An Interleukin-6–Related Systemic Inflammatory Syndrome in Patients Co-Infected with Kaposi Sarcoma–Associated Herpesvirus and HIV but without Multicentric Castleman Disease

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Background. Kaposi sarcoma–associated herpesvirus (KSHV) is the causal agent for Kaposi sarcoma (KS) and multicentric Castleman disease (MCD) in human immunodeficiency virus (HIV)–infected patients. Patients with KSHV-MCD develop fevers, wasting, hypoalbuminemia, cytopenias, and hyponatremia that are related to overproduction of KSHV-encoded viral interleukin (IL)–6 (vIL-6) and human IL-6 (hIL-6).

Methods. We identified 6 HIV-infected patients with KS or serological evidence of KSHV infection who had severe inflammatory MCD-like symptoms but in whom we could not diagnose MCD, and we hypothesized that these symptoms resulted from vIL-6 overproduction. Serum vIL-6 levels were assessed in these 6 patients and compared with levels in 8 control patients with symptomatic KSHV-MCD and 32 control patients with KS. KSHV viral load, serum hIL-6 level, and human IL-10 level were also evaluated.

Results. Patients with inflammatory MCD-like symptoms but without MCD had elevated vIL-6 levels, comparable with levels in patients with symptomatic KSHV-MCD, and had levels that were significantly greater than those in control patients with KS (P = .003). Elevated hIL-6, IL-10, and KSHV viral loads were also comparable to patients with symptomatic KSHV-MCD and significantly greater than those with KS.

Conclusions. A subset of patients with HIV and KSHV co-infection, but without MCD, can develop severe systemic inflammatory symptoms associated with elevated levels of KSHV vIL-6, IL-6, and KSHV viral loads. Excess lytic activation of KSHV, production of the lytic gene product vIL6, and associated immunologic dysregulation may underlie the pathophysiology of these symptoms. This IL-6–related inflammatory syndrome is important to consider in critically ill patients with HIV and KSHV co-infection.

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Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>MCD (n = 8)</th>
<th>MCD-like syndrome (n = 6)</th>
<th>Severe KS (n = 24)</th>
<th>Mild KS (n = 8)</th>
</tr>
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<tbody>
<tr>
<td>Age, median years (range)</td>
<td>41 (34–47)</td>
<td>38 (29–52)</td>
<td>36 (27–46)</td>
<td>36 (30–49)</td>
</tr>
<tr>
<td>Prescribed cART, no. (%) of patients</td>
<td>7 (87.5)</td>
<td>4 (66.7)</td>
<td>21 (87.5)</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>CD4+ cell count, median cells/μL (range)</td>
<td>189 (21–364)</td>
<td>255 (28–492)</td>
<td>228 (12–1060)</td>
<td>287 (52–572)</td>
</tr>
<tr>
<td>HIV load, median copies/mL (range)</td>
<td>&lt;50 (&lt;50–664)</td>
<td>4650 (&lt;50–1.1x10⁶)</td>
<td>930 (&lt;50–1.3 x10⁶)</td>
<td>&lt;50 (&lt;50–27,500)</td>
</tr>
<tr>
<td>Sodium level, median mEq/L (range)</td>
<td>130 (127–136)</td>
<td>132 (127–139)</td>
<td>138 (133–142)</td>
<td>137 (133–138)</td>
</tr>
<tr>
<td>Albumin level, median g/dL (range)</td>
<td>2.7 (1.7–3.9)</td>
<td>2.6 (2.0–3.9)</td>
<td>4 (3.0–4.8)</td>
<td>4.2 (3.3–5.4)</td>
</tr>
<tr>
<td>Platelets, median platelets/μL × 1000 (range)</td>
<td>5.7 (2.7–10.1)</td>
<td>4.5 (2.3–6.4)</td>
<td>4.2 (2.0–10.7)</td>
<td>4.3 (3.2–7.2)</td>
</tr>
<tr>
<td>Hemoglobin level, median g/dL (range)</td>
<td>10.2 (7.4–12.7)</td>
<td>8.7 (7.0–14.8)</td>
<td>13.6 (9–15.5)</td>
<td>13.7 (9.8–15.9)</td>
</tr>
<tr>
<td>Follow-up, median months (range)</td>
<td>36 (4–91)</td>
<td>10 (3–60)</td>
<td>75 (3–148)</td>
<td>34 (6–128)</td>
</tr>
<tr>
<td>Deaths, no. (%) of patients</td>
<td>1 (12.5)</td>
<td>3 (50)</td>
<td>6 (25)</td>
<td>1 (12.5)</td>
</tr>
</tbody>
</table>

NOTE. cART, combined antiretroviral therapy; HIV, human immunodeficiency virus; KS, Kaposi sarcoma; MCD, multicentric Castleman disease.

Figure 1. Comparisons of serum viral interleukin 6 (vIL-6) between multicentric Castleman disease (MCD), MCD-like syndrome, and Kaposi sarcoma (KS) control groups. Comparisons of vIL-6 between MCD (8 patients) MCD-like syndrome (6), severe KS (24) and mild KS (8) groups were performed using Fisher’s exact test (p<2850 vs ≥2850 pg/mL). Values shown are log_10 transformed pg/mL. P values are 2-sided. Horizontal lines indicate median value, and the dashed line indicates the lower limit of detection.

to KSHV-MCD [2, 13, 14]. Therefore, KSHV vIL-6 is considered to be an important factor that causes KSHV-MCD symptoms. Patients with KSHV-MCD also have elevated serum hIL-6 levels, which may contribute to symptoms, and elevated levels of IL-10, the role of which is less clear [2, 15].

Over the past several years, we observed several HIV-infected patients with either KS or serological evidence of KSHV infection who had severe inflammatory MCD-like symptoms and laboratory abnormalities but in whom we could not diagnose MCD. We hypothesized that overproduction of cytokines, particularly vIL-6 from KSHV-infected cells, was the principal cause of these clinical abnormalities. With use of an enzyme-linked immunosorbent assay (ELISA) for vIL-6, we compared serum vIL-6 levels in 6 such cases with levels in control patients with symptomatic KSHV-MCD or HIV-associated KS. Serum hIL-6 level, IL-10 level, KSHV viral load, and other parameters were measured to further evaluate the immunologic and virologic milieu in case patients and control patients.

PATIENTS, MATERIALS, AND METHODS

Patient selection. A retrospective review of 143 patients seen from 1993 through 2006 in the HIV and AIDS Malignancy Branch (Bethesda, MD) adult clinic with HIV and known KSHV co-infection (either KS or serum antibodies to KSHV) identified 6 patients with unexplained MCD-like inflammatory symptoms in whom we were unable to diagnose MCD. All 6 were seen after 2000. Patient histories are provided in the Appendix, which appears only in the online version of the journal. In each case, MCD had been considered. Efforts to diagnose MCD or related diseases included 3 lymph node biopsies, 1 lung biopsy, 3 bone marrow biopsies, 1 thoracentesis, and 1 pericardiocentesis. Pathologic evaluation generally revealed reactive lymphoid or plasmacytoid cells in these tissues, except 1 lymph node in which KS spindle cells were observed. However, no diagnosis of MCD could be made. These patients were designated as having “MCD-like syndrome.” None developed MCD during follow-up of 3–60...
Table 2. Clinical Course of Patients with Multicentric Castleman Disease (MCD)-Like Symptoms and No MCD

<table>
<thead>
<tr>
<th>Patient</th>
<th>CD4+ cell count, cells/μL</th>
<th>Infection(s) at time of evaluation</th>
<th>vIL-6 level, pg/mL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Peak vIL-6 level, pg/mL</th>
<th>hIL-6 level, pg/mL&lt;sup&gt;b&lt;/sup&gt;</th>
<th>KSHV viral load, copies per 10⁶ cells</th>
<th>Clinical course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>324</td>
<td>4254</td>
<td>4254</td>
<td>41.5</td>
<td>248,485</td>
<td>Patient treated with combination chemotherapy, antibiotics and additional therapy with AZT and valganciclovir for suspected MCD-like syndrome. Fevers persisted with intermittently elevated vIL-6. Tracheal aspirate cultures persistently grew S. aureus and yeast.Patient developed thrombocytopenia leading to diffuse alveolar hemorrhage and death from respiratory distress.</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>492</td>
<td>15,372</td>
<td>15,372</td>
<td>2.1</td>
<td>&lt;1</td>
<td>Patient had high antibody titer to KSHV K8.1 but lymph node biopsy failed to reveal MCD. Symptoms and elevated vIL-6 level persisted during 2.5-month evaluation. Patient later treated for possible Lyme disease at another hospital with prolonged antibiotics. At 4.8 years (last contact), patient well and receiving cART and had not developed MCD.</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>118</td>
<td>7102</td>
<td>7102</td>
<td>93.5</td>
<td>51,111</td>
<td>Patient treated with liposomal doxorubicin and IL-12 on clinical study. He had persistent fevers and elevated vIL-6. Chemotherapy stopped after 3 months because of progressive KS, and the patient died shortly thereafter.</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>255</td>
<td>&lt;2,850</td>
<td>3307</td>
<td>27.12</td>
<td>222,000</td>
<td>Patient with cellulitis at time of evaluation was initially treated with antibiotics and liposomal doxorubicin, with improvement in inflammatory symptoms. Patient had elevated vIL-6 in a serum sample obtained 3 weeks after initial evaluation. Patient had improvement KS over 5 months; no subsequent MCD over 13.5 months follow-up.</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>28 initially</td>
<td>39,835</td>
<td>39,835</td>
<td>11.5</td>
<td>12,632</td>
<td>Patient with KS, anemia, and adenopathy was enrolled in a trial of liposomal doxorubicin and IL-12. First cycle complicated by S. aureus bacteremia and fungal cholecystitis. Serum vIL-6 persistently elevated throughout his clinical course. The patient had progressive KS despite 4 cycles of liposomal doxorubicin. The patient opted for hospice care and died at 7.5 months. Autopsy did not reveal MCD.</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>562</td>
<td>3894</td>
<td>10,489</td>
<td>205.7</td>
<td>8697</td>
<td>Patient had waxing and waning vIL-6 over first 2 months after starting cART and receiving a brief course of valganciclovir, with improvement in clinical symptoms. vIL-6 level decreased to c2850 and remained undetectable through month 9. Patient then discontinued cART and was lost to follow-up for 8 months. Patient readmitted with a large pericardial effusion but no evidence of primary effusion lymphoma, and undetectable vIL-6. Patient restarted cART with no subsequent MCD over 3.5 years follow-up.</td>
</tr>
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</table>

NOTE. AZT, zidovudine; cART, combined antiretroviral therapy; HIV, human immunodeficiency virus; IL, interleukin; KS, Kaposi sarcoma; KSHV, Kaposi sarcoma–associated herpesvirus; vIL-6, viral interleukin 6.

<sup>a</sup> Value when initially evaluated. Lower limit of detection, 2850 pg/mL.

<sup>b</sup> Value when initially evaluated. Normal IL-6 value in healthy donors, 2.3 ± 1.1 pg/mL.
months (median follow-up, 10 months). One patient had an autopsy in which MCD was not found. Microbiological assessment was performed in each case. Although several patients had HIV viremia or bacterial infections (possible Lyme disease in a subsequent evaluation done elsewhere in 1 patient, *Staphylococcus aureus* in 2, and clinical cellulitis in 1), these were not felt to fully account for the symptoms.

To explore the hypothesis that this MCD-like syndrome was caused, at least in part, by overproduction of IL-6, particularly vIL-6, we assayed serum vIL-6 level, serum hIL-6 level, and other immunologic and virologic parameters on stored serum, plasma, and peripheral blood mononuclear cell (PBMC) samples obtained at the first time point for which each patient showed the abnormal clinical profile and for which samples were available. In 1 patient, PBMCs for determination of KSHV viral load were obtained 2 days before the serum samples for determination of vIL-6 and human cytokine levels. As control subjects, we selected 8 HIV-infected patients with symptomatic KSHV-MCD and 32 patients with HIV-KS, who had stored clinical samples obtained during 1987–2006 (all but 1 obtained in 1996 or later). KS controls were subcategorized as severe (>50 lesions and/or visceral disease) or mild (<50 lesions, no visceral disease). Importantly, cases and control subjects were selected without knowledge of KSHV viral load, vIL-6 levels, or hIL-6 levels. All patients were enrolled on National Cancer Institute Institutional Review Board–approved protocols that allowed such testing after obtaining informed consent.

**Viral IL-6 assay.** We measured vIL-6 levels by modifying a sandwich ELISA, as described elsewhere [14, 16]. With use of a different mouse monoclonal antibody (clone v6m 31.2.4) to coat 96-well plates, we incubated diluted serum samples overnight at 4°C, then incubated with rabbit polyclonal anti-vIL-6 antibody, followed by affinity-purified human serum protein- absorbed goat antirabbit immunoglobulin G (IgG) (H+L) antibody conjugated to horseradish peroxidase (Bio-Rad) diluted 1:5000 in phosphate-buffered saline–Tween 20/bovine serum albumin. SureBlue TMB Microwell Peroxidase Substrate (KPL) was then added to the wells for 10 min, followed by stop solution (1 N HCl). Plates were read at 450 nm with correction at 630 nm. Standard curves were generated, and vIL-6 levels were calculated as described elsewhere [16]. We used a new monoclonal antibody in this study, because the antibody used previously [14, 16] sometimes bound to a component in normal serum samples from patients not known to be KSHV infected. These modifications reduced the possibility of false- positive detection of vIL-6. Fifty-five serum samples from healthy donors not known to be infected with HIV or KSHV were tested, with the upper 95% confidence limit in these samples (2850 pg/mL) set as the cutoff value for elevated vIL-6 levels. This cutoff value, which was substantially higher than that used in previous studies [14, 16], further increased assay specificity. This assay did not detect hIL-6 added to serum at concentrations up to 10,000 pg/mL.

**KSHV quantitative real-time polymerase chain reaction (PCR).** DNA was extracted from PBMCs using the QIAamp DNA blood mini kit (QiaGen). DNA quality and concentration was assessed by optical density using Nanodrop1000 (Thermo Fisher Scientific).
Other assays. Serum hIL-6, IL-10, and 5 other cytokines (IL-1β, IL-8, IL-12 p70, interferon [IFN] γ, and tumor necrosis factor [TNF] α) were evaluated using the MSD 96-Well Multibead Proinflammatory 7-plex Assay and the Sector Imager (Meso-Scale Discovery). With this assay, the mean serum hIL-6 level (± standard deviation [SD]) in healthy donors was 2.3 ± 1.1 pg/mL [21]. Cross-reactivity of vIL-6 was tested in the Meso-Scale IL-6 assay by diluting vIL-6 in a protein containing kit diluent. The Meso-Scale hIL-6 assay did not detect vIL-6 at concentrations ranging from 0.6 pg/mL through 20,000 pg/mL. Results were confirmed with vIL-6 diluted in pooled normal donor serum, using serum with albumin human or hIL-6 as negative and positive controls. Analysis of IL12 p70 and IFN-γ was performed excluding values from 2 patients (MCD-like patient 3 and 1 control patient with KS) who were receiving IL12 on a research protocol, which would lead to an expected elevation in the levels of these 2 cytokines. CD4+ cell counts were assessed by fluorescent-activated cell sorting. Plasma HIV type 1 (HIV-1) RNA was measured by quantitative RNA PCR with use of Roche Amplicor HIV-1 Monitoring Kits (Roche Diagnostic Systems).

Statistical analysis. Patients were categorized as having MCD, MCD-like syndrome, severe KS, or mild KS. We hypothesized that vIL-6 levels would be elevated in the MCD-like syndrome group, compared with levels in the KS control group. We also analyzed KSHV viral load, hIL-6 level, and hIL-10 level. Fisher’s exact test was used to compare elevated vIL-6 level (>2850 pg/mL) and detectable HIV load between groups. Other cytokine and KSHV viral load comparisons were performed using an exact form of the Wilcoxon rank-sum test. Principal comparisons were between the MCD-like syndrome group and either the combined KS control group or the MCD group. Other than comparison of vIL-6 levels between patients in the MCD-like syndrome group and those with KS, statistical analyses were considered exploratory, with no formal correction for multiple comparisons. All P values ≤.01 were interpreted as statistically significant, P values ≤.05 but >.01 were considered to indicate strong trends, and P values >.05 were not considered to be statistically significant.

RESULTS

Patient characteristics. Patient characteristics are shown in Table 1. Patients in both MCD and MCD-like syndrome groups had low median sodium, albumin, and hemoglobin levels and low platelet counts. Plasma HIV was detectable in 4 of 5 patients with MCD-like syndrome for whom plasma HIV was measured at the time that vIL-6 levels were obtained. HIV was detectable in 2 of 7 patients in the MCD group and 22 of the 35 patients in the KS group were assayed. There was no statistically significant difference in HIV load between patients in the MCD-like syndrome group and those with KS, statistical analyses were considered exploratory, with no formal correction for multiple comparisons. All P values ≤.01 were interpreted as statistically significant, P values ≤.05 but >.01 were considered to indicate strong trends, and P values >.05 were not considered to be statistically significant.

The figure is available in its entirety in the online edition of Clinical Infectious Diseases.

Figure 5. Comparisons of interleukin 1b (IL-1b) between multicentric Castleman disease (MCD), MCD-like syndrome, and Kaposi sarcoma (KS) control groups. Comparisons of IL-1b between MCD (7 patients) MCD-like syndrome (6), severe KS (22), and mild KS (8) groups were performed using an exact form of the Wilcoxon rank-sum test. Values are log10 transformed pg/mL. P values are 2-sided. Horizontal lines indicate median values.
pg/mL) in all patients with MCD flares (median level, 8150 pg/mL; range, 4069–12,931 pg/mL) (Figure 1). Serum vIL-6 was also elevated in the original serum sample in 5 of the 6 patients with MCD-like symptoms (median level, 5678 pg/mL; range, <2850 to 39,835 pg/mL) and was not statistically different from those in the MCD group (P = .43, by Fisher’s exact test). One patient with MCD-like symptoms but initially undetectable vIL-6 had an elevated level (3307 pg/mL) 3 weeks later (Table 2). By contrast, only 5 of the 32 control patients with KS had detectable vIL-6. vIL-6 was significantly elevated in the MCD-like syndrome group, compared with the combined KS control groups (P = .003, Fishers exact test). These results demonstrate that patients with KSHV infection or KS, selected for otherwise unexplained MCD-like inflammatory symptoms, had high serum vIL-6 levels that were comparable to those in patients with an MCD flare. Interestingly, closer evaluation of clinical records showed that all 5 control patients with KS who had elevated vIL-6 levels had visceral KS, and 4 of these 5 patients had clinical abnormalities suggestive of MCD (hemoglobin level <10.0 g/dL in 2 patients, platelet count <150,000 platelets/µL in 1, and albumin level <3.1 g/dL in 1), although none had severe inflammatory symptoms or had been identified as being MCD-like prior to the measurement of vIL-6.

**KSHV viral load.** KSHV viral load was assessed in all but 2 patients with KS (who had inadequate stored material). All but 1 with MCD-like syndrome had quantifiable KSHV (median load, 31,900 copies per 10^6 cells), with levels comparable to those in symptomatic patients in the MCD group (median load, 87,400 copies per 10^6 cells; P = .49) (Figure 2). The MCD-like syndrome group had significantly more KSHV copies than did the combined KS groups (median load, 73 copies per 10^6 cells; P = .003). Interestingly, patients with severe KS had substantially more KSHV than did those with mild KS (P = .02).

**Serum hIL-6, IL-10, and other cytokines.** Serum hIL-6 was also elevated in patients with MCD-like syndrome (median level, 34.3 pg/mL) and was comparable to levels in those with KSHV-MCD flares (median level, 16.4 pg/mL; P = .57) (Figure 3). The hIL-6 levels in the MCD-like syndrome group were substantially greater than levels in the combined KS group (median level, 3.5 pg/mL; P = .003). Similarly, IL-10 levels were elevated in the MCD-like syndrome group (median level, 494 pg/mL), with levels comparable to those in the KSHV-MCD group (median level, 1275 pg/mL; P = .35) and significantly greater than levels in the combined KS group (median level, 4.7 pg/mL; P < .001) (Figure 4). The only other cytokines with levels modestly increased in the MCD-like syndrome group, compared with the combined KS groups, with a trend towards statistical significance, were IL-1β (median level, 1.51 vs 0.48 pg/mL; P = .011) and IFN-γ (median level, 2.62 vs 1.04 pg/mL; P = .046) (Figures 5–9; online only). There were no statistically significant differences between the MCD-like syndrome group and the combined KS group in the levels of IL-8, IL-12 p70, or TNFα.

**Follow-up of MCD-like syndrome patients.** The course of MCD-like syndrome patients is described in Table 2 and the Appendix, which appears only in the online version of the journal. None of the patients subsequently developed MCD. Three patients (patients 1, 3, and 5), each with extensive progressive KS, had persistent MCD-like symptoms and elevated vIL-6 and died during 3–60 months of follow-up. Causes of death were alveolar hemorrhage, progressive KS, and progressive KS complicated by infection. The other 3 patients (patients 2, 4, and 6), had resolution of their MCD-like symptoms and elevated vIL-6 levels when last seen after 4.8 years, 13.5 months, and 3.5 years, respectively. In addition to combination antiretroviral therapy (cART), patient 2 was treated for possible Lyme disease, patient 4 was treated with antibiotics for cellulitis and liposomal doxorubicin for severe KS, and patient 6 was treated with cART and valganciclovir for possible MCD-like syndrome.

**DISCUSSION**

KSHV-MCD is characterized histologically by KSHV-infected plasmablastic cells in the mantle zone of lymphoid organs [22]. These cells express the KSHV antigen LANA, are restricted for expression of lambda light chains, and often express KSHV vIL-6 and other KSHV lytic genes [5, 6, 23]. The clinical profile of patients with MCD is dominated by systemic inflammatory symptoms that are believed to be attributable to excessive production of KSHV vIL-6 by the plasmablasts, as well as over-

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**Figure 6.** Comparisons of interleukin 8 (IL-8) between multicentric Castileman disease (MCD), MCD-like syndrome, and Kaposi sarcoma (KS) control groups. Comparisons of IL-8 between MCD (8 patients), MCD-like syndrome (6), severe KS (22), and mild KS (8) groups were performed using an exact form of the Wilcoxon rank-sum test. Values are log10 transformed pg/mL. P values are 2-sided. Horizontal lines indicate median values.

**Figure 7.** Comparisons of interleukin 12p70 (IL-12p70) between multicentric Castileman disease (MCD), MCD-like syndrome, and Kaposi sarcoma (KS) control groups. Comparisons of IL-12p70 between MCD (8 patients), MCD-like (5), severe KS (21), and mild KS (8) groups were performed using an exact form of the Wilcoxon rank-sum test. Values are log10 transformed pg/mL. P values are 2-sided. Horizontal lines indicate median values.
We report here 6 patients with KSHV infection but without apparent MCD who exhibited symptomatology similar to that for MCD without other obvious etiology. Five of these patients had detectable serum vIL-6 when first tested (the sixth patient subsequently had elevated vIL-6), with vIL-6 levels in this group comparable to those in a control group with symptomatic KSHV-MCD. These patients also had elevated hIL-6 levels that were comparable to those in patients with KSHV-MCD flares, as well as increased IL-10 levels.

Some patients with KS (but without MCD or MCD-like symptoms) have previously been reported to have serum vIL-6 detectable with a somewhat different vIL-6 assay [16], and patients with HIV infection may have some elevations in serum hIL-6 and IL-10 levels [14, 24, 25]. However, the present study demonstrates that serum vIL-6, hIL-6, and IL-10 levels and KSHV viral load were significantly greater in patients with MCD-like symptoms than they were in an HIV-KS control group without inflammatory syndromes at time of evaluation and were similar to those in a group with symptomatic KSHV-MCD. Notably, we used a more specific assay for vIL-6 and employed a substantially higher cutoff value. Based on these findings, vIL-6 and hIL-6 overproduction appears likely to be responsible for at least part of the symptomatology in these patients with MCD-like syndrome. Overproduction of IL-10 or other cytokines may also contribute. Our conservative cutoff value for detection of vIL-6 (2850 pg/mL) does not exclude the possibility that patients could have vIL-6–induced symptomatology, even with vIL-6 levels below the limit of detection.

The question must be asked whether these patients had MCD. We were unable to diagnose MCD despite careful evaluation, and none of the patients subsequently developed MCD. If these patients represented cases of undiagnosed MCD, it would suggest that MCD frequently evades detection and that the incidence of KSHV-MCD is higher than is generally appreciated [1, 26]. A related question is whether increased cytokine levels and MCD-like symptoms may have resulted from other infectious processes. Four of the 6 patients had evidence of some bacterial infection, and their symptoms may have resulted, in part, from these infectious processes. However, increased vIL-6 levels suggest, at a minimum, that other infections may trigger the release of vIL-6 from KSHV-infected cells in patients with extensive KSHV involvement, which may cause or contribute to excessive inflammatory symptoms. Lytic activation of KSHV-infected cells through toll-like receptor signaling is one possible mechanism, although further study is needed to clarify this issue [27]. Finally, a patient in the MCD-like syndrome group (patient 4) had what appeared to be worsening of KS as part of immune reconstitution inflammatory syndrome (IRIS) a month after starting cART [28], and 2 others (patients 1 and 3) were within their first year of cART. It is possible that KSHV activation as part of IRIS can contribute to MCD-like syndrome in some patients.

All but 1 of the 6 patients with MCD-like syndrome had a high KSHV burden, manifested by extensive KS (3 patients) and/or a high viral load of PBMC KSHV (4 patients). Also, all 5 control patients with KS who had detectable vIL-6 had advanced KS with visceral involvement. vIL-6 is a lytic gene, and factors that may activate production of KSHV lytic genes in these patients include underlying cytokine dysregulation from uncontrolled HIV infection or other infections, poor immunologic control, or tissue hypoxia [24, 29–32]. KSHV activation in these patients may also have contributed to overproduction of hIL-6 and IL-10 [33–35].

Three patients with MCD-like syndrome had KS that progressed despite chemotherapy and contributed to their deaths. IL-6 can induce the growth of KS-derived spindle cells [36, 37], and we have noted that, in patients with KS and KSHV-MCD, KS is difficult to control until MCD is brought into clinical remission (R.L. and R.Y., unpublished observation). Substantial overproduction of vIL-6, hIL-6, and other cytokines in these patients likely contributes to tumor growth and resistance to therapy.

This study provides evidence that some patients with a high burden of KSHV may manifest a symptom complex related to IL-6 overproduction even though they do not have MCD. The patients reported here share a common physiologic mechanism for their symptomatology (elevated levels of vIL6, hIL-6, and possibly other cytokines directly or indirectly produced by KSHV), but they are heterogeneous with regard to the mechanism of lytic activation of KSHV and cytokine excess. Phy-

Figure 8. Comparisons of interferon (IFN) γ between multicentric Castleman disease (MCD), MCD-like syndrome, and Kaposi sarcoma (KS) control groups. Comparisons of IFNγ between MCD (8 patients) MCD-like syndrome (5), severe KS (21), and mild KS (n = 8) groups were performed using an exact form of the Wilcoxon rank-sum test. Values are log10 transformed pg/mL. P values are 2-sided. Horizontal lines indicate median values.

Figure 9. Comparisons of tumor necrosis factor (TNF) α between multicentric Castleman disease (MCD), MCD-like syndrome, and Kaposi sarcoma (KS) control groups. Comparisons of TNFα between MCD (8 patients) MCD-like syndrome (6), severe KS (22), and mild KS (8) groups were performed using an exact form of the Wilcoxon rank-sum test. Values are log10 transformed pg/mL. P values are 2-sided. Horizontal lines indicate median values.
sicians should be alert to the possibility that pathological vIL-6 (and hIL-6) overproduction in some patients with HIV and KSHV co-infection, especially those with a large burden of KS or KSHV, may contribute to severe inflammatory symptomatology. This syndrome is particularly worth considering in patients with advanced KS and MCD-like symptoms whose KS progresses despite chemotherapy. It may also possibly mimic or exacerbate manifestations of sepsis in patients with KS.

Additional studies are needed to better understand the pathogenesis of elevated vIL-6 and other cytokines in such patients and to develop diagnostic criteria and treatment strategies. Several approaches have been reported to have utility in MCD, including rituximab, interferon alpha, and ganciclovir [1, 38, 40], but it is unclear how these approaches will work in MCD-like syndrome. Agents like rituximab, for example, that target B cells may not be effective against vIL-6 produced by KS spindle cells. As we learn more about this syndrome, novel therapeutic approaches that address activation of KSHV lytic genes or target vIL-6 and hIL-6 overproduction [31, 38, 40] may be worth evaluating.

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