Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5281–5285, June 1994
Cell Biology

Role of scatter factor in the pathogenesis of AIDS-related Kaposi sarcoma

YATHI M. NAIDU*, ELIOT M. ROSEN†, RALPH ZITNICK†, ITZHAK GOLDBERG‡, MORAG PARK§, MONICA NAUJOKAS§, PETER J. POLVERINI¶, AND BRIAN J. NICKOLOFF*||

*Department of Pathology, University of Michigan Medical School, M4232 Medical Science I, 1301 Catherine Street, Ann Arbor, MI 48109-0602; †Department of Therapeutic Radiology, Yale University School of Medicine HRT 132, New Haven, CT 06510; ‡Department of Radiation Oncology, Long Island Jewish Medical Center, New Hyde Park, NY 11042; §Molecular Oncology Group, Royal Victoria Hospital, McGill University, Montreal, Canada H3A1A1; and ¶Department of Oral Pathology, University of Michigan School of Dentistry, Ann Arbor, MI 48109-1078

Communicated by George F. Vande Woude, January 7, 1994 (received for review September 19, 1993)

ABSTRACT **Kaposi sarcoma (KS) is a complex multicellular neoplasm that is commonly associated with AIDS. The pathogenesis of KS is not well understood. KS tumor cells grow poorly *in vitro* and require medium conditioned by retrovirus-infected T lymphocytes. We observed that conditioned medium (CM) from type II human T-cell leukemia virus (HTLV-II)-infected T cells (HTLV-II CM) induces conversion of endothelial cells (ECs) to a KS tumor cell-like phenotype. ECs grown in HTLV-II CM acquired a spindle-shaped morphology, the ability to express factor XIIIa and other KS cell markers, and a cytokine production profile similar to that of KS cells. We found that HTLV-II CM contains large quantities of scatter factor (SF), an angiogenic cytokine that stimulates cell motility. SF induced ECs to become spindle-shaped and express factor XIIIa. Moreover, SF was found to be a mitogen for KS cells *in vitro* and was identified within KS lesions *in vivo*. SF mRNA was present in KS cells *in vitro*, and antibodies against SF inhibited the growth of KS cells. The receptor for SF, the c-met protein, was expressed by ECs, dermal dendrocytes, and KS tumor cells *in vitro* and *in vivo*. HTLV-II CM was highly angiogenic *in vivo*, which was blocked by antibodies against SF. Based on these findings, we suggest that SF plays a role in the initiation and maintenance of KS lesions.**

Kaposi sarcoma (KS) poses an increasing public health problem because of its frequent association with AIDS. KS lesions contain multiple cellular constituents including proliferating endothelial cells (ECs), an expanded population of dermal dendrocytes that express factor XIIIa, lymphocytes, and a population of spindle-shaped tumor cells (1, 2). The relationships between these cellular constituents have not been delineated clearly. It has been hypothesized that KS tumor cells are derived from ECs (3), dermal dendrocytes (2), and smooth muscle cells (4). ECs, dendrocytes, and KS tumor cells *in vivo* share a number of immunophenotypic features, including expression of CD34, vascular cell adhesion molecule 1 (VCAM-1), and CD31 (5, 6). It has recently become possible to propagate KS tumor cells *in vitro* by the use of KS cell growth medium (KSGM), which contains filtered, conditioned medium (CM) from HTLV-II-infected human T lymphocytes (7, 8). Studies of cultured KS cells have revealed that these cells express the dermal dendrocyte marker factor XIIIa but do *not* express EC markers—factor VIII and EC adhesion molecule 1 (ELAM-1) (9). To further explore the relationship between ECs, dermal dendrocytes, and spindle-shaped tumor cells, we sought to determine if ECs could be *induced* to express dermal dendrocyte/KS tumor cell marker factor XIIIa *in vitro*. We discovered that KSGM could convert ECs to a KS tumor cell-like phenotype. During this study, we also identified high levels of scatter factor (SF; ref. 10) in KSGM. SF is identical to hepatocyte growth factor (HGF) (11, 12), a serum-derived mitogen (13, 14), with potent angiogenic activity (15–19). SF interacts with cells via its receptor, the c-*met* protooncogene (20, 21). Based on the current findings, we suggest that SF and c-met may play a pathophysiologically relevant role in KS lesions.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Lines. Four different KS tumor cell lines were isolated from skin (KS-I and KS-II), ocular conjunctiva (KS-III), and pleural effusion (KS-IV) of AIDS patients and grown in KSGM (9). Human umbilical vein ECs (HUVECs) and human arterial smooth muscle cells (HASMCs) were provided by S. Zaki Salahuddin (University of Southern California, Los Angeles). Madin–Darby canine kidney (MDCK) cells were obtained from Stephen Warren (Yale University School of Medicine). KS cells were isolated and cultured in KSGM, which is composed of RPMI 1640 (GIBCO) plus fetal calf serum [FCS; 15% (vol/vol)] (HyClone), Nutrodoma-Hu [5% (vol/vol)] (Boehringer Mannheim), and CM from the type II human T-cell leukemia virus (HTLV-II)-infected human T lymphocyte line 38-10 [20% (vol/vol)] (provided by Parkash Gill, University of Southern California). HUVECs were cultured in HUVEC growth medium, which consists of Ham's F-12 and Iscove's modified Dulbecco's medium (1:1) (GIBCO) plus 20% FCS, EC growth factor (30 μg/ml), and heparin (20 units/ml) (Sigma). HASMCs and MDCK cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (15). For proliferation assays, KS cells were initially seeded into 48-well dishes in KSGM, allowed to attach overnight, and then washed three times over the next 6 hr with RPMI 1640. Next, KS cells were incubated either in KSGM or in RPMI 1640 plus 10% FCS supplemented with the indicated cytokines or growth factors. After 80 hr, cell counts were manually performed. In some experiments, KSGM was preincubated with a 1:200 dilution of SF neutralizing rabbit antibody (Ab) 978 or control normal rabbit serum for 2 hr at 37°C prior to addition to the washed KS cells. Duplicate counts were performed on duplicate wells, and results were expressed as mean ± SD.

Abbreviations: Ab, antibody; CM, conditioned medium; EC, endothelial cell; ELAM-1, EC adhesion molecule 1; FCS, fetal calf serum; HASMC, human arterial smooth muscle cell; HGF, hepatocyte growth factor; HTLV-II, type II human T-cell leukemia virus; HUVEC, human umbilical vein EC; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; KS, Kaposi sarcoma; KSGM, KS cell growth medium; MDCK, Madin–Darby canine kidney; SF, scatter factor; TGF, transforming growth factor; VCAM-1, vascular cell adhesion molecule 1.

||To whom reprint requests should be addressed.

PCR Analysis. Analysis of mRNA transcripts was performed following standard procedures using the hot start PCR method (22). Validity of the amplified product was ensured by using nested PCR primers specific for factor XIIIa mRNA and by including control reactions lacking various reagents and cDNA. β-Actin was utilized as a control for loading. GenBank base pair sequence numbers (5′ → 3′) for the PCR primers used were factor XIIIa internal primers, 1999–2020 and 2244–2224; β-actin primers, 1259–1278 and 2374–2351. Expected sizes of amplified sequences are 245 bp for factor XIIIa and 531 bp for β-actin. To amplify an HGF/SF-specific product of 612 bp, two primers corresponding to positions 838–856 and 1429–1450 of the sequence HsHGFHL (European Molecular Biology Laboratory data base accession number M60718) were used in a PCR (23). Amplification was performed by 45 cycles at 94°C (1 min), 48°C (2 min), and 72°C (2 min) in a Perkin–Elmer/Cetus DNA thermal cycler. An oligonucleotide corresponding to position 1105–1124 was radiolabeled with [γ-^{32}P]dATP using polynucleotide kinase and hybridized to a blot of the gel used to analyze the fragments. To amplify a c-met-specific fragment, primers corresponding to positions 2257–2274 and 2557–2574 of the sequence HsMETPAO (European Molecular Biology Laboratory data base accession number J02958) were used. For cytokines and adhesion molecules, the GenBank base pair sequence numbers (5′ → 3′) for the PCR primers used were interleukin (IL) 1β, 393–412 and 639–628; IL-6, 148–167 and 342–321; IL-8, 75–95 and 375–343; intercellular adhesion molecule 1 (ICAM-1), 1246–1466 and 1594–1586; IL-10, 313–339 and 665–639; granulocyte/macrophage colony-stimulating factor, 36–56 and 472–449; transforming growth factor (TGF) type α, 35–58 and 522–495; TGF-β, 1678–1697 and 2006–1994.

Immunohistochemical Staining. Cells grown in eight-well Lab-Tek chambers (VWR Scientific) were stained using the avidin-biotin immunoperoxidase technique (Vector Laboratories) (2). The chromogen 3-amino-4-ethylcarbazole produced a red reaction product. Primary Abs used included Abs against factor XIIIa (1:500) (Calbiochem), VCAM-1 (1:50) (Genzyme), factor VIII-related antigen (1:100) (Dako), and anti-smooth muscle α-actin (1A4; Sigma). Control Ab for factor XIIIa was rabbit antiserum against factor XIIIs (Calbiochem). Five-micrometer-thick cryostat sections of AIDS KS lesions from five different patients were immunostained (2) using rabbit polyclonal Abs to human placental SF (Ab 978; 1:1000) (18) or to a C-terminal peptide of the c-met protein (Ab C28; 1:1000) (24) as the primary Ab. Normal rabbit serum (1:1000) served as a negative control.

MDCK Serial Dilution Scatter Assay. SF activity was quantitated using the MDCK scatter assay (15). Samples were serially diluted by factors of two and incubated with 1-day-old colonies of MDCK cells for 20 hr in 96-well plates. Cells were stained and examined for scattering (colony spreading and cell separation). SF activity at the limiting dilution was defined as 0.5 scatter units per ml, allowing calculation of the SF titer in the undiluted sample.

SF ELISA. Immunoreactive SF protein was quantitated using a double Ab ("sandwich") ELISA using Immulon II 96-well plates (Dynatech) and mouse monoclonal Ab to human SF (10C11) (1:4000 of ascites) as well as rabbit Ab to human SF (1:1000) as described (12, 18). The assay was specific for SF; plasminogen, serum, and a variety of growth factors and cytokines were not cross-reactive. The lower limit of detection was about 0.1 ng of SF in the 100-μl assay volume.

Rat Cornea Angiogenesis Assay. Neovascularization was assayed in the avascular rat cornea (25). In some experiments, HTLV-II CM or SF was combined with a 1:200 dilution of SF neutralizing rabbit and chicken Abs (18) for 2 hr at 37°C prior to assay. Hydron implants were placed into a surgical pocket within rat corneas for 7 days. Responses were scored after carbon perfusion as positive only when sustained ingrowth of new vessels was present.

Cytokine Preparations. Mouse SF was purified from *ras*-transformed NIH 3T3 cells, as described (18). Recombinant human SF was provided by George Vande Woude (Frederick Cancer Research and Development Center, Frederick, MD). Recombinant human oncostatin M was obtained from PeproTech (Rocky Hill, NJ). Recombinant human IL-6 was purchased from Collaborative Research.

RESULTS

All KS tumor cell lines appeared morphologically similar and grew with a predominantly spindle-shaped morphology; they expressed strong cytoplasmic immunoreactivity for factor XIIIa and for VCAM-1, two markers expressed by KS cells *in vivo* and *in vitro* (9). KS cells were negative for EC markers—factor VIII antigen, ELAM-1, and factor XIIIs (data not shown). HUVECs cultured in HUVEC growth medium grew with an epithelioid configuration, expressed strong immunoreactivity for factor VIII antigen, and were uniformly negative for factor XIIIa and VCAM-1. However, after incubation for 18–24 hr in KSGM, HUVECs became spindle-shaped and acquired immunoreactivity for factor XIIIa and VCAM-1 (data not shown). No loss of factor VIII expression was detected when HUVECs became spindle-shaped after exposure to KSGM. In contrast, HASMCs were negative for factor XIIIa before *and* after exposure to KSGM. These cells remained positive for the smooth muscle marker α-actin (data not shown). HUVECs could be passaged in KSGM multiple times over 5–6 weeks. These "phenotypically converted" HUVECs appeared healthy and divided, although at a slower rate than the parental cells.

We used PCR analysis to confirm the presence of factor XIIIa mRNA in cells showing positive immunoreactivity. Human peripheral blood monocytes, a cell type known to express factor XIIIa, served as a positive control (26). Three of three KS cell lines tested expressed factor XIIIa mRNA (Fig. 1*A*). HUVECs did *not* constitutively express factor XIIIa mRNA. However, after exposure to KSGM, these cells contained factor XIIIa mRNA (Fig. 1*B*). HASMCs did not contain detectable constitutive XIIIa mRNA and could not be induced to express factor XIIIa mRNA by exposure to KSGM (Fig. 1*B*).

It has been suggested that a disordered cytokine network may contribute (7, 27). We next examined expression of

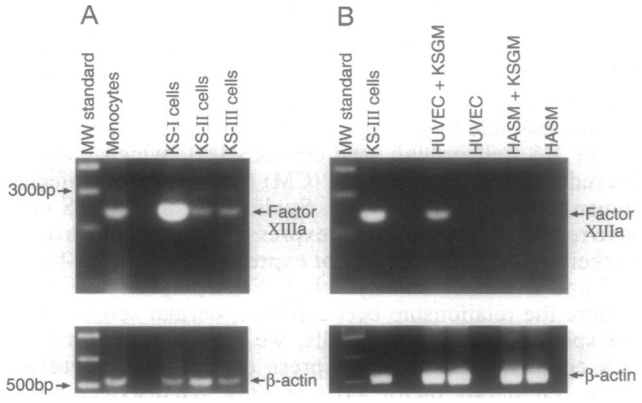

FIG. 1. PCR analysis of factor XIIIa transcripts. (*A*) Constitutive expression of factor XIIIa mRNA in monocytes, a positive control, and in three different lines of KS tumor cells. (*B*) Expression of factor XIIIa mRNA in HUVECs and HASMCs before and after a 24-hr exposure to KSGM.

mRNAs encoding a number of cytokines and cytokine-regulated adhesion and growth factors. When HUVECs were phenotypically converted to KS-like cells by exposure to KSGM, they acquired the ability to express four transcripts (IL-1β, IL-6, IL-8, and ICAM-1) that are also expressed by KS tumor cells (Fig. 2). These mRNAs were *not* expressed constitutively by control HUVECs cultured in HUVEC growth medium. KS cells, HUVECs, and phenotypically converted HUVECs shared the ability to express TGF-β mRNA (Fig. 2), while none of these cell types expressed mRNAs for IL-2, IL-3, IL-4, IL-5, tumor necrosis factor α, or interferon γ (data not shown). However, there were differences in the expression of IL-10, granulocyte/macrophage colony-stimulating factor, and TGF-α among these various cultured cells. Thus, ECs can be converted into cells that clearly resemble cultured KS tumor cells by morphologic and immunologic criteria, but the conversion process by KSGM is not complete.

Since SF converts epithelial cells to a spindle-shaped morphology, analogous to the effect of KSGM on EC morphology, we assayed KSGM for the presence of SF. We detected very high titers of SF activity in CM from the HTLV-II-infected human T-cell line 38-10 (HTLV-II CM), an essential component of KSGM. No SF was detected in any other component of KSGM. Two batches of HTLV-II CM contained 218 and 154 scatter units per ml, using a sensitive and specific bioassay of SF (15). We also found high levels of SF antigen in HTLV-II CM (10.5 ng/ml), using a double-antibody ELISA. SF production rate for 38-10 cells was estimated to be about 120 units per 10^6 cells per 48 hr, as compared with 20–80 units per 10^6 cells per 48 hr for six different human fibroblast lines. PCR analysis confirmed that 38-10 cells express SF mRNA (data not shown). In contrast, CM from purified, resting human T cells and from the HUT 78 human T-cell line contained no detectable SF by bioassay or ELISA.

Phenotypic conversion of ECs by KSGM, which required HTLV-II CM, could be reproduced by SF in the absence of other components of HTLV-II CM. After exposure to purified native mouse SF or recombinant human SF for 12–18 hr, HUVECs became spindle-shaped and expressed immunoreactive factor XIIIa and factor XIIIa mRNA (data not shown). Rabbit Ab to human SF blocked KSGM-induced phenotypic conversion of HUVECs (data not shown). Thus, SF appears to be the essential factor required for the morphologic and immunologic conversion of ECs to a KS cell-like phenotype. We next examined KS lesions for the presence of SF *in vivo*.

KS lesions from five patients showed positive immunostaining for SF in round lymphoid cells, perivascular dendritic cells, and interstitial spindle-shaped cells, but not in ECs (Fig. 3 *A* and *B*). Similar results were obtained using rabbit polyclonal Ab or mouse monoclonal Ab (10C11) (20) against human placental SF. The receptor for SF, c-met, is a transmembrane tyrosine kinase growth factor receptor (20, 21). A variety of cell types in the KS lesions stained positively for c-met protein, including pericytes, pili-erector muscle bundles, ECs, dermal dendritic cells, and interstitial spindle-shaped tumor cells (Fig. 3*F*). Moreover, cytospin preparations of cultured KS tumor cells (Fig. 3*C*) and HUVECs (Fig. 3*D*) were positively stained by antibody to c-met but *not* by control antibody (Fig. 3*E*). In addition, cultured bovine pericytes (provided by P. D'Amore, Harvard Medical School), HUVECs, and three out of three KS tumor cell lines expressed c-*met* mRNA by PCR analysis (data not shown). The three KS tumor cell lines also expressed SF mRNA, along with MRC5 cells, but not HUT 78 T cells used as a negative control (Fig. 4).

The presence of SF in KSGM and in KS lesions *in vivo* and KS cell lines *in vitro* together with c-met expression suggested that SF might be a growth factor for KS cells. Three of three KS tumor cell lines tested were stimulated to proliferate by both mouse and human SF (Fig. 5). Dose–response studies showed little or no stimulation of proliferation of KS cells at 10 units/ml, near maximal stimulation at 100 units/ml, and maximal stimulation at 250 units/ml of human SF. SF appeared to be at least as potent as two known

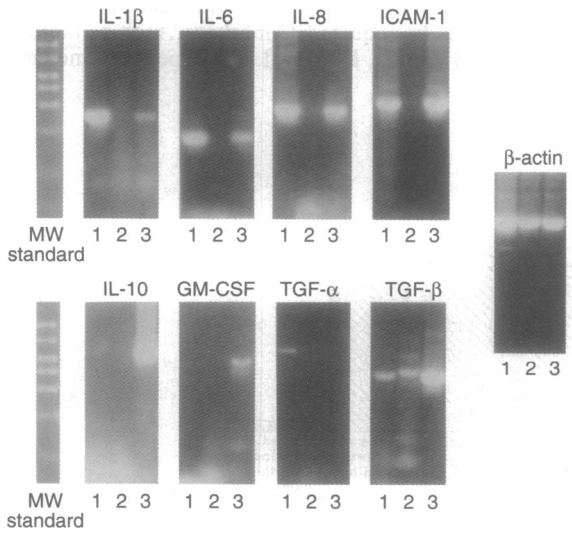

FIG. 2. Cytokine production profiles of AIDS KS tumor cells, normal ECs, and phenotypically converted ECs. PCR-based results are shown for KS tumor cells that were isolated and cultured in KSGM (lane 1), control HUVECs that were cultured in HUVEC growth medium (lane 2), and phenotypically converted HUVECs following exposure to KSGM for 24 hr (lane 3).

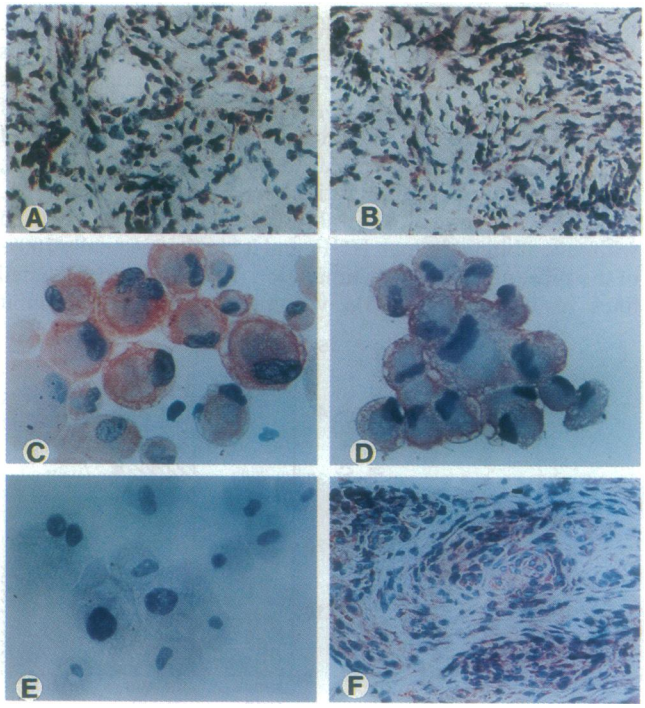

FIG. 3. Immunohistochemical detection of SF and its cell surface receptor, the c-met protein, in KS lesions *in vivo* and in cultured cells. Cryostat sections of AIDS KS lesions were immunostained using polyclonal rabbit Abs to human placental SF (*A* and *B*) or to a C-terminal peptide of the c-met protein (*F*) as the primary Abs. Cytospin preparations of cultured KS tumor cells (*C* and *E*) and HUVECs (*D*) were stained with anti-c-met Ab (*C* and *D*) or with normal rabbit serum as a control (*E*). Results portrayed are representative of five different KS lesions and three different KS cell lines.

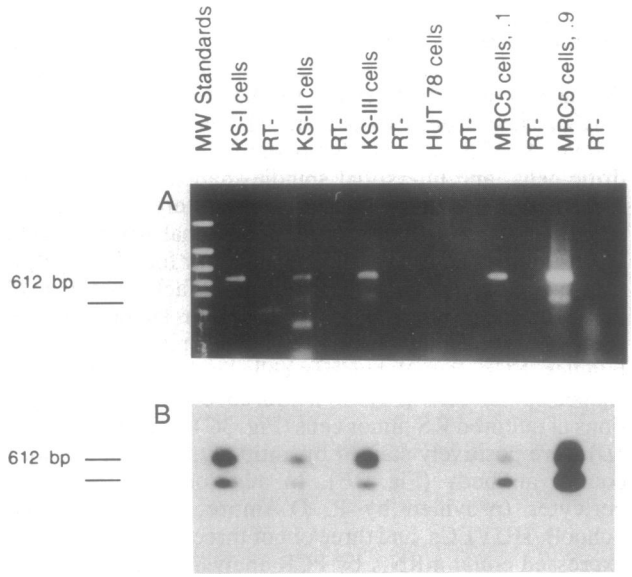

FIG. 4. KS tumor cells express SF mRNA. PCR amplification of mRNAs for SF was performed as described in *Materials and Methods*. (*A*) Ethidium bromide-stained gel showing the expression of SF mRNA in MRC5 cells, a positive control (0.1 of a full PCR and 0.9 of the same PCR), in three different lines of KS tumor cells, and no expression in HUT 78 cells, a negative control. First-strand cDNA synthesis was performed with and without reverse transcriptase (RT-). The last lane (RT-) represents a control where no cDNA template was used in the PCR amplification. (*B*) Same gel after blotting and hybridizing with an internal radiolabeled SF oligonucleotide primer. The 612-bp SF specific product is indicated, as well as a fragment that is consistently detected in different cell lines and that is thought to be derived from a smaller isoform of SF mRNA.

KS tumor cell mitogens, IL-6 (28) and oncostatin M (29) (Fig. 5), although none of these three factors was as potent as KSGM. Combinations of saturating concentrations of SF, oncostatin M, and IL-6 did *not* yield additional stimulation of proliferation. To assess the relative mitogenic contribution of SF in KSGM, neutralizing Ab to SF was added to KSGM, and the subsequent proliferation of KS cells was measured. As a positive control, the rabbit neutralizing Ab was used with recombinant human HGF, and it blocked virtually 100% of the subsequent growth stimulation (Fig. 5). In three KS cell lines tested, when KSGM was preincubated with rabbit anti-SF Ab (at 1:40), the KSGM-induced proliferation was reduced between 29% and 46%. A representative experiment of tumor cell line KS-III is shown in Fig. 5. Two other KS tumor cell lines tested revealed similar responses (data not shown). There was no inhibition of KSGM-induced proliferation when normal rabbit serum was substituted at a 1:20 dilution. Also, when the rabbit anti-SF Ab was further diluted beyond 1:400, no inhibition of KS cell growth was observed (data not shown).

The angiogenic activity of KSGM and relevant positive/negative control cytokines was investigated. Compared to known angiogenic cytokines such as basic fibroblast growth factor and HGF, KSGM HTLV-II CM was highly angiogenic in the angiogenesis assay (25), with four out of four strongly positive responses. This angiogenic activity was reduced by 75% (two out of eight positive responses) in the presence of rabbit antibody to human SF and reduced by 60% using the chicken antibody to SF. Both anti-SF Abs could neutralize recombinant human HGF induced angiogenesis and did not cross-react with basic fibroblast growth factor. Thus, SF appears to be a key factor in KSGM, with growth-promoting activity for KS tumor cells as well as proangiogenic activity.

DISCUSSION

CM of T lymphocytes infected with human retrovirus stimulates AIDS KS-derived tumor cells (7). In this report we demonstrate that ECs can be converted to a KS tumor cell-like phenotype by treatment with CM from HTLV-II-infected T lymphocytes. The resemblance between phenotypically converted ECs and KS tumor cells was striking, based on similarities in morphologic and immunologic features. Because ECs exposed to KSGM, together with KS tumor cells and dermal dendrocytes, all share factor XIIIa expression, this immunophenotypic link suggests that these cell types may be more interrelated than previously thought (1). Indeed a growing body of evidence points to phenotypic diversity and biological potential of ECs derived from skin (30). Despite the similarities between phenotypically converted ECs and KS tumor cells, these two cell types were *not* identical. Converted ECs continued to express factor VIII antigen, which is not expressed by KS cells, and showed a few differences in the cytokine production profile relative to that of KS cells. We have not yet found conditions that would induce KS cells to acquire EC markers, such as factor VIII or ELAM-1 (9). Thus, HTLV-II CM appears to mediate the

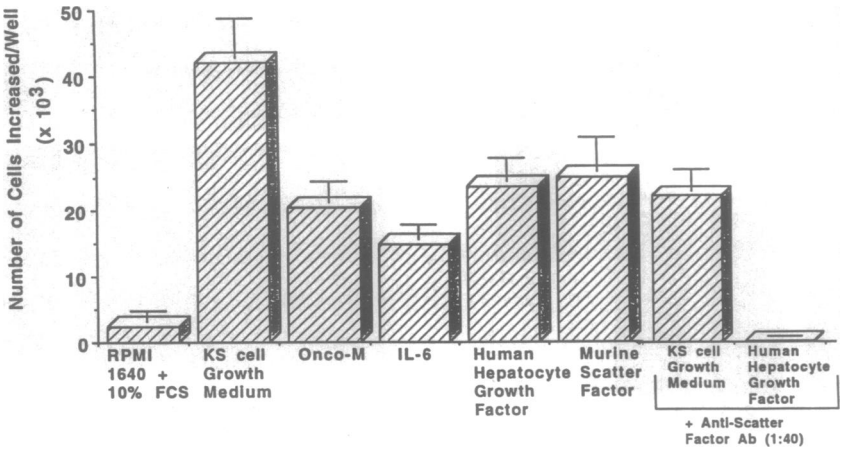

FIG. 5. Importance of SF in KS tumor cell proliferation. KS cells (2.5×10^4) were seeded into 48-well plates and allowed to attach overnight. Cells were washed three times, incubated in 0.5 ml of RPMI 1640 plus 10% fetal calf serum containing different factors for 80 hr, and counted. Cytokines were used at doses sufficient to give maximal proliferative responses: recombinant human SF (HGF), 250 units/ml; purified native mouse SF, 250 units/ml; recombinant human oncostatin M (Onco-M), 100 ng/ml; recombinant human IL-6, 500 units/ml. For blocking studies, the growth medium was preincubated with rabbit anti-SF Ab (2 hr, 37°C), prior to initiating the proliferation assay. Values are means ± SD of duplicate assays. Results portrayed are representative of three different experiments.

initial phases of the transdifferentiation of ECs into KS tumor cells but is not, by itself, capable of producing *fully* transformed tumor cells. Cytokines and/or cell–cell interactions missing from the culture environment may be required to complete the transformation process.

We detected large quantities of SF in the CM from HTLV-II-infected T cells, but *not* from resting or uninfected T cells. We showed that the ability of HTLV-II CM to convert ECs to a KS cell phenotype is due, in part, to SF. We further demonstrated that HTLV-II CM is highly angiogenic and that SF is responsible for most of this angiogenic activity. In this study, we found SF to be an important growth factor contained within KSGM, a potent mitogen for cultured KS-derived cells, and that SF is present in KS lesions *in vivo*. SF should thus be added to other KS cell mitogens such as oncostatin M and IL-6 (28, 29, 31).

This study established that T cells activated by retrovirus infection can produce significant amounts of SF. In preliminary studies, type 1 human immunodeficiency virus-infected HUT 78 T cells can also produce SF mRNA and protein, although at much lower levels compared to the HTLV-II-infected 38-10 T cells. We detected immunoreactive SF in round lymphoid cells, dendritic cells, and KS tumor cells, but *not* in ECs, *in vivo*. This finding is consistent with our previous observation that perivascular round mononuclear cells and spindle-shaped cells in psoriatic lesions stain positively for SF, but that cells of the blood vessel wall do not stain for SF (18). Psoriasis, like KS, is increased in AIDS patients (1, 2), and both share a prominent angiogenic response (32), as well as accumulation of factor XIIIa-positive dermal dendrocytes. The presence of SF in dermal dendrocytes and KS tumor cells within KS lesions could result from the uptake or production of SF by these cells. To further distinguish between these possibilities, we observed that three different KS cells *in vitro* could express SF mRNA. Autocrine production of, and response to, SF by epithelial cells and other human sarcoma cell lines has been described (33, 34). For KS cells, it is also likely that they utilize such an autocrine, as well as paracrine, mechanism of SF-induced growth stimulation because these tumor cells express high levels of c-met. In a murine model system, NIH 3T3 cells overexpressing c-met were shown to be tumorigenic via an autocrine mechanism (35).

Based on this study, we propose the following hypothesis: retroviral infection of T cells causes these cells to produce SF locally, which stimulates ECs to migrate into adjacent perivascular sites. These stimulated ECs undergo phenotypic conversion and accumulate within perivascular interstitium as factor XIIIa-positive spindle-shaped tumor cells. These c-met-expressing cells produce cytokines that further expand the ongoing neovascularization process and promote the autocrine and paracrine-mediated growth of KS tumor cells. In conclusion, despite the cellular heterogeneity within KS lesions, these results suggest that SF and c-met may be important in potential interconversion (i.e., transdifferentiation) between ECs, dermal dendrocytes, and spindle-shaped tumor cells. A recent report proposed that SF/c-met signaling is critically important in mesenchymal to epithelioid cell conversion (36). Additional studies of SF and c-met in KS are indicated to determine if this previously enigmatic tumorigenic process can be inhibited by targeting this ligand–receptor pathway.

This work was supported by U.S. Public Health Service Grants HL39926 (P.J.P.), AR40065, AR01823, and AR40488 (B.J.N.). E.M.R. is an Established Investigator of the American Heart Association.

1. Nickoloff, B. J. & Griffiths, C. E. M. (1989) *Science* **243**, 1736–1737.
2. Nickoloff, B. J. & Griffiths, C. E. M. (1989) *Am. J. Pathol.* **135**, 793–800.
3. Dorfman, R. F. (1984) *Hum. Pathol.* **15**, 1013–1017.
4. Weich, H. A., Salahuddin, S. Z., Gill, P., Nakamura, S. & Folkman, J. (1991) *Am. J. Pathol.* **139**, 1251–1258.
5. Nickoloff, B. J. (1991) *Arch. Dermatol.* **127**, 523–529.
6. Nickoloff, B. J. (1993) *Arch. Dermatol.* **129**, 250–251.
7. Nakamura, S., Salahuddin, S. Z., Biberfeld, P., Ensoli, B., Markham, P. D., Wong-Staal, F. & Gallo, R. C. (1988) *Science* **242**, 425–430.
8. Salahuddin, S. Z., Nakamura, S., Biberfeld, P., Kaplan, M. H., Markham, P. D., Larsson, L. & Gallo, R. C. (1988) *Science* **242**, 430–433.
9. Huang, Y., Friedman-Kien, A. E., Li, J. J. & Nickoloff, B. J. (1993) *Arch. Dermatol.* **129**, 1291–1296.
10. Stoker, M., Gherardi, E., Perryman, M. & Gray, J. (1987) *Nature (London)* **327**, 238–242.
11. Weidner, K. M., Arakaki, N., Vandekerckhove, J., Weingart, S., Hartmann, G., Rieder, H., Fonatsch, C., Tsubouchi, H., Hishida, T., Daikuhara, Y. & Birchmeier, W. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 7001–7005.
12. Bhargava, M., Joseph, A., Knesel, J., Halaban, R., Li, Y., Pang, S., Goldberg, I., Setter, E., Donovan, M. A., Zarnegar, R., Michalopoulos, G. A., Nakamura, T., Faletto, D. & Rosen, E. M. (1992) *Cell Growth Differ.* **3**, 11–20.
13. Nakamura, T., Nishizawa, T., Hagiya, M., Seki, T., Shimonishi, M., Sugimura, A. & Shimizu, S. (1989) *Nature (London)* **342**, 440–443.
14. Miyazawa, K., Tsubouchi, H., Naka, D., Takahashi, K., Okigaki, M., Arakaki, N., Nakayama, H., Hirono, S., Sakiyama, O., Gohda, E., Daikuhara, Y. & Kitamura, N. (1989) *Biochem. Biophys. Res. Commun.* **163**, 967–973.
15. Rosen, E. M., Meromsky, L., Setter, E., Vinter, D. W. & Goldberg, I. D. (1990) *Proc. Soc. Exp. Biol. Med.* **195**, 34–43.
16. Rosen, E. M., Grant, D., Kleinman, H. K., Jaken, S., Donovan, M. A., Setter, E., Luckett, P. M. & Carley, W. (1991) in *Cell Motility Factors*, eds. Goldberg, I. D. & Rosen, E. M. (Birkhauser, Basel), pp. 76–88.
17. Rubin, J. S., Chan, A. M.-L., Bottaro, D. P., Burgess, W. H., Taylor, W. G., Cech, A. C., Hirschfield, D. W., Wong, J., Miki, T., Finch, P. W. & Aaronson, S. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 415–419.
18. Grant, D. S., Kleinman, H. K., Goldberg, I. D., Bhargava, M. M., Nickoloff, B. J., Kinsella, J. L., Polverini, P. & Rosen, E. M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 1937–1941.
19. Bussolino, F., DiRenzo, M. F., Ziche, M., Bocchietto, E., Oliver, M., Naldini, L., Gandino, G., Tamagnon, L. & Comoglio, P. M. (1992) *J. Cell Biol.* **119**, 629–641.
20. Bottaro, D. P., Rubin, J. S., Faletto, D. L., Chan, A. M.-L., Kmiecik, T. E., Vande Woude, G. F. & Aaronson, S. A. (1991) *Science* **251**, 802–804.
21. Naldini, L., Vigna, E., Narsmhan, R. P., Gandino, G., Zarnegar, R., Michalopoulos, G. K. & Comoglo, P. M. (1991) *Oncogene* **6**, 501–504.
22. Chou, Q., Russell, M., Birch, D. E., Raymond, J. & Bloch, W. (1992) *Nucleic Acids Res.* **20**, 1717–1720.
23. Yang, X. M. & Park, M. (1993) *Dev. Biol.* **157**, 308–320.
24. Gonzatti-Haces, M., Seth, A., Park, M., Copeland, T., Oroszlan, S. & Vande Woude, G. F. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 21–25.
25. Polverini, P. J. & Leibovich, S. J. (1984) *Lab. Invest.* **51**, 635–642.
26. Henriksson, P., Becker, S., Lynch, G. & McDonagh, J. (1985) *J. Clin. Invest.* **76**, 528–534.
27. Vogel, J., Henrichs, S. H., Reynolds, R. K., Luciw, P. A. & Jay, G. (1988) *Nature (London)* **335**, 606–611.
28. Miles, S. A., Rezai, A. R., Salazar-Gonzalez, J. F., Vander Meyden, M., Stevens, R. H., Logan, D. M., Mitsuyasu, R. T., Taga, T., Hirano, T., Kishimoto, T. & Martinez-Maza, O. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4068–4072.
29. Miles, S. A., Martinez-Maza, O., Rezai, A., Magpantay, L., Kishimoto, T., Nakamura, S., Radka, S. F. & Linsley, P. S. (1992) *Science* **255**, 1432–1434.
30. Karasek, M. A. (1992) in *Dermal Immune System*, ed. Nickoloff, B. J. (CRC, Boca Raton, FL), pp. 149–162.
31. Nair, B. C., DeVico, A. L., Nakamura, S. & Copeland, T. D. (1992) *Science* **255**, 1430–1432.
32. Folkman, J. (1972) *J. Invest. Dermatol.* **59**, 40–43.
33. Adams, J. C., Furlong, R. A. & Watt, F. M. (1991) *J. Cell Sci.* **98**, 385–394.
34. Rong, S., Jeffers, M., Resau, J. H., Tsarfaty, I., Oskarsson, M. & Vande Woude, G. F. (1993) *Cancer Res.* **53**, 5355–5360.
35. Rong, S., Bodescot, M., Blair, D., Nakamura, T., Mixuno, K., Park, M., Chan, A., Aaronson, S. & Vande Woude, G. F. (1992) *Mol. Cell. Biol.* **12**, 5152–5158.
36. Tsarfaty, J., Rong, S., Resau, J. H., Rulong, S., Pinto De Silva, P. & Vande Woude, G. F. (1993) *Science* **263**, 98–101.