Expression of Adhesion Molecules, Platelet-Activating Factor, and Chemokines by Kaposi's Sarcoma Cells[1]

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The present study was designed to investigate whether cells cultured from Kaposi's sarcoma (KS), a vascular tumor with a prominent leukocyte infiltration, express molecules important for the recruitment and activation of leukocytes. KS cells expressed intercellular adhesion molecule-1, which was augmented by exposure to IL-1β or TNF-α. Unlike endothelial cells, resting or cytokine-activated KS cells did not express appreciable levels of intercellular adhesion molecule-2, vascular cell adhesion molecule-1, and E-selectin on their surface. Weak expression of vascular cell adhesion molecule-1 mRNA was detectable by Northern blot analysis and, most clearly, by PCR analysis. Upon exposure to inflammatory cytokines, KS cells produced the attractant/activating lipid platelet-activating factor. KS cells expressed appreciable levels of the chemotactic cytokines, monocyte chemotactic protein-1 (MCP-1) and IL-8, as determined by Northern blot analysis, immunoassay, or bioassay. Chemokine production was augmented by IL-1β or TNF-α. MCP-1 expression was also detected in KS lesions by in situ hybridization. The set of molecules identified in the present study is probably important in determining the prominent leukocyte infiltration observed in KS. Tumor-associated leukocytes may amplify autocrine/paracrine circuits that sustain KS proliferation and contribute to recruitment of host vascular cells. *The Journal of Immunology,* 1994, 153: 4816.

Kaposi's sarcoma (KS)[4] is a tumor composed of spindle-shaped cells with elongated nuclei, sheets of endothelial-like cells, and, particularly early in the disease, a prominent infiltrate of host leukocytes (for review, see Refs. 1 and 2). KS behaves as an opportunistic tumor, its incidence and aggressiveness increasing in immunodeficient individuals, in particular during HIV infection (3). The histogenetic origin and pathogenesis of KS is still unclear. KS spindle cells can be cultured in vitro (4–7), where they produce and respond to various cytokines (8–13). In particular, cytokines such as oncostatin M (10, 11), IL-6 (12), and PDGF (14), as well as the HIV-1 *tat* protein (9, 15), can stimulate their growth. When injected into nude mice, KS-derived spindle cells cause vascular lesions by recruiting host elements (16).

We are interested in leukocyte infiltration in tumor tissues (for review, see Ref. 17). There is evidence that tumor-derived mediators, such as monocyte chemotactic protein-1 (MCP-1; alternative acronyms are MCAF, TDCF, and JE; Refs. 17–19) play an important role in the regulation of leukocyte infiltration in tumors (17). Tumor-associated macrophages (TAM) have complex functions in the immunobiology of neoplastic tissues, including promotion of tumor growth and angiogenesis (17). It was therefore of interest to define whether KS-derived cells, which cause tumors characterized by conspicuous leukocyte infiltration and neovascularization (16), express molecules important for recruiting, retaining, and activating host leukocytes.

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[4] Abbreviations used in this paper: KS, Kaposi's sarcoma; MCP-1, monocyte chemotactic protein-1; TAM, tumor-associated macrophages; ICAM, intercellular adhesion molecule; VCAM-1, vascular cell adhesion molecule-1; PAF, platelet-activating factor; FBS, fetal bovine serum; EC, endothelial cell; RT, reverse transcriptase.

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We found that in vitro-cultured KS-derived cells express intercellular adhesion molecule-1 (ICAM-1), and, after exposure to inflammatory cytokines, low levels of VCAM-1 mRNA, but not other adhesion molecules such as intercellular adhesion molecule-2 (ICAM-2) and E-selectin, typical of endothelial cells (EC; for review, see Refs. 20 and 21). KS cells were also induced to produce the activating/attractant lipid platelet-activating factor (PAF) (for review, see Ref. 22) by inflammatory cytokines (IL-1β and TNF-α). Finally, KS cells expressed substantial amounts of the chemoattractant cytokines IL-8 and MCP-1 (17–19). Leukocytes, recruited and retained in KS lesions via the set of molecules described here, may amplify growth and vascularization of these tumors.

Materials and Methods

Cell culture and reagents

The following reagents were used for culture and separation of cells: pyrogen-free saline and distilled water (Bieffe, Bergamo, Italy) for clinical use; DMEM (Life Technologies, Inc., Glasgow, Scotland); RPMI 1640 medium (Seromed Biochron KG, Berlin, Germany); glutamine (Life Technologies); gentamicin (Life Technologies); aseptically collected fetal bovine serum (FBS; HyClone Laboratories, lot 1111960, Logan UT; Irvine Scientific, lot 1432, Santa Ana, CA). The routinely employed cell culture medium was RPMI 1640 or DMEM with 2 mM glutamine, 50 mg/ml gentamicin, and 10% FBS, hereafter referred to as complete medium. All reagents contained less than 0.125 U/ml of endotoxin as checked by the *Limulus* amoebocyte lysate assay (Microbiologic Associates, Walkersville, MD). Serum was tested after 1/3 dilution and heating at 100°C.

PBLs

Buffy coats from blood donations (courtesy of Centro Trasfusionale, Ospedale Sacco, Milan, Italy) were used in most experiments as a source of PBMC. Alternatively, we obtained heparinized venous blood from healthy laboratory donors. Blood was diluted 1/5 with saline, and 40 ml was then placed on 10 ml Ficoll (Seromed, Berlin, FRG), in 50-ml conical tubes (Falcon, Becton Dickinson Labware, Lincoln Park, NJ) for centrifugation at 400 $\times$ *g* for 20 min at room temperature. PBMC were collected at the interface, washed with saline, and suspended in complete medium at 2 to 5 $\times$ 10^6 cells/ml in 50-ml conical tubes (23).

Cells

Cultures of KS-derived cells were established from cutaneous biopsies of patients as previously described (7, 24–26). Briefly, the vascularized lesion was minced into small pieces and extensively washed to eliminate blood cells. The minced tissue was put into gelatin-coated plastic flasks (NUNC, Mascia Brunelli, Milano, Italy) and allowed to adhere for 3 h. DMEM supplemented with 20% FBS was then added carefully and the flasks monitored for outgrowth of cells from explants. Subcultures were made by trypsinization of the cells and subsequent plating (ratio 1:3) on gelatin-coated plastic dishes. The experiments presented here were performed on cultures at the fourth to seventh passage. KS-derived cells have the elongated morphology typical of the spindle-shaped KS cells (4–6); immunohistochemical characterization shows positivity for vimentin, collagen I, laminin, smooth muscle α-actin, and desmin, but not for CD45, von Willebrand factor, or EN4 Ag, in agreement with previous reports (4, 6, 8, 27, 28). As expected (14), KS lines, unlike normal fibroblasts, caused vascular lesions in nude mice (25, 26). The cultures, originating from three different HIV-seropositive patients, were named AIDS-IST-KS3, AIDS-IST-KS4, and AIDS-IST-KS11, abbreviated KS3, KS4, and KS11, respectively; the culture originating from a classical KS of an HIV-seronegative patient was named KS8.

EC (second to eighth passage) from human umbilical veins were cultured in medium 199 (Life Technologies) supplemented with 20% FBS and were used in the study as described in detail in previous reports (29, 30).

Cytokines and chemoattractants

rIL-1β (sp. act., 10^6 U/mg) was obtained from Dompè (L'Aquila, Italy). Human rTNF-α was obtained from BASF/Knoll (Ludwigshafen, Germany; sp. act., 8.1 $\times$ 10^6 U/mg) or from Genentech (South Francisco, CA; sp. act., 10^7 U/mg). Human rIFN-γ was obtained from Hoffman-La Roche (Basel, Switzerland; sp. act., 10^7 U/mg protein). A rabbit antiserum raised against MCP-1/JE was kindly donated by Dr. B. Rollins (Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA). JE was identified as a gene expressed in stimulated mouse fibroblasts; human JE was shown to be identical to MCP-1 (19). The Ab was used as recently described for vascular cell supernatants (31). A protein A-Sepharose-immobilized anti-MCP-1/JE micro-column was prepared as described by Leonard and Skeel (32). The monocyte supernatant was passed through the column and then assayed for chemotactic activity for monocytes. Normal rabbit serum was used for the preparation of a control column. In one experiment, a commercially available anti-MCP-1 mAb (Peprotech, Rocky Hill, NJ) and, as a control, an anti-IFN-γ mAb were used. FMLP was purchased from Sigma Chemical Co. (St. Louis, MO).

mAbs to adhesion molecules

mAbs, used in the present study as recently described (31), were obtained through the courtesy of the following persons: anti-ICAM-1, clone LB2 (IgG2b), Dr. N. Hogg, Imperial Cancer Research Fund, London, UK; anti-ICAM-2, clone 6D5 (IgG1), Dr. C. G. Gahmberg, Helsinki, Finland; anti-E-selectin, clone BBIG-E6 (IgG1), British Biotechnology Products (BBP, Oxford, UK); anti-P-selectin, clone RUU-SP 2.18 (IgG1), kindly donated by M. Metzelaer (University Hospital, Utrecht, The Netherlands); anti-VCAM-1, clone 4B9 (IgG1), Dr. J. Harlan, (Washington University, Seattle, WA). Control Abs (e.g., anti-IFN-γ and anti-CD8) were used for flow cytometric analysis with the use of a FACStar^{Plus} apparatus as described (33).

Chemotaxis assay

KS or EC were incubated with medium, IL-1β (10 ng/ml), or TNF-α (10 ng/ml) for 4 h. Then the stimulus was removed and fresh medium was added. Supernatants were harvested after 18 h of incubation at 37°C in 5% CO_2 in air. Leukocyte chemotaxis was assessed by a microchamber technique (34). In the lower compartment, 25 μl of different medium dilutions of the supernatants were seeded. The two compartments were separated by a 5-μm pore size polycarbonate filter (Neuroprobe, Cabin John, MD). Chambers were incubated at 37°C for 90 min; at the end of the incubation, filters were removed, fixed, and stained with Diff-Quick (Harleco, Gibbstown, NJ), and 5 oil immersion fields were counted after coding samples (three replicates per group). FMLP was used as a reference chemoattractant. Under these conditions, only the migration of monocytes was measured. The statistical significance of migration toward stimulus vs medium control was assessed by Dunnett's test.

Measurement of cytokines and PAF

The levels of immunoreactive IL-8 were measured by using either a commercially available ELISA (Amersham, Buckinghamshire, UK) or a RIA with a rabbit anti-IL-8 antiserum generated in the laboratory of Dr. A. Mantovani. MCP-1 activity was evaluated as the capacity of KS supernatants to induce directional migration of monocytes (see *Chemotaxis assay* section, above). An anti-MCP-1 antiserum or mAb was used to identify MCP-1 as the chemoattractant cytokine present in KS supernatants (see *Chemokines* section under *Results*). MCP-1 was also measured by using a recently developed ELISA assay (35). The assay, which is based on a rabbit polyclonal antiserum for capture and a novel mAb for detection, has a sensitivity of 30 pg/ml in medium. It is highly specific for MCP-1 in that it does not detect MCP-2, MCP-3, RANTES, MIP-1α, MIP-1β, IP-10, IL-8, or NAP-2.

PAF extracted from KS-derived cells, EC, and their supernatants was purified by TLC followed by HPLC, characterized by physicochemical treatments and sensitivity to phospholipases, and quantified by aggregation of washed rabbit platelets as described (27, 30).

Northern blot analysis

Northern blot analysis was conducted according to standard procedures (36). Total RNA was isolated by using the guanidine isothiocyanate method. Five to eight micrograms of total RNA were analyzed by electrophoresis through 1% agarose formaldehyde gels in the presence of ethidium bromide (Sigma Chemical Co.), followed by Northern blot transfer to Gene Screen Plus membranes (DuPont NEN, Boston, MA). The plasmids containing the full length cDNA human MCP-1 probe (0.672-kb fragment; Ref. 37) or the human IL-8 probe (0.3-kb fragment; Ref. 38), the VCAM-1 (2.2-kb fragment; Ref. 39) and the E-selectin (1.4-kb fragment; Ref. 40) cDNA were nick translated or random primed with [^{32}P]dCTP (5000Ci/mmol; Amersham, Buckinghamshire, UK). Membranes were pretreated and hybridized in 50% formamide (Merck, Darmstadt, Germany) with 10% dextran sulfate (Sigma Chemical Co.) and washed twice with 2X SSC, then twice with 2X SSC plus 1% SDS (Merck) at 60°C for 30 min, and finally twice with 0.1X SSC at room temperature for 30 min. The membranes were exposed for 12 to 24 h at −80°C with intensifying screens. RNA loading and transfer to membrane were checked by examination of filters under UV light.

RT-PCR

One microgram of total RNA was reverse transcribed and then amplified by PCR. RNA was mixed with 2.5 mmol/liter $MgCl_2$, 25 mmol/liter KCl, 10 mmol/liter Tris-HCl pH 8.3, 1 mmol/liter each dNTP and 2.5 U/μl Moloney murine leukemia virus RT (Perkin-Elmer Cetus, Norwalk, CT) in a final volume of 20 μl. Reverse transcription was conducted at 42°C for 15 min. This reaction was then amplified by adding in a final volume of 100 μl 2 mmol/liter $MgCl_2$, 50 mmol/liter KCl, 10 mmol/liter Tris-HCl, pH 8.3, 2.5 U Taq DNA polymerase, and 0.15 mol/l each of specific primers. VCAM-1 primers were as described (41, 42): oligomer forward is 5′-GGAACCTTGCAGCTTACAGTGACAGAGCTCCC-3′ and oligomer backward is 5′-CAAGTCTACATATCACCCAAG-3′. Samples were amplified in a thermal cycler (Perkin-Elmer Cetus) by using 30 cycles at 95°C (1.30 min), 55°C (1.30 min), and 72°C (1.30 min). Ten microliters of each RT-PCR were subjected to electrophoresis through a 1.5% agarose gel, which was stained, photographed, and blotted onto a ζ Probe Membrane (Bio-Rad, Richmond, CA). This was then hybridized to a VCAM-1 probe according to standard procedures for Southern blotting.

In situ hybridization

For in situ hybridization experiments, five biopsies from skin lesions of five HIV-seropositive patients were removed and immediately transferred to a solution of a freshly prepared 4% paraformaldehyde PBS. The procedure for dehydration and embedding was performed as described (43). Subsequently, thin sections (5 to 10 μm) were prepared and subjected to in situ hybridization. For synthesis of RNA hybridization probes specific for MCP-1, the transcription plasmid pBS-MCP-1 was used. A 670-bp cDNA fragment coding for the full length human MCP-1 gene was inserted into the transcription vector pBluescript SK II. The identity of the inserted sequences was verified by sequence analysis. Hybridization specificity was examined by Northern blot analysis by using the respective fragments labeled by nick translation as probes.

Synthesis of ^{35}S-labeled complementary RNA probes and in situ hybridization were conducted as described (43). RNA probes (sp. act., 10^9 cpm/μg) were applied to tissue sections at a final concentration of 50,000 cpm/μl in 50% (v/v) deionized formamide, 0.3 M NaCl, 20 mM Tris-HCl, pH 7.4, 5 mM EDTA, 10 mM sodium phosphate, pH 8, 10% (w/v) dextran sulfate, and Denhardt's solution containing total yeast RNA (50 μg/ml). After hybridization at 50°C for 16 h, coverslips were floated off in 5X SSC/10 mM DTT at 50°C. Subsequently, tissue was subjected to a stringent washing at 65°C in 50% formamide/10 mM DTT. After photographic development, slides were fixed and stained with hematoxylin and eosin. Parallel to each in situ hybridization, a negative control was conducted by using the sense strand probe on sections consecutive to those in which the antisense probe was applied.

Results

Adhesion molecules

In a series of experiments we examined cultured KS cells for the expression of adhesion molecules and compared them with EC. Figure 1 shows typical fluorescence profiles for KS4 cells, but similar results were obtained with KS3, KS8, and KS11. KS-derived cells expressed appreciable amounts of ICAM-1, and the levels of this adhesion molecule were augmented by in vitro exposure to inflammatory cytokines (IL-1β, TNF-α ± IFN-γ). KS cells showed no detectable expression (constitutive or cytokine induced) of ICAM-2, E-selectin, and VCAM-1 after 6 h (not shown) and 24 h of stimulation (Fig. 1). In contrast, under the same conditions, ICAM-2, E-selectin, and VCAM-1 were readily observed on EC (Fig. 1, *panel C*) and VCAM-1 was also expressed on melanoma and mesothelial cells (44, 33). Expression of adhesion molecules was also investigated at the mRNA level by Northern blot or PCR analysis in three cell lines: KS3, KS8, and KS11. As shown in Figure 2, E-selectin (*panel A*) and VCAM-1 (*panel B*) were undetectable by Northern blot analysis in KS cells. After prolonged exposure of the blot, a faint hybridization band was visible with a VCAM-1 probe. In an effort to further define VCAM-1 mRNA expression in KS lines, PCR was used. As shown in Figure 3, VCAM-1 cDNA was visible only in KS cells treated with IL-1β or with TNF-α, as well as in EC cells treated with IL-1β, used as positive control. The specificity of these results was confirmed with Southern analysis of the gel by hybridization with a VCAM-1-specific probe (Fig. 3, *panel C*). The seven Ig domains (631 bp) was the predominant form of VCAM-1.

Chemokines

Expression of the chemoattractant cytokines IL-8 and MCP-1 was investigated by Northern blot analysis as well as by immunoassay and bioassay. Sufficient RNA for Northern blot analysis could be obtained from KS3, KS8, and KS11 cells. As illustrated in Figure 4, *panel A*, KS cells expressed appreciable levels of chemokine (IL-8 and MCP-1) mRNA in the absence of deliberate stimulation. Exposure to IL-1β or TNF-α resulted in a 5- to 10-fold increase in mRNA expression.

Transcript expression was associated with actual production of chemokines. As shown in Figure 5, supernatants of resting KS cultures contained low but appreciable levels of immunoreactive IL-8 (approximately 1 to 20 ng/ml). Exposure to inflammatory cytokines (IL-1β or TNF-α) markedly augmented IL-8 production by KS-derived cells, with values ranging from 13 to 360 ng/ml.

In an effort to define whether expression of MCP-1 mRNA was associated with protein production, monocyte chemotaxis was first studied with KS3, KS4, KS8, and KS11 lines. As shown in Figure 6, KS released substantial amounts of monocyte chemotactic activity in the absence

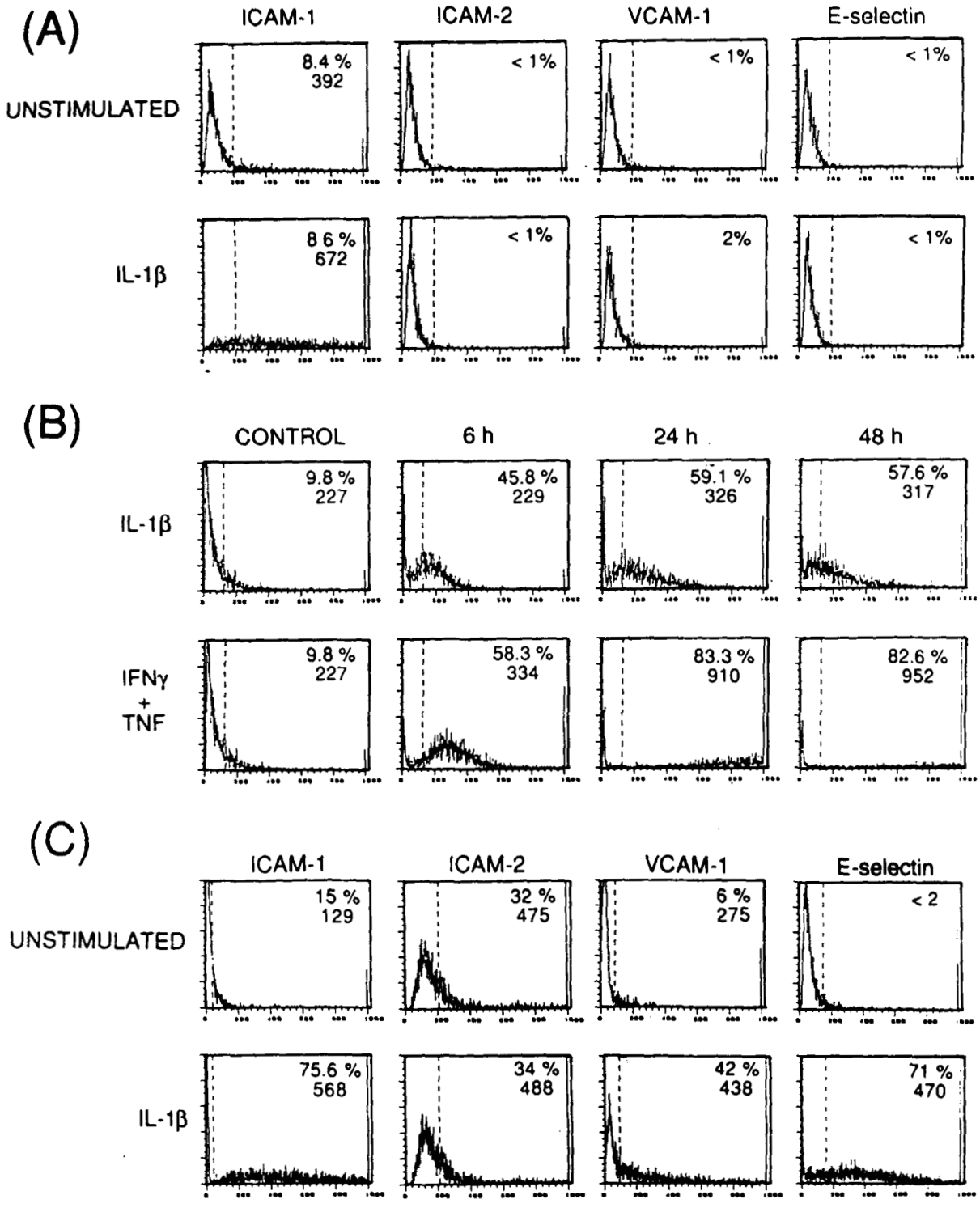

FIGURE 1. Expression of adhesion molecules on KS cell surface. KS4 cells were evaluated either resting or after exposure to IL-1β (10 ng/ml) or TNF-α (10 ng/ml) and IFN-γ (500 U/ml). Expression of adhesion molecules was assessed by using appropriate mAb and flow cytometry. Numbers indicate the percent of positive cells and the mean channel of fluorescence. Similar results were obtained with KS3, KS8, and KS11. *Panel A*: Expression in a representative KS4 culture after 24 h of incubation with medium or with IL-1β (10 ng/ml). *Panel B*: Expression of ICAM-1 in KS4 cells after different times of exposure to TNF-α + IFN-γ or IL-1β. *Panel C*: Expression of adhesion molecules in EC is used as reference positive cell population. Resting or IL-1β activated EC were exposed to IL-1β (10 ng/ml) for 4 (E-selectin) or 24 h (ICAM-1, ICAM-2, VCAM-1).

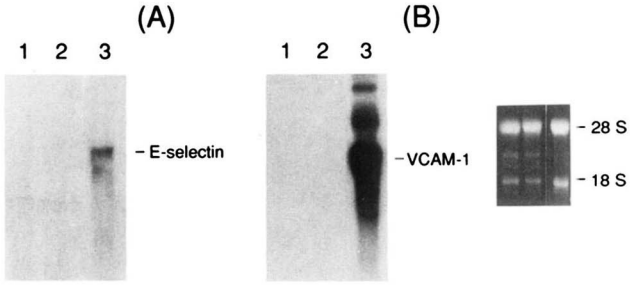

FIGURE 2. Northern blot analysis of expression of adhesion molecules in KS cells. KS cells were evaluated either resting (*lane 1*) or after exposure TNF-α (10 ng/ml) for 4 h (*lane 2*). Results refer to KS11 and IL-1β-stimulated EC (10 ng/ml, 4 h) used as positive control (*lane 3*). The exposure time was 24 h. Overexposure of the VCAM-1 blot (120 h) revealed a faint hybridization band in TNF-stimulated KS cells (not shown, see *Results*). Quality and quantity of RNA blotted is shown in the ethidium bromide-stained panel.

of deliberate stimulation, except for the supernatant of KS4 cells that had minimal activity. Exposure to IL-1β or TNF-α significantly increased the levels of monocyte chemotactic activity in KS supernatants. In some experiments (e.g., Fig. 6, *panel B*), conditioned medium at high dilution caused some reduction of baseline migration, which may be related to metabolic effects or inhibitors (17). Abs directed against MCP-1 substantially reduced the monocyte chemotactic activity present in KS supernatants (Fig. 7). In an effort to obtain a quantitative estimate of MCP-1 production by KS cells, a recently developed ELISA assay (35) was used (Fig. 8). KS cells released detectable amounts of MCP-1 in the absence of deliberate stimulation, with 0.18, 0.36, 29.6, and 1.2 ng/ml for KS3, KS4, KS8, and KS11 over a period of 24 h. Exposure to TNF increased MCP-1 to 3.6, 17.3, 100, and 14 ng/ml for the four lines, respectively. In the same conditions, EC, treated in parallel with TNF, released 5.8 ng/ml of MCP-1. The demonstration of MCP-1 production by KS cells does not exclude that they may concomitantly produce other monocyte chemoattractants.

PAF

The experiments shown in Figure 9 established that KS-derived cells produce and partially release PAF in response to IL-1β and TNF-α. KS-derived cells incubated for 6 h with 50 and 10 ng/ml of IL-1β and TNF-α respectively, under optimal conditions for PAF synthesis by vascular cells (30, 45, 46), produced considerable amounts of PAF, which was approximately two- to threefold higher than production from EC (Fig. 9). The level of PAF released in the supernatant was approximately 30% of that associated with the cells.

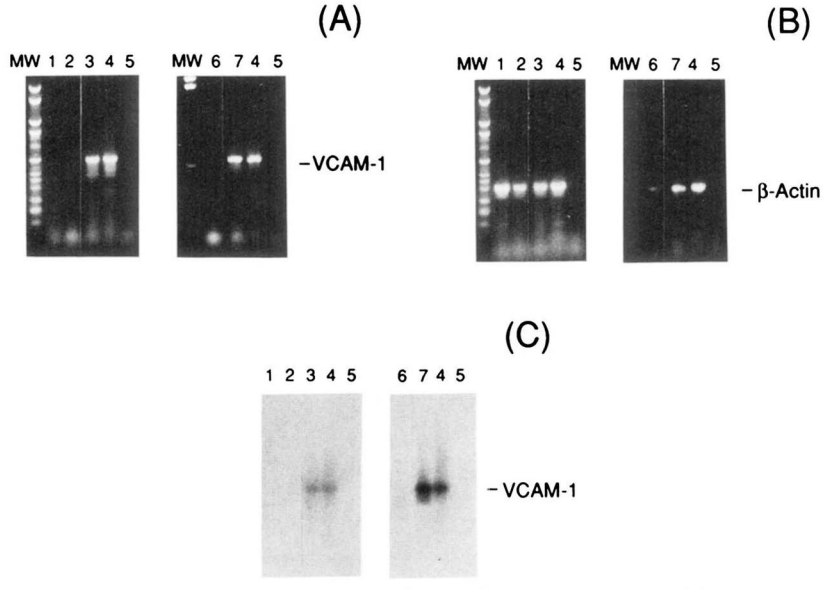

FIGURE 3. RT-PCR analysis of VCAM-1 expression in KS cells. Total RNA was extracted from resting or IL-1β- (10 ng/ml, 4 h) or TNF-α- (10 ng/ml, 4 h) stimulated KS cells. IL-1β- (10 ng/ml, 4 h) stimulated EC served as a positive control. Total RNA was reverse transcribed, the same cDNA preparation was divided into two tubes, and each of them was amplified, respectively, with specific pairs of primers designed for VCAM-1 (*panel A*) and β-actin (*panel B*). *Panel A*: Ethidium bromide-stained agarose gel analysis of VCAM-1-amplified products from KS cells and EC. MW, molecular weight standard; *lane 1*, KS3 resting; *lane 2*, KS8 resting; *lane 3*, KS8 stimulated with IL-1β; *lane 4*, EC stimulated with IL-1β; *lane 5*, no RNA; *lane 6*, KS11 resting; *lane 7*, KS11 stimulated with TNF-α. *Panel B*: Ethidium bromide-stained agarose gel analysis of β-actin transcripts in KS cells and EC. The same RNAs of *panel A* were amplified with β-actin-specific primers. *Panel C*: Southern analysis of RT-PCR with a VCAM-1-specific probe. Numbers of the lanes indicate the same samples described in *panel A*.

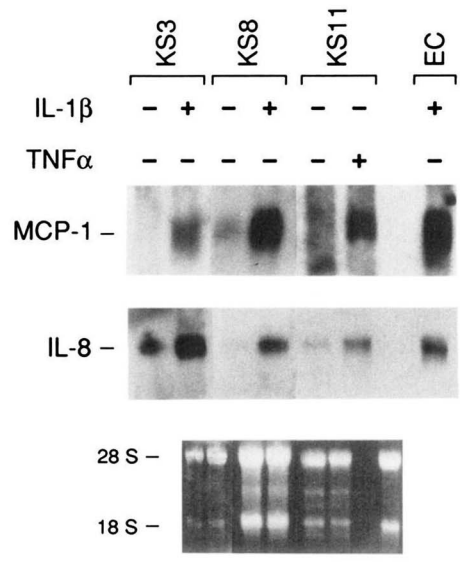

FIGURE 4. Expression of IL-8 and MCP-1 mRNA in KS cells. KS3, KS8, KS11, and EC were exposed to IL-1β (10 ng/ml) or TNF-α (10 ng/ml) for 4 h; the RNA was extracted and cytokine gene expression was studied by Northern blot analysis. Comparable amounts of total RNA were transferred to filters, as shown by ethidium bromide staining.

In situ analysis

The MCP-1 expression by KS cells, a major observation of this study, was investigated in tissue sections of KS biopsies by using in situ hybridization. Five different AIDS-KS lesions derived from skin biopsies of AIDS-KS patients were used for in situ hybridization studies. Strong expression of MCP-1 was found in the KS spindle cells (Fig. 10, *panels A* and *C*, black *arrows*). In addition, signals were obtained from the basal cells of the epidermis overlaying KS lesions (Fig. 10, *panel A*, white *arrow*). A negative control was conducted by using the respective sense strand probe on consecutive sections (Fig. 10, *panels B* and *D*). No signals were obtained in these control experiments. By immunohistochemistry, spindle cells in KS lesions expressed low levels of ICAM-1, but not VCAM-1 and E-selectin (47).

Discussion

The present study was designed to characterize KS cells in terms of fundamental mechanisms of leukocyte recruitment and activation. It was found that in vitro-cultured KS cells have ICAM-l on their surface, but not ICAM-2, E-selectin, and VCAM-l. Expression of ICAM-1 was augmented by IL-1β and TNF-α. Inflammatory cytokines induced production of PAF in KS cells. Finally, and perhaps most importantly, KS-derived cells produced substantial amounts of IL-8 and MCP-1; these were augmented by exposure to IL-1β or TNF-α.

Adhesion molecules are crucial determinants of the interaction of leukocytes with endothelial or mesothelial cells (20, 21, 33). A variety of cell types express ICAM-1, whereas expression of ICAM-2, VCAM-1, and E-selectin is more restricted. In addition to EC, synoviocytes (48) and mesothelial cells (33) also have VCAM-1. It is likely that VCAM-1 on these nonvascular cells subserves the same function of regulating leukocyte traffic as in vascular endothelium. Expression of E-selectin and ICAM-2 is restricted to EC (20, 21). The results presented here show that KS-derived cells express in their surface a repertoire of adhesion molecules distinct from that of EC or other cells with a "barrier" function (mesothelium and synoviocytes), including ICAM-1 but not ICAM-2, VCAM-1, and E-selectin. However, by PCR analysis we obtained unequivocal evidence of expression of VCAM-1 mRNA in KS cells.

While this report was in preparation, Yang et al. reported that cultured KS cells express ICAM-1 but not VCAM-1 or E-selectin (49). However, in contrast to data reported herein, they found induction of surface VCAM-1 and E-selectin by TNF-α or IL-1β, albeit at substantially lower levels than in EC. The reason for this partial discrepancy is unclear, but most likely it is related to culture selection, conditions, and characterization, which for the lines studied herein included the generation of vascular lesions in immunodeficient mice (24–26). Immunohistochemical analysis of E-selectin expression in KS has

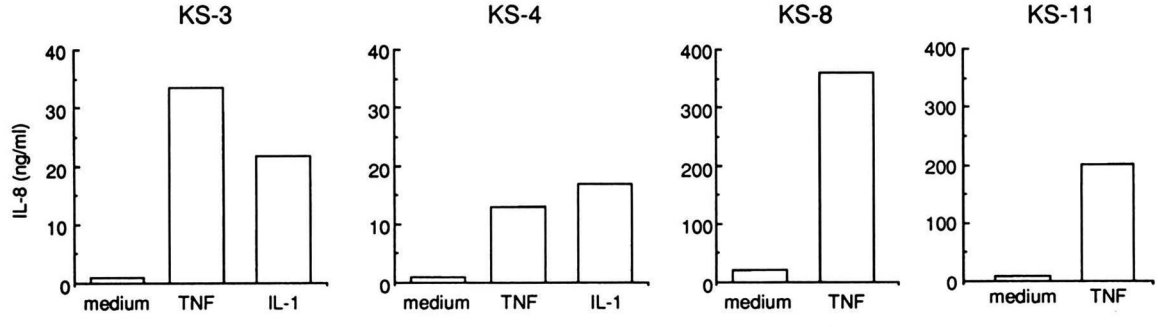

FIGURE 5. Release of IL-8 by KS cells. KS cells (KS3, KS4, KS8, and KS11) were cultured for 20 h with medium, IL-1β (10 ng/ml) or TNF-α (10 ng/ml). IL-8 was measured by ELISA. Stimulated EC released 55.8 $\pm$ 12.3 ng/ml IL-8 under the same conditions.

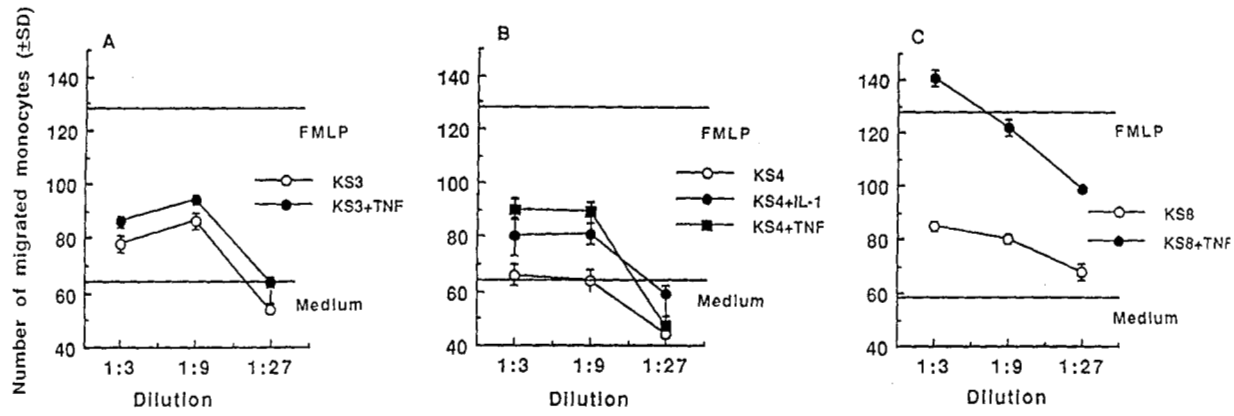

FIGURE 6. Chemotactic activity for monocytes of supernatants from KS cells. KS cells (KS3, KS4, KS8) were cultured for 4 h with medium, IL-1β (10 ng/ml) or TNF-α (10 ng/ml). The cells were washed and the supernatants were collected after 18 h of culture. FMLP (10^{-8} M) was used as reference chemoattractant. Results are mean ± SD of three replicates.

yielded conflicting results: although in one study widespread expression was observed in spindle cells (50), in other reports, E-selectin (47, 49, 51) and VCAM-1 (47, 49) were detected in EC lining blood vessels or in a minor subset of spindle cells. In all studies ICAM-1 was found in KS lesions (47, 49, 51).

The ontogenetic origin of KS has not been unequivocally defined (for recent reviews, see Refs. 1 and 2). A widely held view is that KS cells are of endothelial origin, though they express markers typical of smooth muscle cells (1, 2, 7, 28, 52, 53). Alternatively, the existence of a mesenchymal progenitor of vascular cells, which could be stimulated to grow as a consequence of a cytokine imbalance, has been postulated (28). In support of these views, it has recently been shown that KS spindle cells express in vitro and in vivo the vascular endothelium cadherin (47), a molecule restricted to vascular endothelium (54). It is of interest in this context that the repertoire of adhesion molecules of KS cells does not include E-selectin and ICAM-2, two structures restricted to EC (20, 21). Thus, if KS cells indeed have an ontogenetic relationship to the endothelial differentiation pathway, as seems most likely, this must occur at a stage that does not include expression of ICAM-2 and E-selectin.

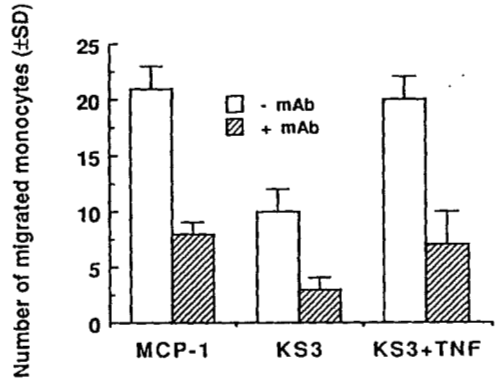

FIGURE 7. Inhibition of the chemotactic activity for monocytes of the supernatant of KS3 cells by anti-MCP-1 mAb. KS3 cells were cultured for 4 h with medium or TNF-α (10 ng/ml). After washing, the supernatant was collected after 18 h of culture. The supernatant was absorbed on an anti-MCP-1 microcolumn or a control column with an irrelevant mAb (anti-IFN-γ). As a positive control for immunoabsorption, rMCP-1 (50 ng/ml) was treated similarly. The KS supernatant was tested at a 1:9 dilution. Immunoabsorption did not affect the chemotactic activity of FMLP or of a control medium (not shown). Results are the number of migrated monocytes after subtraction of spontaneous migration.

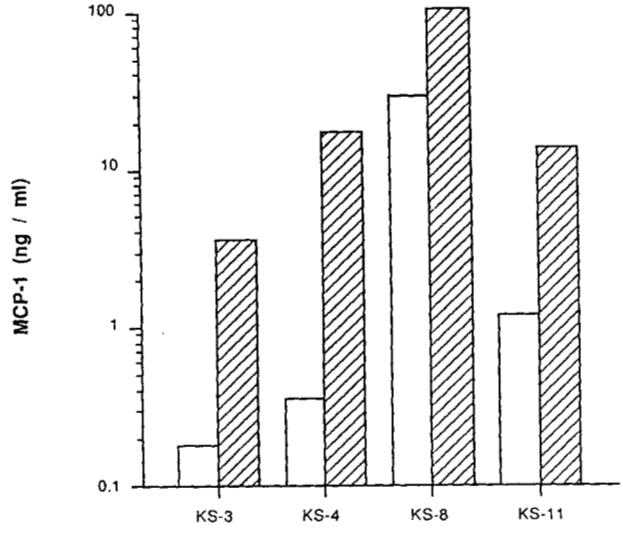

FIGURE 8. Immunoreactive MCP-1 in KS cell supernatants. Supernatants from resting (open column) or IL-1β- (KS3 and KS4) or TNF-α- (KS8 and KS11) stimulated (hatched columns) KS cells were evaluated for MCP-1 with the use of an ELISA assay. IL-1β-stimulated EC released 5 to 8 ng/ml MCP-1 over the same period of time.

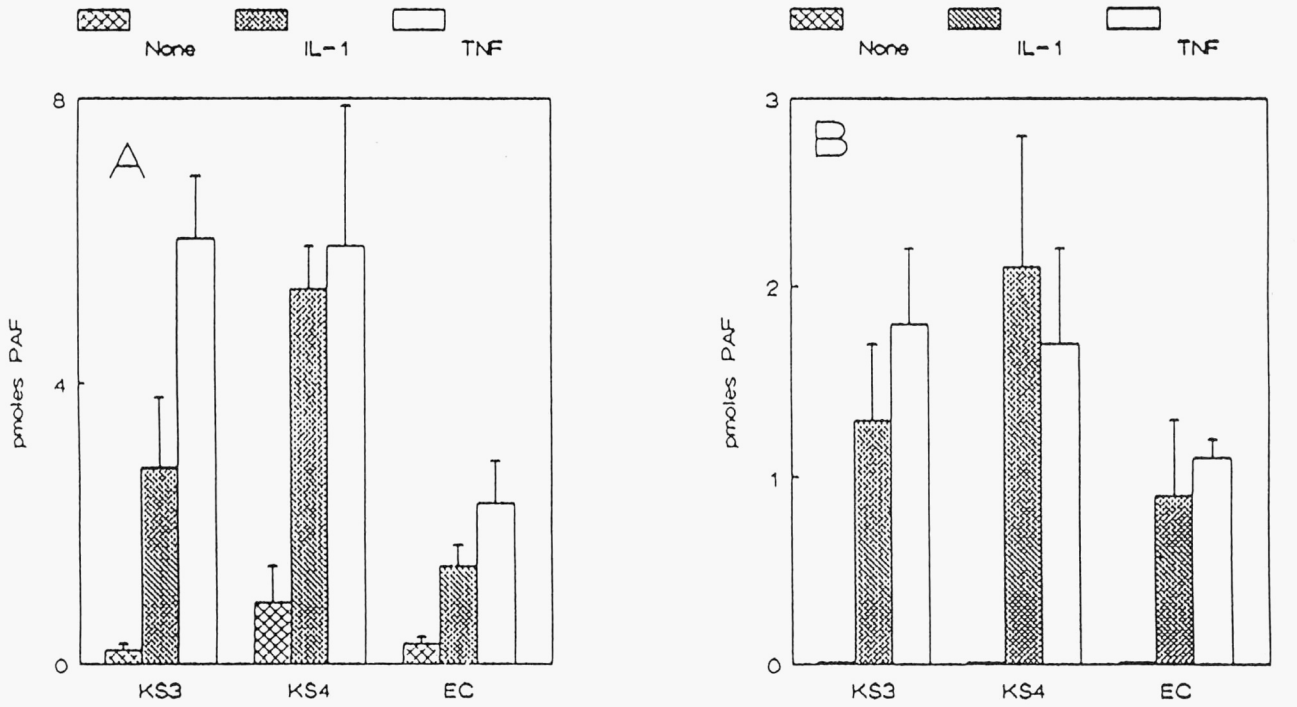

FIGURE 9. Production of PAF by KS3 cells, KS4 cells, and EC. Cells were cultured for 6 h with medium, IL-1β (50 ng/ml), or TNF-α (10 ng/ml). PAF, extracted and purified from the cells (*panel A*) and from the supernatant (*panel B*), was quantified by bioassay on washed rabbit platelets. Mean ± SD of three determinations in one typical experiment, expressed as pmols PAF/5 × 10^5 cells.

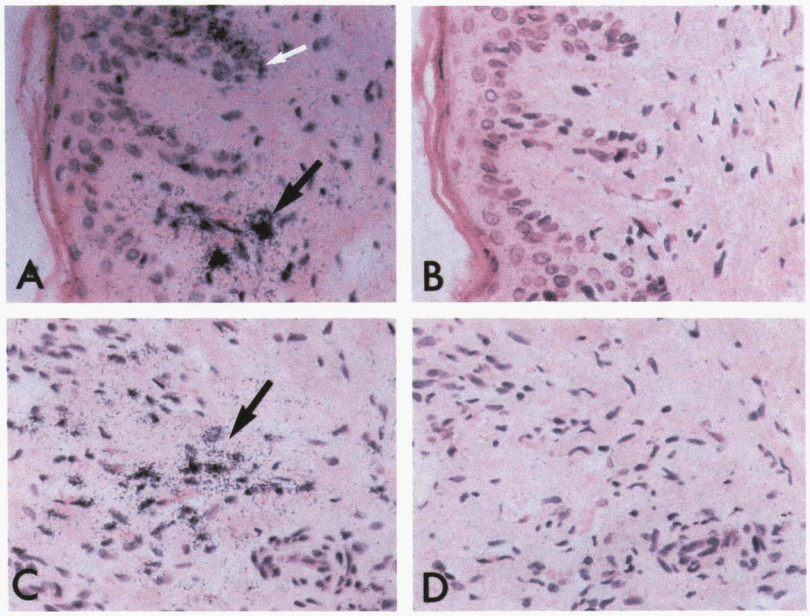

FIGURE 10. In situ hybridization with the use of a MCP-1-specific probe on tissue sections of KS. The figure shows the results obtained in two different KS lesions. Hybridization was conducted with a ^{35}S-labeled RNA hybridization probe complementary to MCP-1 mRNA (*panels A* and *C*). Prominent expression of MCP-1 was found in the spindle cells of KS (*panels A* and *C*, black arrows) and in the basal cells of epidermis overlaying KS lesions (*panel A*, white arrow). As a control for background staining, the sense strand hybridization probe was applied to consecutive sections (*panels B* and *D*).

MCP-1 (C-C family) and IL-8 (C-X-C family) belong to a recently identified superfamily of polypeptide mediators collectively called chemokines, many of which induce directional migration of different leukocyte populations (for recent reviews, see Refs. 18 and 19). Various cell types can produce IL-8 and MCP-1. Cells of vascular origin, including EC (31), smooth muscle cells (55), and mesangial cells (56) are among the most efficient producers of these chemokines. PAF is a phospholipid autocoid, able to activate and recruit leukocytes, including monocytes and T cells, EC, and smooth muscle cells (revised in Ref. 22). IL-1β and TNF-α are agonists able to induce PAF synthesis in EC (30,

45, 46) and in monocytes/macrophages (57, 58). The results presented here extend these observations to KS-derived cells that are most likely of vascular origin, though their ontogenetic relationship to vascular elements remains undefined.

The spectrum of action of IL-8 is not restricted to circulating leukocytes. Melanoma cells (59) and EC (60, 61) have been reported to respond to IL-8. In particular, it has recently been reported that IL-8 induces migration and proliferation of EC and is angiogenic in vivo (60). Thus, IL-8 produced by KS cells may contribute to vascularization of these lesions.

Cells of the monocyte macrophage lineage are a major component of the lymphoreticular infiltrate of tumors. TAM derive from circulating monocytic precursors (17). The chemokine MCP-1, identified also as tumor-derived chemotactic factor (62), is one important mechanism of regulation of TAM in several mouse and some human tumors (17). The results presented here suggest that MCP-1, identified by Northern blot analysis, bioassay, and immunoassay in cultured cell lines and by in situ hybridization in KS lesions, can play an important role in recruiting mononuclear phagocytes in this tumor, characterized by a conspicuous inflammatory infiltrate.

Infiltrating host leukocytes, and TAM in particular, can play an important role in the regulation of tumor growth and progression. TAM are potent producers of growth factors and promote angiogenesis (17). For instance, TAM have been shown to release high amounts of IL-6 (63), a KS growth factor (12). Thus, leukocytes recruited and retained in situ via the molecules described herein may amplify growth and vascularization of KS lesions.

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