Interleukin 6 and AIDS-Associated Kaposi's Sarcoma: A Nested Case Control Study within the Multicenter AIDS Cohort Study

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ABSTRACT

Since the beginning of the AIDS epidemic, there has been considerable research on the etiology of Kaposi's sarcoma (KS) among HIV-infected individuals. A number of studies have confirmed that HIV or HIV-encoded products can interact with human cells to induce the production of cytokines, including interleukin 6 (IL-6). *In vitro* observations have indicated that AIDS-KS cells can produce significant levels of IL-6 and also respond to this cytokine. Preliminary data suggested that IL-6 may be elevated among HIV-infected individuals that subsequently develop AIDS-KS. The objective of this study was to determine if elevated levels of IL-6 are associated with an increased incidence of AIDS-KS compared to other AIDS-defining illnesses such as opportunistic infections (OIs). Serum IL-6 levels were determined by ELISA in frozen sera collected from participants in the Multicenter AIDS Cohort Study (MACS) at 6 months prior to AIDS diagnosis, in 73 cases (AIDS-KS), and 152 controls (OI). Elevated IL-6 levels were more prevalent among men with AIDS-OI than those with AIDS-KS: crude odds ratio (OR), 0.4 (95% CI, 0.2–0.9). Models of multivariate logistic regression were used to study potential confounders. Sexual behavior variables did not seem to confound the association between IL-6 and AIDS-KS. The higher prevalence of IL-6 among controls could be explained by the association of higher levels of IL-6 with lower levels of CD4 T cell number. IL-6 may be a marker of severe immune dysfunction among HIV-infected individuals.

INTRODUCTION

PRIOR TO THE AIDS EPIDEMIC, Kaposi's sarcoma (KS) was a rare tumor in North America and Europe. The disease was mostly reported in elderly men, 50 years or more of age, of eastern European and Mediterranean origin such as Italians and Jews of Polish and Russian ancestry.^{1–5} In 1979, a number of cases of the aggressive, disseminated, fatal form of KS were recognized among young to middle-aged homosexual men. It soon became clear that KS is one of the most frequent clinical manifestations of HIV infection, and that KS is the most com-

mon AIDS-associated cancer.⁶ It occurs much more frequently among homosexual and bisexual HIV-infected men than it does in other risk groups. Kaposi's sarcoma may be the direct manifestation of the HIV-induced impairment of the immune system, or may be caused by some other process, pathogenic organisms, or a combination of factors seen among HIV-infected individuals.

Some time ago, Ensoli *et al.*⁷ proposed a model to explain the mechanisms involved in the association between KS and HIV infection, based on results from *in vitro* and *in vivo* studies with transgenic mice. According to this model, in HIV-in-

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fectured individuals, the combination of factors released from activated lymphocytes and factors released from infected T cells, e.g., the HIV Tat protein, induce the proliferation of specific types of normal cells (endothelial, smooth muscle cells, and/or fibroblasts) that later can be transformed into spindle-shaped cells. These KS precursor cells can produce several cytokines including interleukin 6 (IL-6), IL-1 β , and tumor necrosis factor (TNF) that may induce their own growth (autocrine pathway). Also, proliferation of KS spindle cells might be enhanced by cytokines produced by other cells (paracrine pathway). The interaction between normal and spindle cells undergoing uncontrolled proliferation and growth may lead to the development of a KS lesion. As with other lesions, if secondary genetic changes take place, the KS lesion can be transformed into a tumor and/or malignancy.

While HIV-encoded products can promote the growth of KS cells, this does not explain the higher risk of KS among homosexual and bisexual men.⁸ This observation raised important questions regarding the etiology of AIDS-associated KS (AIDS-KS): possibly, a cofactor that is more prevalent among homosexuals than heterosexuals with HIV infection was involved in the induction of KS. The lifestyle of these individuals, who have frequent episodes of sexually transmitted diseases, may predispose them to chronic antigenic stimulation differentially from other risk groups. The combination of HIV infection and chronic antigenic stimulation may contribute to the development of KS via the production of "inflammatory" cytokines (IL-1, TNF, IL-6) released from activated immune cells. These cytokines have potent cell growth-promoting activity and have been found in increased levels in the sera or in the supernatant of blood cells from HIV-1-infected individuals.⁹⁻¹¹

It also is possible that homosexual and bisexual men may be at greater risk for the acquisition of a unique, sexually transmitted agent that can induce KS.¹²⁻¹⁵ Presumably, such an agent would be transmitted more efficiently sexually than perinatally, through needle sharing or through blood products. The agent may have been endemic in the homosexual population before HIV became prevalent, and the immunodeficiency that accompanies HIV infection allowed the agent to express itself. Work performed by Moore *et al.*¹⁶⁻¹⁸ suggests that a previously unrecognized herpesvirus is associated with, and may contribute to, the pathogenesis of AIDS-associated KS: DNA sequences for a previously uncharacterized human herpes-type virus were detected in KS lesions. Sequences characteristic of this new KS-associated herpesvirus (KSHV or HHV-8) are present in most KS lesions, including those from people who are not HIV positive; however, KS-derived cell lines with spindle cell morphology generally do not contain KSHV, and various B cell lines have been seen to be KSHV positive.¹⁷⁻²² While the association of KSHV with KS lesions has been established clearly, the role of this new virus in the pathogenesis of AIDS-KS is not clear. Direct infection of cells involved in KS lesions may be involved in the establishment of these cancers. However, the means by which KSHV infection contributes to KS have not been determined. Perhaps, KSHV enhances the development or growth of KS lesions by enhancing cytokine production, either by the primary tumor cells (autocrine), or by infiltrating immune system cells (paracrine). The interaction of KSHV with endothelial cells, B lymphocytes, or monocytes/macrophages might lead to the production of cytokines, including cellular IL-

6 (cIL-6), known to enhance KS cell growth, although this remains to be determined experimentally. Interestingly, reports indicate that the KSHV/HHV-8 genome encodes a viral version of human IL-6 (vIL-6).^{23,24} The biological properties of vIL-6, and the effects of this molecule on KS cell growth, have not been defined.

Initial interest in examining the role of IL-6 in AIDS-KS was stimulated by *in vitro* observations that AIDS-KS cell lines can produce various cytokines and growth factors.²⁵⁻³⁴ These studies demonstrated enhanced growth of AIDS-KS cells when they were cultured with conditioned media from T cells infected with human retroviruses, suggesting that a growth factor for AIDS-KS cells was present. Interleukin 6 was considered to be a potential growth factor for AIDS-KS cells because its characteristics are similar to those of the growth factor isolated from the T cell supernatant. Subsequent work demonstrated that various AIDS-KS cell lines can produce significant amounts of IL-6 and can respond to IL-6.^{26,27,32,33} It is not known whether the IL-6 produced by these KS cells is of cellular or viral origin. Furthermore, exposure of AIDS-KS cells to IL-6 antisense oligodeoxynucleotides, which diminish the production of endogenous IL-6, resulted in decreased KS cell proliferation.^{27,28} Other cytokines, including TNF, IL-1 β , oncostatin M, and basic fibroblast growth factor (bFGF), as well as the HIV Tat protein, also seem to be able to induce AIDS-KS cell proliferation and IL-6 production by KS cells.^{29,30} These results suggested that various factors, particularly oncostatin M, can independently, or together with IL-6, promote the proliferation and/or growth of spindle-shaped KS cells.^{28,30,31}

Despite the association of IL-6 with the growth of AIDS-KS cells in *in vitro* studies, it had not been determined if elevated serum IL-6 levels could predict the development of KS among HIV-infected individuals. We, therefore, designed a nested case control study to investigate if elevated levels of serum IL-6 are associated with an increased incidence of AIDS-KS compared to other AIDS-defining illnesses such as opportunistic infections (OI).

MATERIALS AND METHODS

Study population

The study used a nested case control design to estimate the effect of serum IL-6 level on the risk of AIDS-KS among HIV-infected men. An important feature of the nested case control design is that cases and controls come from the same source population. In addition, the exposure was ascertained prior to knowledge of outcome. These features reduce the potential for selection and ascertainment bias. It is also a more cost-efficient design than a design using the entire cohort, since we need to test only the case group and a sample of controls for IL-6.

The Multicenter AIDS Cohort Study (MACS) is one of the largest ongoing cohorts of homosexual and bisexual men, 18 to 68 years old, who did not have AIDS at the time of entry into the study. This closed cohort was initiated in 1984 and included men from four different cities in the United States: Los Angeles, Chicago, Baltimore, and Pittsburgh. A total of 4954 men was recruited and agreed to participate voluntarily in the study. The Los Angeles (LA) Center (UCLA School of Public Health)

recruited 1637 study participants 18–50 years of age. The participants have been seen approximately every 6 months for completion of a questionnaire; physical examination; and collection of specimens for HIV antibody testing, CD4 T cell measurements, and storage in local and national repositories. Cases of AIDS have been defined by the 1987 Center for Disease Control (CDC) criteria.³⁵ These cases are identified in the MACS cohort through ongoing surveillance. Their diagnoses are confirmed by medical records and/or death certificates. The complete description of the MACS is available elsewhere.^{36,37} For the present analysis, we used the LA Center data through 1992.

Kaposi's sarcoma cases are self-reported during the follow-up interviews. The control group is composed of men with other AIDS opportunistic infections. The following opportunistic infections were included in the control group: *Pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cryptosporidiosis, isosporiasis, cytomegalovirus (CMV), candidiasis, mycobacterial infection, cryptococcal meningitis, herpes simplex infection, coccidioidomycosis, and salmonellosis. Both KS and OI are confirmed by medical records and/or death certificates or autopsy: 95.6% of the KS cases in the LA-MACS have been verified, by biopsy or clinically.

Measurement of Interleukin 6

Interleukin 6 was measured in the sera of study subjects at three time points: 6 months, 1 year, and 2 years before AIDS. Serum IL-6 level was determined by an enzyme-linked immunosorbent assay (ELISA) (Fujirebio, Tokyo, Japan) able to detect 4 pg of IL-6 per milliliter of serum.

Analysis

Frequency distributions were determined for each variable, including mean and standard deviations for continuous variables. The information on selected variables was taken for the same time point that IL-6 was measured. Crude odds ratios and 95% confidence intervals for the association of IL-6, other selected variables, and AIDS-KS were calculated from 2 × 2 tables.^{38,39} Interleukin 6 was considered both as a continuous and as a categorical variable. An IL-6 level greater than 4 pg/ml was the cutoff point in determining when to consider a study subject as exposed. We choose to use 4 pg/ml because it was the lowest consistent level of IL-6 detected using the Fujirebio ELISA. Age was considered as a categorical variable (18–34 and 35–50 years). CD4 T cell number was considered both as a continuous and as a categorical variable (<200 cells/mm³ and ≥200 cells/mm³). The selected sexual behavior information was determined from reported sexual activities within the previous 6 months. Sexually transmitted disease (STD) status (syphilis and gonorrhea) in the previous 6 months was categorized as yes or no. The reported number of sexual partners was dichotomized at 0–20 and greater than 20 as used by Archibald *et al.*¹⁴ Each of the other sexual behavior variables in this analysis was categorized as experiencing this behavior with “no partners and/or a single partner” (reference category) or with “multiple partners.” β_2 -Microglobulin (β_2 M) and neopterin were dichotomized as “low” or below the medium value versus “high” or above the median value for both. The median value for β_2 M was 3.01 ng/liter and for neopterin was 17 nmol/liter. Linear regression analysis was used to verify the correlation of

IL-6 with CD4 cell number, β_2 M, and neopterin. A multiple logistic regression model was used to obtain the adjusted estimates of the IL-6–AIDS-KS disease odds ratio. The statistical package SAS⁴⁰ was used to perform the different steps of the analysis.

RESULTS

Within the entire LA-MACS cohort, the distribution of first AIDS diagnosis for selected diseases through December 1992 was as follows: 117 cases of KS (28.7%), 163 cases of PCP (40%), 27 cases of CMV (6.6%), and 101 cases of other opportunistic infections (24.8%). A large percentage of individuals experiencing an AIDS outcome were tested at each time point. Not everyone was tested at each visit because there was no serum available at every time point for all men for two reasons: either the participant had not come for that follow-up visit, or there was only one vial of serum left for the subject for that visit. Of the KS cases diagnosed through December 1992, we tested 73 subjects (62.4%). Among the OI controls we tested the following percentages: PCP, 49.1% (80 of 163); CMV, 48.1% (13 of 27); and other OI, 58.4% (59 of 101).

There were virtually no detectable levels of serum IL-6 at 2 years or 1 year prior to AIDS. In contrast, there were detectable levels of serum IL-6 seen at 6 months pre-AIDS. The 6 months prior to AIDS is the time point that we, therefore, chose to analyze in terms of the association of IL-6 as a predictor of AIDS-KS. This analysis includes 73 cases (AIDS-KS) and 152 controls (AIDS-OI combined).

Cases and controls were similar with respect to race, ethnicity, and education at baseline. The case group was slightly older than the control group: 58.6% of cases and 62.7% of controls were between 15 and 34 years of age and 41.4% of cases and 37.3% of controls were between 34 and 48 years of age. The mean age was 33.2 years for the cases and 32.7 years for the controls at study entry ($p = 0.46$, t test for difference in means).

Elevated serum IL-6 (>4 pg/ml) was more prevalent among the control group than among the cases (Table 1). The crude odds ratio (OR) and 95% confidence intervals (CI) for the association between IL-6 and AIDS-KS at 6 months prior to AIDS was 0.4 (0.2–0.9). This OR is below the null value, which indicates a possible inverse association of IL-6 and AIDS-KS,

TABLE 1. UNADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR THE ASSOCIATION OF SERUM IL-6 AT 6 MONTHS PRIOR TO AIDS-KS AND OI

Risk factor	AIDS-KS (N)	OI (N)	OR	95% CI
IL-6 (2 levels)				
>4 pg/ml	11	45	0.4	0.2–0.9
0–4 pg/ml	62	107		
IL-6 (3 levels)				
0–4 pg/ml	62	107	1.0	
4–10 pg/ml	8	33	0.4	0.2–1.0
>10 pg/ml	3	12	0.4	0.1–1.7

i.e., IL-6 (serum levels >4 pg/ml) was more prevalent among men with opportunistic infections than among men with AIDS-KS. We also considered IL-6 as an ordinal variable in order to investigate a potential dose-response relationship. However, we found no change in the odds ratios from one level to the other. The 95% confidence interval for the ">10 pg/ml" stratum included the null value.

Also, we examined the association of IL-6 with each individual group of diseases (Table 2). The odds ratios for AIDS-KS compared with PCP, CMV, and other OI were consistently below the null value, suggesting that people with these other diseases had higher levels of serum IL-6 than did those with AIDS-KS (Table 2). We examined the levels of IL-6 for each individual disease in the control group, as well. The disease groups that had elevated serum IL-6 levels (>4 pg/ml) were as follows: PCP, CMV, and other OI, including cryptosporidiosis, *Candida* esophagitis, *Mycobacterium avium* infection, disseminated mycobacterial tuberculosis, cryptococcal infection, and wasting syndrome. These diseases did not show a specific pattern in the distribution of IL-6.

Table 3 summarizes the comparisons of the study groups for known risk factors for AIDS. The odds ratio for age was 1.4 (0.8–2.6), suggesting the possibility that younger MACS men are at greater risk of AIDS-KS as compared to AIDS-OI. The odds ratio, however, was not statistically significant. Higher levels of CD4 cell numbers were found to be associated with AIDS-KS [OR 1.8 (1.0–3.2)]. In fact, in our data, the mean CD4 cell number was higher for the cases than for the controls. The mean $\pm$ standard deviation was 203 ± 168 for the cases and 158 ± 142 for controls ($p = 0.049$; test for difference). β_2 -Microglobulin and neopterin appeared to be associated with a "protective" odds ratio for AIDS-KS, but the confidence intervals included the null value.

Oral-genital receptive intercourse and anal-insertive intercourse were the only two sexual behaviors that were significantly associated with the risk of AIDS-KS as compared to OI. Associations that were not statistically significant included hav-

TABLE 3. COMPARISON OF POTENTIAL RISK FACTORS FOR AIDS-KS AND OI AT 6 MONTHS PRIOR TO AIDS: UNADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS

Risk factor	AIDS-KS (N)	OI (N)	OR	95% CI
Age (years)				
15–34	31	52	1.4	0.8–2.6
35–48	42	100		
CD4 cells/mm ³				
>200	31	44	1.8	1.0–3.2
≤200	42	108		
β_2 M (ng/liter)				
>3.01	17	48	0.7	0.3–1.6
1.3–3.01	22	47		
Neopterin (nmol/liter)				
>17	19	50	0.7	0.3–1.6
5–17	25	45		
Syphilis				
Yes	5	4	2.7	0.7–9.8
No	67	143		
Gonorrhea				
Yes	8	9	1.9	0.7–5.1
No	64	138		
Sexual Part ^a				
>20	6	6	1.6	0.4–5.3
0–20	44	71		
Oral-genital Ins ^a				
Multiple	38	60	1.7	0.9–2.9
Single/none	35	92		
Oral-genital Rec ^a				
Multiple	41	61	1.9	1.1–3.4
None/single	32	91		
Anal sex Ins ^a				
Multiple	23	29	2.0	1.0–3.7
None/single	50	123		
Anal sex Rec ^a				
Multiple	24	36	1.6	0.9–2.9
None/single	49	116		
Rimming Ins ^a				
Multiple	9	11	1.8	0.7–4.5
None/single	64	141		
Rimming Rec ^a				
Multiple	11	13	1.9	0.8–4.4
None/single	62	139		

^aRisk activities reported at 6 months prior to this visit: Part, Number of partners; Ins, insertive; Rec, receptive; Rimming, oral-anal behavior; β_2 M, β_2 -microglobulin.

TABLE 2. UNADJUSTED ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR THE ASSOCIATION OF IL-6 AT 6 MONTHS PRIOR TO AIDS AND AIDS-KS, COMPARED WITH DIFFERENT CONTROL DISEASES

IL-6 level (pg/ml)	AIDS-KS (N)	PCP (N)	OR	95% CI
>4	11	21	0.5	0.2–1.1
0–4	62	59		
		Other OI (N)		
>4	11	19	0.4	0.2–0.9
0–4	62	40		
		CMV (N)		
>4	11	5	0.3	0.1–0.98
0–4	62	8		

ing syphilis or gonorrhea during the 6 months prior to the 6 months pre-AIDS and reported number of sexual partners.

Given that CD4⁺ cell levels were higher in AIDS-KS cases, we plotted the levels of CD4 cell number against the values of IL-6 for the whole study group and for cases and controls separately. The scatterplot suggested that higher levels of serum IL-6 were associated with lower levels of CD4 T cell numbers (Fig. 1). The regression line suggested an inverse linear relationship between IL-6 and CD4 cell number among cases and controls (Figs. 2 and 3). The estimated regression coefficient was as follows: $\beta = -0.0037$ with a p value of 0.08 that tested the hypothesis of independence between IL-6 and CD4. How-

FIG. 1. Scatterplot of serum IL-6 and CD4 T cell number at 6 months prior to AIDS.

ever, the inverse linear relationship of IL-6 and CD4 seems to hold only among the controls. The correlation coefficient among controls was: ($r = -0.15$, $p = 0.04$) and among cases: ($r = 0.032$; $p = 0.80$). We also plotted the values of serum β_2 M and neopterin against the values of IL-6. β_2 -Microglobulin and neopterin had a weak positive correlation with IL-6 among the cases (β_2 M: $r = 0.12$, $p = 0.45$; neopterin: $r = 0.17$, $p = 0.24$), but neither was statistically significant. Among the controls, however, β_2 M and neopterin were both positively significantly correlated with IL-6 (β_2 M, $r = 0.26$; $p = 0.01$; neopterin, $r = 0.50$; $p = 0.0001$).

Table 4 shows logistic regression models for the IL-6-AIDS-KS association. The IL-6 odds ratios are the same for models 1 and 2 but changed slightly in models 3 and 4. The inclusion of biological variables such as CD4 cell number, β_2 M, and neopterin decreases the magnitude of the IL-6 association such that the odds ratios are no longer statistically significant. Oral-genital insertive intercourse, anal sex insertive and receptive, β_2 M, and neopterin are not significantly associated with case/control status. On the other hand, oral-genital receptive intercourse and CD4 cell number are significantly associated with AIDS-KS in models 1 and 2 and at borderline statistical significance in models 3 and 4.

FIG. 2. Scatterplot of serum IL-6 and CD4 T cell number at 6 months prior to AIDS, among cases (AIDS-KS); $r = 0.032$; $p = 0.80$.

FIG. 3. Scatterplot of serum IL-6 and CD4 T cell number at 6 months prior to AIDS among controls (AIDS-OI); $r = 0.15$, $p = 0.04$.

Because of the limited sample size, to evaluate effect modification we fit the data to a logistic regression model that incorporated the preceding variables as product terms with the exposure variable in the model. The coefficients for these product terms did not have a value statistically significant from zero.

DISCUSSION

The results presented here confirm other findings that HIV-infected individuals have elevated serum concentrations of IL-6.^{41,42} However, consistently elevated levels of serum IL-6 were seen only at 6 months pre-AIDS. Possible explanations for these findings are that there is no elevation in serum IL-6 at 1 or 2 years prior to AIDS or, alternatively, that there was measurement error in testing for IL-6. It is possible that some or all of the samples from the MACS repository have undergone freeze/thaw procedures before they were examined in this study. However, it is not likely that this affected the IL-6 levels detected in this study: serum samples from the MACS, known to have high levels of IL-6, and frozen and thawed many times, did not show a significant loss in IL-6 levels detected with the same ELISA that was used in this study (data not shown). This suggests that the negative results seen at 2 years and 1 year prior to AIDS were in fact true negatives.

When AIDS-KS patients were compared to AIDS-OI patients, fewer AIDS-KS patients had detectable levels of serum IL-6 at 6 months prior to AIDS. A hypothesis that could explain this inverse association is that the control group had more advanced HIV disease than the KS group. Evidence to support this hypothesis is that the cases had, on average, a higher CD4 T cell level than the controls. Individuals who have more advanced HIV infection probably have greater immune dysfunction. Therefore, elevated levels of IL-6 may represent greater immune dysfunction among HIV-infected individuals. In fact, results presented here indicate an association between low levels of CD4 T cells and higher serum IL-6 levels. Furthermore, our results suggest a potential association between markers of immune activation (neopterin, β_2 M) and IL-6 among controls. Another study⁴³ that has investigated the association of IL-6 and CD4 did not find a correlation between these two markers.

TABLE 4. LOGISTIC REGRESSION MODELS OF IL-6-AIDS-KS ASSOCIATION

Adjusting for:	Model 1		Model 2		Model 3		Model 4	
	OR ^a	95% CI ^a	OR	95% CI	OR	95% CI	OR	95% CI
IL-6	0.4	0.1–0.9	0.4	0.1–1.1	0.5	0.1–2.3	0.5	0.1–2.5
Oral-genital Ins ^b	0.4	0.1–1.1	0.3	0.0–1.0	0.4	0.0–2.0	0.4	0.0–2.0
Oral-genital Rec ^b	3.1	1.0–9.7	3.3	1.1–10.7	3.5	0.7–17.8	3.4	0.6–18.1
Anal sex Ins ^b	1.0	0.4–2.2	1.1	0.5–2.5	1.0	0.3–3.0	1.0	0.3–3.0
Anal sex Rec ^b	0.8	0.3–1.7	0.7	0.3–1.6	0.5	0.2–1.6	0.5	0.1–1.6
CD4			2.0	1.0–4.1	2.2	0.8–5.8	2.6	0.8–5.9
β_2 -Microglobulin					0.5	0.2–1.5	0.6	0.1–1.6
Neopterin							0.9	0.3–2.7

^aOR, Odds ratio; CI, confidence interval.

^bIns, insertive; Rec, receptive.

However, their study group incorporated HIV-infected individuals with no signs of clinical disease. Furthermore, the serum IL-6 concentrations were reported to be similar for healthy HIV antibody positives and HIV antibody negatives. These results support the hypothesis that IL-6 may be a marker of relatively advanced disease and of immune dysfunction among HIV-infected individuals.

Even though an inverse association was found between serum IL-6 and AIDS-KS at 6 months prior to AIDS, it is still possible that IL-6 exerts a growth effect on pre-KS cells. Interleukin 6 may be overproduced chronically and consumed at the nascent KS lesion sites, with the emerging KS cells binding available IL-6. In such a scenario, IL-6 would not appear in increased levels in the circulation.

An alternative explanation for these findings can be drawn from the fact that infections included in an AIDS diagnosis have an incubation period that can be several weeks to months in length. It is, therefore, likely that the individuals in the control group were already infected for up to 6 months before they were diagnosed with an AIDS-OI. The infection process may stimulate the production of IL-6. The preceding explanation could certainly hold for some or most of the diseases in our control group. For instance, in those with CMV retinitis, it is likely that there was shedding of CMV as long as 6 months before the development of retinitis. *Mycobacterium* infections are usually chronic infections with longer incubation periods. The incubation period for cryptococcal meningitis is not known but a person can have the infection for months before a brain infection is manifested. *Pneumocystis carinii* pneumonia, *Mycobacterium avium*, cryptosporidiosis, and *Candida* esophagitis are diseases associated with immunodeficiency in HIV/AIDS. The infection can be asymptomatic until the immunodeficiency becomes more serious. Therefore it is likely that subjects in the control group had active infections in the 6 months prior to diagnosis.

The production of IL-6 in response to different infections may enhance HIV viral replication and contribute to the AIDS pathogenesis. Cytokines can induce HIV expression, converting latent or chronic infections to productive ones, and/or by contributing to the generation of activated T cells, thereby facilitating infection with HIV and propagating the spread of the virus.⁴⁴ In fact, IL-6 has been seen to enhance HIV replication directly in infected monocytes.⁴⁵ If IL-6 in HIV infection does

enhance HIV replication, using IL-6 inhibitors in monocytes may reduce HIV viral replication and consequently improve survival among HIV-infected subjects.

New information has pointed to KSHV/HHV-8 as an infectious etiologic agent for AIDS-KS.^{12,16,17,46,47} While it is clear that KSHV is associated with the presence of KS, the biological mechanism(s) by which this herpesvirus contributes to the pathogenesis of AIDS-KS have not been defined. It is possible that KSHV infection can induce cytokine production, thereby enhancing autocrine/paracrine growth of KS cells. The observation^{23,24} that KSHV encodes a viral homolog (vIL-6) of cellular IL-6 (cIL-6) is exciting, and suggests that vIL-6 may play some role in KS pathogenesis. While there is clear homology between vIL-6 and cIL-6, there are significant differences in amino acid composition between these two versions of IL-6.^{23,24} This suggests that vIL-6 would not be detected by the IL-6 ELISA used in this study, which was optimized for the detection of cIL-6. Studies to confirm this, and to examine the biological properties of vIL-6, are underway.

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