Supernatants of Acquired Immunodeficiency Syndrome-related Kaposi's Sarcoma Cells Induce Endothelial Cell Chemotaxis and Invasiveness

Erik W. Thompson, Shuji Nakamura, Thomas B. Shima, Antonella Melchiori, George R. Martin, S. Zaki Salahuddin, Robert C. Gallo, and Adriana Albini

Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research; and Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20892, and Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 10, 16132 Genoa, Italy

ABSTRACT

Kaposi's sarcoma (KS) in general, and acquired immunodeficiency syndrome-related KS (AIDS-KS) in particular, is a highly invasive and intensely angiogenic neoplasm of unknown cellular origin. We have recently established AIDS-KS cells in long-term culture and reported the development of KS-like lesions in nude mice inoculated with these cells. Here, we have examined the in vitro invasiveness of basement membrane by AIDS-KS cells, as well as the effect(s) of their supernatants on the migration and invasiveness of human vascular endothelial cells. AIDS-KS cells were highly invasive in the Boyden chamber invasion assay and formed invasive, branching colonies in a 3-dimensional gel (Matrigel). Normal endothelial cells form tube-like structures on Matrigel. AIDS-KS cell-conditioned media induced endothelial cells to form invasive clusters in addition to tubes. KS-cell-conditioned media, when placed in the lower compartment of the Boyden chamber, stimulated the migration of human and bovine vascular endothelial cells across filters coated with either small amounts of collagen IV (chemotaxis) or a Matrigel barrier (invasion). Basic fibroblast growth factor could also induce endothelial cell chemotaxis and invasion in these assays. However, when antibodies to basic fibroblast growth factor were used the invasive activity induced by the AIDS-KS-cell-conditioned media was only marginally inhibited, suggesting that the large quantities of basic fibroblast growth factor-like material released by the AIDS-KS cells are not the main mediators of this effect. Specific inhibitors of laminin and collagenase IV action, which represent critical determinants of basement membrane invasion, blocked the invasiveness of the AIDS-KS cell-activated endothelial cells in these assays. These data indicate that KS cells appear to be of smooth muscle origin but secrete a potent inducer of endothelial cell chemotaxis and invasiveness which could be responsible for angiogenesis and the resulting highly vascularized lesions. These assays appear to be a model to study the invasive spread and angiogenic capacity of human AIDS-related KS and should prove useful in the identification of molecular mediators and potential inhibitors of neoplastic neovascularization.

INTRODUCTION

Classically, KS is a highly vascularized but relatively benign neoplasm arising on the lower extremities of elderly males in Mediterranean and African countries (1–4). A clinically more virulent form of Kaposi’s sarcoma develops in 20–30% of individuals with AIDS (5–8), and in some individuals suffering immunosuppression due to other causes (9–12). This histopathology of Kaposi’s sarcoma is highly complex, mostly displaying conspicuous vascularization in a stroma of spindle-shaped cells of mesenchymal origin (13–16). Recently, long-term cultures of cells from AIDS-KS lesions have been established utilizing media conditioned by human T-cell leukemia/lymphoma virus type II-transformed T+, positive lymphocytes (17). This medium contains novel factor(s) that promote the growth and survival of the AIDS-KS cells. When implanted s.c. into the nude mouse, the AIDS-KS cells induce a highly vascularized lesion which is composed almost entirely of murine cells (18). This lesion forms in response to strong angiogenic factors secreted by metabolically active AIDS-KS cells, since lightly fixed AIDS-KS cells elicit no response (18). Live AIDS-KS cells also induce an angiogenic response to the choroidal membrane of the 9-day-old chicken embryo (18).

Basement membranes represent significant barriers to the spread of normal and nonmalignant tumor cells (19, 20) and also appear to have potent morphogenetic effects on the induction and maintenance of differentiated phenotypes (21). The ability of cells to penetrate through a barrier of reconstituted basement membrane correlates with metastatic potential (22, 23) and requires specific proteinases shown to be more abundant in malignant cells than in nonmetastatic tumor cells or normal cells (24, 25).

We have examined invasiveness of the AIDS-KS cells, their morphological responses to Matrigel, and the effects of their secreted products on the migration and invasiveness of normal vascular endothelial cells. Our studies indicate that the AIDS-KS cells show invasive behavior comparable to other malignant tumor cells, resemble smooth muscle cells in their initial morphological response to Matrigel, and secrete factors that stimulate the invasiveness of vascular endothelial cells. Although it has been found that basic FGF induces endothelial cell invasion when added to basement membrane in the amnion invasion assay (26), the invasiveness induced by AIDS-KS-CM was not inhibited by anti-bFGF antibodies.

MATERIALS AND METHODS

Reagents. Matrigel, a mixture of basement membrane components extracted from the EHS tumor (21), was kindly provided by Dr. Hynda Kleinman, NIH. Collagen IV, also purified from the EHS tumor (27), was kindly provided by Dr. Roy Ogle, NIH. Acidic FGF was from Collaborative Research (Bedford, MA) and we used recombinant basic FGF from Amgen Biologicals (Thousand Oaks, CA). Antibodies to bFGF were a gift of Dr. Andrew Baird, La Jolla, CA. Antisera to bFGF used on bovine aorta endothelial cells were obtained from Dr. Marco Presta, Brescia, Italy. Anti-IL-1 antibodies were purchased from Endogen (Boston, MA) and anti-platelet-derived growth factor antibodies from R&D (Minneapolis, MN).

Cell Culture. AIDS-KS cells (AIDS-KS-3) (17) were maintained as described previously in RPMI 1640 containing 15% FCS (Gibco, New York, NY) and 20% conditioned medium from human T-cell leukemia/lymphoma virus type II-transformed T+, positive lymphocytes. HuVE
cells, isolated from full-term umbilical cords, were supplied by Advanced Biotechnology Incorporated (Silver Spring, MD) and maintained in RPMI 1640 containing 15% FCS supplemented with 30 μg/ml endothelial cell growth supplement (Collaborative Research, Bedford, MA) and 45 μg/ml heparin (Sigma, St. Louis, MO). BAEC were the gift of Dr. Elisabetta Dejana, Torino, Italy, and were cultured in DMEM-10% FCS. Human aortic SM cells from the abdominal aorta (passage 8), a gift of Dr. Jerry Winkels, American Red Cross, Holland Research Laboratories, Rockville, MD, were maintained in M199 containing 10% FCS and used at passage 9. Human skin fibroblasts (MRC-5) were obtained from the ATCC, Rockville, MD, and maintained in DMEM containing 10% FCS. Conditioned media were obtained by washing confluent cell monolayers twice with phosphate-buffered saline and incubating them for 24 h with serum-free minimal essential medium.

Chemoinvasion Assay. The Boyden chamber chemoinvasion assay was performed as described previously (22). Polycarbonate filters (12-μm pore, polyvinylpyrrolidone free; Nucleopore, Pleasanton, CA) were coated with Matrigel (25 μg/filter) which was dried and then reconstituted at 37°C into a solid, even gel on the surface of the filter. Filters were precoated with 5 μg of collagen IV. Conditioned media or bFGF (1–25 ng/ml) were used as chemoattractants. Cells were harvested with trypsin, washed twice with DMEM containing 0.1% bovine serum albumin (Gibco, New York, NY) and added to the top chamber (3 × 10^5 cells/chamber). Chambers were incubated in a humidified incubator at 37°C in 5% CO₂ in air for 6 h. The cells remaining on the top filter surface were mechanically removed, while those which had traversed the Matrigel and attached to the lower filter surface were stained (Diff-Quick; American Scientific Products, McGaw Park, IL) and either counted under microscope or quantitated electronically with the Optronic Lab 3200.

Chemotaxis Assay. Chemotaxis assays were performed as described for the chemoinvasion studies with the single exception that the filters were coated with 5 μg of collagen IV. This coats the interstices of the filter but does not form a barrier over the surface, and it improves the ability of the cells to attach to and migrate through the filter (28). Chemotaxis assays were performed in parallel to the chemoinvasion assays using similar aliquots of cells and chemoattractant.

Matrigel Cultures. Cells were seeded either on top of a Matrigel (10 mg/ml) gel, inside the gel or “sandwiched” between two layers of gel (Matrigel embedded cultures) (22, 29–31).

Cells were harvested with trypsin, resuspended in the growth media used for monolayer culture, and plated onto a layer of Matrigel (0.5 ml in a 16-mm-diameter tissue culture well) already polymerized for 30 min at 37°C in the bottom of the wells of a Costar 24 plate. For growth inside the gel, the cells were mixed with liquid Matrigel and the gel was polymerized afterwards. For Matrigel-embedded cultures, cells were plated on a thin layer (0.2 ml) of polymerized Matrigel. Cells were allowed to attach to the surface of the Matrigel for 60 min at 37°C. A second layer of Matrigel (0.1 ml) was applied over the cells (“sandwiched”) and allowed to set (37°C, 30 min). Five × 10^5 cells/well were used. In all three assays culture medium was added carefully to the wells, and Matrigel cultures were incubated at 37°C. After 48 h or 7 days the cultures were photographed under Hofmann optics, using a Leitz photomicroscope. In some experiments serum-free supernatants of fibroblasts, KS cells, and HUVE cells were added 1:1 with culture media for HUVE cells to observe their effects on HUVE cell morphology.

RESULTS

Morphological Responses to Matrigel. The responses of the AIDS-KS cells when growing on a gel of reconstituted basement membrane may provide clues to their origins and state of malignancy. We examined the behavior of cells cultured either on top of or within Matrigel (Fig. 1). When embedded as single cells within Matrigel, the AIDS-KS cells proliferated and spread into the gel forming large branching colonies (Fig. 1, 2a). Under these conditions, both the HUVE (Fig. 1, 3a) and SM (Fig. 1, 3a) cell populations remained as single cells dispersed throughout the gel. When plated on top of Matrigel, HUVE cells underwent rapid alignment (1 h) and formed an interconnected system of tube-like structures after 48 h (Fig. 1, 3b), consistent with recent reports (32, 33). Under the same conditions, AIDS-KS (Fig. 1, 2b) and aortic SM cells (Fig. 1, 1b) aggregated into tight clusters. After 3 days, the AIDS-KS cells remained in spherical aggregates, anchored to the Matrigel layer by a number of penetrating cells at the base (Fig. 1, 2b, left) and formed invasive branching colonies after 7 days (Fig. 1, 2b, right). At the same time the aortic SM cells flattened out and spread onto the Matrigel surface (Fig. 1, 1b, right). We also examined the morphology of these cell types when plated onto Matrigel and then covered by another layer of Matrigel after 60 min (“sandwiched”). Under these conditions, the HUVE cells formed a more anastomosed network of tubes (Fig. 1, 3c), while the AIDS-KS (Fig. 1, 2c) and SM (Fig. 1, 1c) cells aggregated into clusters of similar appearance. The invasive behavior of AIDS-KS cells when plated on top of the Matrigel gels was comparable to that of other malignancies previously investigated (22–31). These Matrigel responses indicate that the AIDS-KS cultures behave differently than HUVE cells and have a closer resemblance to SM cells.

Effect of Conditioned Media on HUVE Cell Morphology on Matrigel. Because of the high vascularization of the AIDS-KS lesions, we were interested in studying the effect of secreted AIDS-KS products on endothelial cell invasive behavior. We first analyzed the effect of AIDS-KS-conditioned medium on HUVE cell morphology on Matrigel. Serum-free supernatants (CM) of fibroblasts, AIDS-KS, and HUVE cells were added 1:1 with HUVE cell-culture media to HUVE cells seeded on Matrigel (Fig. 2). In the presence of HUVE-CM (Fig. 2a) the endothelial cells formed tube structures as with regular tissue culture media (see Fig. 1, 3b). In the presence of KS-CM, the anastomosed tube structures were observed with three-dimensional invasive clusters at their crossing points (Fig. 2b). FB-CM, on the contrary, inhibited tube formation and numerous small cell aggregates with no tubes departing were observed (Fig. 2c). KS cells appear to produce specific factors which not only promote HUVE cell growth in the matrix but also induce an invasive morphology in these cells.

Cultured AIDS-KS Cells Secrete Potent Chemoattractants. We initially compared the serum-free conditioned media from AIDS-KS cell cultures (KS-CM) to that collected from fibroblast cultures under similar conditions (FB-CM), the latter being a reliable source of potent chemoattractants including matrix factors, degraded matrix components, and growth factors (28). Similar levels of chemotaxis were induced by KS-CM and FB-CM for each cell type examined (fibroblast, smooth muscle cells, HUVE cells, and the AIDS-KS cells) (Fig. 3). The aortic SM cells showed a higher level of mobility to each CM than the AIDS-KS or HUVE cells, the latter sharing approximately equal levels of migratory activity with FB-CM and KS-CM. HUVE-CM chemotactic activity was also investigated. HUVE-CM activity was comparable to that of the other CMs for fibroblasts but was less active than the other CMs in inducing migration of SM and KS cells. It was inactive with respect to autocrine stimulation of HUVE cells.

Stimulation of Endothelial Cell Invasiveness by Conditioned Media from Cultured AIDS-KS Cells. In the chemoinvasion assay the AIDS-KS cells showed a much higher invasiveness of the Matrigel barrier than either the SM or HUVE cells inde-
Fig. 1. Comparative morphologies of normal HUVE, SM, and AIDS-KS cells grown in and on Matrigel. Primary cultures of human aortic smooth muscle (passage 9, Row 1), AIDS-KS-3 cells (Row 2), and HUVE cells (Row 3) were either embedded throughout Matrigel as single cells (Column a), plated on top of gelled Matrigel (Column b), or allowed to aggregate for 60 min on top of a Matrigel bed and then sandwiched between a subsequent layer of Matrigel (Column c). Photographs were taken under Hofmann optics on days 3 (Column b, left and Column c), and 7 (Column a and right of Column b). Column 1 and left of Column 2, × 100; right of Column b and Column c, × 200.

Fig. 2. Effect of conditioned media on HUVE cell morphology on Matrigel. Serum free supernatants of HUVE cells (a), AIDS-KS cells (b), and fibroblasts (c) were added 1:1 with culture media for HUVE cells. In the presence of HUVE-CM (a) the endothelial cells formed tube structures as with regular tissue culture media (see Fig. 1). Cells were photographed on day 3. The experiment was done in duplicate and repeated twice.
Fig. 3. Chemotactic responses of each cell type to conditioned media from AIDS-KS cells (KS-CM), fibroblasts (FB-CM), and HUVE (HUVE-CM) cells and bovine serum albumin controls. Experiment was run in triplicate and repeated three times. Columns, means from an experiment done in triplicate; bars, SD. Five fields were counted on each triplicate filter.

Fig. 4. Chemoinvasive responses of each cell type to conditioned media from AIDS-KS cells (KS-CM), fibroblasts (FB-CM), and HUVE (HUVE-CM) cells. Columns, means from an experiment done in triplicate; bars, SD. The experiment was repeated twice more and the same results were obtained. The data used from each experiment was the mean cell number/field from 5 fields counted on each duplicate filter. Invasion in the presence of bovine serum albumin alone was 10-20% of the KS-CM-induced HUVE cell invasion (not shown).

Table 1 Inhibition of basement membrane invasion

<table>
<thead>
<tr>
<th>Endothelial cells (KS-CM)</th>
<th>Chemotaxis</th>
<th>Invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>chemoattractant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No inhibitor</td>
<td>100 ± 7*</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>+anti-laminin antibodies</td>
<td>104 ± 11</td>
<td>7 ± 3*</td>
</tr>
<tr>
<td>+collagenase inhibitor</td>
<td>130 ± 10</td>
<td>10 ± 2*</td>
</tr>
<tr>
<td>SC44463</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KS cells (FB-CM chemoattractant)</th>
<th>Chemotaxis</th>
<th>Invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inhibitor</td>
<td>100 ± 14</td>
<td>100 ± 17</td>
</tr>
<tr>
<td>+collagenase inhibitor</td>
<td>96 ± 23</td>
<td>5 ± 1*</td>
</tr>
<tr>
<td>SC44463</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data are expressed as a percentage of migrated cells as compared to untreated controls. Mean ± SEM for 10 fields counted on duplicate filters.
* Significantly less than control, P < 0.01. Experiment was repeated twice.

Fig. 5. Effect of antibodies to growth factors on KS-CM induced HUVE cell chemotaxis and invasion. Purified bFGF (25 ng/ml) was used as a control chemoattractant. Affinity-purified antibodies to bFGF, IL-1, and platelet-derived growth factor (at a 1:200 dilution) were used as potential inhibitors of migration and invasion. Columns, means from an experiment done in triplicate; bars, SD. The experiment was repeated twice more and the same results were obtained.

Secretive factors that promote cell migration but also specifically stimulate basement membrane invasiveness in vascular elements, particularly endothelium.

Inhibition of AIDS-KS Cell and Endothelial Cell Invasion. We have examined the effect of a specific inhibitor of collagenase (SC44463; Searle Research and Development) on the invasiveness of HUVE and AIDS-KS cells. A previous report has shown that this compound inhibits type IV collagenase activity (23). SC44463 prevented the KS-CM-stimulated HUVE cells, as well as the AIDS-KS cells, from invading through the reconstituted basement membrane barrier (Table 1), suggesting an involvement of type IV collagenase in this process. Since laminin binding is essential for cells to cross basement membrane compartments (19, 20, 34) we also tested the effect of anti-laminin antibodies on the KS-CM-induced HUVE cell migration and invasion. The antibodies did not effect migration but virtually abolished invasiveness (Table 1). These data show that invasiveness of AIDS-KS cells as well as HUVE cells induced by KS-CM occurs through the same mechanisms as for invasive tumor cells.

Induction of Endothelial Cell Invasiveness by Basic Fibroblast Growth Factor (bFGF). Basic FGF has been shown to be involved in vitro angiogenesis of the human amniotic membrane (26). To determine if bFGF could also stimulate endothelial cells to invade through our reconstituted basement membranes, we utilized it as a chemoattractant and in the invasion assay (Fig. 5). bFGF was chemotactic for the HUVE cells (Fig. 5) and significantly increased the penetration of Matrigel by the HUVE cells. Acidic FGF was also tested and was found to be able to stimulate the HUVE cell invasion but was approximately 10-fold less active than basic FGF, the two factors showing maximal effects at 10 and 100 ng/ml, respectively (data not shown). Such activity is consistent with the known angiogenic effects of these molecules and their capacity to induce plasminogen activator production and migration in bovine capillary and aortic endothelial cells (35-37).

To determine the extent to which bFGF contributed to HUVE cell invasion induced by KS-CM, we performed the assay in the presence of antibodies to bFGF (38). An antibody concentration 10-fold higher than that able to inhibit KS-CM stimulation of HUVE cell growth (39) was added along with the cells to the migration chambers. While the antibodies were able to block invasion stimulated by purified bFGF, they could...
inhibit only 10% of KS-CM-stimulated HUVE cell invasion (Fig. 5). This indicates that bFGF is not the major factor inducing HUVE cell invasion. IL-1 is a cytokine also expressed at high levels in our AIDS-KS cells (39). Antibodies against IL-1 were also tested at 10-fold higher concentration than that required to inhibit proliferation in these cells. These antibodies appeared to induce a partial reduction of KS-CM-induced HUVE cell invasion (about 30%). Anti-bFGF and anti-IL-1 antibodies were also tested on BAEC, which are also induced to be invasive by KS-CM (Table 2). Again, very little inhibition was observed. A combination of the two antibodies together showed that they did not act synergistically (Table 2). Antibodies to platelet-derived growth factor, a chemoattractant for smooth muscle cells (40), fibroblasts, but not endothelial cells (41), were ineffective in inhibiting KS-CM-induced endothelial cell invasion (Fig. 5). Of the three antibodies tested, none was able to inhibit the formation of invasive clusters by HUVE cells in Matrigel in the presence of KS-CM (data not shown). These experiments suggest that a potent inducer of endothelial cell invasiveness, other than bFGF or IL-1, is produced by AIDS-KS cells.

DISCUSSION

The cellular origins of Kaposi's sarcoma, as gauged from the literature, remain largely unresolved. The predominant occurrence of vascular channels and the presence of various cellular markers suggest an endothelial origin (15, 42, 43). However, enzyme histochemical studies and factor VIII staining have been inconclusive in terms of the precise cellular origins, with observations in favor of either vascular endothelium (43-46) or lymphatic endothelium (16, 47-50) as the originating tissue. The relative abundance of basement membrane components associated with the spindle-shaped cells of the KS lesions in all 3 histological stages (51-54) is consistent with the electron microscopic visualization of basal laminae in these lesions (42, 55) and supports a vascular origin for the Kaposi's sarcoma lesion. The presence of basement membrane in the lesion has led to speculation that the KS spindle-shaped cells may originate from pericytes, smooth muscle cells, or myofibroblasts (14, 42, 55, 56).

Matrigel induces profound morphological responses in a variety of cellular systems including rat seminiferous tubule explants (57) and isolated alveoli from the midpregnant mouse (58), and individual cell types assume different characteristic morphologies in Matrigel. Neurite outgrowth from neuroblastoma cell lines (59) and tube formation in endothelial cells (32, 33) have been observed. Recently, we have also found that invasive and/or metastatic cancer cells adopt a stellate, branching morphology when grown on Matrigel (22, 23). In this study, we compared the morphological responses to Matrigel of AIDS-KS cells, normal HUVE cells, and normal human vascular SM cells. The responses observed suggest that AIDS-KS cells are morphologically distinct from normal endothelial cells but resemble normal vascular smooth muscle cells. While this contrasts with their lack of desmin staining (18), it is consistent with previous reports of spindle-shaped cells isolated from AIDS-KS skin biopsies which showed SM cell-like growth factor chemoinvasion specificities (41) and cell surface markers. AIDS-KS cell behavior was more aggressive than that of SM cells; the AIDS-KS cells showed an invasive phenotype similar to that of other malignant cells (22, 30, 31), forming stellate colonies when grown on Matrigel and substantial invasion in the chemoinvasion assay. This is in agreement with data obtained with other cultures of AIDS-KS cells (41) and is consistent with the relatively progressive nature of the dermal and visceral Kaposi's sarcoma in AIDS-infected humans (7, 8).

Invasion by AIDS-KS cells was inhibited by agents which have been found to inhibit the invasion of other malignant cells (23, 34), indicating the AIDS-KS cells utilize similar mechanisms for invasion into those found in other malignancies. Previously published observations which best support the notion that KS is a true malignancy are the isolation of an FGF-related oncogene hst from KS DNA (60, 61), and an earlier report of transforming capacity of DNA isolated from KS (62). Balanced against these findings are the tendency for KS to regress in cases where immunocompetence is restored, the apparent lack of aneuploidy in the spindle-shaped cells, and its onset as a multifocal or multi-centric disease (63-65).

The AIDS-KS cells also secrete potent activators of endothelial cells, inducing these cells to become invasive and stimulating their chemotaxis. A similar effect, although to a lesser extent, was also noted for SM cells. On the contrary, there was no enhancement of fibroblast cell invasion by KS-CM, indicating cell type specificity. Because local invasion and migration are prerequisites for angiogenesis (66), the induction of endothelial cells by the AIDS-KS-secreted factors is likely to result in local angiogenesis. Secretion of potent angiogenic factors by KS cells is in agreement with the highly vascular nature of these lesions. This would also account for the ability of cultured KS cells to induce vascular lesions which consist almost entirely of murine cells when injected in nude mice (18).

Antibodies to laminin and the collagenase IV inhibitor SC44463 blocked the invasiveness of the AIDS-KS cell-activated endothelial cells in these assays. Since laminin and type IV collagen represent critical determinants of basement membrane invasion by malignant cells, it appears that KS-CM-activated endothelial cells invade through a similar mechanism.

The components of the KS-CM which activate endothelial cells are not yet clear. Both basic and acidic FGF are known to be potent angiogenic factors (67-70). The potential role of FGF, or FGF-related molecule(s), in mediating the effects of AIDS-KS cells on endothelia was suggested by the recent isolation of the FGF-related hst oncogene from a KS lesion (60, 61) and by the discovery that the AIDS-KS cells described here produce high levels of a basic FGF-related molecule in culture (39). It has been found that antibodies to bFGF can inhibit the endothelial cell growth induced by KS-CM. However, antibodies to bFGF were unable to substantially inhibit chemoinvasion of KS-CM-activated HUVE cells as well as BAEC, and had no effect on the invasive cluster formation induced by KS-CM in HUVE cells plated onto Matrigel. These same antibodies were effective in inhibiting HUVE cell invasion induced by bFGF.

References:

1. A. E. Wittek et al., unpublished observations.
2. S. Nakamura, personal communication.
Therefore it appears unlikely that FGF or related molecules are responsible for the KS-CM activation of HUVE cells. KS cells have also been found to produce high levels of IL-1 (39). IL-1 elicits a complex reprogramming of endothelial cells (71), including changing their morphology and interactions with the extracellular matrix (72). Antibodies to IL-1 were able to inhibit only 30% of HUVE and BAEC KS-CM-induced invasion and were unable to inhibit stellate, invasive colony formation induced by KS-CM in HUVE cells plated onto Matrigel. IL-1 is also produced by endothelial cells, particularly in the absence of serum (73). However, serum-free endothelial cell supernatants are also produced by endothelial cells, particularly in the absence of serum. Therefore it appears unlikely that FGF or related molecules are contributing to the activation of HUVE cells by KS-CM. Angiogenesis is a critical factor in tumor growth and progression (66, 74). Identification and inhibition of tumor factors inducing angiogenesis can be targeted as key points in developing anti-tumor therapies. These assays could prove very useful for the identification of new angiogenic factors produced by the KS cells.

Taken together, the results presented here indicate that KS cells are invasive cells which morphologically resemble smooth muscle cells but produce potent activators which induce endothelial cell invasion and chemotaxis. The highly vascular nature of KS lesions is likely due to recruitment of endothelial cells into the lesion by secreted factors produced by the KS cells.

ACKNOWLEDGMENTS

We thank Dr. Douglas Noonan (Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy) and Peter Biberfeld (Department of Pathology, Karolinska Institute, Stockholm, Sweden) for helpful discussion and revision of the manuscript. We are grateful to Dr. Michele De Luca and Dr. Gabriella Allavena (Istituto Nazionale per la Ricerca sul Cancro) for their critical reading of the manuscript.

REFERENCES

STIMULATION OF ENDOTHELIAL CELL INVASION BY AIDS-KS CELLS


Supernatants of Acquired Immunodeficiency Syndrome-related Kaposi’s Sarcoma Cells Induce Endothelial Cell Chemotaxis and Invasiveness

Erik W. Thompson, Shuji Nakamura, Thomas B. Shima, et al.