Our working concept is that one or more factors released by retrovirus-infected T cells are critical in the development of KS because they initiate cellular events that lead to the production of cytokines. These cytokines, in turn, have autocrine and paracrine effects and thus produce histological changes characteristic of AIDS-KS (*17*). Immunological and molecular probes for Oncostatin M will be valuable tools to study its role in KS development.

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12. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Oncostatin M as a Potent Mitogen for AIDS-Kaposi's Sarcoma–Derived Cells

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Oncostatin M, a cytokine produced by activated lymphoid cells, regulates the growth and differentiation of a number of tumor and normal cells. In contrast to its effects on normal endothelial and aortic smooth muscle cell cultures, Oncostatin M was a potent mitogen for cells derived from acquired immunodeficiency syndrome–related Kaposi's sarcoma (AIDS-KS). After exposure to Oncostatin M, AIDS-KS cells assumed a spindle morphology, had an increased ability to proliferate in soft agar, and secreted increased amounts of interleukin-6. Oncostatin M RNA and immunoreactive Oncostatin M protein were found in AIDS-KS–derived cell isolates. These results suggest that Oncostatin M may play a role in the pathogenesis of AIDS-KS.

AIDS-RELATED KAPOSI'S SARCOMA (AIDS-KS) is a malignant, neoplastic proliferation of mesenchymal cells. Kaposi's sarcoma (KS) is the most common malignancy complicating human immunodeficiency virus (HIV) infection and is the source of significant morbidity and occasional mortality (*1*). Although effective therapy exists for mild to moderate cases of KS (*2*), many patients develop aggressive cases of the disease that complicate treatment of both the underlying HIV infection and the associated opportunistic infections (*3*). New therapeutic modalities are needed to more effectively treat AIDS-KS.

The introduction of routine cell culture of AIDS-KS–derived spindle cells (AIDS-KS cells) (*4*) has made possible the in vitro study of cytokines that may be involved in the pathogenesis and regulation of proliferation of AIDS-KS cells. By means of these culture systems, interleukin-6 (IL-6) (*5*) and the transactivating protein of HIV, Tat (*4, 6*), were identified as important growth factors for AIDS-KS–derived cells. A variety of other cytokines, including interleukin-1β (IL-1β), tumor necrosis factor–α (TNF-α), gamma-interferon, transforming growth factor–β (TGF-β), platelet factor 4, and interleukin-4 (IL-4), have been shown to modulate IL-6 and to have effects on the proliferative rate of cultured AIDS-KS cells (*6, 7*). Alteration in the levels of one or more of these cytokines has been demonstrated in patients with various stages of HIV infection (*8*), and the potential role of these cytokines in the pathogenesis and proliferation and progression of KS has been discussed (*8*).

Oncostatin M is a 28,000- to 36,000-kD polypeptide cytokine produced by activated T lymphocytes and phorbol 12-myristate 13-acetate (PMA)–treated monocytic cell lines (*9–11*). Although originally identified by its ability to inhibit the growth of A375 melanoma cell lines (*10*), Oncostatin M has a wide variety of in vitro activities including growth regulation of malignant and normal cell lines (*9, 11*). Oncostatin M also increases the expression of IL-6 in cultured human endothelial cells (*12*). Because of the effects of Oncostatin M on these cells, its production by both activated T lymphocytes and PMA-treated U937 cells, and the belief that AIDS-KS–derived cells are mesenchymal-derived cells, we investigated the effects of Oncostatin M on AIDS-KS–derived cell lines.

Recombinant Oncostatin M at low concentrations was found to be one of the most potent mitogens for AIDS-KS–derived cell lines (Fig. 1). For example, for the mitogenesis of AIDS-KS cell lines the half-maximal concentration of Oncostatin M was ~2 ng/ml of medium (70 pM) in short-term culture. Long-term cultures of AIDS-KS cells show that the optimal concentration for proliferation is approximately 250 pg/ml (*6*). These results are less than the half-maximal concentration for mitogenesis of approximately 20 ng/ml for IL-1β and TNF-α. Similar to the effects of Oncostatin M on endothelial cells, mitogenesis of KS

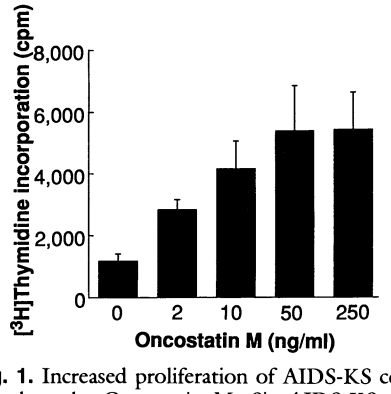

Fig. 1. Increased proliferation of AIDS-KS cells in culture by Oncostatin M. Six AIDS-KS cell lines (KSL1, KSL2, KSL4, KSL5, KSL6, KSL7) were exposed to recombinant Oncostatin M at concentrations from 1 to 1500 ng/ml in serum-free media; representative data from one cell line (KSL4) performed in quadruplicate are presented (mean ± SD). Proliferation was measured by [^{3}H]thymidine incorporation over 18 hours, and proliferation assays were performed as described (*5*).

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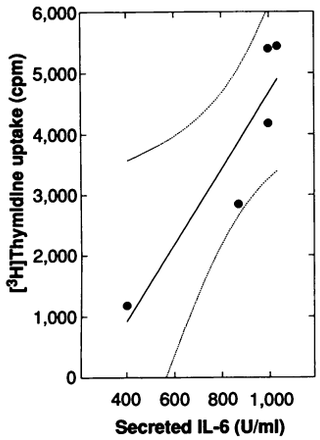

Fig. 2. Relationship of increased AIDS-KS cell proliferation with Oncostatin M to secreted IL-6. The proliferation of four cell lines was measured by thymidine incorporation at five concentrations of Oncostatin M. Each cell isolate was tested in quadruplicate (Fig. 1). IL-6 levels were quantified on the pooled quadruplicate supernatants using an IL-6–specific enzyme-linked immunosorbent assay (*5*). The mean of the proliferation of the four cell isolates and mean of the corresponding IL-6 levels are plotted (circles). The first-order regression line (solid) and 95% confidence intervals (dotted lines) are shown.

cells is associated with increases in IL-6 secretion (Fig. 2). However, although antisense IL-6 molecules inhibit IL-1 and TNF-induced mitogenesis completely, antisense IL-6 oligonucleotides inhibit approximately 50% of the mitogenic effects of Oncostatin M (*13*). This result suggests that only part of the effects of Oncostatin M may be modulated by IL-6 and that other mechanisms not dependent on IL-6 may also be involved.

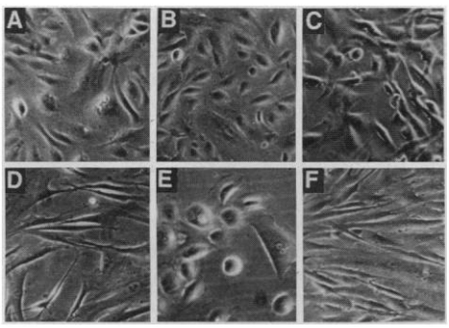

Fig. 3. Morphologic effects of Oncostatin M on freshly cloned AIDS-KS cells. (**A**) Normal aortic endothelia. (**B**) Fresh KSL2 cells (passage 2) isolated on gelatin in the presence of media lacking endothelial growth supplement (Collaborative Research, Boston, Massachusetts), heparin, conditioned media, or recombinant Oncostatin M. (**C**) The same isolate as grown with media and recombinant Oncostatin M (250 pg/ml). (**D**) Normal smooth muscle cells. (**E**) Cells from (B) harvested by trypsin, split evenly, and replated in the same media. (**F**) Trypsinized cells simultaneously replated in the presence of Oncostatin M (250 pg/ml).

Table 1. Number of colonies of AIDS-KS cells in soft agar with Oncostatin M, IL-1β, or TNF-α. Results are given for two experiments. Values represent the number of colonies of KSL1 cells in soft agar. Concentration of IL-1β and TNF-α was 200 ng/ml; TNTC, too numerous to count. Cells (5×10^4) were suspended in 1 ml total volume of 0.3% agar with 30% fetal bovine serum, media, and Oncostatin M. The semisolid agar was layered onto 0.5% hard agar and incubated on scored petri dishes in a humidified, 5% CO_2 atmosphere at 37°C. Colony formation was counted with a dissecting microscope. Clusters of more than 20 cells were considered colonies.

Days	Oncostatin M (ng/ml)					IL-1β	TNF-α
	0	2	10	50	250		
3	0, 0	26, 14	27, 25	27, 39	41, 42	10, 13	5, 5
7	5, 4	110, 74	141, 145	140, 162	198, 236	10, 10	12, 42
10	14, 12	112, 108	200, 194	300, 325	TNTC	20, 10	12, 12

Oncostatin M also changed the morphology of AIDS-KS cells in culture. Fresh, primary KS-derived cells isolated in the absence of Oncostatin M and other mitogens have a cobblestone-like, round shape characteristic of endothelial cell cultures. These cells grow poorly and do not survive in long-term culture. In contrast, KS cells isolated in the presence of Oncostatin M are spindle-shaped like smooth muscle cell cultures (Fig. 3) and proliferate readily. This spindle shape is indistinguishable from the morphology of AIDS-KS cells in histologic sections. When cell lines grown in the absence of Oncostatin M are replated in media containing Oncostatin M, the cells immediately assume the spindle morphology with frequent development of vertical plumes of semiattached cells.

These observations suggested that Oncostatin M may be important in maintaining the transformed phenotype of AIDS-KS–derived cells. To examine this possibility, we plated AIDS-KS–derived cells at low concentration in semisolid agar. Spreading sheets of KS cells were observed in cultures containing Oncostatin M (Table 1), and the density and rate of growth were dependent on the concentration of Oncostatin M. Although some growth was observed in the cultures without Oncostatin M, growth of the cells in semisolid agar was enhanced by Oncostatin M but not by IL-1β or TNF-α.

The proliferative effects of Oncostatin M on AIDS-KS–derived cells are completely different from the effects of Oncostatin M on other normal human mesenchymal cell cultures examined. This result was not anticipated since Oncostatin M increases IL-6 production in endothelial cells (*12*). For example, normal human aortic endothelia, aortic smooth muscle, and umbilical vein endothelia were all inhibited by low (<1 pmol/liter) concentrations of Oncostatin M (*6*). This effect was specific and not related to the toxicity of the recombinant Oncostatin M preparation used because a neutralizing monoclonal antibody (MAb) to Oncostatin M (OM2) partially blocked the inhibitory effects of the recombinant protein.

To investigate whether Oncostatin M was synthesized in KS cells, we examined the expression of Oncostatin M RNA and protein in AIDS-KS–derived cells. Northern (RNA) blot analysis revealed a single 2-kb messenger RNA in cells from the AIDS-KS cell line KSL1 and immunoreactive Oncostatin M on chemiluminescence-enhanced protein immunoblots. Utilizing an Oncostatin M–specific MAb [immunoglobulin G2b (IgG2b) isotype, OM3], we detected 36,000-, 32,000-, and ~28,000-kD proteins in AIDS-KS cell lines KSL1 and KSL4. In the AIDS-KS cell line KSL2 and the control U937 cells, only the ~32,000-kD protein was observed. These bands were competed away in the presence of excess exogenous Oncostatin M and were not observed in the absence of primary antibody (Ab). Two other KS cell isolates examined

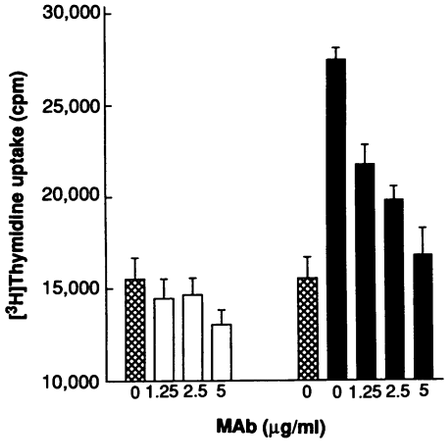

Fig. 4. Effects of a neutralizing MAb to Oncostatin M on basal proliferation of AIDS-KS cells and their response to exogenous Oncostatin M. AIDS-KS cells (KSL5) were plated (Fig. 1) in quadruplicate in serum-free media in the presence (solid bars) or absence (open bars) of Oncostatin M (20 ng/ml). Increasing concentrations of a neutralizing MAb, OM2, were added. Basal proliferation in the absence of Oncostatin M and the MAb is shown (control, crosshatched bar). A mouse isotype IgG1 control MAb had no effect (*14*).

did not have immunoreactive protein. We also looked to see if endogenous Oncostatin M could act in an autocrine growth loop. Although the neutralizing Oncostatin M MAb OM2 had no significant effect on basal proliferation of KSL1 cells at concentrations up to 5 μg/ml (Fig. 4), it reduced by more than 90% the stimulation of proliferation seen with 20 ng/ml of exogenous Oncostatin M. These results, in cells producing Oncostatin M, suggest that the major mitogenic stimulus from Oncostatin M in vivo is exogenous Oncostatin M.

Together, these results suggest that Oncostatin M may contribute to the pathogenesis and progression of AIDS-KS. Oncostatin M is one of the most potent mitogens for KS cells in culture and has several properties not shared by other mitogens such as IL-1β or TNF-α. These properties include the ability to transform the morphologic phenotype of cells to spindle cells as well as to facilitate the proliferation of AIDS-KS–derived cells in semisolid agar. Thus, Oncostatin M may help maintain the transformed phenotype of AIDS-KS–derived cells. Its ability to increase IL-6 and proliferation correlates with the effects of many other cytokines studied (*6*). Paradoxically, Oncostatin M also inhibits the growth of normal mesenchymal cells. Because of the restricted response of KS cells to the HIV Tat protein (*4*), IL-6 (*5*), and Oncostatin M, we suggest that these cells may be functionally different from their normal mesenchymal counterparts. With respect to their in vitro phenotype, these cells could be considered "transformed" but not "immortalized."

As with several other cytokines, Oncostatin M RNA as well as protein is found in some AIDS-KS–derived cells and may be functionally active. As a result, Oncostatin M could act as both a paracrine and autocrine growth factor in vivo. However, the response of all cell lines tested to exogenous Oncostatin M suggests that the principal site of action is through exogenous Oncostatin M. Although the heterogeneity in expression of Oncostatin M in cultured AIDS-KS cells could be the result of the isolation and culture process, it is also possible that expression of Oncostatin M is variable in vivo. If so, this variable expression could explain some of the wide range of growth characteristics of KS observed in patients with HIV infection.

Compared to the effects of other more modest growth-promoting proteins such as the HIV Tat protein (*4*), the effects of Oncostatin M are far more potent. While exposure of AIDS-KS cells to the HIV Tat protein gives a 50 to 100% increase in proliferation, proliferation increases from Oncostatin M in soft agar can be as high as tenfold. In addition, because activated T cells as well as PMA-stimulated U937 cells produce Oncostatin M, it is possible that the HIV Tat protein or other activating factors for monocytes and T lymphocytes could increase the production of Oncostatin M. This situation could amplify the effects of the HIV Tat protein and indicate a potential role for Tat-based inhibitors in AIDS-KS therapy. This situation also leaves open the possibility that other sexually transmitted agents could alter Oncostatin M secretion and thereby participate in the pathogenesis of AIDS-KS. Given the potent activity of Oncostatin M, inhibitors of Oncostatin M expression or biologic activity should be explored as potential therapeutic agents in AIDS-KS.

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13. Two oligonucleotides of 15 bases in length were constructed (TCCTGGGGGTACTGG and CCAGTACCCCCAGGA) (Operon Technologies). These constructs were homologous to the sense and antisense orientations of the first coding exon of the mature IL-6 protein. The antisense oligonucleotide has been shown to inhibit IL-6 secretion of AIDS-KS cells in an orientation-specific manner (*5*). The oligonucleotides were added at a concentration of 15 μM to serum-free media simultaneously with Oncostatin M as previously described (*5*). Proliferation was measured by [^{3}H]thymidine uptake. At this oligonucleotide concentration, approximately 50% of the stimulation by Oncostatin M was inhibited.
14. Data not shown.

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The IL-6 Signal Transducer, gp130: An Oncostatin M Receptor and Affinity Converter for the LIF Receptor

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Leukemia inhibitory factor (LIF) and interleukin-6 (IL-6) are multifunctional cytokines with many similar activities. LIF is structurally and functionally related to another cytokine, Oncostatin M (OSM), that binds to the high-affinity LIF receptor but not to the low-affinity LIF receptor. A complementary DNA was isolated that encodes the high-affinity converting subunit of the LIF receptor. The converter conferred high-affinity binding of both LIF and OSM when expressed with the low-affinity LIF receptor and is identical to the signal transducing subunit of the IL-6 receptor, gp130. The gp130 subunit alone confers low-affinity binding of OSM when expressed in COS-7 cells. This receptor system resembles the high-affinity receptors for granulocyte-macrophage colony-stimulating factor, IL-3, and IL-5, which share a common subunit.

LEUKEMIA INHIBITORY FACTOR (LIF) acts as an inducer or inhibitor of differentiation and promotes either survival, proliferation, or activation of a wide array of target cells, including hemopoietic, hepatic, adipogenic, osteogenic, renal, neuronal, and embryonic cells (*1, 2*). LIF action appears to be mediated through specific cellular receptors of high affinity (dissociation constant, $K_d = 1 \times 10^{-11}$ to 20×10^{-11} M) and low affinity ($K_d = 1 \times 10^{-9}$ to 3×10^{-9} M) (*3*). LIF receptor cDNA confers specific low-affinity binding of LIF when expressed in COS-7 cells (*1*) and both low-affinity and high-affinity binding when expressed in murine B9 cells (*4*).

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