Interleukin 8 Is an Autocrine Growth Factor and a Surrogate Marker for Kaposi’s Sarcoma

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ABSTRACT
Kaposi’s sarcoma (KS) is the most common tumor associated with HIV-1 infection. Here, we report the expression, regulation, and biological effect of interleukin (IL)-8 in KS. AIDS-KS cell lines expressed higher levels of IL-8 than either human umbilical vein endothelial cells (HUVECs), human aortic smooth muscle (AoSM) cells or fibroblast cells (T1). The inflammatory cytokine IL-1β up-regulated IL-8 expression in a time- and concentration-dependent manner in KS cell lines. IL-8 antisense oligonucleotides specifically reduced IL-8 mRNA and protein levels and inhibited KS cell growth in a dose-dependent manner. In addition, supernatant from a KS cell line induced the growth of HUVECs and angiogenesis in chicken chorioallantoic membrane assays, both of which were inhibited by IL-8 neutralizing antibody. Serum levels of IL-8 were also elevated in KS cases compared with matched controls. Modulation of IL-8 may thus be of therapeutic value in this disease.

INTRODUCTION
KS is the most common tumor associated with HIV-1 infection (1). The tumor frequently involves the skin and mucous membranes and can lead to tumor-associated edema and ulceration. Visceral involvement occurs in nearly one-third of KS patients and can lead to death (1).

A number of growth factors have been shown to regulate the growth of cells derived from AIDS-KS tumors. Transforming growth factor-β is an autocrine inhibitory factor (2), whereas IL-1β, IL-6, oncostatin-M, platelet-derived growth factor, bFGF, and VEGF promote cell growth (3-12). bFGF and VEGF are also angiogenic factors that can be induced by IL-1β and IL-6 (13-15). In addition, the HIV-tat protein binds to VEGF receptor to induce KS cell growth (16).

KSHV/HHV-8 is associated with KS, even in the HIV-negative classic form of the disease (17, 18). Nearly all KS tumor biopsies show evidence of viral KSHV/HHV-8 DNA, compared with low or undetectable levels in a variety of other tissues from the same patients. Several genes induced during the lytic phase of the virus have potential roles in KS pathogenesis and include vGPCRs, cyclin D, FLIP, MIP-1, and IL-6 (19-29). vGPCR transforms the target cells and is constitutively activated (30). Furthermore, vGPCR transgenic mice develop angiogenic tumors (31). Both transformed cell lines and the transgenic mice show increased production of VEGF, which is a mitogen for both endothelial cells and KS cells (12, 32). KSHV/HHV-8-infected endothelial cells similarly show induction of VEGF and VEGF receptors (33), and inhibition of VEGF leads to inhibition of cell growth. It is thus clear that VEGF plays an important role in HHV-8-mediated effects. However, it is highly likely that other cellular genes are also regulated by HHV-8 infection and specifically by the expression of vGPCR. IL-8 has been shown to bind to vGPCR and enhance its activity (34). We thus studied the effect of IL-8 on KS cell and measured the serum levels of IL-8 of a prospectively controlled study of classic KS and their controls.

IL-8 is a member of a family of related proinflammatory cytokines that has a molecular mass of ~10 kDa. IL-8 is synthesized and secreted by lipopolysaccharide-stimulated monocytes, macrophages, and by numerous other nonleukocytic cell types, including epithelial and endothelial cells, lymphocytes, and keratinocytes (35-37). IL-8 was initially identified as a chemoattractant for neutrophils but not monocytes (38) and has been shown to activate neutrophils in inflammatory sites (39). IL-8 has also been shown to induce angiogenesis (40). In nude mice xenografts, IL-8 expression correlates directly with metastatic potential in a variety of tumors (41). However, the expression of IL-8 depends on the organ microenvironment (42).

IL-8 receptors belong to the superfamily of seven transmembrane, G protein-coupled receptors. Two types of IL-8 receptors have been reported, IL-8R-A and IL-8R-B, also called CXCR1 and CXCR2, respectively. These two receptors share 77% amino acid identity. IL-8 receptors are found on neutrophils, basophils, T lymphocytes, monocytes, and keratinocytes. Both receptors are also expressed on endothelial cells (43).

Here, we report that: (a) IL-8 and IL-8 receptors are expressed in KS cells and primary tumor tissue; (b) inhibition of IL-8 leads to inhibition of KS cell growth; and (c) supernatants of KS cells exert a mitogenic effect on endothelial cells and induce angiogenesis, both of which are blocked by IL-8 antibodies. In addition, serum IL-8 levels are significantly elevated.

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2 To whom requests for reprints should be addressed, at USC/Norris Comprehensive Cancer Center, Room 3458, 1441 Eastlake Avenue, Los Angeles, CA 90089-9172. E-mail: masood@hsc.usc.edu.
3 The abbreviations used are: KS, Kaposi’s sarcoma; IL, interleukin; rhIL, recombinant human IL; IL-8, IL-8 sense; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; KSHV/HHV-8, KS-associated herpes virus/human herpes virus-8; vGPCR, viral homologue of G protein-coupled receptor; CAM, chorioallantoic membrane; HUVEC, human umbilical vein endothelial cell; AoSM, aortic smooth muscle; AS, antisense; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RT-PCR, reverse transcription-PCR.
in patients with KS compared with controls and thus may serve as a surrogate marker. Modulation of IL-8 may thus be important in KS, especially when combined with VEGF inhibitors.

**MATERIALS AND METHODS**

**Cells.** KS cell lines were grown on gelatin-coated plates in culture medium containing RPMI 1640, 2% FCS, 1% sodium pyruvate, 1% essential amino acids, 1% nonessential amino acids, 1 mM glutamine, and 1% penicillin-streptomycin (Life Technologies, Inc., Gaithersburg, MD). Long-term spindle isolates (KSC10, KSC29, KSC-59, KSC13, and KS-38) were also established from KS lesions of AIDS-KS patients as described previously (44). These isolates have been maintained in RPMI supplemented with 15% FCS, 2 mM glutamine, 0.5% sodium pyruvate, 1% essential amino acids, 0.5% nonessential amino acids, 1 mM sodium pyruvate, and 1% Nutridoma HU (Boehringer Mannheim, Indianapolis, IN) in the absence of conditioned medium from transformed T-cell lines (45). KS-SLK cells (46) express VEGFR-2 and vascular smooth muscle cell-specific endothelial cells markers (such as CD31, Tie-1, Tie-2, and CD34) from transformed T-cell lines (45). KS-SLK cells (46) express VEGFR-2 and vascular smooth muscle cell-specific endothelial cells markers (such as CD31, Tie-1, Tie-2, and CD34).

**Chemokines and Antibodies.** Human recombinant IL-1β, IL-4, IL-6, IL-8, IL-8 ELISA kits, human IL-8 monoclonal neutralizing antibody (clone 6217.111), and oncostatin-M neutralizing polyclonal antibody were purchased from R&D Systems (Minneapolis, MN). Monoclonal antibodies to IL-8 receptors A and B (CXCR1 and CXCR2; clones B-1 and E-2, respectively) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz Biotechnology, CA). Glucocorticoid was purchased from Sigma Chemical Co. (St. Louis, MO).

**Northern Blot.** Total RNA was extracted from several AIDS-KS cell lines, HUVEC, AoSM, and T1 by guanidine isothiocyanate (RNAzol; Tel-Test, Inc., Friendswood, TX). An aliquot (15 μg) of the total RNA from each sample was electrophoresed in a 1% agarose formamide gel and transferred to nylon membranes. A probe for human IL-8 was generated by PCR amplification of the region corresponding to nucleotides −3 to +377 of the coding region of IL-8 with primers as shown in Table 1. The amplified IL-8 product was subcloned in the TA cloning vector (Invitrogen, Carlsbad, CA) to give pcRII IL-8. The IL-8 plasmid was digested with EcoRI, and the IL-8 insert was further purified using a QiAquick Gel extraction kit (Qiagen, Santa Clarita, CA). Radiolabeled IL-8 DNA probe was prepared using a nick translation kit (Life Technologies, Inc.) with [α-32P]dCTP (3000 Ci/mmol; DuPont, Boston, MA). RNA blots were sequentially hybridized to IL-8 and β-actin probes. The radiolabeled signal was also quantitated by using the Molecular Dynamics phosphorimager 445SI (Sunnyvale, CA).

**Cell Proliferation Assay.** AIDS-KS cells, HUVECs, and AoSM cells were seeded at a density of 1 × 10⁴ cells/well in gelatin-coated, 24-well plates on day 0. The cells were then treated with various concentrations of oligonucleotides ranging from 1 to 10 μM or with IL-8 monoclonal neutralizing antibody (10–1000 ng/ml) on days 1 and 3. Cell proliferation was measured on day 5 using MTT at a final concentration of 0.5 mg/ml. Cells were incubated for 2 h, medium was aspirated, and the cells were dissolved in acidic isopropanol (90% isopropanol, 0.5% SDS, and 40 mM HCl). Developed color was read in an ELISA reader at 490 nm using the isopropanol as blank (Molecular Devices, Sunnyvale, CA). The assays were performed twice in quadruplicate. These experiments were repeated in AIDS-KS cell lines with or without the addition of rhIL-8 (10 ng/ml). For HUVEC growth studies, HUVECs (2 × 10⁵) were seeded on gelatin-coated, 24-well plates in endothelial cell me-

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dium as described earlier. The next day, the medium was replaced by KS culture supernatant with and without various concentrations of IL-8 antibody. Cell proliferation was measured on day five.

**ELISA for Human IL-8.** IL-8 levels in culture supernatants were determined by a solid phase double-ligand ELISA obtained from R&D Systems. A monoclonal antibody specific for IL-8 was precoated onto a microtiter plate. Standards, samples, and IL-8 conjugate to horseradish peroxidase were pipetted into the wells, and any IL-8 present was sandwiched by the immobilized antibody and the enzyme-linked polyclonal antibody specific for IL-8. After removal of excess of any unbound substances and/or antibody-enzyme reagent, a substrate solution (hydrogen peroxide and chromogen tetramethylbenzidine) was added to the wells, and color was developed in proportion to the amount of IL-8 bound. The color development was stopped after 30 min at room temperature, and the intensity of the color was measured at 450 nm in a microplate reader (Molecular Devices).

**RT-PCR for IL-8 and IL-8 Receptors.** KS cells (1 × 10^5 cells/well) were treated with various concentrations (0, 1, 5, and 10 μM) of IL-8-AS and IL-8-S oligonucleotides on days 1 and 2. Cells were harvested on day 3, and total RNA was extracted. cDNAs were synthesized by reverse transcriptase using a random hexamer primer in a total volume of 20 μl (Superscript; Life Technologies, Inc.). Five μl of the cDNA reaction were used for PCR. Primer pairs for amplification of IL-8, CXCR1, and CXCR2 are shown in Table 1. Each PCR cycle consisted of denaturation at 94°C for 1 min, primer annealing at 60°C for 2 min, and extension at 72°C for 3 min. The samples were amplified for 35 cycles. Amplified products were visualized on a 1.5% agarose gel containing ethidium bromide. RT-PCR for β-actin was performed to determine the integrity and quantity of RNA.

**Immunohistochemistry.** Cultured KS, HUVECs, and fibroblast cells were trypsinized and centrifuged onto glass slides using a Cytospin centrifuge (Shandon, Astmoor, United Kingdom) and fixed in 1% paraformaldehyde in PBS for 30 min. The slides were then washed twice in PBS and incubated with 0.2% Triton X-100 in PBS and 1% FCS for 20 min. The slides were then preincubated with 50% FCS for 30 min and again with monoclonal antibodies to CXCR1 and CXCR2 with 1:25 dilution in PBS and 10% FCS for 45 min at 37°C. After washing three times, the slides were incubated with secondary anti-IgG mouse antibody. Tissue sections were also stained using the same antibodies by standard methods.

**CAM Assay.** Chicken CAM assay was used to test the effect of KS cells supernatants (5 × 10^3 and 10 × 10^3) with or without neutralizing monoclonal IL-8 antibody (1 μg/ml) on angiogenesis. Ten-day-old fertilized chicken eggs were prepared by creating a window, and filter paper discs saturated with test substances were placed on the CAM. VEGF (400 ng) was used as positive control. Concentrated KS cell supernatants alone or with neutralizing IL-8 antibody or an equal amount of carrier buffer made up the test group and negative controls, respectively. CAMs were harvested after 48 h and analyzed using an Olympus stereomicroscope. The number of new branching blood vessels infiltrating under the disc was counted and photographed. Eight CAMs were studied for each test group, and the experiments were repeated twice. Blood vessels counts were performed, and the results represent the mean ± SD of an experiment performed in duplicate.

**Serum Collection from Cases with Classic KS and Controls.** Cases of classic KS (i.e., HIV-negative cases) were identified from the Cancer Surveillance Program in Los Angeles County between 1989–1993. For each classic case, one neighborhood control matched on age, race, and sex was sought. Serum from 23 patients with classic KS and 28 control cases were tested for various cytokines.

**In Vivo Studies.** Human KS tumor cell line KS-SLK (2 × 10^6 cells) was injected s.c. in the lower back of 5-week-old male BALB/c Nu−/Nu− athymic mice. Twelve mice were randomized into two groups of 6 mice. The experimental group received IL-8 monoclonal antibody (100 μg/mouse, i.p.) on days 4, 8, and 11. Controls received an equal volume (100 μl) of PBS. The length (L) and width (W) of tumors were measured three times a week using calipers. Tumor volume was calculated with the formula V = 0.52LW^2. Mice were sacrificed at the conclusion of the study. All mice were maintained in accordance with the University of Southern California institutional guidelines governing the care of laboratory mice.

**RESULTS**

**KS Cell Lines Express High Levels of IL-8.** We examined expression of IL-8-specific mRNA in several AIDS-KS cell lines (KS Y-1, KSC10, KSC29, KSC59, KSC13, and KS-38). Fig. 1A shows that a single band for IL-8 mRNA transcripts was expressed at high levels in all AIDS-KS cell lines. HUVECs and AoSM cells also expressed very low levels of IL-8 mRNA compared with all KS cell lines; a fibroblast cell line (T1) expressed the lowest levels of IL-8 mRNA. Supernatants from equal numbers of five KS cell lines, an HTLV-II transformed T-cell line 38–10 (45), and T1 fibroblasts were also examined for IL-8 protein levels. The levels of IL-8 protein were substantially higher in AIDS-KS cells (KS Y-1, KS-SLK, KSC-10, KSC13, and KSC59) in comparison with levels in 38–10, HUVEC, and T1 cell lines (Fig. 1B).

**KS Cells and Tissues Express CXCR1 and CXCR2.** Expression of both IL-8 receptors was examined by immunohistochemistry in KS cell lines. A high expression of CXCR1 was observed in KS cells compared with CXCR2, as shown in Fig. 2A. Early passage of HUVECs showed a low level of both IL-8 receptors. Expression of either IL-8 receptor was undetectable in T1 fibroblasts (data not shown). Three KS cell lines (KS Y-1, KS-SLK, and KSC-59) and HUVECs were also examined for CXCR1 and CXCR2 expression by RT-PCR. All three KS cell lines showed high expression of both IL-8 receptors. HUVECs expressed both IL-8 receptors, but the level of expression of both CXCR1 and CXCR2 in HUVECs was lower than in KS cells (Fig. 2B).

We also examined the expression of CXCR1 and CXCR2 in KS biopsies and skin biopsies of the same patients. Immunohistochemistry showed high levels CXCR1 in the KS biopsies from three patients. Cells expressing the receptor revealed spindle-shaped morphology characteristic of the KS cells. (Fig. 2A). The skin biopsies from the same patients did not show the expression of either IL-8 receptor (data not shown). The data
IL-8 Activity in Kaposi’s Sarcoma

Fig. 1  IL-8 is expressed in AIDS-KS cells. A, Northern blot of total RNA (15 µg) from several AIDS-KS cell lines, HUVEC, AoSM, and T1 human fibroblasts. AIDS-KS cell lines: KS Y-1, KSC10, KSC29, KSC59, KSC13, and KS-38. Shown is the same membranes hybridized to the human IL-8 probe, stripped and re-probed with the β-actin probe. The β-actin signal shows integrity of RNA and equivalent loading of samples. B, IL-8 protein levels in 48-h supernatants from equal numbers of KS Y-1, KS-SLK, KSC10, KSC13, KSC59, 38–10, and T1 cells cultured in RPMI and 10% FCS. Supernatants were analyzed for IL-8 protein by ELISA.

from cell lines were consistent with that of the primary tumor tissue, thus validating the in vitro studies.

IL-1β Up-Regulates IL-8 Expression in KS Cells. A number of growth factors have been shown to regulate the growth of KS cells. We investigated whether growth factors (oncostatin-M), cytokines (IL-1β, IL-4, and IL-6), or glucocorticoids have any role in the up-regulation of IL-8 expression in KS cells. KS cells were incubated in medium alone or medium containing 10 ng/ml of either recombinant IL-1β, IL-4, or IL-6 for 24 h, and culture supernatants were analyzed for IL-8 protein by ELISA. IL-1β treatment of KS cells significantly enhanced IL-8 production (40-fold) compared with KS cells incubated with medium alone (Fig. 3, control). KS cells treated with other growth factors (IL-4, IL-6, oncostatin-M, and hydrocortisone) did not show a significant effect on the expression of IL-8.

Effect of IL-8-AS Oligonucleotides on AIDS-KS Cell Growth. IL-8-AS corresponding to IL-8 cDNA was tested for activity against AIDS-KS cell growth at several concentrations. The growth of two KS cell lines (KSC-59 and KS-SLK) was inhibited by IL-8-AS in a dose-dependent manner (Fig. 4A). The IC50 of IL-8-AS oligonucleotides were 3.2 µM in KSC-59 and 4.4 µM in KS-SLK. IL-8-S used as a control had minimal inhibitory effect on the growth of both KS cell lines (Fig. 4A). We have also examined the effect of IL-8-AS and IL-8-S on the growth of various control cell lines. No growth-regulatory effects were observed on fibroblast (T1), 23-1 (B lymphoma cell line), or HUVECs with IL-8-AS (data not shown). IL-8-S had no significant effect on any of the cell types examined (data not shown).

Exogenous rhIL-8 Blocks IL-8-AS Effects on AIDS-KS Cell Growth. To demonstrate the specificity of the IL-8-AS oligonucleotide on KS cell growth, KS cells were treated with IL-8-AS alone or in combination with rhIL-8. The dose-dependent inhibition of KS cell growth mediated by IL-8-AS was blocked by addition of exogenous recombinant IL-8. These data confirm the specificity of the IL-8-AS oligonucleotides (Fig. 4A).

Effect of IL-8-AS on IL-8 mRNA Level. IL-8 mRNA was also measured by RT-PCR in KS cells treated with various concentrations of IL-8-AS and IL-8-S oligonucleotides. The PCR product of cycles ranging from 20 to 32 cycles was analyzed by agarose gel electrophoresis. There was a significant inhibition of IL-8 mRNA after treatment with IL-8-AS at a concentration of 5 µM in KS cells. In contrast, IL-8-S had no effect of the expression of IL-8 (Fig. 4B).

Effect of IL-8-AS on IL-8 Protein. IL-8 protein levels in the supernatants of KS-SLK cells treated with various concentrations of IL-8-AS and IL-8-S oligonucleotides for 24 h were determined by ELISA. IL-8 protein decreased in a dose-dependent manner in cells treated with IL-8-AS (Fig. 4C). No appreciable effect on IL-8 was seen when the cells were treated with IL-8-S.

IL-8 Antibody Inhibits KS Cell Proliferation. The requirement of IL-8 as a growth factor for KS cells was confirmed using IL-8 neutralizing monoclonal antibody. Dose-dependent inhibition of cell growth was observed in the KS-SLK cell line in response to incubation with IL-8 monoclonal antibody (Fig. 4D). The same line had shown growth inhibition with IL-8-AS. Unrelated polyclonal antibody (to perforin) had no effect on the viability of KS-SLK cells.

KS Cell Supernatant Induces the Growth of HUVECs. HUVECs in culture require growth supplements to survive. KS spindle cell isolates share many endothelial cell markers yet do not require supplements for growth. We determined whether KS cells produce factors that support the growth of nontransformed HUVECs. HUVECs cultured in the presence of KS cell supernatant used as conditioned medium showed a 40% increase in growth compared with control (Fig. 5). Because IL-8 is produced by the KS cells, we tested whether IL-8 contributed to the growth of HUVECs by using IL-8 neutralizing antibodies. Induction of HUVEC growth was blocked by the presence of IL-8 antibody in the KS cell conditioned medium but not by antibody to IL-10 (Fig. 5).

IL-8 Antibody Inhibits KS Cell Supernatant-induced Angiogenesis in CAM Assays. To further investigate the angiogenic activity in KS cells supernatants, CAM assays were performed. Induction of angiogenesis by KS cell supernatants was seen, and with 10×-concentrated supernatant was as effective as the positive control of 400 ng of VEGF (Fig. 6). IL-8 neutralizing antibody (1 µg disc) was able to fully block formation of blood vessels in the presence of 5×-concentrated supernatant and partially block the effect of 10×-concentrated supernatant. This is consistent with the presence and angiogenic activity of IL-8 in the KS cell supernatants. VEGF is also
produced in significant amounts by KS cells (12) and could account for the residual angiogenesis in the presence of IL-8 neutralizing antibody in the 10× KS cell supernatant.

Serum Levels of IL-8 Were Higher in KS Than Controls.
We also measured serum IL-8 levels from patients with the classic form of KS and controls. KS patients showed significantly higher levels of IL-8, mean value 142.6 pg/ml. In contrast, the levels of IL-8 in controls were significantly lower, mean value 70.8 pg/ml (Table 2). Similarly, the levels of VEGF were higher in KS than the controls, whereas the serum IL-6 levels were similar in both groups. These results are in agreement with the in vitro data for both VEGF and IL-8 but not IL-6. These results are the first to show that among various cellular factors associated with KS, IL-8 and VEGF are elevated in the serum in addition to locally in the tumor tissue.

IL-8 Monoclonal Antibody Inhibits in Vivo KS Tumor Growth.
KS-SLK grown as a s.c. tumor xenografts in BALB/c nude mice is sensitive to IL-8 ablation. The growth of established tumors was inhibited by twice-weekly treatment of the mice with neutralizing monoclonal antibody to human IL-8 (Fig. 7). After three treatments with IL-8 antibody, mean tumor volume was 33% of controls, a result that was significant (P = 0.027). The differences between the tumor sizes of IL-8 antibody-treated mice and controls became significant after the second dose of antibody (P = 0.014). These data support the in vivo requirement for IL-8 in KS tumor growth.

DISCUSSION
There is substantial information on the role of cytokines and chemokines in the etiology and pathogenesis of KS (3–12). A notable feature of the histology of KS is the marked infiltration with mononuclear cells, which suggests the role of chemokines in its pathogenesis. IL-8 was originally isolated as a neutrophil-activating protein (47) and is therefore likely to be containing ethidium bromide and photographed. RT-PCR for β-actin is included to show the equivalence and integrity of the input RNA. Specific bands for CXCR1 and CXCR2 are seen in all cells tested, with a relatively lower level expression of both receptors in HUVECs.
important to the KS disease state. In addition, two other features of the disease suggest other roles for IL-8. Angiogenesis is known to play an important role in KS. Lesions are characterized by dense vascularization, and the tumor (spindle) cells express many important endothelial markers (48, 49). We have shown that inhibition of the most potent angiogenic factor, VEGF, can lead to KS cell death in vitro and strong inhibition of tumor growth in murine xenografts (12). IL-8 is a CXC chemokine that contains the ELR motif and acts as a proangiogenic factor (43). This chemokine may therefore contribute to KS lesion development through its angiogenic activity. In addition, the gamma herpesvirus KSHV/HHV-8 has been implicated in the etiology of KS (17, 18). The product of HHV-8 ORF74, vGPCR is a constitutively activated CXC chemokine receptor that is capable of transforming certain cells (30). It is of particular interest then, that IL-8 acts a ligand and enhances...
Our finding that IL-8 is expressed in KS is in agreement with Sciacca et al. (50). We also show the significance of IL-8 in KS by demonstrating the expression of the receptors CXCR-1 and CXCR-2 in cell lines and primary tumor tissue (51). Expression of the IL-8-specific CXCR-1 was clearly detectable in the spindle cells of primary tumor tissues and KS cells in vitro, whereas expression of the broader specificity CXCR-2 was barely seen in the biopsies but was clearly present in cultured KS cells, as shown by immunohistochemistry and RT-PCR. We also have shown a function for IL-8 in KS cells in vitro. IL-8 ablation by specific AS oligonucleotides inhibited the proliferation of KS cells. Failure of the KS cells to proliferate in the presence of IL-8-AS but not sense oligodeoxynucleotide could be directly attributed to a concurrent decrease in IL-8 mRNA and protein. Because the addition of exogenous recombinant IL-8 rescued cell growth, we conclude that the growth inhibition seen in response to IL-8-AS was attributable to an AS-induced decrease in IL-8 protein levels. The presence of IL-8 and its receptors CXCR-1 and CXCR-2 in KS cells, together with the demonstration that KS cell growth is inhibited by ablation of IL-8, demonstrated that this chemokine is an autocrine growth factor for KS. The presence of the same cellular machinery in vivo, as seen in the immunostaining of KS biopsies, indicates that the in vitro results are relevant to the disease in humans.

### Table 2: Serum levels of IL-8, VEGF, and IL-6 in classical KS and controls

<table>
<thead>
<tr>
<th></th>
<th>Mean&lt;sup&gt;a&lt;/sup&gt; (pg/ml)</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-8</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic KS</td>
<td>142.6</td>
<td>95–215</td>
<td>0.027</td>
</tr>
<tr>
<td>Controls</td>
<td>70.8</td>
<td>37–137</td>
<td></td>
</tr>
<tr>
<td><strong>VEGF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic KS</td>
<td>419.9</td>
<td>337–523</td>
<td>0.019</td>
</tr>
<tr>
<td>Controls</td>
<td>195.6</td>
<td>112–340</td>
<td></td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic KS</td>
<td>8.5</td>
<td>6–12</td>
<td>0.41</td>
</tr>
<tr>
<td>Controls</td>
<td>10.0</td>
<td>7–14</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Geometric mean.
IL-8 Activity in Kaposi’s Sarcoma

Part of a cascade of chemokine production in these cells. Endogenous IL-1β may thus be responsible for the enhanced expression of IL-8.

Having established that IL-8 is produced and required by KS cells in vitro, we next verified that IL-8 secreted from these cells was an active angiogenic factor. To illustrate this, HUVEC were exposed to conditioned medium from the KS cell cultures known to secrete IL-8. Consistent with the presence of an angiogenic factor, the KS cell supernatant stimulated the proliferation of the endothelial cells in a dose-dependent manner. We demonstrated that this activity was attributable, at least in part, to IL-8 because IL-8 neutralizing antibody was able to inhibit HUVEC growth in the presence of KS cell supernatant. The angiogenic activity of KS cell supernatants was confirmed using chicken CAMs.

IL-8 was also identified as contributing to new blood vessel growth in ovo, because specific neutralizing antibody substantially blocked angiogenesis. Previously, we have shown high levels of VEGF production by these KS cells (12). We infer that VEGF is responsible for a portion of the residual angiogenic activity seen in the presence of IL-8 antibody.

We showed that treatment of nude mice bearing established KS tumor xenografts with neutralizing monoclonal antibody to human IL-8 inhibited the growth of the tumors significantly. Data presented in this report show that the KS tumor cells used to establish the xenograft (KS-SLK) synthesize IL-8 and require an angiogenic factor, the KS cell supernatant, to establish the xenograft (31). Synthesis of IL-8 and VEGF was also observed in tissue sections of KS biopsies. Consistent with the hypothesis that IL-8 and VEGF are important in KS biology, we have shown that levels of IL-8 and VEGF may serve as surrogate markers for KS.

The case for the importance of IL-8 and VEGF in KS is strengthened by our observation that levels of IL-8 and VEGF but not IL-6 are elevated in the serum of KS patients relative to controls. This suggests that IL-8 and VEGF are central to the pathogenesis of KS. Inhibition of these factors may result in prevention or treatment of KS. There is already limited evidence that VEGF inhibitors can induce response in some patients with KS (57–59). Combined use of VEGF and IL-8 inhibitors may be even more effective. Combination with agents already shown to have high activity in KS, such as low-dose Taxol, can possibly enhance the antitumor activity even further. Furthermore, serum levels of IL-8 and VEGF may serve as surrogate makers for KS.

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Interleukin 8 Is an Autocrine Growth Factor and a Surrogate Marker for Kaposi's Sarcoma

Rizwan Masood, Jie Cai, Anil Tulpule, et al.