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22. Stimulation of DNA synthesis and cell number were measured as follows: KS cells (3×10^3 cells per well) or H-UVE or BCE cells (5×10^3 cells per well) were incubated in 24-well plates with 15% fetal calf serum (FCS) in RPMI 1640 medium in the absence or presence of test factors or CM. Plating efficiency was approximately 70% for each KS cell culture and 50% for H-UVE and BCE cells. Medium and specimens were replaced at day 3 (KS cells) and on day 2 (H-UVE and BCE cells) with 1 μCi per well of [^{3}H]thymidine and incubated overnight. The stimulation index was calculated as: [^{3}H]thymidine uptake in the presence of growth factors divided by [^{3}H]thymidine uptake in control medium. The cell number was determined with a Coulter particle counter after trypsinization of cells on day 6 of culture. For fibroblast growth determination, the cells (3×10^3 per well) were incubated with RPMI 1640 medium supplemented with FCS (10%) in the presence or absence of test CM in 24-well plates. Cells were incubated overnight with [^{3}H]thymidine (1 μCi per well) on day 4 of culture; uptake was measured on day 5. The AIDS-KS cells were subcultured and maintained on gelatinized plastic ware with the use of complete culture medium with HTLV-II CM (20% v/v). The H-UVE and BCE cells were also cultured in the gelatinized flasks with ECGS (30 μg/ml) and heparin (45 μg/ml) in addition to the complete culture medium.
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Angiogenic Properties of Kaposi's Sarcoma–Derived Cells After Long-Term Culture in Vitro

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Cells derived from lung biopsies and pleural effusions from AIDS patients with Kaposi's sarcoma (KS) of the lungs were established in long-term culture with the aid of conditioned medium from HTLV-II–transformed T cells (HTLV-II CM). These AIDS-KS cells were similar to the so-called spindle cells in KS lesions and had some of their features. They produced factors that supported their own growth (autocrine) and the growth of other cells (paracrine), including umbilical vein endothelium and fibroblasts. That the AIDS-KS cells also expressed potent angiogenic activity was demonstrated by the chorioallantoic membrane assay and by subcutaneous inoculation of AIDS-KS cells into nude mice, which resulted in the development of angiogenic lesions composed of mouse cells and showing histological features similar to those of human KS lesions. These data suggest that AIDS-associated KS and possibly other types of KS may be initiated by signals that induce the growth of particular cells (spindle cells of lymphatic or vascular origin) and the expression of autocrine and paracrine activities.

KAPOSI'S SARCOMA WAS FIRST described more than 100 years ago (*1*). The disease was rare in Caucasians and usually limited to elderly men, although an aggressive form was subsequently found to occur in young people in Africa. This latter form of KS has several features in common with the KS associated with infection by human immunodeficiency virus type 1 (HIV-1) (*2, 2a*) and the KS that occurs in transplant recipients (*3*) and other patients receiving immunosuppressive therapy (*4*). KS lesions have a complex histology characterized by abnormal vascularization and the presence of proliferating endothelium and "spindle" cells, fibroblasts, and infiltrating leukocytes (*5*). Studies of the origin of the KS have been inconclusive, in part because of the complex nature of the lesions but also because of the lack of appropriate culture systems in vitro.

Several features of the lesions of KS suggest that they represent polyclonal proliferations rather than true tumors. Genetic factors (*2*) as well as certain herpesviruses (*6*) and drugs (*2*), acting independently or in concert, have all been suggested as contributing to KS. However, none of these factors have convincingly been linked to the pathogenesis of the disease (*7, 8*). Although there is a clear correlation between HIV-1 infection and the aggressive, epidemic form of AIDS-KS, no genomic sequences of HIV-1 or of any other virus have been detected in KS tissues (*8*). We have now characterized AIDS-KS cells cultured in the presence of conditioned medium (CM) from HTLV-II–infected and –transformed T cell lines (*9*), and have obtained data that are of relevance to the understanding of the origin and biology of KS.

Six KS cell lines were used: AIDS-KS1, AIDS-KS3, and AIDS-KS4 were initiated from lung biopsies; the other lines were derived from pleural effusions from male AIDS patients with lung KS (*9*). Most of the cells had some morphologic features characteristic of KS "dendritic" spindle cells (Fig. 1) (*5*). Ultrastructural examination indicated that they were undifferentiated proliferating cells with prominent smooth (SER) and rough endoplasmic reticulum (RER), gap junctions and "foot" junctions, vermiform and branched mitochondria (Figs. 2), and often prominent, clustered, interchromatin granules in the nucleus. The appearance of the RER and SER was suggestive of well-developed secretory functions. The cells lacked the Weibel-Palade bodies (*10*) that are characteristic of cultures of normal vascular endothelial (H-UVE) cells but are not usually seen in spindle cells of primary KS tissues (*5*). No specific chromosomal abnormalities have been described in cells from KS tissues (*8*), and the cultured AIDS-KS cells also had a normal, human male diploid karyotype (46XY). The most common variation observed was a missing Y chromosome, which frequently occurs in cultured cells.

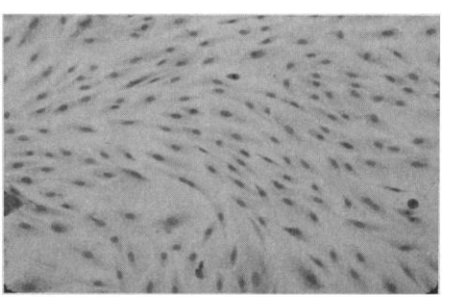

Fig. 1. Long-term cultured confluent AIDS-KS cells stained with Wright-Giemsa. Cells obtained from tissue biopsies from KS lesions by digestion with trypsin (0.05%) and EDTA (0.5 m*M*), as well as cells obtained from pleural effusion from AIDS patients, were grown in a growth medium (RPMI 1640, 15% fetal calf serum) supplemented with HTLV-II CM in 96-well plates by limiting dilution methods.

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It was previously suggested that the spindle cells in KS tissues are endothelial cells, possibly of lymphatic origin (*5*). To further elucidate the lineage of the AIDS-KS cells, we examined their immunophenotypic expression specific for endothelial cells and other cell types (Table 1). Factor VIII–related antigen (FVIIIR:Ag), commonly found in endothelial cells of vascular origin (*11*) but with a variable expression in primary KS tissues (*12*), was present in the H-UVE cells but not in the cultured AIDS-KS cells. The monoclonal antibody PAL-E (*13*), which recognizes cells of the vascular but not lymphatic endothelium or KS spindle cells in primary tissues (*5*), reacted with H-UVE but not AIDS-KS cells; monoclonal antibody EN-4 (*13*), which reacts strongly with vascular and lymphatic endothelial cells and weakly with KS spindle cells in situ (*5*), also did not react with the AIDS-KS cells. Both H-UVE and AIDS-KS cells reacted with monoclonal antibody to tissue plasminogen activator inhibitor (TPAI) (*14*), which is secreted by endothelial cells, as well as with monoclonal antibodies to intermediate filaments, that is, vimentin and fibronectin, which are commonly expressed in endothelial cells (*11*). However, neither desmin, which is expressed in skeletal, visceral, and certain vascular smooth muscle cells, nor keratin, which is expressed in ectodermal and mesothelial cells (*15*), were detected in either cell type.

Cytochemical assays were used to test for specific enzymatic activities commonly associated with endothelial tissues. Both adenosine triphosphatase (ATPase) (*5*) and angiotensin converting enzyme (ACE) (*16*), known to be present in endothelial cells, were detected only in H-UVE. Two cytochemical markers routinely used to distinguish endothelial cells from other cell types, that is, lectin-binding sites specific for *Ulex europaeus* (UEA-1) (*5, 11, 17*) and acetylated low density lipoprotein (Ac-LDL) (*18*), were found at high levels in both H-UVE and AIDS-KS cells. The pattern of reactivities summarized in Table 1 suggests that the AIDS-KS cells have features of endothelial cells, possibly of lymphatic rather than vascular origin.

In vitro, several growth stimulatory activities were induced by the AIDS-KS cells, suggesting that such cells might play an important role in the development and maintenance of KS lesions. Thus, CM and cell extracts from AIDS-KS cells contained an activity (or activities) that stimulated and supported their own growth and, to an even greater extent, the growth of normal vascular endothelium (H-UVE) (Fig. 3). Some of this activity could be attributed to the presence of interleukin-1 (IL-1) in these cultures; however, other growth factors such as basic fibroblast growth factor (bFGF) are probably more important and, in fact, these AIDS-KS cells do indeed release high levels of bFGF-like factor (*9, 20*). We have found that the AIDS-KS cells produce and respond to their own chemotactic and chemoinvasive activities (*21*).

We also studied the angiogenic properties of the AIDS-KS cells and the medium conditioned by these cells. When AIDS-KS cells

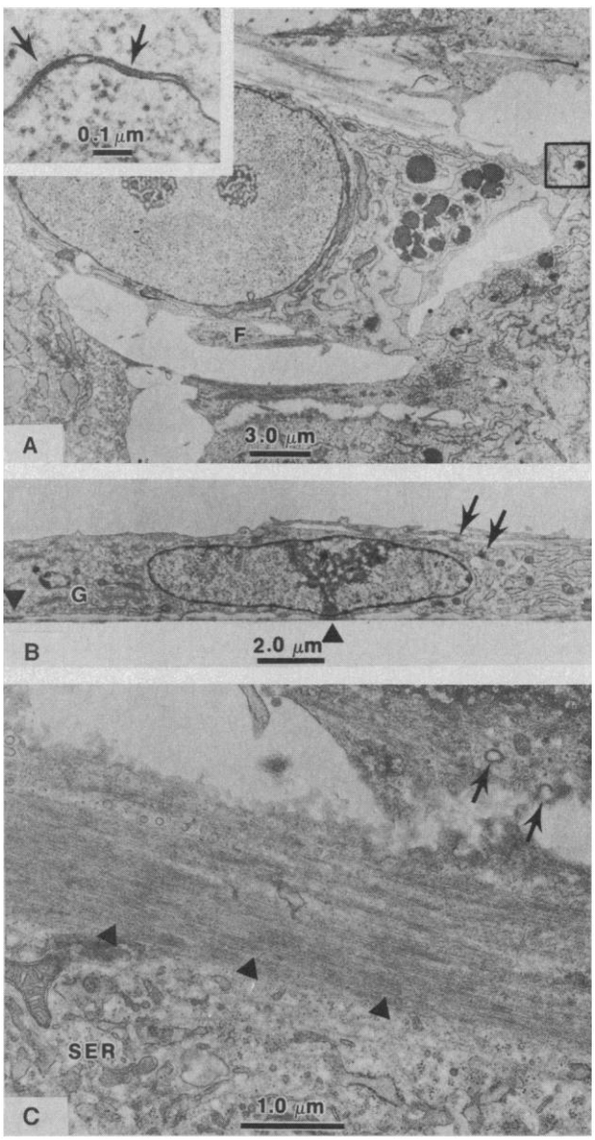

Fig. 2. Electron micrographs of AIDS-KS3 cells from a confluent cell culture. The cells were fixed in situ with 1.25% glutaraldehyde and then with 1% osmium tetroxide, then dehydrated and embedded in Epon. Sections were cut parallel to the growth surface after separating the resin from the plastic surface by freeze-cracking the flask in liquid nitrogen. (**A**) AIDS-KS cells with prominent RER, vermiform mitochondria, some lipid bodies, and microfilament (F). (Inset top left) Higher magnification of junctional complexes with adjacent cell (←) shown in right inset. (**B**) Perpendicular section of AIDS-KS3 cells showing large nucleus, several Golgi complexes (G), and foot junction structures (▲). Small tight junctions (↙↙) with the processes of the adjacent cells present on the upper surface. (**C**) Perpendicular section showing bundles of microfilaments beneath the cell surface (▲), and micropinocytotic coated pits (↗↗).

Table 1. Characteristics of AIDS-KS cells and human umbilical vein endothelial cells (H-UVE). Reactivities are shown as: −, negative; +, weak positive; ++, moderate positive; +++, strong positive.

Assay	AIDS-KS	H-UVE
Immunohistochemical assays*		
FVIIIR:Ag	−	++
HLA Class II	−	−
EN-4	−	+++
PAL-E†	−	+
Tissue plasminogen activator inhibitor	++	+
Fibronectin, vimentin‡	+	+
Cytokeratin	++	−
Desmin	−	−
Keratin	−	−
Enzyme histochemical assays‡		
Adenosinetriphosphatase	−	++
Angiotensin converting enzyme	−	++
Ó-Napthyl acetate esterase	+	+
Acid phosphatase	++	+
Alkaline phosphatase§	−	−
5′ Nucleotidase	+	+
Cytochemical stains for		
Ulex europaeus (UEA-I)	+++	+++
Acelylated low density lipoprotein (Ac-LDL) uptake	++	+++

*See (*27*). †Reaction to PAL-E MAb gave variable degree of staining in primary H-UVE cells. ‡KSF cells were negative in all tests except for fibronectin and vimentin. §Alkaline phosphatase was positive in freshly cultured primary cells of H-UVE.

were placed on the chorioallantoic membrane (CAM) of 9-day-old fertilized chicken eggs (*22*), extensive vascularization occurred within 3 days (Table 2 and Fig. 4A). The cell-free culture medium did not give a corresponding angiogenic reaction, probably because of rapid dissipation of the inductive signal. No angiogenic reaction occurred when fixed AIDS-KS cells were used.

When nude mice (Beige, BALB/c, Swiss, and NCr) were inoculated subcutaneously with 4×10^6 AIDS-KS cells, a strong angiogenic reaction developed at the site of inoculation within 5 days. The highly vascular and hemorrhagic histology of these lesions was more reminiscent of KS than of an inflammatory or granulomatous reaction (Fig. 4, C and D). The lesions increased in size until day 6 and gradually regressed with a demonstrable decrease in inoculated KS cells. No tumor growth was observed in the inoculated animals.

Chromosomal analysis of 34 metaphases of proliferating cells from these lesions showed that they were all of mouse origin. Fibroblastic cells obtained from the same KS lesions as AIDS-KS3 (KSF) stimulated the formation of a lesion with minimal vascularization compared to the lesions produced by AIDS-KS cells.

To test the possibility that a known infectious agent might be involved in KS pathogenesis, we tested the six AIDS-KS cell cultures with specific probes for several human retroviruses and human DNA viruses. No evidence of nucleotide sequences for HTLV-I (*23*) and -II (*24*), HIV-1 (*2a*), HHV-6/HBLV (*25*), HHV-4/EBV, HHV-5/HCMV, HHV-1 and -2, papova, and polyoma viruses, was found. Nor did we find evidence of such viruses in cell cultures by electron microscopy or by virus isolation techniques.

Our results indicate that the AIDS-KS cells have features common to, yet distinct from, umbilical vein endothelium. The differences may reflect heterogeneity among the endothelial cells or acquired properties intrinsic to cells of the KS lesions. They could also suggest a possible nonvascular origin of the spindle cells of the KS lesion (*5*). The data presented here and elsewhere (*9, 20*) suggest that AIDS-KS may develop in separate stages. In the first stage, certain cells of vascular or lymphatic origin might be induced by a factor (or factors) produced by infected (HIV, other viruses?) lymphocytes or by another inductive signal (*9*). Such cells might subsequently produce factors that stimulate the growth of various cell types in an autocrine as well as a paracrine fashion. Immunodeficiency would have a dual role in KS development, since it would both facilitate the development of infections and suppress effector cell functions that nor-

Table 2. Tests for angiogenic activity of AIDS-KS cells and other cells in the CAM assay (*22*) and in NCr nude mice. Cells tested include: KSF, KS-derived fibroblasts; SK-HEP-1, human hepatoma cells, producer of bFGF; and MS180, mouse sarcoma cells; ND, not done.

Specimen	No. positive/ No. tested in CAM assay	No. positive/ No. tested in nude mouse assay
AIDS-KS1	13/16	3/3
Fixed cells	0/9	0/3
AIDS-KS2	10/12	9/9
Fixed cells	0/8	0/6
AIDS-KS3	7/10	8/10
Fixed cells	0/14	0/3
AIDS-KS4	6/8	3/3
Fixed cells	2/9	0/3
AIDS-KS5	12/12	2/2
Fixed cells	ND	ND
AIDS-KS6	12/12	2/2
Fixed cells	ND	ND
KSF	1/5	2/2
SK-HEP-1	1/10	0/4
MS180	6/10	0/4
BAE, HT-29, H-UVE	8/25	0/8

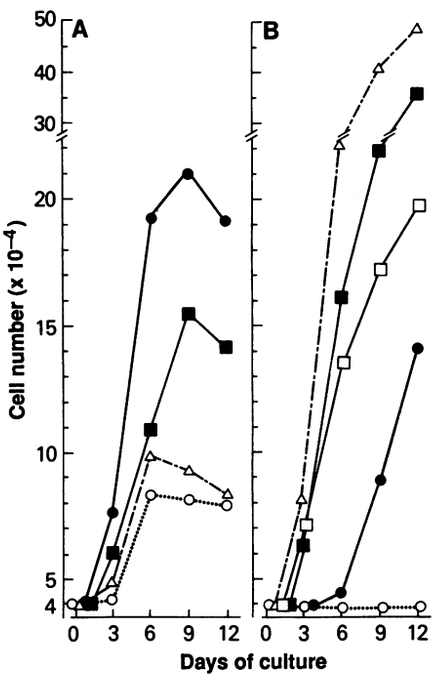

Fig. 3. Growth kinetics of (**A**) AIDS-KS cells and (**B**) H-UVE cells in (○), RPMI 1640 with 15% FCS (△), endothelial cell growth supplement (ECGS), 30 μg/ml, plus heparin, 45 μg/ml; (●) HTLV-II CM (20% v/v); (■), extract of AIDS-KS cells, and (□), CM from AIDS-KS cells alone (25% v/v). Initial seeding density was 4×10^4 cells in each case. Repeated experiments with these cells and growth factors yielded similar results (negligible variation in cell numbers at each time point).

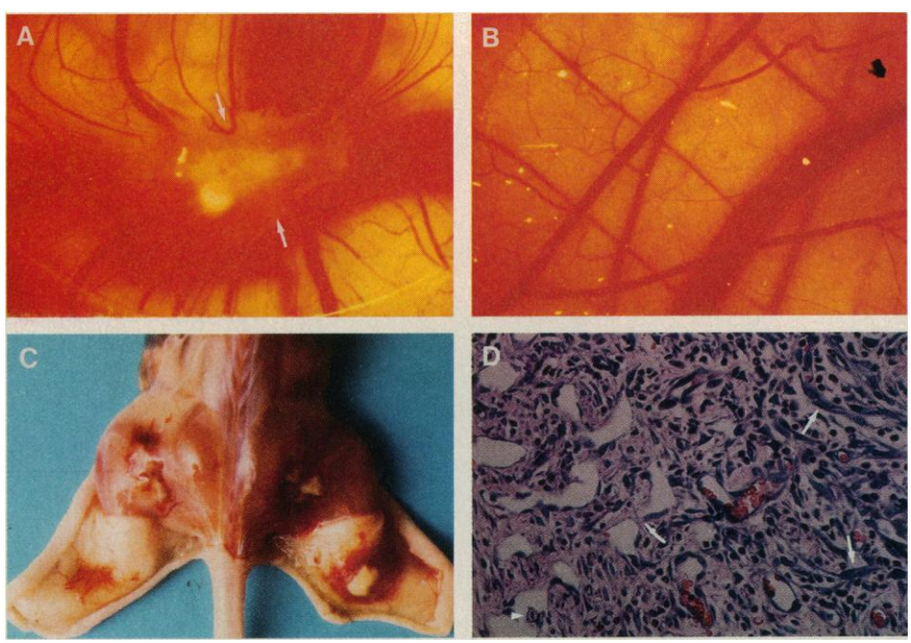

Fig. 4. (**A**) Induction of angiogenic response in a CAM fertilized for 12 days (*22*) by 2×10^4 AIDS-KS cells. Numerous newly formed small vessels (→). (**B**) Control CAM showing no angiogenic response. (**C**) NCr-nude mouse injected subcutaneously in the lower back with AIDS-KS cells [4×10^6 live (right) or fixed (left) cells per site]. Note the angiogenesis on the site injected with live AIDS-KS cells and absence of such reaction when fixed (0.00125% glutaraldehyde) cells were injected. (**D**) Histological appearance of the subcutaneous lesion in NCr-nude mouse injected with AIDS-KS cells. Marked angiogenic response was evident 5 days after injection. Note the presence of numerous slits and small vessels with and without red blood cells and spindle-like cells (→). Evidence of endothelial cell division (▲) (mitosis) is shown.

mally prevent "tumor" cell outgrowth. Other homeostatic mechanisms that participate in the control of angiogenesis might also be affected by a deficient immune system. The development of KS would thus depend on the balance and strength of any combination of these mechanisms. According to this model, and consistent with previous suggestions, the KS lesion may develop and "spread" without implying a metastatic process (*26*). This is also consistent with observations that AIDS-KS is usually a polycentric disease in which the lesions are composed of various cells with a normal diploid chromosomal pattern and lacking obvious, biological tumor properties. Further studies of AIDS-KS cells in the growth systems should lead to a better understanding of the pathophysiology of KS and to the development of treatments that do not depend on cytotoxic chemotherapy.

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Heat Shock Is Lethal to Fibroblasts Microinjected with Antibodies Against hsp70

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Synthesis of a small group of highly conserved proteins in response to elevated temperature and other agents that induce stress is a universal feature of prokaryotic and eukaryotic cells. Although correlative evidence suggests that these proteins play a role in enhancing survival during and after stress, there is no direct evidence to support this in mammalian cells. To assess the role of the most highly conserved heat shock protein (hsp) family during heat shock, affinity-purified monoclonal antibodies to hsp70 were introduced into fibroblasts by needle microinjection. In addition to impairing the heat-induced translocation of hsp70 proteins into the nucleus after mild heat shock treatment, injected cells were unable to survive a brief incubation at 45°C. Cells injected with control antibodies survived a similar heat shock. These results indicate that functional hsp70 is required for survival of these cells during and after thermal stress.

IN ALL ORGANISMS AND CULTURED cells examined, a small group of evolutionarily conserved proteins is synthesized in response to heat and a variety of other stress-inducing agents. Most of these agents promote protein denaturation (*1, 2*). Of the several size classes of stress or heat shock proteins (hsps), those referred to as the hsp70 family are the most conserved and the best characterized (*1, 2*). In most mammalian cells there are two prominent forms of hsp70, an abundant constitutive member, hsp73, and a highly stress-inducible member, hsp72 (*3*). In cells grown at 37°C the majority of ~70,000 kD (70K) hsps are found in the cytoplasm. In response to heat shock, these proteins are rapidly sequestered in the nucleus with high levels accumulating in that region of the nucleolus involved in the assembly of small ribonucleoproteins and preribosomes (*4–6*). During recovery from heat shock, the 70K hsps accumulate in the cytoplasm where a portion colocalizes with ribosomes and polysomes (*7*).

One major line of evidence supports the idea that hsps, and in particular the 70K hsps, are important in protecting or facilitating the recovery of cells from the adverse effects of heat shock. Cells given a mild heat shock treatment sufficient to elevate the expression of the heat shock proteins (or alternatively to "activate" preexisting hsps) exhibit significantly higher survival rates to a second, and what would otherwise be lethal, heat shock challenge (*8*). This phenomenon, referred to as acquired thermotolerance, can be elicited by pretreatment with agents other than heat, all of which have in common the ability to induce stress protein synthesis (*9, 10*). A number of studies have shown a strong correlation between the expression and decay of thermotolerance and the induction, accumulation, and degradation of hsps (*10–12*). Moreover, an inability to produce functional hsps precludes the acquisition of

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