A Multiplex Panel of Plasma Markers of Immunity and Inflammation in Classical Kaposi Sarcoma

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Kaposi sarcoma (KS) risk is affected by perturbed immunity. Herein, we compared plasma from 15 human immunodeficiency virus (HIV)–negative classic KS cases to plasma from 29 matched controls, using a multiplex panel of immunity markers. Of 70 markers, CXCL10 (IP-10), sIL-1RII, sIL-2RA, and CCL3 (MIP-1A) were strongly and significantly associated with KS, after adjustment for age and smoking status. These and previous observations are consistent with a tumor-promoting role for these cytokines, particularly CXCL10, but the small sample size and case-control design preclude firm conclusions on KS risk or pathogenesis. Larger, well-designed prospective studies are needed to better assess the association of these markers with KS.

Keywords: classical Kaposi sarcoma; IP-10; sIL-1RII; human herpesvirus 8.

The primary cause of Kaposi sarcoma (KS) is infection with KS-associated herpesvirus (KSHV; also called human herpesvirus 8), but the immune perturbations associated with emergence of KS have not been fully characterized [1]. In population-based case-control studies, we reported previously that the risk of classic KS (KS) in Italy was significantly elevated with older age and male sex and, particularly, in nonsmokers [2]. We further found that classic KS cases differed from matched controls in common variants in immune-response genes [3]; in delayed-type hypersensitivity, especially in the legs where the disease typically originates [4]; and in T-cell responses to KSHV peptides [5]. Classic KS cases had significantly elevated serum levels of β2-microglobulin and neopterin in a case-control study in Greece [6], but elevations of plasma β2-microglobulin and neopterin were not statistically significant among Italian classic KS cases [7]. Other putative plasma markers of immunity or inflammation, such as levels of Epstein–Barr virus antibodies and of soluble CD26, CD23, and CD30, yielded ambiguous findings [8].

Herein, we used a newly developed and qualified multiplex immune marker panel [9] with plasma from classic KS cases and controls to further explore the association between circulating immune perturbations and classic KS.

METHODS

Study Participants

Recruitment and characteristics of study participants have been reported previously [2, 4]. Briefly, for a study of risk factors, cases of classic KS and population-based matched controls were enrolled from the entire island of Sicily from July 2002 through June 2006 [2]. In 2009, 44 participants (15 cases and 29 controls) were recalled for immunologic studies [4, 5]. Histopathologically confirmed KS originated on the skin in all cases (foot/leg in 14 and trunk in 1); 1 case developed metastatic KS on the arm. Nine cases currently had no KS lesions, 3 had 1–2 lesions, and 3 had approximately 20 lesions [4]. Controls without KS were recruited from physicians’ rosters within the study area, using 2-stage random selection methods and matching by age. All subjects provided signed informed consent and tested negative for human immunodeficiency virus antibody. Ethical approval was obtained from the US National Cancer Institute, the University of Palermo, and the coordinating center. Subjects were interviewed using a standardized questionnaire about demographic characteristics, socioeconomic variables, medical history, and smoking status. Participants were defined as smokers if they had smoked at least 1 cigarette per week; otherwise, they were classified as nonsmokers.

Serology

An ethylenediaminetetraacetic acid–anticoagulated blood sample was obtained and chilled (at approximately 4°C) until centrifugation and aliquotting within 2 hours. Aliquots of plasma were stored at −80°C until testing. As reported previously
KSHV antibodies in plasma were detected by immunofluorescence assay (IFA), performed at a 1:120 dilution with uninduced BCBL-1 cells, plus an enzyme immunosassay with recombinant K8.1 structural glycoprotein at a 1:20 plasma dilution. Subjects were considered KSHV seropositive if they had uninduced IFA positivity or an K8.1 optical density of >1.2. KSHV-seronegative patients had uninduced IFA negativity plus a K8.1 optical density ≤1.2.

**Immune Markers**

We used Luminex bead-based assays (Millipore, Billerica, MA) to measure levels of 71 markers, most related to inflammation or immunity, based on satisfactory performance and reproducibility in plasma (detectability, ≥10%; coefficients of variation, <20%) [9]. Concentrations were calculated using either a 4- or 5-parameter standard curve. Samples were assayed in duplicate and averaged to calculate concentrations. One marker (sIL-1RI) with <10% detectability was excluded from analysis.

**Statistical Analyses**

Markers detected in <25% of subjects were compared as detectable/nondetectable. For markers detected in ≥25% of subjects, tertiles of plasma levels were constructed using levels in all 44 subjects. Cases and controls were compared for age and marker levels, using the Student t test and the Mann–Whitney U test, respectively. Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of the association between immune markers and classic KS and to determine a trend in the ORs for classic KS across tertile levels, without and with adjustment for age and smoking status. $P_{\text{trend}}$ were estimated by treating categorized markers as ordinal variables. We did not adjust for sex because all participants except 1 case were male. In a sensitivity analysis (not presented), exclusion of the female case had no substantial effect on the results presented. Analyses of the associations between classic KS and natural log–transformed continuous marker levels were also performed using logistic regression. Two-sided $P$ values of <.05 were considered statistically significant. Statistical analyses were performed using Stata (version 13, StataCorp, College Station, TX).

**RESULTS**

We compared categorized levels of 59 high-prevalence plasma markers of immunity and inflammation, as well as detection/nondetection of 11 lower-prevalence markers, in 15 classic KS cases and 29 controls without classic KS. Age distributions were similar in cases and controls and in smokers and nonsmokers (data not shown). Four markers met nominal statistical significance for association with classic KS when adjusted for age and smoking status ($P_{\text{trend}} \leq .05$; Supplementary Table 1). Median levels of these classic KS–associated markers were higher in cases than in controls (Figure 1A), whereas median levels did not differ between smokers and nonsmokers (Figure 1B).

Figure 1. Comparison of median levels of markers by case or control (A) and smoking status (B). $P$ values (by the Mann–Whitney U test) indicate difference in marker levels between cases and controls (A) and between smokers and nonsmokers (B). Note the different scales along the y-axes for each marker.
The 4 classic KS-associated markers are shown in Table 1. Age- and smoking-adjusted, highest-tertile versus lowest-tertile ORs were as follows: for CXCL10, adjusted OR, 7.8 (95% CI, 1.0–62.6); for sIL-1RII, adjusted OR, 8.0 (95% CI, 1.2–54.3); for sIL-2RA, adjusted OR, 12.9 (95% CI, 1.3–129.4); and for CCL3, adjusted OR, 14.4 (95% CI, 1.4–144.7). The associations for middle- versus lowest-tertile ranged from an adjusted OR of 3.6 to an adjusted OR of 10.4. On a continuous log-transformed scale (Supplementary Table 2), the association with classic KS was strongest with CXCL10 (adjusted OR, 28.8/log; \( P_{\text{trend}} = .01 \)) followed by sIL-1RII (adjusted OR, 21.5/log; \( P_{\text{trend}} = .04 \)), modest with CCL3 (adjusted OR, 14.9/log; \( P_{\text{trend}} = .02 \)), and not significant with sIL-2RA (adjusted OR, 14.0/log; \( P_{\text{trend}} = .10 \)).

KSHV antibody serostatus among controls (14 positive and 15 negative) was not associated with any of these 4 classic KS-associated markers (Supplementary Table 3).

**DISCUSSION**

KS risk is profoundly sensitive to perturbations of immunity, which resulted in the epidemic of HIV-associated AIDS KS, but the multiple immune defects that underlie KS risk have not been firmly established [1]. The current study found that classic (ie, non–AIDS related) KS risk was significantly increased with high levels of 4 circulating immune mediators, CXCL10, sIL-1RII, sIL-2RA, and CCL3, each of which could reflect host response to the tumor or, possibly, play a role in KS pathogenesis.

Individuals with high levels of CXCL10 (also called interferon-\( \gamma \)-induced protein 10) were approximately 8 times as likely to have classic KS than those with low CXCL10 levels. CXCL10 is a proinflammatory pleiotropic chemokine that is induced by KSHV infection and that interacts with the KSHV G protein-coupled receptor to stimulate migration of infected endothelial cells [10]. Elevation of serum CXCL10 levels has also been observed in patients with HIV-associated KS [11]. Thus, CXCL10 might contribute to KS pathogenesis and/or might merely reflect host response to an established tumor.

The classic KS cases were approximately 8 times as likely as controls to have high levels of sIL-1RII. These results were unexpected given that sIL-1RII is a decoy that binds interleukin 1 (IL-1) and reduces its strongly proinflammatory activity [12]. Cancer cells exhibit constitutive production of IL-1\( \beta \) protein, to which sIL-1RII preferentially binds [12]. Therefore, the high levels of sIL-1RII observed in the current study could have been a response to high levels of IL-1\( \beta \) in the classic KS cases. If so, then the association with sIL-1RII would be a consequence and not a cause of KS.

sIL-2RA, a protein expressed on the surface of certain immune cells, including lymphocytes, also was strongly associated with classic KS. It binds and mediates the activity of interleukin 2, and it is induced and expressed only following activation of mononuclear cells, including T cells, B cells, monocytes, and natural killer cells [13]. Increased serum sIL-2RA levels were reported in patients with lymphoid malignancies and associated with lower survival in patients with non-Hodgkin lymphoma [13].

### Table 1. Crude and Adjusted Odds Ratios (ORs) for Associations of Markers With Classic Kaposi Sarcoma, by Case or Control Status and Smoking Status

| Marker | Tertile | Cases, no. | Controls, no. | Crude OR (95% CI) | \( P_{\text{trend}} \) | Adjusted OR (95% CI)\( ^a \) | \( P_{\text{trend}} \) |
|--------|--------|-----------|---------------|-------------------|----------------|--------------------------|----------------|
| CXCL10 | 1      | 2         | 12            | Reference         | .03            | Reference                 | .05            |
|        | 2      | 5         | 10            | 3.0 (0.5–18.9)    |                | 3.6 (0.5–26.3)            |                |
|        | 3      | 8         | 7             | 6.9 (1.1–41.8)    |                | 7.8 (1.0–62.6)            |                |
| sIL-1RII| 1      | 2         | 12            | Reference         | .03            | Reference                 | .03            |
|        | 2      | 5         | 10            | 3.0 (0.5–18.9)    |                | 2.8 (0.4–18.7)            |                |
|        | 3      | 8         | 7             | 6.9 (1.1–41.8)    |                | 8.0 (1.2–54.3)            |                |
| sIL-2RA| 1      | 1         | 13            | Reference         | .01            | Reference                 | .02            |
|        | 2      | 6         | 9             | 8.7 (0.9–84.8)    |                | 8.7 (0.9–87.0)            |                |
|        | 3      | 8         | 7             | 14.9 (1.5–144.2)  |                | 12.9 (1.3–129.4)          |                |
| CCL3   | 1      | 1         | 14            | Reference         | .02            | Reference                 | .02            |
|        | 2      | 6         | 7             | 12.0 (1.2–120.1)  |                | 10.4 (1.0–107.2)          |                |
|        | 3      | 8         | 8             | 14.0 (1.5–133.2)  |                | 14.4 (1.4–144.7)          |                |

*Abbreviation: CI, confidence interval.*

\( ^a \) Adjusted for smoking status and age.
The chemokine CCL3 (also known as macrophage inflammatory protein-1α) is associated with the synthesis and release of proinflammatory cytokines such as IL-1, interleukin 6, and tumor necrosis factor α from fibroblasts and macrophages. Elevated serum CCL3 levels occur in patients with multiple myeloma [14], but it has not been reported previously with KS.

Inflammatory cytokines have been shown to reactivate KSHV lytic replication and might potentially increase risk of KS development [15], but it is unknown whether high plasma levels of cytokines, chemokines, or their receptors are markers of classic KS risk or activity. Given that KS cases have elevated KSHV antibody titers and viral load in the peripheral blood [1], the higher levels of markers observed in classic KS cases may reflect an immune response to the KS tumor or its related burden of KSHV infection and expression.

Like most studies of conditions that are rare, the power of this study was limited by the small sample size. And because classic KS is primarily a disease of elderly men, we did not have enough women to analyze or adjust for sex. Another limitation is that the associations observed would withstand statistical adjustment for multiple comparisons with the 70 markers in the panel.

In summary, we are the first to report elevated plasma levels of CXCL10, sIL-1R1, sIL-2RA, and CCL3 in classic KS cases, compared with controls. All 4 markers should be assessed for possible diagnostic, prognostic, or etiologic importance for KS in larger, prospective studies, including those involving HIV-infected patients with AIDS-related KS [11].

### Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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