Title

Immunosenescence is associated with presence of Kaposi's sarcoma in antiretroviral treated HIV infection

Journal

AIDS, 27(11)

ISSN

0269-9370

Authors

Unemori, Patrick
Leslie, Kieron S
Hunt, Peter W
et al.

Publication Date

2013-07-17

DOI

10.1097/qad.0b013e3283601144

Peer reviewed

Published in final edited form as:
AIDS. 2013 July 17; 27(11): 1735–1742. doi:10.1097/QAD.0b013e3283601144.

Immunosenescence is associated with presence of Kaposi's sarcoma in antiretroviral treated HIV infection

Patrick Unemori[a], **Kieron S. Leslie**[a], **Peter W. Hunt**[b], **Elizabeth Sinclair**[c], **Lorrie Epling**[c], **Ronald Mitsuyasu**[d], **Rita B. Effros**[d], **Jeffrey Dock**[d], **Sheila G. Dollard**[e], **Steven G. Deeks**[b], **Jeffrey N. Martin**[f], and **Toby A. Maurer**[a]

[a]Department of Dermatology, University of California, San Francisco

[b]Department of Internal Medicine, University of California, San Francisco

[c]Center for AIDS Research Core Immunology Laboratory, University of California, San Francisco

[d]Department of Pathology & Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, California

[e]Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases, Atlanta, Georgia

[f]Department of Epidemiology and Biostatistics, University of California, San Francisco, California, USA

Abstract

Objective—Some antiretroviral treated HIV-infected patients develop Kaposi's sarcoma despite long-term suppression of HIV replication. These Kaposi's sarcoma lesions are consistent with Kaposi's sarcoma observed in the elderly uninfected population (`classical Kaposi's sarcoma'). We investigated potential mechanisms for this phenomenon, focusing on measures of immune activation and T-cell senescence.

Design—We compared markers of immunosenescence, naive T cells, activation, and inflammation in $CD4^+$ and $CD8^+$ T cells from antiretroviral-treated participants with new-onset Kaposi's sarcoma (cases, $n = 19$) and from treated individuals without Kaposi's sarcoma (controls, $n = 47$).

Results—There was increased frequency of $CD4^+$ and $CD8^+$ T cells with an immunosenescence phenotype ($CD57^+$ and $CD28^-$) in cases vs. controls ($CD4^+$T cells: $CD57^+$ 7.4 vs. 3.7%, $P = 0.025$; $CD28^-$ 9.1 vs. 4.8%, $P = 0.025$; $CD8^+$ T cells: $CD57^+$ 41.5 vs. 27.7%, $P = 0.003$; $CD28^-$ 60.5 vs. 51.3%, $P = 0.041$). Cases had lower proportions of naïve T cells ($CD27^+CD28^+CD45RA^+$) in $CD4^+$ (23.0 vs. 32.2%, $P = 0.023$) and $CD8^+$ (11.3 vs. 20.7%, $P <$

Correspondence to Patrick A. Unemori, San Francisco General Hospital, Ward 92, 1001 Potrero Avenue, San Francisco, CA 94110, USA. Patrick_unemori@yahoo.com.

Conflicts of interest The authors have no conflicts of interest.

P.U., K.L., and T.M. contributed to study concept. P.U., K.L., P.H., R.M., S.D., J.M., and T.M. contributed to study design. P.U., E.S., L.E., R.E., J.D., and S.D. contributed to clinical/experimental procedures. P.U., P.H., K.L., and T.M. contributed to data analysis. P.U. contributed to initial article draft. P.U., K.L, P.H., E.S., R.M., R.E., S.D., J.M., and T.M contributed to article revisions.

0.001) T-cell compartments. CCR5 was more highly expressed in $CD4^+$ (16.3 vs. 11.0%, P = 0.025), and $CD8^+$ (43.1 vs. 28.3%, P <0.001) T-cell compartments in cases vs. controls. There was no difference in telomere length or telomerase activity in peripheral blood mononuclear cells, or in T-cell expression of activation markers ($HLADR^+CD38^+$).

Conclusion—Among antiretroviral-treated patients, increased frequencies of T cells with an immunosenescence phenotype and lower frequencies of naive T cells were associated with presence of Kaposi's sarcoma among effectively treated patients. These data suggest that certain immunologic perturbations –including those associated with aging – might be causally associated with development of Kaposi's sarcoma.

Keywords

aging; CCR5; CD28; CD57; immunosenescence; Kaposi's sarcoma; senescence

Introduction

Traditionally, Kaposi's sarcoma in the general population has been associated with advanced age, in which the lesions are often isolated, indolent, and rarely systemic (classical Kaposi's sarcoma). Kaposi's sarcoma is also found in patients with advanced immunodeficiency, in which the lesions can be far more aggressive [1–3]. Although the advent of antiretroviral therapy (ART) may have greatly decreased the incidence of Kaposi's sarcoma in HIV-infected adults, Kaposi's sarcoma continues to be the most prevalent AIDS-defining malignancy worldwide and carries with it significant morbidity and mortality [4–6].

In spite of the clinical relevance of Kaposi's sarcoma, mechanisms behind development of Kaposi's sarcoma in the elderly have yet to be fully elucidated. We previously reported an atypical cohort of patients who developed cutaneous Kaposi's sarcoma despite having undetectable plasma HIV RNA levels and relatively high $CD4^+$ T-cell counts [7]. The Kaposi's sarcoma lesions seen in these patients ran an indolent course, and were morphologically and clinically reminiscent of classical Kaposi's sarcoma, or the Kaposi's sarcoma seen in the HIV-negative elderly patients. Given emerging evidence suggesting that prolonged periods of untreated HIV infection result in accelerated and perhaps irreversible `aging' of the immune system [8–12], we hypothesized that gradual loss of immunologic function during advancing age, or `immunosenescence,' may account for the development of Kaposi's sarcoma in these individuals.

Given that immunologic aging may present in both populations which develop classical Kaposi's sarcoma – namely very old uninfected adults and long-term antiretroviral treated adults – we hypothesized this group of patients who developed Kaposi's sarcoma in spite of well controlled HIV would carry an immunosenescent phenotype, which would include increased proportions of $CD28^-$ and $CD57^+$ T cells, decreased telomere length, decreased proliferative capacity, increased T-cell activation, and/or lower levels of naïve T cells.

Methods

Design

This is a case–control study comparing global immunosenescence markers ($CD28^{-}$ and $CD57^{+}$), naive T-cell markers ($CD27^{+}CD28^{+}CD45RA^{+}$), activation markers ($HLADR^{+}CD38^{+}$ and $CCR5^{+}$), proliferative response (carboxyfluorescein diacetate succinimidyl ester, CFSE), and telomere length and telomerase activity in antiretroviral treated HIV-positive individuals with active Kaposi's sarcoma (cases) vs. those without Kaposi's sarcoma (controls). In order to determine whether any observed differences in immune response might be due to presence or absence of human herpesvirus 8 (HHV8), we also determined the HHV8 serology of the controls.

Participants

We identified 19 individuals on ART who developed or had active biopsy proven, unremitting Kaposi's sarcoma after an interval of at least 24 months with viral loads less than 75 copies RNA/ml, and peripheral CD4 cell counts more than 300 cells/μl. We also recruited 47 HIV-positive and Kaposi's sarcoma-negative controls on ART with viral loads less than 75 copies RNA/ml and peripheral CD4 cell counts more than 300 cells/μl. For controls, absence of Kaposi's sarcoma in history was determined by self-report. Thus, cases and controls were matched in terms of viral load (all <75 copies RNA/ml), and in terms of CD4 cell threshold (>300 cells/μl). All cases and controls were HIV-positive, as determined by standard ELISA documented at any time in the past, or by documented viral load within 6 months of enrollment, and had no history of autoimmune disease, systemic fungal or mycobacterial infection, immunomodulatory or chemotherapy use within 1 year of enrollment, or current other active malignancy. Women were excluded because of potential gender effects on immune responses [13].

Recruitment and follow-up

Cases and controls were recruited from Dermatology and HIV Primary Care Practices in San Francisco and adjacent counties, University of California, Los Angeles (UCLA) related HIV primary care clinics, and from the Study of the Consequences of the Protease Inhibitor Era (SCOPE), a clinic-based cohort of over 1500 HIV-infected individuals at the University of California, San Francisco (UCSF). This study was reviewed and approved by institutional review boards at UCSF and UCLA.

T-cell immunophenotyping

Cryopreserved T cells were characterized by flow cytometry as previously described [14]. Briefly, cryopreserved peripheral blood mononuclear cells (PBMCs) were rapidly thawed washed and assessed for cell count and viability using the Viacount assay on a Guava Personal Cell Analysis system (Guava Technologies Inc., Hayward, California, USA). Cells were stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen, Grand Island, New York, USA), and then stained with the following combination of fluorescently conjugated monoclonal antibodies: panel 1: CD3-Pacific Blue and CCR5-PE CY5 (BD Biosciences Pharmigen, San Diego, California, USA), CD4-PE CY7, HLA-DR-FITC and

CD38-PE (BD Biosciences), CD57-Alexa Fluor 647 (BioLegend, San Diego, California, USA), and CD8-Q-Dot605 (custom conjugate; Invitrogen); Panel 2: CD3-Pacific Blue and CD28-APC (BD Biosciences Pharmigen), CD45RA-PECY7 (BD Biosciences), CD27-APC-ALEXA 750 and CD4 PE-TR (Invitrogen), and CD8-Q-Dot605 (custom conjugate; Invitrogen).

Stained cells were washed, re-suspended in 0.5% formaldehyde (Polyscience, Warrington, Pennsylvania, USA), stored at 4°C, and run on a customized BD Biosciences LSR II Flow Cytometer within 18 h. Rainbow beads (Spherotec, Lake Forest, Illinois, USA) were used to standardize instrument settings between runs. At least 200 000 lymphocytes were collected for each sample. CD28 and CD57 were not run in concurrent panels. Data were compensated and analyzed in FlowJo using standard gating methods to define subsets of $CD4^+$ and $CD8^+$ T cells.

Carboxyfluorescein diacetate succinimidyl ester

PBMCs were stained with CFSE, suspended in media, and stimulated as previously described [15]. Thereafter, cells were stained for flow cytometric analysis.

Cell collection, culture, and stimulation protocol for telomerase activity and telomere length assays

Cryogenically preserved PBMC aliquots were thawed, washed, and re-suspended in `complete RPMI 1640,' (10% fetal bovine serum, 10 mmol/l HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 2 mmol/l glutamine, and 50 IU/ml penicillin/streptomycin). From each thawed aliquot, 1×10^6 cells were collected and pelleted for the PBMC ex-vivo telomerase activity (Telomerase Repeat Amplification Protocol, TRAP) assay, between 1×10^6 and 5×10^6 cells were collected and pelleted for telomere length measurements, and 2×10^6 cells were stimulated in culture for the activation-induced TRAP assay, using CD2/CD3/CD28 Ab-coated beads (T Cell Expansion Kit- Miltenyi) at a bead : cell ratio of 0.5 : 1. Every 48–72 h, half the media in the wells was removed and replaced with fresh media and 4 μl of IL-2 (10 000 U/ml) was added. At day 7, 1×10^6 PBMCs were collected and pelleted for telomerase activity measurements. In some experiments, purified CD8 T cells were obtained using Pan T isolation (Pan T Isolation Kit- Miltenyi) followed by a CD4 depletion (CD4 T Cell Isolation Kit- Miltenyi).

Telomere length real-time PCR

Telomere lengths were measured by quantitative real-time PCR, as described previously [16]. Cells were washed twice with phosphate-buffered saline (PBS) and DNA was isolated using the DNeasy kit (Qiagen, Valencia, California, USA) and diluted to 50 ng/100 μl in dilution buffer. Each milliliter of buffer consists of 40 μl of *Escherichia coli* DNA (100 ng/μl), 100 μl of 10×*Taq* polymerase buffer, and 860 μl of H_20. DNA samples were boiled at 95°C for 30 min. Samples were run in triplicate in a 96-well plate in the I-CycleriQMulticolor Real-Time Detection System (Bio-Rad, Hercules, California, USA). Separate plates were used for the telomere PCR and the control Human β Globin (HBG) PCR. In the telomere PCR plate, each well contained 10 μl of DNA, 10 μl of iQ SYBR Green Super Mix, 0.2 μl of Tel primer 1 (20 μmol/l stock), and 0.8 μl of Tel primer 2 (20

μmol/l stock), for a total of 21 μl. In the HBG PCR plate, each well contained 10 μl of DNA, 10 μl of iQ SYBR Green Super Mix, 0.3 μl of HBG primer 1 (20 μmol/l stock), and 0.7 μl of HBG primer 2 (20 μmol/l stock), for a total of 21 μl. The sequences of the primers used were as follows: Tel primer 1 (5′ CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT 3′), Tel primer 2 (5′ GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT 3′), HBG primer 1 (5′ GCTTCTGACACAACTGTGTTCACTAGC 3′), and HBG primer 2 (5′ CACCAACTTCATCCACGTTCACC 3′). The IQcycler program for the telomere PCR consisted of initial denaturation at 94°C for 1 min, followed by 25 PCR cycles at 95°C for 15 s and 56°C for 1 min (single fluorescence measurement). The IQcycler program for the HBG PCR consisted of initial denaturation at 94°C for 1 min, followed by 36 PCR cycles at 95°C for 15 s, 58°C for 20 s, and 72°C for 20 s (single fluorescence measurement). Values were calculated as a ratio of telomere DNA to HBG DNA for each sample.

Telomerase activity measurements

Telomerase activity was determined using a modified version of the TRAP as previously described [17]. Briefly, for each sample, 1×10^6 live cells were pelleted and washed twice with PBS and cell pellets were lysed in 100 μl of lysis. To control for intersample cell number variance, activity was normalized by nucleic acid concentration, which was determined using the Quant-iT Ribogreen RNA Assay Kit (Molecular Probes, Eugene, Oregon, USA). Telomeric addition and amplification was performed (BioRad iCycler) using the following primers: Cy5-TS primer (5′Cy5 label AATCCGTCGAGCAGAGTT 3′) and ACX primer (5′ GCGCGGCTTACCCTTACCCTTACCCTAACC 3′). Each sample was mixed with 25 μl of Bromothenol Blue loading dye and 25 μl of sample and loading dye were loaded and run using 10% nondenaturing PAGE in 1X TBE buffer. Gels were run at approximately 300 V for 80 min. Gels were scanned on a STORM 865 (GE Healthcare, Piscataway, New Jersey, USA) and quantified using the software ImageQuant 5.2 (GE Healthcare).

Human herpesvirus 8 antibody testing

All control blood samples were tested for antibodies to HHV8 with two enzyme immunoassays (EIAs) and one indirect immunofluorescence assay (IFA). The two EIAs (EIA-K8.1-synthetic and EIA-ORF65-synthetic) target antibodies to open reading frame (ORF) K8.1 and ORF 65, respectively, with use of synthetic peptides as antigen substrates [18]. The IFA uses HHV8-infected BCBL-1 cells as an antigen substrate, in which HHV8 is induced to its replicative phase [19]; specimens were evaluated at a dilution of 1 : 80. Specimens that were reactive in any two tests or were positive in the IFA alone were classified as HHV8 antibody-positive. Specimens that were non-reactive in all tests or reactive in only one EIA were categorized as HHV8 antibody-negative. This serologic algorithm has an estimated specificity of 97.5%, as evidenced by testing blood donors [20], and a sensitivity of 96.3%, as evidenced by testing patients with Kaposi's sarcoma [21].

Statistical methods

Mann–Whitney comparisons were used to compare variables between HIV-positive and Kaposi's sarcoma-positive cases vs. HIV-positive and Kaposi's sarcoma-negative controls.

Linear regression and quartile analysis were used to further control for potential confounding by age.

Results

Age and HIV markers of infection

The cases and controls were not significantly different with regard to $CD4^+$ T-cell count, $CD8^+$ T-cell count, and viral load, although the cases were significantly older than the controls (medians of 54 and 43 years of age, respectively, $P<0.001$, see Table 1).

Global immunosenescence markers CD28 and CD57

The frequency of $CD8^+$ T cells expressing CD57 was higher in the cases than controls (median of 41.5 vs. 27.7%, $P = 0.003$, Table 2). This difference remains significant after adjusting for age and CD4 cell count (age-adjusted $P=0.005$, age-adjusted and CD4 cell count-adjusted $P=0.009$). As compared with the controls, the cases also had a higher frequency of $CD28^- CD4^+$ cells (median of 9.1 vs. 4.8%, $P=0.025$, age-adjusted $P=0.030$, age-adjusted and CD4 cell-adjusted $P=0.06$, Table 2) and $CD28^-CD8^+$ cells (median of 60.5 vs. 51.3%, $P=0.041$, age-adjusted $P=0.044$, age-adjusted and CD4 cell count-adjusted $P=0.05$, Table 2) vs. controls. There was a trend suggesting that the cases had a higher frequency of $CD57^+CD4^+$ T cells than controls (median of 7.4 vs. 3.7%, $P=0.045$, age-adjusted $P=0.07$, age-adjusted and CD4 cell count-adjusted $P=0.12$, Table 2).

T-cell maturation

The proportion of naive ($CD27^+CD28^+CD45RA^+$) $CD8^+$ T cells was lower in the cases than the controls (median of 11.3 vs. 20.7%, $P<0.001$, age-adjusted $P=0.022$, age-adjusted and CD4 cell count-adjusted $P=0.01$, Table 2). In addition, the proportion of T-effector cells ($CD27^-CD28^-CD45RA^-$) was greater in cases than the controls in both $CD4^+$ (median of 6.7 vs. 3.0%, $P=0.014$, age-adjusted $P=0.022$, age-adjusted and CD4 cell count-adjusted $P=0.05$, Table 2) and $CD8^+$ (35.0 vs. 19.5%, $P=0.005$, age-adjusted $P=0.05$, age-adjusted and CD4 cell count-adjusted $P=0.01$, Table 2) T-cell compartments. There was a trend suggesting cases had a lower frequency of naïve ($CD27^+CD28^+CD45RA^+$) $CD4^+$ T cells than controls (median of 23.0 vs. 32.2%, age-adjusted $P=0.11$, age-adjusted and CD4 cell count-adjusted $P=0.07$, Table 2).

CCR5 expression

The median percentage $CD4^+$ T cells expressing CCR5 was 16.3 for Kaposi's sarcoma-positive cases vs. 11.0 for Kaposi's sarcoma-negative controls, ($P=0.025$, age-adjusted $P=0.05$, age-adjusted and CD4 cell count-adjusted $P=0.04$, Table 2) and the median percentage $CD8^+$ T cells expressing CCR5 was 43.1 for Kaposi's sarcoma-positive cases vs. 28.3 for Kaposi's sarcoma-negative controls (unadjusted, age-adjusted, and age-adjusted and CD4 cell count-adjusted $P<0.001$, Table 2).

Measures for activation, proliferation, telomere length, and telomerase activity

There were no differences between cases and controls for the HLA-DR and CD38 activation markers, proliferation as determined by CFSE assays, nor were telomerase activity and telomere lengths different between the groups. However, we observed a significant negative correlation between the overall PBMC telomere length and the proportion of more differentiated $CD8^+CD28^-$T cells ($\rho=-0.41$, $P=0.001$), as has been observed in healthy individuals [22].

Age adjustment via quartile analysis

As additional means of controlling for age as a potential confounding variable, we employed quartile adjustment. When quartile analysis was applied, differences between Kaposi's sarcoma-positive cases and Kaposi's sarcoma-negative controls were preserved for measures of global immunosenescence, T-cell maturation, and CCR5 expression (Table 2).

The impact of human herpesvirus 8 serology on immune responses

To further examine the effect of HHV8 itself, the Kaposi's sarcoma-negative controls were divided by HHV8 status (HHV8-positive and HHV8-negative). These two groups were compared to each other and finally, compared to Kaposi's sarcoma-positive cases.

Of the 47 Kaposi's sarcoma-negative controls, 26 had a positive serology. Notably, when comparing HHV8-positive vs. HHV8-negative subgroups with one another, there were no differences in age, markers of HIV infection, global markers of immunosenescence, T-cell maturation markers, CCR5 markers of expression, or measures of activation, telomere length, and telomerase activity.

Human herpesvirus 8 subgroups: age and HIV markers of infection

When comparing Kaposi's sarcoma-positive cases vs. HHV8-positive and HHV8-negative subgroups, there were no differences in HIV markers of infection. Kaposi's sarcoma-positive cases were older than the HHV8-positive subgroup (median 54 and 45 years of age, $P=0.003$), as well as the HHV8-negative subgroup (median 54 and 43 years of age, $P<0.001$).

Human herpesvirus 8 subgroups: global markers of immunosenescence

Kaposi's sarcoma-positive cases had a higher frequency of $CD8^+CD57^+$ T cells than the HHV8-positive subgroup (median 41.5 and 30.4%, respectively, $P=0.01$, age-adjusted $P=0.02$), and HHV8-negative subgroup (median 41.5 and 26.2%, respectively, $P=0.04$, age-adjusted $P=0.05$). There was a trend suggesting Kaposi's sarcoma-positive cases had a higher frequency of $CD8^+CD28^-$ T cells than the HHV8-positive subgroup (median 60.5 and 52.1%, respectively, $P=0.06$, age-adjusted $P=0.08$). As well, there was a trend

Human herpesvirus 8 subgroups: T-cell maturation markers

Naive CD4 and CD8 T cells were lower in the Kaposi's sarcoma-positive cases as compared with the Kaposi's sarcoma-negative controls, a comparison that was preserved when Kaposi's sarcoma-negative controls were further subdivided into HHV8-positive and HHV8-

negative subgroups. The Kaposi's sarcoma-positive cases had a lower frequency of naïve ($CD27^+CD28^+CD45RA^+$) $CD8^+$ T cells than the HHV8-positive subgroup (median 11.3 and 23.9%, respectively, $P = 0.04$, age-adjusted $P = 0.05$, Table 3) and the HHV8-negative subgroup (median 11.3 and 19.1%, respectively, $P = 0.01$, age-adjusted $P=0.02$, Table 3). The Kaposi's sarcoma-positive cases had a lower frequency of naïve ($CD27^+CD28^+CD45RA^+$) $CD4^+$ T cells than the HHV8-negative subgroup (median 23.0 and 28.0% respectively, $P = 0.06$, age-adjusted $P = 0.05$, Table 3).

Human herpesvirus 8 subgroups: CCR5 expression

The Kaposi's sarcoma-positive cases had a higher frequency of $CCR5^+$ $CD8^+$T cells than the HHV8-positive subgroup (median 43.1 and 29.0%, respectively, $P <0.01$, age-adjusted $P <0.01$, Table 3) and the HHV8-negative subgroup (median 43.1 and 27.5%, respectively, $P <0.01$, age-adjusted $P = 0.03$, Table 3).

Human herpesvirus 8 subgroups: measures of activation, telomere length, and telomerase activity

There were no significant differences between Kaposi's sarcoma-positive cases and HHV8 control subgroups when looking at measures of activation, proliferation, telomere length, and telomerase activity.

Discussion

The incidence and prevalence of systemic, life-threatening Kaposi's sarcoma has declined dramatically since the advent of highly effective ART. Despite this unquestioned success, some apparently well treated individuals still develop Kaposi's sarcoma, although the clinical presentation is much less aggressive than that which was observed among untreated individuals with advanced disease. Indeed, as we have previously described, the Kaposi's sarcoma that occurs during effective therapy has many characteristics of that seen in elderly HIV-uninfected men (i.e. classical Kaposi's sarcoma). Given that HIV affects the immune system in a manner comparable to that observed in aging, we hypothesized that certain markers of immunologic aging (immunosenescence) may be associated with Kaposi's sarcoma in the context of effective therapy. Here, we present data that are largely consistent with this hypothesis.

Specifically, we observed increased frequencies of T cells with an immunosenescent phenotype ($CD28^-$ and $CD57^+$) in those with Kaposi's sarcoma vs. those without Kaposi's sarcoma. We also found evidence of lower frequencies of naive T cells and higher frequencies of effector T cells in those with Kaposi's sarcoma than those without Kaposi's sarcoma. These associations were preserved even when controlling for effects of age (age was controlled via both regression and quartile analysis with preservation of results). These associations remained significant regardless of HHV8 status. This suggests that HHV8 infection itself does not explain the difference in immunosenescence patterns observed in this comparison of cases with clinical Kaposi's sarcoma vs. Kaposi's sarcoma-negative controls. These findings support the hypothesis that immune aging may play a role in increasing vulnerability to and/or as a consequence of Kaposi's sarcoma.

While this study begins to look at the relationship between immune aging and Kaposi's sarcoma, further investigation is needed to see how duration and severity of HIV infection may be influencing immune aging in these cohorts. At this time, very few cohorts can provide precise information regarding the duration of infection – mainly those cohorts enrolling individuals with primary infection. These cohorts examining primary infection, at this time, are too small to address the pathogenesis of relatively more unusual events such as development of clinical Kaposi's sarcoma. CD4 nadir was thought of as a possible surrogate for duration or severity of HIV infection but a significant proportion of our Kaposi's sarcoma-positive cases were HIV-infected prior to widespread clinical availability of CD4 cell count assay, and were, thus, already immune reconstituted prior to our being able to accurately assess their CD4 cell count.

Another way to isolate the effect of HIV from the acquisition of clinical Kaposi's sarcoma would be to include non-HIV-infected patients with Kaposi's sarcoma in these analyses. Comparisons involving this HIV-negative, `classical Kaposi's sarcoma' group would certainly be of great interest, but this is a rare group of patients in the United States. It is hoped that this work may provide the impetus to explore issues of immunosenescence in other Kaposi's sarcoma cohorts.

Although many of our measurements of immunologic aging were higher in our cases than controls, there were exceptions. Specifically, telomere length and telomerase activity did not differ between the groups. Our analysis was limited, however, by the use of total PBMCs for these analyses. It is possible that telomeric effects may be predominant in certain T cell or other cellular subpopulations not evaluated. Although not directly related to our study question, we found that having a higher frequency of more differentiated $CD28^{-}$ $CD8^{+}$T cells was associated with shorter telomere length. This observation is consistent with the known biology of $CD8^{+}$ T cells, and is consistent with findings in uninfected individuals [22]. This study is one of the first to demonstrate this association in persons with HIV disease.

The elevated CCR5 expression in the Kaposi's sarcoma cohort carries potential implication for Kaposi's sarcoma disease pathogenesis. CCR5, a chemokine receptor, has been shown to interact with viral macrophage inflammatory protein (vMIP), one of the proteins encoded by HHV8, the virus necessary for Kaposi's sarcoma development [23–25]. The interaction of CCR5 with vMIP has been shown in monocytes to effect calcium influx [26], which may indicate there are potential downstream effects to the inflammatory process involved with Kaposi's sarcoma lesion formation and persistence.

This study does not address how the relationship between ART (or lack thereof), duration and severity of HIV infection, duration and severity of HHV8 infection, potential interaction between HIV and HHV8, and the nonlinear inflammation associated with Kaposi's sarcoma oncogenesis, might affect these immunosenescence and activation markers – arenas that should be addressed and controlled for in future studies. Although we did subgroup analysis involving HHV8 status, it is important to note that this study was not originally powered for these tertiary outcome analyses and, thus, conclusions with regard to lack of differences found between these groups must be carefully considered. As well, other future studies

would include investigation of other HIV malignant disease states with immunosenescence markers, as well as prospective tracking of these immunosenescence markers during changes in disease course. Finally, exploration of CCR5's potential role in Kaposi's sarcoma pathogenesis and in other modes of Kaposi's sarcoma oncogenesis (e.g. classical Kaposi's sarcoma, low CD4 cell count Kaposi's sarcoma, and transplant Kaposi's sarcoma) may yet shed more light on the mechanisms behind Kaposi's sarcoma oncogenesis.

This is one of the first studies documenting an association between immunosenescence and Kaposi's sarcoma even when controlling for confounders including age, CD4 cell count, gender, and HHV8 status. Our findings add to the increasing evidence supporting the notion that HIV disease may constitute a model of accelerated aging [27]. Our data also suggest that immunosenescence may be causally associated with development of Kaposi's sarcoma in uninfected elderly adults. Future studies in that population are needed.

Acknowledgments

Funding is provided by the National Center for Research Resources (NCRR, Grant Number UL1RR024131), a component of National Institutes of Health (NIH) Roadmap for Medical Research, the UCSF-GIVI Center for AIDS Research (P30AI027763), the Penn Center for AIDS Research (CFAR), an NIH-funded program (P30 AI 045008), and UCLA Center for AIDS Research (CFAR), an NIH-funded program (AI 28697).

The SCOPE cohort was also supported by the NIAID (RO1 AI087145, K24AI069994), the Center for AIDS Prevention Studies (P30 MH62246), and the CFAR Network of Integrated Systems (R24 AI067039).

References

1. Abbaszadeh S, Taheri S. Kaposi's sarcoma after renal transplantation. Saudi J Kidney Dis Transpl. 2009; 20:775–778. [PubMed: 19736472]
2. Aronovitch SA. Dermatological management of Kaposi sarcoma. Dermatol Nurs. 1995; 7:191–195. [PubMed: 7779523]
3. Eltom MA, Jemal A, Mbulaiteye SM, Devesa SS, Biggar RJ. Trends in Kaposi's sarcoma and non-Hodgkin's lymphoma incidence in the United States from 1973 through 1998. J Natl Cancer Inst. 2002; 94:1204–1210. [PubMed: 12189223]
4. Gallafent JH, Buskin SE, De Turk PB, Aboulafia DM. Profile of patients with Kaposi's sarcoma in the era of highly active antiretroviral therapy. J Clin Oncol. 2005; 23:1253–1260. [PubMed: 15718323]
5. Serraino D, De Paoli A, Zucchetto A, Pennazza S, Bruzzone S, Spina M, et al. The impact of Kaposi sarcoma and non-Hodgkin lymphoma on mortality of people with AIDS in the highly active antiretroviral therapies era. Cancer Epidemiol. 2010; 34:257–261. [PubMed: 20413362]
6. Polesel J, Franceschi S, Suligoi B, Crocetti E, Falcini F, Guzzinati S, et al. Cancer incidence in people with AIDS in Italy. Int J Cancer. 2010; 127:1437–1445. [PubMed: 20049835]
7. Maurer T, Ponte M, Leslie K. HIV-associated Kaposi's sarcoma with a high CD4 count and a low viral load. N Engl J Med. 2007; 357:1352–1353. [PubMed: 17898112]
8. Appay V, Almeida JR, Sauce D, Autran B, Papagno L. Accelerated immune senescence and HIV-1 infection. Exp Gerontol. 2007; 42:432–437. [PubMed: 17307327]
9. Appay V, Papagno L, Spina CA, Hansasuta P, King A, Jones L, et al. Dynamics of T cell responses in HIV infection. J Immunol. 2002; 168:3660–3666. [PubMed: 11907132]
10. Emu B, Sinclair E, Favre D, Moretto WJ, Hsue P, Hoh R, et al. Phenotypic, functional, and kinetic parameters associated with apparent T-cell control of human immunodeficiency virus replication in individuals with and without antiretroviral treatment. J Virol. 2005; 79:14169–14178. [PubMed: 16254352]

11. Le Priol Y, Puthier D, Lecureuil C, Combadiere C, Debre P, Nguyen C, et al. High cytotoxic and specific migratory potencies of senescent CD8+ CD57+ cells in HIV-infected and uninfected individuals. J Immunol. 2006; 177:5145–5154. [PubMed: 17015699]
12. Pommier JP, Gauthier L, Livartowski J, Galanaud P, Boue F, Dulioust A, et al. Immunosenescence in HIV pathogenesis. Virology. 1997; 231:148–154. [PubMed: 9143314]
13. Meier A, Chang JJ, Chan ES, Pollard RB, Sidhu HK, Kulkarni S, et al. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. Nat Med. 2009; 15:955–959. [PubMed: 19597505]
14. Kaplan RC, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, et al. T cell activation and senescence predict subclinical carotid artery disease in HIV-infected women. J Infect Dis. 2011; 203:452–463. [PubMed: 21220772]
15. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. Blood. 2003; 101:2711–2720. [PubMed: 12433688]
16. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002; 30:e47. [PubMed: 12000852]
17. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994; 266:2011–2015. [PubMed: 7605428]
18. Pau CP, Lam LL, Spira TJ, Black JB, Stewart JA, Pellett PE, et al. Mapping and serodiagnostic application of a dominant epitope within the human herpesvirus 8 ORF 65-encoded protein. J Clin Microbiol. 1998; 36:1574–1577. [PubMed: 9620379]
19. Dollard SC, Nelson KE, Ness PM, Stambolis V, Kuehnert MJ, Pellett PE, et al. Possible transmission of human herpesvirus-8 by blood transfusion in a historical United States cohort. Transfusion. 2005; 45:500–503. [PubMed: 15819669]
20. Pellett PE, Wright DJ, Engels EA, Ablashi DV, Dollard SC, Forghani B, et al. Multicenter comparison of serologic assays and estimation of human herpesvirus 8 seroprevalence among US blood donors. Transfusion. 2003; 43:1260–1268. [PubMed: 12919429]
21. Cannon MJ, Operskalski EA, Mosley JW, Radford K, Dollard SC. Lack of evidence for human herpesvirus-8 transmission via blood transfusion in a historical US cohort. J Infect Dis. 2009; 199:1592–1598. [PubMed: 19385734]
22. Lin J, Epel E, Cheon J, Kroenke C, Sinclair E, Bigos M, et al. Analyses and comparisons of telomerase activity and telomere length in human T and B cells: insights for epidemiology of telomere maintenance. J Immunol Methods. 2010; 352:71–80. [PubMed: 19837074]
23. Navenot JM, Wang ZX, Trent JO, Murray JL, Hu QX, DeLeeuw L, et al. Molecular anatomy of CCR5 engagement by physiologic and viral chemokines and HIV-1 envelope glycoproteins: differences in primary structural requirements for RANTES, MIP-1 alpha, and vMIP-II binding. J Mol Biol. 2001; 313:1181–1193. [PubMed: 11700073]
24. Shao W, Fernandez E, Sachpatzidis A, Wilken J, Thompson DA, Schweitzer BI, et al. CCR2 and CCR5 receptor-binding properties of herpesvirus-8 vMIP-II based on sequence analysis and its solution structure. Eur J Biochem. 2001; 268:2948–2959. [PubMed: 11358512]
25. Nicholas J, Ruvolo VR, Burns WH, Sandford G, Wan X, Ciufo D, et al. Kaposi's sarcoma-associated human herpesvirus-8 encodes homologues of macrophage inflammatory protein-1 and interleukin-6. Nat Med. 1997; 3:287–292. [PubMed: 9055855]
26. Nakano K, Isegawa Y, Zou P, Tadagaki K, Inagi R, Yamanishi K. Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded vMIP-I and vMIP-II induce signal transduction and chemotaxis in monocytic cells. Arch Virol. 2003; 148:871–890. [PubMed: 12721796]
27. Effros RB, Fletcher CV, Gebo K, Halter JB, Hazzard WR, Horne FM, et al. Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. Clin Infect Dis. 2008; 47:542–553. [PubMed: 18627268]

Table 1

Age and markers of HIV infection in HIV-positive and Kaposi's sarcoma-positive cases and HIV-positive and Kaposi's sarcoma-negative controls.

	HIV-positive and Kaposi's sarcoma-positive cases: [median (range)]	HIV-positive and Kaposi's sarcoma-negative controls: [median, (range), *P* value*]
Number of patients (*N*)	19	47
Age (years)	54 (39–74)	43 (29–60), <0.001
CD4 cell count (cells/μl)	701 (338–1205)	521 (311–1194), 0.08
CD8 cell count (cells/μl)	933 (429–2543)	1200 (425–3485), 0.41
Viral load (copies/ml)	<75(<75 to <75)	<75(<75 to <75), >0.5

Women were excluded due to potential gender effects on immune responses [13].

* P values involve comparison between given measure and Kaposi's sarcoma-positive cases.

Table 2

Selected immune markers for HIV-positive and Kaposi's sarcoma-positive cases vs. HIV-positive and Kaposi's sarcoma-negative controls.

	Kaposi's sarcoma-positive cases [median (range)] (*n* = 19)	Kaposi's sarcoma-negative controls [median (range)] (*n* = 47)	Age-adjusted *P* values (linear, quartile)
Percentage of $CD8^+$ T cells			
$CD57^+$	41.5 (30.2–49.3)	27.7 (19.4–38.3)	0.005, 0.005
$CD28^-$	60.5 (46.5–72.9)	51.3 (38.7–60.7)	0.044, 0.07
$CD27^+CD28^+CD45RA^+$	11.3 (6.8–14.8)	20.7 (14.1–30.1)	0.022, 0.025
$CD27^-CD28^-CD45RA^-$	35.0 (19.9–37.8)	19.5 (12.8–28.1)	0.05, 0.05
$CCR5^+$	43.1 (36.6–60.2)	28.3 (23.5–36.3)	<0.001, <0.001
Percentage of $CD4^+$ T cells			
$CD57^+$	7.4 (3.9–17.3)	3.7 (1.6–10.4)	0.07, 0.09
$CD28^-$	9.1 (5.0–20.9)	4.8 (2.0–11.9)	0.030, 0.05
$CD27^+CD28^+CD45RA^+$	23 (14.3–34.0)	32.2 (20.0–43.1)	0.11, 0.05
$CD27^-CD28^-CD45RA^-$	6.7 (3.9–20.4)	3.0 (1.2–9.0)	0.022, 0.04
$CCR5^+$	16.3 (9.4–28.9)	11.0 (7.7–16.1)	0.05, 0.008

Table 3

Selected immune markers for HIV-positive and Kaposi's sarcoma-positive cases, HIV-positive, Kaposi's sarcoma-negative, and human herpesvirus 8-positive controls, and HIV-positive, Kaposi's sarcoma-negative and human herpesvirus 8-negative controls.

	Kaposi's sarcoma-positive cases: [median (range)] (*n* = 19) Reference	**Kaposi's sarcoma-negative and HHV8-positive controls [median, (range), age-adjusted *P* value] (*n* = 26)**	**Kaposi's sarcoma-negative and HHV8-negative controls: [median, (range), age-adjusted *P* value] (*n* = 21)**
Percentage of $CD8^+$ T cells			
$CD57^+$	41.5 (30.2–49.3)	30.4 (19.5–42.1), 0.02	26.2 (16.3–38.0), 0.05
$CD28^-$	60.5 (46.5–72.9)	52.1 (45.5–62.0), 0.06	48.7 (36.1–61.1), 0.24
$CD27^+CD28^+CD45RA+$	11.3 (6.8–14.8)	23.9 (14.1–31.9), 0.05	19.1 (10.7–34.5), 0.02
$CD27^-CD28^-CD45RA^-$	35.0 (19.9–37.8)	17.2 (8.9–27.5), 0.18	20.0 (12.2–31.1), 0.07
$CCR5^+$	43.1 (36.6–60.2)	29.0 (23.3–36.2), <0.001	27.5 (23.4–42.2), 0.003
Percentage of $CD4^+$ T cells			
$CD57^+$	7.4 (3.9–17.3)	3.7 (1.2–14.4), 0.15	5.0 (1.7–10.4), 0.32
$CD28^-$	9.1 (5.0–20.9)	4.8 (1.5–17.0), 0.09	5.3 (1.8–12.1), 0.20
$CD27^+CD28^+CD45RA^+$	23 (14.3–34.0)	38.8 (20.8–56.2), 0.33	28.0 (17.7–42.0), 0.05
$CD27^-CD28^-CD45RA^-$	6.7 (3.9–20.4)	2.9 (0.5–8.7), 0.08	4.0 (1.3–9.8), 0.09
$CCR5^+$	16.3 (9.4–28.9)	8.2 (5.5–15.3), 0.19	12.2 (8.0–17.7), 0.1