropositive at dilutions greater than 1:160, as were all serum samples from 11 (100%) Italian and the single Ugandan HIV-seronegative KS patients (Table 2). Thus, the IFA assay can detect KSHV-related antibodies in both HIV-seronegative and HIV-seropositive patients from geographically dispersed areas.

The KSHV seroprevalence among Italian blood donors appears to be slightly higher than that of US blood donors (0 of 122 versus 4 of 107, two-tailed Fisher's exact test, $P = 0.046$). Nevertheless, seropositivity rates are still low as determined by our immunoassays and not consistent with nested polymerase chain reaction (PCR)-based studies suggesting near-universal KSHV infection among Italian populations[12]. Blood donor populations from Uganda were not available for comparison. Instead, serum samples from HIV-seropositive and HIV-seronegative control patients with or without cancer but no KS were used. These serum samples had more nonspecific staining, but the nuclear staining pattern was clearly either present or absent at 1:160 dilution. Fifty-one percent of both HIV-seropositive and HIV-seronegative patients were seropositive by IFA. Differences in epidemiologic characteristics preclude direct comparison to the US and Italian data; however, this staining pattern suggests that KSHV infection rates are likely to be dramatically higher in Central African populations than in North American and European populations[13]. In this respect, the KSHV seroprevalence resembles geographic differences in seroprevalence to herpes simplex type II (ref. 14).

Immunoglobulins IgA, IgG and IgM were examined by IFA using serum samples collected longitudinally from 39 of the US AIDS-KS patients[6]. Fourteen (36%) were consistently seropositive (>1:160) from study entry (13 to 103 months before KS onset) until KS diagnosis, and four (10%) patients were seronegative (<1:160) at all the time points examined. IFA titers for one additional patient fell from 1:640 to 1:40 and remained below 1:160 until development of KS 14 months later. The remaining 20 pa-

Table 1 End-point dilution IFA titers for five pairs of serum samples matched by nuclear staining titer from homosexual AIDS patients with and without KS, before and after adsorption with the CB33 lymphoblastoid cell line

Patient no.	Disease status	Cytoplasmic staining		Nuclear staining	
		Unadsorbed	Adsorbed	Unadsorbed	Adsorbed
1	KS	80	<20	<20	<20
2	non-KS	40	<20	<20	<20
3	KS	80	<20	320	160
4	non-KS	80	<20	320	160
5	KS	80	<20	320	320
6	non-KS	80	<20	320	320
7	KS	80	<20	640	640
8	non-KS	80	<20	640	640
9	KS	80	<20	640	640
10	non-KS	80	<20	640	640

Although nuclear staining did not change (except for twofold dilution decrease for patients 3 and 4), CB33 adsorption markedly decreased the end-point titers for cytoplasmic staining consistent with cytoplasmic staining being due to nonspecific cross-reactivity.

tients (51%) IgG-seroconverted from a negative (<1:160) to a positive test (>1:160) before KS onset, with all patients having fourfold or higher rise in titer. Seventeen of these patients seroconverted as determined by LNA immunoassay and the remaining three patients were previously found to be seronegative by LNA immunoassay[6]. Of these 17 patients, 6 seroconverted at an earlier time point as seen with the IFA and 3 seroconverted at an earlier time point as seen with the LNA immunoassay[6].

It is possible that KSHV is a ubiquitous infection of adults and that the IFA identifies antibodies generated during virus reactivation but not during primary infection. In this case, antibody titers should vary over time as antigen load varies; however, the end-point IgG antibody titers did not decrease between seroconversion and KS onset for the six patients with more than three samples drawn after seroconversion (the remaining patients had two or fewer samples drawn after seroconversion, Fig. 3). IgG titers for these patients remained persistently elevated after seroconversion for 36–93 months until KS onset. None of the patients had detectable IgA antibodies by IFA, and only three patients had detectable KSHV-specific IgM antibodies (range 1:40 1:160) at the seroconversion time point, with one patient also having an 1:80 IgM titer 13 months after seroconversion. These data are not consistent with IFA titers reflecting virus reactivation, but are consistent with primary infection occurring in these adult patients.

Examination of the cumulative KSHV-seropositivity rate (Fig. 4, solid line) for the AIDS-KS patients before KS onset shows a linear increase in seroprevalence over time (χ^2 for linear trend = 17, $P = 0.00003$, $r^2 = 0.93$) similar to the seroprevalence trend (Fig. 4, dashed line) found previously using the LNA immunoassay[6]. Fifty percent of the patients were seropositive, as detected by IFA, 46 months before KS onset compared with 33 months previously reported for the LNA immunoassay[6]. This suggests that infection rates have been relatively constant among gay men in this cohort and, unlike the risks of being diagnosed with some virus-associated cancers (for example, hepatocellular carcinoma), the risk of getting KS after KSHV infection may be less dependent on duration of infection (a minimum incubation period of six months or less cannot be excluded by our data). One reasonable explanation for these results is that the expression of KS is tightly regulated by host immunity among persons infected with KSHV. Thus, the risk of getting KS is largely dependent on immunologic integrity in those who are KSHV infected, regardless of the length of time of their infection.

Table 2 Detection of antibodies to KSHV antigens by BCP-1 IFA and LNA-immunoblot assay in persons with or without KS from the United States, Italy and Uganda

Group	No. tested	No. (%) positive by BCP-1 IFA	No. (%) positive by LNA-immunoblot
North America			
Homosexual AIDS patients with KS	40	35 (88)	32 (80)[a]
Homosexual AIDS patients without KS	40	12 (30)	7 (18)[a]
HIV-seropositive hemophiliacs	20	0	0[a]
EBV-positive blood samples[b]	69	0	0[c]
General blood donors	122	0	0[a]
Italy			
AIDS-KS patients	14	10 (71)	11 (79)
HIV-seronegative patients with KS	11	11 (100)	11 (100)
General blood donors	107	4 (4)	4 (4)
Uganda			
AIDS-KS patients	18	14 (78)	16 (89)
HIV-seronegative patients with KS	1	1 (100)	1 (100)
HIV-seropositive patients without KS	35	18 (51)	25 (71)
HIV-seronegative patients without KS	47	24 (51)	29 (62)

[a]From ref. 6.
[b]Geometric mean antibody titer to EBV viral capsid antigen by indirect immunofluorescence antibody assay 1378, range 320 to 2560.
[c]Results of 22 patients are from ref. 6.

Methods

Patient serum samples. All case and control serum samples were evaluated in a blinded fashion. Case and control serum samples obtained from patients (enrolled in 1984–1985) in the Multicenter AIDS Cohort Study (MACS) have been previously described[6] and in-

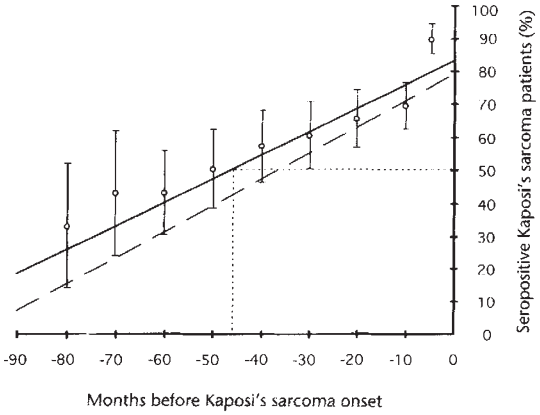

Fig. 4 Comparison of the prevalence of BCP-1 IFA and LNA-immunoassay seropositivity for 39 homosexual AIDS-KS patients before KS onset. Date of seroconversion is estimated to be the midpoint between last negative and first positive serologic test. For comparison, the KSHV seropositivity by LNA immunoassay[6] (dashed line) is plotted with the seropositivity by BCP-1 IFA (solid line). Fifty percent of the KS patients were seropositive 46 months before KS onset by BCP-1 IFA. Error bars are s.e.m. calculated from a binomial distribution.

clude 40 HIV-seropositive homosexual men who developed AIDS-KS and 40 control serum samples from homosexual men with AIDS but without KS collected at the study visit immediately before AIDS diagnosis. One AIDS-KS patient with only two samples was excluded from the longitudinal evaluation. Additional case serum samples were from HIV-seronegative or HIV-seropositive patients with KS seen at Luigi Sacco Hospital, Milan, Italy, and at Mulago Hospital, Kampala, Uganda. Additional controls included serum samples from 20 HIV-seropositive hemophiliacs, 69 persons with high EBV viral capsid antigen antibody titers, 122 general blood donors from various sites in the United States, 107 general blood donors from Milan, Italy, 35 HIV-seropositive patients without KS and 47 HIV-seronegative patients without KS from Uganda. Blood donors from the United States and Italy were seronegative for HIV, hepatitis B virus, hepatitis C virus and *Treponema pallidum* infection.

Results of the LNA immunoassay for 40 US AIDS-KS patients, 40 AIDS controls without KS, 20 HIV-seropositive hemophiliac controls, 122 US blood donors and 22 EBV-infected patients were reported previously[6].

Serologic assays. BCP-1 is maintained in RPMI 1640 with 20% fetal bovine serum and passaged every 2 to 3 days. BC-1 and CB33 were previously described[6,7]. BCP-1 IFA was performed essentially as previously described[7]. Serial dilutions of serum samples were examined starting at 1:20 dilution to determine the end-point titer, and a 1:160 dilution was used as the cutoff for a positive assay (see results). LNA immunoassay was performed as previously described[6].

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Enhancement of vowel coding for cochlear implants by addition of noise

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Profoundly deaf people, who gain no benefit from conventional hearing aids, can receive speech cues by direct electrical stimulation of the cochlear nerve[1,2]. This is achieved by an electronic device, a cochlear implant, which is surgically inserted into the ear. Here we show physiological results from the isolated sciatic nerve of the toad *Xenopus laevis*, used to predict the response of the human cochlear nerve to vowels coded by a cochlear implant. These results suggest that standard analogue cochlear implants do not evoke the patterns of neural excitation that are normally associated with acoustic stimulation. Adding noise to the stimulus, however, enhanced distinguishing features of the vowel encoded by the fine time structure of neural discharges. On the basis of these results, and those concerning stochastic resonance[3–5], we advocate a cochlear implant coding strategy in which noise is deliberately added to cochlear implant signals.

In normal hearing, hair cells in the inner ear transduce sound vibrations into electrical signals. These signals are transmitted to higher auditory centers by a tonotopic array of cochlear nerve fibers. Potentially, the frequency of an acoustic stimulus can be coded both in terms of which fibers are active (place coding) and by the time structure of the neural discharges (time coding)[6]. Hair cells and other cochlear mechanisms are, however, physiologically vulnerable and may be completely lost because of disease or the side effects of some pharmaceuticals[7,8]. Such loss causes profound deafness, but hearing may be partially restored by spatio-temporal electrical stimulation of surviving nerve fibers using a multichannel cochlear implant[1,2].

One approach to cochlear implant coding is to try to evoke the same patterns of excitation with electrical stimulation that would normally have been evoked by acoustic stimulation. Whole-animal experiments have provided knowledge about the neural response to acoustic stimulation[8,9]. Far less, however, is