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AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6

(proliferation/autocrine growth factor/paracrine growth factor/interleukin-6 receptor/endothelial cells)

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ABSTRACT **Cell lines derived from Kaposi sarcoma lesions of patients with AIDS (AIDS-KS cells) produce several cytokines, including an endothelial cell growth factor, interleukin 1β, and basic fibroblast growth factor. Since exposure to human immunodeficiency virus increases interleukin 6 (IL-6) production in monocytes and endothelial cells produce IL-6, we examined IL-6 expression and response in AIDS-KS cell lines and IL-6 expression in AIDS Kaposi sarcoma tissue. The AIDS-KS cell lines (N521J and EKS3) secreted large amounts of immunoreactive and biologically active IL-6. We found both IL-6 and IL-6 receptor (IL-6-R) RNA by slot blot hybridization analysis of AIDS-KS cells. The IL-6-R was functional, as [^{3}H]thymidine incorporation by AIDS-KS cells increased significantly after exposure to human recombinant IL-6 (hrIL-6) at >10 units/ml. When AIDS-KS cells (EKS3) were exposed to IL-6 antisense oligonucleotide, cellular proliferation decreased by nearly two-thirds, with a corresponding decrease in the production of IL-6. The decrease from IL-6 antisense in AIDS-KS cell proliferation was reversed by the addition of hrIL-6. We confirmed that AIDS-KS cells produced IL-6 *in vivo* by preparing RNA and tissue sections from involved and uninvolved skin from a patient with AIDS Kaposi sarcoma. We detected immunoreactive IL-6 in the involved tumor areas and to a lesser extent in the surrounding normal epidermis. Slot blot hybridization showed a great excess of IL-6 and IL-6-R RNA in involved skin compared to uninvolved skin. These results show that both IL-6 and IL-6-R are produced by AIDS-KS cells and that IL-6 is required for optimal AIDS-KS cell proliferation, and they suggest that IL-6 is an autocrine growth factor for AIDS-KS cells.**

Kaposi sarcoma is a multifocal vascular lesion that commonly complicates infection with the human immunodeficiency virus (HIV). It is also seen in other immunosuppressed states such as in patients receiving renal or cardiac transplants (1–3). The pathogenesis of Kaposi sarcoma is not well understood and the cell of origin is unknown. Because of the multifocal nature of the tumor, the anti-neoplastic response with biologic agents, and the frequent association with clinical states characterized by derangements in immune function, it is postulated that Kaposi sarcoma is a nonmalignant proliferative lesion of endothelial cells. Because these tumor cells do not have all the immunohistochemical characteristics of vascular endothelia, it is postulated that the cell of origin is a mesenchymal cell, possibly a lymphatic endothelial cell, that may grow in response to disordered regulation and expression of one or several growth factors. Presumably, the altered immunologic system of the host provides both disordered regulation of growth factor production and inadequate surveillance of the "pseudo"-malignant Kaposi sarcoma cells.

Prior *in vitro* studies of AIDS Kaposi sarcoma-derived cell lines (AIDS-KS cells) showed that these cells produce several cytokines and growth factors, including interleukin 1β (IL-1β) and basic fibroblast growth factor (bFGF) (4). The growth of these cell lines can be increased by culturing them in the presence of medium from cells infected with human T-lymphotropic virus type II ("HTLV-II-conditioned medium") as well as medium from several CD4$^+$ cell lines infected with other human retroviruses (5, 6), suggesting the presence of a soluble growth factor in the medium. A 28- to 30-kDa heparin-binding growth factor for AIDS-KS cells is found in the supernatants of these retrovirus-infected CD4$^+$ cell lines (4). This growth factor is also synthesized by AIDS-KS cell lines. The growth factor has autocrine and paracrine properties for AIDS-KS cells *in vitro* and its altered regulation *in vivo* is postulated to be responsible for the development of Kaposi sarcoma.

One possible cytokine with properties that are consistent with those attributed to the Kaposi sarcoma partially purified growth factor is interleukin 6 (IL-6). IL-6 is a pleiotropic cytokine with a variable molecular mass of 26–30 kDa. It has many biological effects, including both growth- and differentiation-inducing activities. IL-6 can induce B-cell differentiation, stimulate the production of acute response proteins by hepatocytes, induce proliferation of cytotoxic T cells, and augment hybridoma/plasmacytoma growth (7–10). IL-6 is produced by several types of cells, including lymphocytes, monocytes, epidermal cells, and various tumor cells (7–10). Excess production of IL-6 may play a role in the pathogenesis of several diseases, including Castleman disease (7, 8, 11), a disease that is occasionally associated with Kaposi sarcoma (12).

Also, IL-6 is elevated in HIV infection (13) and exposure of monocytes to HIV can induce IL-6 production *in vitro* (14). When the IL-6 receptor (IL-6-R) is simultaneously expressed in IL-6-producing cells, IL-6 can act as an autocrine growth factor. This is seen sometimes in multiple myeloma (15). In addition, several cytokines produced by AIDS-KS cells, including IL-1β, alter IL-6 secretion by normal human um-

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Abbreviations: HIV, human immunodeficiency virus; IL-6, interleukin 6; hrIL-6, human recombinant IL-6; IL-6-R, IL-6 receptor; IL-1β, interleukin 1β; bFGF, basic fibroblast growth factor; PBMC, peripheral blood mononuclear cells; HTLV-II, human T-lymphotropic virus type II; ECGS, epithelial cell growth supplement.

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bilical vein and bovine aortic endothelium in fresh cell cultures (16). This suggests that IL-6 may be the unidentified autocrine growth factor for AIDS-KS cells. This report details studies examining the possibilities that IL-6 is a growth factor for AIDS-KS cells and that it plays a role in the development of Kaposi sarcoma *in vivo*.

METHODS

Maintenance of Cell Lines. N521J cells (5) (passage 8, received from S. Nakamura (National Institutes of Health, Bethesda, MD) on March 16, 1986, and first defrosted on May 1, 1989), or EKS3 cells (passage 21, received from C.-Y. Kang, Ottawa Regional Cancer Center, Ontario, Canada) were continuously passaged on culture flasks coated with 0.1% gelatin (Sigma). N521J cells were passaged on gelatin and human fibronectin (10 $\mu g/cm^2$, Collaborative Research). Cells were harvested after digestion with trypsin/EDTA and replated at sub-confluent density (approximately 0.2×10^6 cells per ml) on gelatin- and fibronectin-coated tissue culture flasks or 96-well plates. Cells were grown in Iscove's modified Dulbecco's medium (IMDM) with penicillin, streptomycin, 10% fetal bovine serum (Hyclone), heparin (100 USP units/ml), endothelial cell growth supplement (ECGS, 30 μg/ml; Collaborative Research), and 10% HTLV-II-conditioned medium.

Quantification of IL-6. IL-6 was quantified by using an IL-6-specific ELISA and an IL-6 bioassay. In the IL-6 ELISA, flat-bottom 96-well plates were coated with a murine monoclonal antibody against human IL-6 (α-BSF2-166) (17), exposed to supernatants of unknown concentration or standard concentration of hrIL-6 diluted in medium, washed, incubated with rabbit anti-IL-6 serum (Genzyme), washed, incubated with goat anti-rabbit serum coupled to horseradish peroxidase, and developed with a standard substrate (*o*-phenylenediamine dihydrochloride). IL-6 levels were determined by comparison of experimental absorbance with a standard curve obtained with known quantities of hrIL-6 in the same assay.

The biologic activity of the secreted IL-6 in the supernatants was measured with a cell proliferation bioassay. This assay is based on the proliferation and incorporation of [^{3}H]thymidine in the IL-6-dependent murine hybridoma MH60.BSF-2 line (14, 17). Growth of this cell line is absolutely dependent on IL-6. The IL-6 activities in the supernatants are expressed as equivalent amounts of hrIL-6 (units/ml required for the same biological activity).

Preparation of Total RNA and Northern Slot Blot Hybridization. AIDS-KS cells (N521J), a human monocyte cell line (THP-1), and peripheral blood mononuclear cells (PBMC) were cultured at 0.2×10^6 cells per ml in complete medium. *Escherichia coli*-derived lipopolysaccharide (1 μg/ml) was added to some cultures to induce IL-6 production. Total RNA was obtained by extraction with guanidine isothiocyanate and ultracentrifugation in cesium chloride (18). For the IL-6-R studies, total RNA was isolated from AIDS-KS cells (N521J), Jurkat cells (a human T-cell line), and U937 cells (a human monocyte cell line). Total RNA (2.5, 1.2, 0.62, and 0.31 μg) from each sample was blotted onto a nylon membrane (GeneScreen*Plus*, NEN). The membrane was baked, pre-hybridized, and then hybridized at 42°C for 24 hr. The hybridization solution was 50% (vol/vol) formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate, and denatured salmon sperm DNA at 150 μg/ml with a ^{32}P-labeled 440-base-pair (bp) *Taq* I–*Ban* II fragment of IL-6 cDNA (pBSF2.38) (19), ^{32}P-labeled 1700-bp *Xho* I fragment of IL-6-R cDNA (pBSF2R.236) (20), or ^{32}P-labeled β-actin cDNA. For *in vivo* studies, total RNA was obtained by guanidine isothiocyanate extraction from brain, spleen, and skin from a patient with AIDS Kaposi sarcoma within 1 hr of his death. Portions of skin that were involved and uninvolved with Kaposi sarcoma were analyzed with the same slot blot hybridization technique described above.

Immunoperoxidase Stains of Cells and Tissue. AIDS-KS were grown on gelatin- and fibronectin-coated slides. After culture, cells were washed in phosphate-buffered saline (PBS; GIBCO), fixed in precooled acetone for 10 min, and air dried. Prior to staining, cells were washed in PBS for 10 min and incubated with blocking reagent (mouse serum) for 20 min at room temperature. Primary antibody (murine monoclonal anti-IL-6, 80 μg/ml) or isotype control (mouse IgG1) was added, and the cells were incubated for 90 min and washed with PBS for 10 min. The biotinylated antibody (horse anti-mouse IgG) was added, and the cells were incubated for 30 min and washed with PBS for 10 min. After this, the ABC kit (Vector Laboratories) reagents were added according to the manufacturer's instructions, and the cells were incubated for 30 min and washed with PBS for 10 min. A freshly prepared substrate solution was added and incubated for 10 min, with color development checked by light microscopy. The enzyme reaction was stopped with a tap water wash to remove substrate. Similarly, paraffin-embedded sections were stained for intracellular IL-6 after sectioning.

Cell Proliferation Assays. AIDS-KS cells were harvested by digestion with trypsin/EDTA, plated in 96-well plates, and allowed to grow for 24 hr in the presence of 20% HTLV-II-conditioned medium (supernatant of passage 107 of Mo T cells, with 10% fetal bovine serum in IMDM). To reduce background stimulation from the growth medium, the growth medium was removed and replaced with serum-free medium [IMDM with ITS+ (Collaborative Research) and ECGS at 30 μg/ml, heparin at 10 USP units/ml, and human fibronectin]. After a 6-hr wash-out period, the medium was removed and replaced with serum-free medium containing hrIL-6 (0–30 units/ml) for 24 hr at 37°C in a 5% CO_2 atmosphere. [^{3}H]Thymidine was added at 1 μCi per well (1 μCi = 37 kBq) for 18 hr. Cells were released with trypsin/EDTA and harvested onto glass filter strips, and radioactivities were measured in a liquid scintillation fluor.

Treatment of AIDS-KS Cells with IL-6 Sense and Antisense Oligonucleotides. EKS3 AIDS-KS cells were plated in 96-well plates and cultured for 24–72 hr as described above. After the 6-hr wash-out period in serum-free IMDM, cells were exposed to IL-6 sense or antisense oligodeoxynucleotides for 12 hr. In this case, a 15-base antisense oligodeoxynucleotide (TCCTGGGGGTACTGG) specific for a sequence in exon II of the IL-6 gene (21) was added to AIDS-KS cells. A control, sense oligodeoxynucleotide was also used. [^{3}H]Thymidine was added for 18 hr, and cells were harvested. Supernatants for IL-6 determinations were collected immediately prior to harvesting. Various concentrations (0.15–20 μM) of IL-6 antisense oligonucleotides were tested; preliminary experiments indicated that maximal effects were seen at 15–20 μM, the concentration range used for the experiments presented here.

RESULTS

IL-6 Production by AIDS-KS Cells. Half-confluent N521J cells from passages 9–13 or EK3 cells grown in the presence of HTLV-II-conditioned medium were used in these experiments. HTLV-II-conditioned medium is known to enhance the growth of these cells and is required for long-term passage (5, 6). There was no IL-6 in the complete culture medium used to culture the N521J cells that was detected by using an IL-6 ELISA (<0.05 ng of IL-6 per ml, $n = 3$). But, after short-term passage, the supernatants of AIDS-KS cell cultures had high IL-6 (50 ± 7.7 ng/ml; mean ± SEM, $n = 16$). Subsequent experiments showed that the IL-6 concentration

in the HTLV-II-conditioned medium that is added as a mitogenic stimulus for these KS cells varies with the passage number of Mo T cells, phytohemagglutinin stimulation, and use of calf serum (data not shown). Thus, sometimes, this conditioned medium has detectable IL-6. For example, the complete medium used to culture EKS3 cells contained substantial amounts of IL-6 (108 ± 52 ng/ml, n = 2). However, after 2 days of culture with EKS3 AIDS-KS cells, culture supernatants had markedly elevated levels of IL-6 (406 ± 133 ng/ml, n = 3). In all cases, medium IL-6 was greatly increased after culture with N521J or EKS3 AIDS-KS cells.

IL-6 production by the AIDS-KS cells was confirmed by two different methods. IL-6 RNA expression was visualized by Northern blot hybridization and intracellular IL-6 was detected by using immunoperoxidase staining with a monoclonal anti-IL-6 antibody. As shown in Fig. 1, the N521J AIDS-KS cells constitutively expressed high levels of IL-6 RNA while THP-1 cells (a human monocytic cell line) or PBMC expressed detectable IL-6 only when stimulated with lipopolysaccharide. No notable differences were seen in the expression of β-actin RNA, which was used as a control (Fig. 1). By immunoperoxidase staining, AIDS-KS cells were strongly positive for IL-6 production, while the same cell line exposed to an isotype control IgG monoclonal antibody showed no detectable staining (data not shown). As a positive control, AIDS-KS cells were also examined by using an antibody to major histocompatibility complex class I molecules (not shown).

Biologic Activity of the Secreted IL-6. The biologic activity of the culture supernatants was examined by quantifying the proliferation of IL-6-dependent MH60.BSF2 with [^{3}H]thymidine incorporation. Supernatants of N521J AIDS-KS cells cultured in the presence of HTLV-II-conditioned medium contained 41 ± 13 units of IL-6 activity per ml (n = 4), while the HTLV-II-conditioned medium used to culture these cells contained 0.07 ± 0.01 unit/ml (n = 2). Therefore, the IL-6 secreted by these AIDS-KS cells was biologically active. EKS3 AIDS-KS cells also secreted biologically active IL-6 (data not shown).

Proliferation of AIDS-KS Cells in Response to hrIL-6. In initial experiments, modest levels of hrIL-6 were used (0.03–30 units/ml). In these experiments, only the 30 units/ml hrIL-6 concentration significantly ($P > 0.001$) increased the proliferation (>220%) of the N521J AIDS-KS cells, as detected by increased thymidine uptake (Fig. 2). In subsequent experiments, concentrations of exogenous hrIL-6 between 19 and 300 units/ml moderately increased (26–53% increase) proliferation of the AIDS-KS cells. These IL-6 levels are equivalent to, or higher than, the levels typically detected in AIDS-KS cell supernatants. The addition of rabbit anti-IL-6 serum partially suppressed exogenous hrIL-6-induced mitogenesis and resulted in a slight reduction (18%) in the basal growth of AIDS-KS cells in the absence of additional hrIL-6. Equivalent amounts of normal rabbit serum did not affect growth (data not shown).

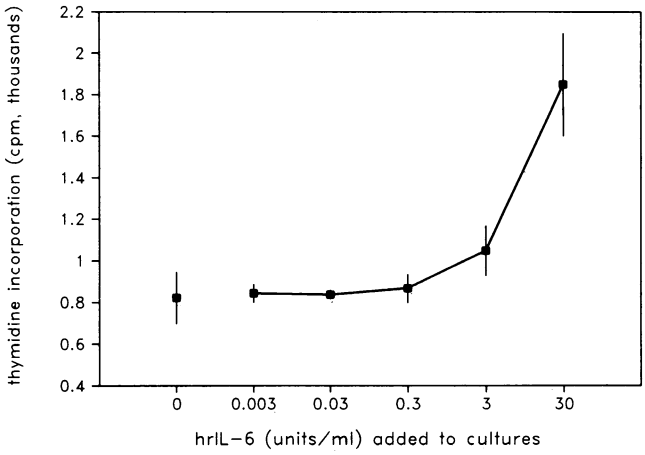

FIG. 2. Addition of exogenous IL-6 increased the proliferation of AIDS-KS cells. Assays were done in quadruplicate, and the results are expressed ± SD. This figure contains the results of one representative experiment.

AIDS-KS Cells Express the Receptor for IL-6. Since AIDS-KS cells appeared to respond to exogenous hrIL-6 with an increase in cellular proliferation, we examined the expression of IL-6-R RNA in the cells. AIDS-KS cells (N521J) expressed substantial levels of IL-6-R RNA (Fig. 3). Jurkat cells and U937 cells expressed considerably less IL-6-R RNA than the AIDS-KS cells (Fig. 3).

Production of IL-6 and IL-6-R *in Vivo* by Kaposi Sarcoma Tissue. We detected the expression of IL-6 in Kaposi sarcoma tissues *in vivo* by several techniques. Total RNA was obtained from freshly isolated Kaposi sarcoma tissue and from uninvolved normal appearing skin, spleen, and brain from an AIDS patient with extensive Kaposi sarcoma. When RNA slot blot hybridization and a probe for IL-6 (18) were used, strong IL-6 RNA expression was detected only in the Kaposi sarcoma-involved skin (Fig. 4). Little IL-6 RNA was detected in uninvolved skin, spleen, or brain. Similarly,

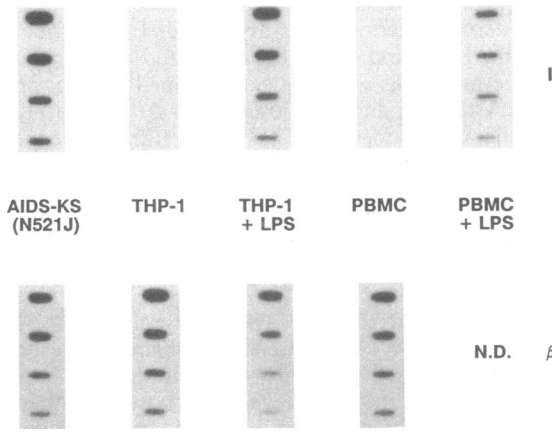

FIG. 1. AIDS-KS cells expressed IL-6 mRNA. Slot blot hybridizations of total RNA from cells were done with probes for either IL-6 or the control β-actin. THP-1 is a monocytoid cell line. THP-1 and PBMC were also cultured for 7 days in the presence of lipopolysaccharide, a known inducer of IL-6 production, at 1 μg/ml (+LPS). N.D., not done.

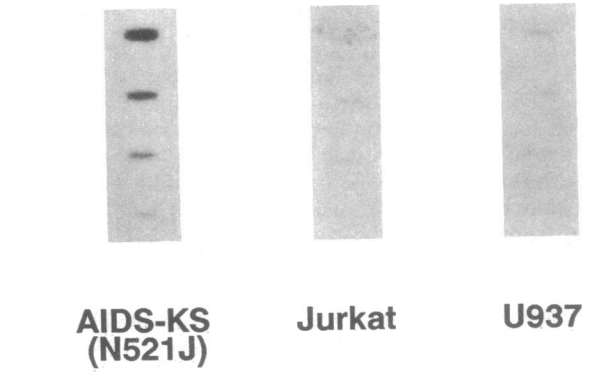

FIG. 3. AIDS-KS cells expressed IL-6-R mRNA. Total RNA was isolated from AIDS-KS cells (N521J), Jurkat cells (a human T-cell line), and U937 cells (a human monocyte cell line). RNA from each sample was blotted onto a nylon membrane (GeneScreen*Plus*) and probed with a ^{32}P-labeled 1700-bp *Xho* I fragment of IL-6-R cDNA (pBSF2R.236).

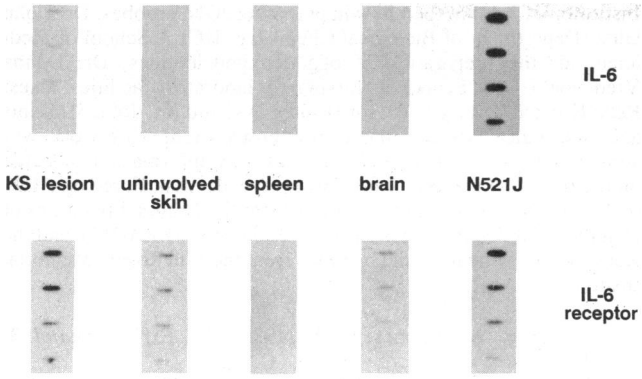

FIG. 4. AIDS Kaposi sarcoma biopsy tissue expressed elevated IL-6 and IL-6-R RNA. Total RNA was obtained by guanidine isothiocyanate extraction from brain, spleen, and skin from a patient with AIDS Kaposi sarcoma within 1 hr of his death. Portions of skin that were involved and uninvolved with Kaposi sarcoma were analyzed with the same RNA slot blot hybridization technique described for Figs. 1 and 3. Total RNA from the N521J cell line was used as the positive control.

substantial IL-6-R RNA expression was seen in Kaposi sarcoma tissue from the same individual (Fig. 4), while considerably less IL-6-R RNA expression was seen in uninvolved skin, brain, or spleen from the same individual.

When a monoclonal mouse antibody to IL-6 and immunoperoxidase staining were used, IL-6 was readily detected in paraffin-embedded sections of Kaposi sarcoma lesions and, to a lesser degree, in the adjacent normal-appearing epithelial cells (data not shown).

IL-6 Antisense, but Not Sense, Oligodeoxynucleotides Inhibited AIDS-KS Cell Growth and IL-6 Secretion. AIDS-KS cells (EKS3) were cultured in serum-free IMDM, containing 15–20 μM IL-6 antisense or sense oligonucleotides. Exposure to IL-6 antisense, but not sense, oligonucleotides led to marked decreases in AIDS-KS cells proliferation (7630 ± 3599 cpm in medium-control cultures, 3033 ± 1574 cpm in IL-6 antisense oligonucleotide-treated cultures, and 6800 ± 3150 cpm in IL-6 sense oligonucleotide-treated cultures, $n = 5$) and in IL-6 secretion (3.7 ± 3.2 ng of IL-6 per ml in medium-control cultures, 1.8 ± 1.5 ng/ml in IL-6 antisense oligonucleotide-treated cultures, and 4.6 ± 4.2 ng/ml in IL-6 sense oligonucleotide-treated cultures, $n = 2$) by AIDS-KS cells. Treatment with IL-6 antisense oligonucleotides resulted in a significant decrease in AIDS-KS cell proliferation ($P \leq 0.001$) and in AIDS-KS cell IL-6 secretion ($P \leq 0.02$), expressed as the percent of the proliferation or IL-6 secretion seen in medium control cultures, when compared to IL-6 sense oligonucleotide-treated control cultures (Fig. 5). No significant differences in AIDS-KS cell proliferation or IL-6 production were seen between medium-control (untreated) cultures and IL-6 sense oligonucleotide-treated cultures.

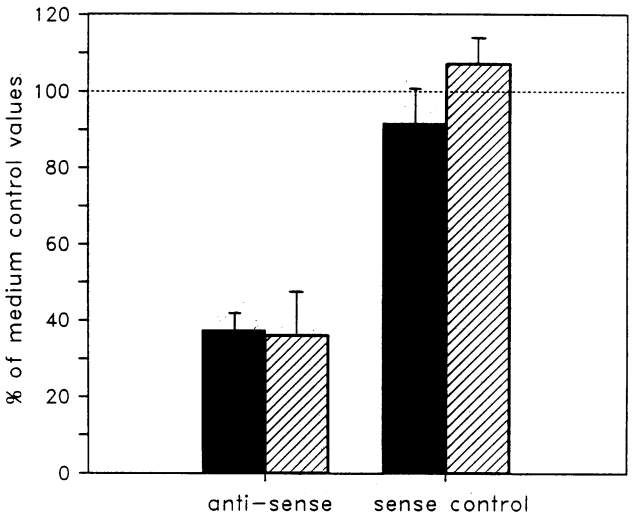

FIG. 5. Exposure of AIDS-KS cells to IL-6 antisense oligonucleotides inhibited cellular proliferation and IL-6 production. AIDS-KS cells (EKS3) were cultured with 15–20 μM IL-6 antisense or sense oligonucleotides. Solid bars represent cellular proliferation, and hatched bars represent IL-6 levels in culture supernatants, expressed as the percentage of values seen in medium-control cultures (cultures not exposed to either sense or antisense oligonucleotides). These results represent the mean ± SEM of five experiments.

When exogenous hrIL-6 was added to IL-6 antisense oligonucleotide-treated AIDS-KS cells, cellular proliferation was restored from a significantly decreased level ($P \leq 0.001$, comparing IL-6 antisense oligonucleotide-treated cultures to control IL-6 sense oligonucleotide-treated cultures) to the level seen in control IL-6 sense oligonucleotide-treated cultures ($P \leq 0.05$, comparing IL-6 antisense oligonucleotide-treated cultures to IL-6 antisense oligonucleotide-treated cultures with added exogenous hrIL-6) (Fig. 6). Addition of hrIL-6 also resulted in a significant ($P \leq 0.05$) increase in cellular proliferation in IL-6 sense oligonucleotide-treated control cultures (Fig. 6).

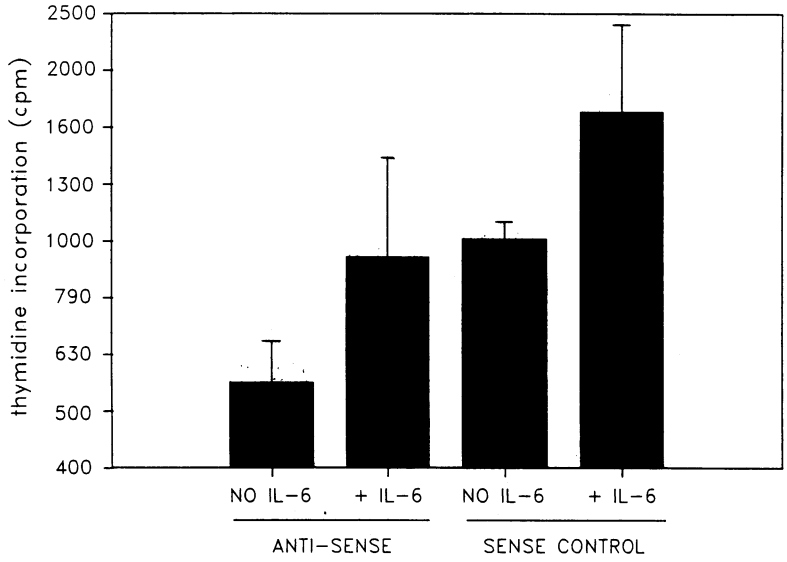

FIG. 6. Exogenous hrIL-6 reversed the IL-6 antisense oligonucleotide-induced suppression of AIDS-KS cell growth. AIDS-KS cells (EKS3) were cultured with 15 μM IL-6 antisense or sense oligonucleotides, with or without hrIL-6 at 300 units/ml. These results represent those obtained in one representative experiment, with [^{3}H]thymidine incorporation measured in quadruplicate cultures and expressed as mean cpm ± SD.

DISCUSSION

IL-6 is a potent growth- or differentiation-inducing stimulus for many cells, including bone marrow stem cells, hepatocytes, and B lymphocytes (7, 8), as well as several neoplastic cell lines, including multiple myeloma (15). However, previous studies failed to show that hrIL-6 is a mitogenic stimulus for normal human endothelial cells at doses up to 500 units/ml (16). Because virus-transformed cells and several tumor cell lines respond to IL-6 (22) and because AIDS-related Kaposi sarcoma tissue may represent transformed endothelial cells, we examined the production and response of AIDS-KS cells to IL-6.

We detected significant IL-6 production by AIDS-KS cells by immunoassay and bioassay. Also, using immunoperoxidase staining, we detected IL-6 expression in paraffin-embedded Kaposi sarcoma tissue. As expected, small amounts of IL-6 expression were seen in the surrounding normal-appearing epidermis. The production of IL-6 by AIDS-KS cells was clearly disordered, as we found a large increase in IL-6 RNA by Northern blot in involved skin compared to uninvolved skin. IL-6 production by endothelia is not unique and is a well-recognized phenomenon (16). If AIDS-KS cells are derived from endothelia, these results would be expected. What we did not expect was the proliferative response of the AIDS-KS cells to high concentrations of hrIL-6 and the detection of IL-6-R RNA in the areas of the skin involved with Kaposi sarcoma. These characteristics appear to be unique to the AIDS-KS cells (16).

We showed that AIDS-KS cells synthesized, released, and responded to biologically active IL-6. Also, we found that AIDS-KS cells, in which IL-6 protein translation arrest was induced by an IL-6 antisense oligodeoxynucleotide, did not proliferate optimally unless exogenous hrIL-6 was added. In addition, biopsy tissue from AIDS Kaposi sarcoma lesions contained large amounts of IL-6 and IL-6-R, compared to uninvolved skin, confirming our *in vitro* studies. Because IL-1β alters IL-6 production from normal endothelia, it is possible that the previously described mitogenic activity of IL-1β, and perhaps bFGF, for AIDS-KS cells might be by inducing IL-6 production or altering the IL-6 responsiveness of these AIDS-KS cells. Possibly, the unidentified growth factor from $CD4^+$ T cells for AIDS-KS cells is IL-6, or a factor that increases IL-6 production or response in endothelial cells.

Because IL-6 is elevated in many clinical conditions where Kaposi sarcoma is not seen, other processes besides the constitutive expression of IL-6 must be involved in the development of Kaposi sarcoma. Clearly, the abnormal expression of IL-6-R in endothelial cells may be one of these processes. Intense local production of IL-6 by adjacent epithelial cells in response to viral infection, including cytomegalovirus or HIV infection (13, 23), is another possibility. The production of altered forms of cytokines that act as mitogens or increase IL-6 production is also possible. Perhaps one or more of these events, occurring in the setting of intense immunosuppression where these changes can go undetected, results in what we recognize as Kaposi sarcoma.

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