Detection of viral interleukin-6 in Kaposi sarcoma–associated herpesvirus–linked disorders

Yoshiyasu Aoki, Robert Yarchoan, Kathleen Wyvill, Shin-ichiro Okamoto, Richard F. Little, and Giovanna Tosato

Expression of a viral interleukin-6 (vIL-6) has been detected in certain Kaposi sarcoma (KS)–associated herpesvirus positive (KSHV+1) lesions. The release of vIL-6 systemically and its contribution to the pathogenesis of HIV-related malignancies was studied. Serum vIL-6 was detected in 13 (38.2%) of 34 HIV+ patients with KS, in 6 (85.7%) of 7 HIV+ patients with primary effusion lymphoma (PEL) and/or multicentric Castleman disease (MCD), and in 18 (60.0%) of 30 HIV+, mostly homosexual, individuals without KS, MCD, or PEL. By contrast, serum vIL-6 was detected in only 3 (23.1%) of 13 patients with classic KS, 1 (2.5%) of 40 blood donors from the United States, and 4 (19.0%) of 21 blood donors from Italy. Circulating vIL-6 levels were associated with HIV+ status (P < .0001). However, within the HIV+ cohort, serum vIL-6 levels were not associated with the occurrence of KSHV-associated malignancies (P = .43). (Blood. 2001;97:2173-2176)

Introduction

Kaposi sarcoma (KS)–associated herpesvirus (KSHV; also known as human herpesvirus 8) has been linked to 3 AIDS-related disorders: KS, primary effusion lymphoma (PEL), and multicentric Castleman disease (MCD).1,2 Molecular piracy of potentially useful cellular genes has emerged as a characteristic feature of this virus.3 Viral interleukin-6 (vIL-6), produced predominantly during lytic viral replication, exhibits approximately 25% amino acid identity to cellular IL-6.3-5 Similar to certain mouse and human cell lines, recombinant vIL-6 accelerated the hematopoiesis and induced vascular endothelial growth factor, which in turn has been implicated in the pathogenesis of KS, MCD, and PEL.5-12 Constitutive expression of vIL-6 has been identified in PEL cells and in the immunoblastic cells within the mantle zone of MCD lymph nodes.13-15 By contrast, expression of vIL-6 has been undetectable or restricted to few lytically infected cells in KS lesions.15,16 To investigate if vIL-6 is released into the circulation and if, through systemic distribution, vIL-6 contributes to the development of KSHV-associated disorders, we assayed vIL-6 in sera from HIV-infected and noninfected individuals.

Study design

Sera from blood donors, HIV-infected individuals, and patients with KS, PEL, or MCD were collected from consent blood banks and clinical centers in the United States, Italy, and Japan. Samples were stored at −70°C prior to testing.

Enzyme-linked immunosorbent assay (ELISA) for vIL-6 (assay sensitivity, 30 pg/mL) was performed as described elsewhere.14 All samples were diluted 1:10 prior to assay, and the lower limit of ELISA sensitivity for serum vIL-6 was thus 300 pg/mL. hIL-6 was measured using an hIL-6 Quantikine kit (R&D Systems, Minneapolis, MN) that does not detect vIL-6.14 HIV RNA load was determined by Amplicor HIV test (Roche Diagnostic Systems, Basel, Switzerland). Counts for CD4+ and CD8+ cells were determined by flow cytometry. Severity of KS in patients was assessed by both the TIS staging system17 and counting the total number of lesions. Serum antibodies to KSHV were detected using the whole-virus lysate ELISA kit18 (Advanced Biotechnologies, Columbia, MD) according to the manufacturer’s instructions. All statistical analyses were performed with StatView (version 5.0.1) software.

Results and discussion

Using a recently established vIL-6–specific ELISA that does not detect hIL-6,14 serum vIL-6 was detected in 1 (2.5%) of 40 blood donors from the United States (range, < 300-927 pg/mL; median, < 300 pg/mL; and 75th percentile, < 300 pg/mL) and 4 (19.0%) of 21 blood donors from Italy (range, < 300-2284 pg/mL; median, < 300 pg/mL; and 75th percentile, < 300 pg/mL) (Figure 1A). In control experiments, all positive sera tested negative when the plates were not coated with antibody, demonstrating that positive reactions were not attributable to nonspecific binding (data not shown). Immunoglobulin G (IgG) antibodies against KSHV were detected in 4 (19.0%) of 21 Italian and 0 (0%) of 40 US blood donors (Figure 1B).

We measured circulating vIL-6 levels in patients with classic (HIV−) KS and HIV-associated KS (Figure 1A). Serum vIL-6 was detectable in 3 (23.1%) of 13 patients with classic KS...
evaluate the results in HIV

1

P
types of KS compared with blood donors (antibody titers were markedly elevated in patients with both

1

serum vIL-6 in 30 HIV

was detected in 18 (60.0%) of these 30 HIV

als without clinically apparent KS, PEL, or MCD. Serum vIL-6

P
1564 pg/mL). There was no significant difference in the

percentile, (range,

300-1445 pg/mL; median,

300 pg/mL; and 75th percentile,

300 pg/mL; and 75th

3

Whitney test. Statistical significance of group differences was determined by the Mann-

3

levels were significantly elevated in US HIV

P
levels and numbers of KS lesions (P = .13, Fisher exact test

P
5
(n = 64)); KS severity by the TIS staging system17 (v = 0.307, Cramer test [n = 64]); treatment with antiretroviral agents

P
.99, Fisher exact test [n = 64]); or antiviruses against

P
.27, Fisher exact test [n = 64]), HIV RNA load (P = .95,

Mann-Whitney test [n = 35]), CD4 cell counts (P = .38, Mann-Whitney test [n = 64]), or lymphadenopathy (P = .15, Fisher exact test [n = 64]). These results are consistent with previous studies showing that KSHV infects a substantial subset of homosexual HIV

P
.0001, as measured by Mann-Whitney test), but

levels were not associated with the occurrence of

levels and numbers of KS lesions (P = .13, Mann-Whitney test) and the presence of anti-KSHV IgG

P
.99, Fisher exact test 

Spearman rank correlation). These results suggest that cellular IL-6 and vIL-6 are differently regulated and perhaps play different roles in the pathogenesis of MCD.

Overall, serum vIL-6 was detected in 46 (52.3%) of 88 serum samples from 71 HIV

P
1.0-116 pg/mL, and median, 2.4 pg/mL). We evaluated
correlations between serum vIL-6 detection and a number of parameters in HIV infection. Our analysis extended to all 64 HIV

P
.0001, Fisher exact test) and levels of vIL-6

vIL-6 remained relatively stable for a period of months (Table 1). hIL-6 was undetectable or was detected at low levels (range,

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HIV-induced immunodeficiency may allow for increased KSHV replication and vIL-6 expression in KSHV-infected patients with AIDS. In spite of considerable effort, the sites of initial KSHV infection and replication and the sites of viral latency and subsequent reactivation are incompletely characterized. Many of the currently available serological assays for the detection of KSHV antibodies have inadequate concordance with each other. Thus, when suspecting KSHV infection, vIL-6 testing may provide useful and complementary information on the occurrence of KSHV replication.

### Table 1. Clinical events and laboratory findings in patients with HIV infection

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sampling mo/y</th>
<th>KS</th>
<th>vIL-6 pg/mL</th>
<th>hIL-6 pg/mL</th>
<th>Anti-KSHV IgG OD ratio*</th>
<th>CD4/CD8 cells/μL</th>
<th>Antiretroviral therapy</th>
<th>Anti-KS treatment</th>
<th>CD4/CD8</th>
<th>vIL-6</th>
<th>hIL-6</th>
<th>Anti-KSHV IgG OD ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12/87</td>
<td>−</td>
<td>&lt; 300</td>
<td>&lt; 1.0</td>
<td>1.0</td>
<td>19/477</td>
<td>AZT, ddC</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>9/93</td>
<td>+</td>
<td>370</td>
<td>60.7</td>
<td>1.0</td>
<td>2/317</td>
<td>AZT</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>6/87</td>
<td>+</td>
<td>&lt; 300</td>
<td>3.8</td>
<td>5.92</td>
<td>242/591</td>
<td>AZT</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>5/89</td>
<td>+</td>
<td>&lt; 300</td>
<td>1.0</td>
<td>3.71</td>
<td>972/1458</td>
<td>AZT</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>3/98</td>
<td>+</td>
<td>470</td>
<td>25.0</td>
<td>&lt; 1.0</td>
<td>502/2279</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td>9/93</td>
<td>+</td>
<td>300</td>
<td>62.6</td>
<td>6.26</td>
<td>601/1328</td>
<td>AZT, 3TC</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>3/88</td>
<td>+</td>
<td>714</td>
<td>6.6</td>
<td>7.41</td>
<td>714/1006</td>
<td>AZT, 3TC</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>6/98</td>
<td>+</td>
<td>&lt; 300</td>
<td>569</td>
<td>5.74</td>
<td>275/1132</td>
<td>D4T, 3TC</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>4/99</td>
<td>+</td>
<td>&lt; 300</td>
<td>&lt; 1.0</td>
<td>4.78</td>
<td>287/670</td>
<td>D4T, 3TC</td>
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<td>−</td>
<td>−</td>
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<tr>
<td>10</td>
<td>3/88</td>
<td>+</td>
<td>470</td>
<td>25.0</td>
<td>&lt; 1.0</td>
<td>502/2279</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*OD ratios were determined by dividing the OD reading of each sample by cut-off value (average of negative control) × 3. Samples with OD ratio 1.0 were interpreted as positive.

KS indicates Kaposi sarcoma; vIL-6, viral interleukin-6; hIL-6, human IL-6; KSHV, Kaposi sarcoma-associated herpesvirus; IgG, immunoglobulin G; OD, optical density; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI; PI, protease inhibitor; AZT, zidovudine; ddC, zalcitabine; ABV, abacavir; NVP, nevirapine; IDV, indinavir; ADM, liposomed adriamycin; NFV, nelfinavir; 3TC, lamivudine; D4T, stavudine; CDV, cidofovir; SQV, saquinavir; RTV, ritonavir; F-ddA, lodenosine; ddI, didanosine.

HIV-induced immunodeficiency may allow for increased KSHV replication and vIL-6 expression in KSHV-infected patients with AIDS. In spite of considerable effort, the sites of initial KSHV infection and replication and the sites of viral latency and subsequent reactivation are incompletely characterized. Many of the currently available serological assays for the detection of KSHV antibodies have inadequate concordance with each other. Thus, when suspecting KSHV infection, vIL-6 testing may provide useful and complementary information on the occurrence of KSHV replication.

### Table 2. Human and viral interleukin-6 in sera from patients with multicentric Castleman disease and/or primary effusion lymphoma

<table>
<thead>
<tr>
<th>Case no.</th>
<th>HIV status</th>
<th>MCD</th>
<th>PEL</th>
<th>Related diseases</th>
<th>Time of 2nd/3rd sampling</th>
<th>vIL-6 pg/mL</th>
<th>hIL-6 pg/mL</th>
<th>Anti-KSHV IgG OD ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>11 days later</td>
<td>1076</td>
<td>5.8</td>
<td>9.01</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>13 days later</td>
<td>2628</td>
<td>1964.4</td>
<td>9.63</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>5795</td>
<td>&lt; 1.0</td>
<td>6.24</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>300</td>
<td>6096.7</td>
<td>2.36</td>
<td></td>
</tr>
<tr>
<td>15</td>
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<td>2549</td>
<td>6432.8</td>
<td>2.38</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>11 833</td>
<td>134.0</td>
<td>9.87</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>448</td>
<td>1.0</td>
<td>9.73</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>300</td>
<td>31.7</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>300</td>
<td>38.8</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>300</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>300</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Samples with OD ratio 1.0 were interpreted as positive.

MCD indicates multicentric Castleman disease; PEL, primary effusion lymphoma; AIHA, autoimmune hemolytic anemia; NHL, non-Hodgkin lymphoma; CNS, central nervous system; for other abbreviations, see Table 1.
Acknowledgments

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References

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