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.

REFERENCES AND NOTES

1. R. T. Mitsuyasu, *Blood Rev.* **2**, 222 (1988).
2. P. A. Volberding, in *AIDS Pathogenesis and Treatment*, J. A. Levy, Ed. (Dekker, New York, 1989), p. 345.
3. L. J. Laubenstein, R. L. Krigel, C. M. Odajnk, *J. Clin. Oncol.* **2**, 1115 (1984).
4. B. Ensoli, G. Barillari, S. Z. Salahuddin, R. C. Gallo, F. Wong-Staal, *Nature* **345**, 84 (1990); S. Z. Salahuddin *et al.*, *Science* **242**, 430 (1988); S. Nakamura *et al.*, *ibid.*, p. 426.
5. S. A. Miles *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4068 (1990).
6. S. A. Miles *et al.*, unpublished data.
7. B. Ensoli *et al.*, *Science* **243**, 223 (1989).
8. A. J. Ammann *et al.*, *J. Clin. Immunol.* **7**, 481 (1987); M. A. Berman, C. I. Sandborg, B. S. Calabia, B. S. Andrews, G. J. Friou, *Clin. Immunol. Immunopathol.* **42**, 133 (1987).
9. D. Horn *et al.*, *Growth Factors* **2**, 157 (1990).
10. J. M. Zarling *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9739 (1986).
11. P. S. Linsley, J. Kallestad, V. Ochs, M. Neubauer, *Mol. Cell. Biol.* **10**, 1882 (1990); N. Malik *et al.*, *ibid.* **9**, 2847 (1989); P. S. Linsley *et al.*, *J. Biol. Chem.* **264**, 4282 (1989); T. J. Brown, M. N. Lioubin, H. Marquardt, *J. Immunol.* **139**, 2977 (1987).
12. T. J. Brown, J. Rowe, L. Jingwen, M. Shoyab, *J. Immunol.* **147**, 2175 (1991).
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.

15. We acknowledge funding from the State of California as directed by the Universitywide Task Force for AIDS (R87LA039, R88LA078, R90LA121, and R91LA149) and the NIH (CA 01588 and AI27660). We also thank R. Mitsuyasu and S. Z. Salahuddin for technical comments, L. Souza for IL-6, and D. Slamon for laboratory facilities.

10 October 1991; accepted 27 January 1992

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

DAVID P. GEARING,* MICHAEL R. COMEAU, DELLA J. FRIEND, STEVEN D. GIMPEL, CATHERINE J. THUT, JACKIE MCGOURTY, KELLE K. BRASHER, JULIE A. KING, STEVEN GILLIS, BRUCE MOSLEY, STEVEN F. ZIEGLER, DAVID COSMAN

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*).

Immunex Research and Development Corporation, 51 University Street, Seattle, WA 98101.

*To whom correspondence should be addressed.

The LIF receptor is a member of the hematopoietin receptor superfamily (*5*) and resembles most closely the beta subunit (gp130) of the IL-6 receptor (*6, 7*) and the granulocyte–colony-stimulating factor (G-CSF) receptor (*8*). LIF and a second cytokine, Oncostatin M (OSM), are related in structure, chromosomal localization, and biological activity (*9*). Analysis of the binding of human OSM (hOSM) to native and recombinant LIF receptors on a variety of cell types (*4*) led to the following conclusions: (i) the high-affinity, but not the low-affinity, LIF receptor is also an OSM receptor; (ii) distinct OSM-specific receptors exist on A375 melanoma cells and H2981 lung carcinoma cells; and (iii) OSM may have all of the biological activities of LIF and also a variety of activities mediated by the OSM-specific receptor.

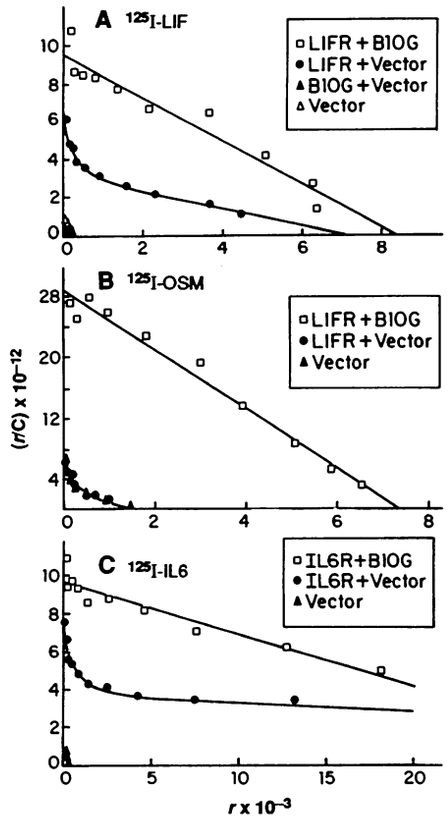

Fig. 1. Binding of human LIF, OSM, and IL-6 to COS-7 cells transfected with the LIF receptor, clone B10G, and the IL-6 receptor. COS-7 cells were transfected with the following plasmids alone (2 μg) or in combination (1 μg each): pHLIFR-65, B10G, pIL-6R, or empty vector (Vector). (**A**) Binding of ^{125}I-labeled hLIF to cells transfected with pHLIFR-65 and B10G, pHLIFR-65 and vector, B10G and vector, or vector alone. (**B**) Binding of ^{125}I-labeled hOSM to cells transfected with pHLIFR-65 and B10G, pHLIFR-65 and vector, or vector alone. (**C**) Binding of ^{125}I-labeled hIL-6 to cells transfected with pIL-6R and B10G, pIL-6R and vector, or vector alone. Experimental conditions were as described (*19*). r = molecules bound per cell; C = concentration (M). The calculated equilibrium dissociation constant (K_d ± SD) and the upper limit value of r (r_{max} ± SD), which is the number of receptors per cell, for each binding curve were (A) pHLIFR-65 and B10G, $K_d = 9.1 \pm 0.1 \times 10^{-10}$ M, $r_{max} = 8450 \pm 200$; pHLIFR-65 and vector, $K_{d1} = 3.3 \pm 2.0 \times 10^{-11}$ M, $r_{max\ 1} = 105 \pm 40$, $K_{d2} = 2.4 \pm 0.3 \times 10^{-9}$ M, $r_{max\ 2} = 7350 \pm 370$. (B) pHLIFR-65 and B10G, $K_d = 2.4 \pm 0.4 \times 10^{-10}$ M, $r_{max} = 7170 \pm 400$. (C) pIL6R and B10G, $K_d = 3.3 \pm 0.3 \times 10^{-9}$ M, $r_{max} = 35{,}000 \pm 4{,}000$; pIL6R and vector, $K_{d1} = 7.4 \pm 3.5 \times 10^{-11}$ M, $r_{max\ 1} = 380 \pm 150$, $K_{d2} = 3.5 \pm 2.3 \times 10^{-8}$ M, $r_{max\ 2} = 130{,}000 \pm 70{,}000$.

We expected that a second subunit of the LIF receptor might confer high-affinity binding of LIF and OSM to the cloned LIF receptor. Because hOSM does not bind to the low-affinity hLIF receptor, we used hOSM to screen for this subunit. We expressed the cloned low-affinity hLIF receptor and pools of cDNA clones from a human placental cDNA library in COS-7 cells and screened for binding of ^{125}I-labeled hOSM (*10*). A pool of cDNAs was identified that conferred hOSM binding in the presence of the hLIF receptor, and a single cDNA clone (B10G) with the same properties was isolated. The cDNA insert of this clone was nearly identical to that of gp130, the high-affinity converter and signal transducing component (beta subunit) of the human IL-6 receptor complex, which does not bind IL-6 alone but associates with the low-affinity IL-6 receptor alpha subunit to form a high-affinity complex (*6, 7*). Clone B10G is an incomplete cDNA of gp130 that is missing the COOH-terminal 209 amino acids of the cytoplasmic domain and terminates in vector-encoded sequences. It also has a single conservative substitution in the signal sequence (Val^8 to Leu) that is presumed to be a polymorphism.

Expression of the hLIF receptor cDNA in COS-7 cells increased the number of LIF binding sites of low affinity ($K_d = 2.4 \times 10^{-9}$ M) with no detectable change in OSM binding as compared to that seen with COS-7 cells transfected with vector alone (Fig. 1). Expression of the hLIF receptor cDNA with clone B10G resulted in hOSM binding ($K_d = 2.4 \times 10^{-10}$ M; Fig. 1B) and increased the affinity of hLIF binding ($K_d = 9 \times 10^{-10}$ M; Fig. 1A) without affecting the number of LIF binding sites. These results indicate a conversion of low-affinity binding sites to higher affinity binding sites rather than addition of a new class of high-affinity sites. The affinity of LIF binding to COS-7 cells expressing the hLIF receptor and B10G was somewhat lower than that to high-affinity LIF receptors on responsive cell lines ($K_d = 1 \times 10^{-11}$ to 20×10^{-11} M) (*3*). Expression of clone B10G with a cDNA encoding the low-affinity IL-6 receptor ($K_d = 3.5 \times 10^{-8}$) (*11*) also resulted in formation of a higher affinity hIL-6 receptor complex ($K_d = 3.3 \times 10^{-9}$; Fig. 1C), but again, this affinity was lower

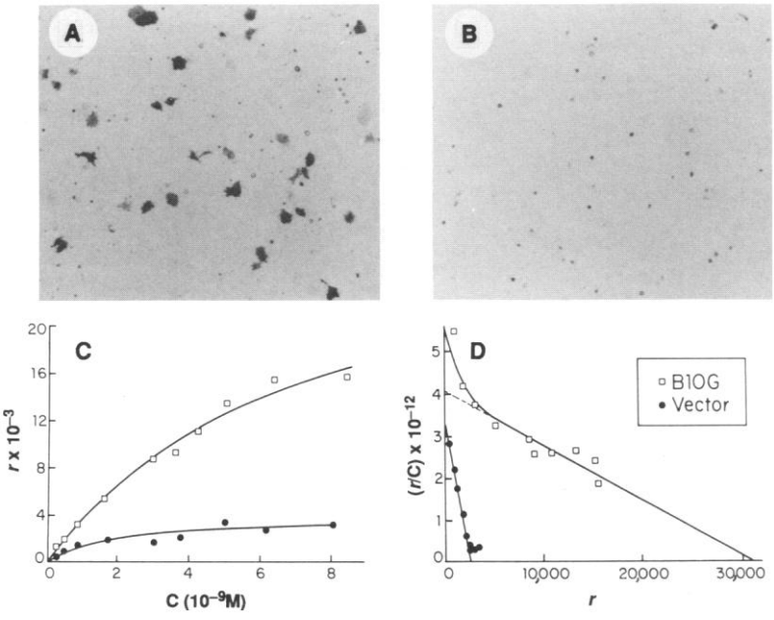

Fig. 2. Low-affinity binding of OSM to COS-7 cells transfected with B10G. COS-7 cells on microscope slides were transfected with (**A**) clone B10G or (**B**) empty vector and incubated with 5 nM ^{125}I-labeled hOSM for 1 hour at room temperature, washed, fixed, and dipped in photographic emulsion as described (*1*). Slides were exposed for 2 days and then developed. (**C**) Direct analysis of binding to transfected COS-7 cells in suspension. (**D**) Scatchard transformation of data presented in (C). Untransfected COS-7 cells had hOSM binding indistinguishable to cells transfected with the vector. Calculated equilibrium dissociation constants and site numbers (±SD) were as follows: B10G, $K_d = 7.7 \pm 1.4 \times 10^{-9}$ M, $r_{max} = 31{,}000 \pm 5{,}000$. Vector, $K_d = 7.6 \pm 2.0 \times 10^{-10}$ M, $r_{max} = 2{,}500 \pm 600$.

than that previously described (K_d = 1.3 × 10^{-10} to 6.7 × 10^{-11} M) (*7, 12*). The high-affinity LIF-OSM receptor and IL-6 receptor may require the presence of a third subunit (which is present in limiting amounts in COS-7 cells) for full affinity conversion. A similar three-subunit model has been proposed for the IL-2 receptor (*13*). Alternatively, the truncations present in the cytoplasmic domains of our LIF receptor and B10G clones may have contributed to the lower affinities.

Transfection experiments in COS-7 cells (*14*) also demonstrated the following features of the binding of the three ligands to the three receptor subunits: (i) coexpression of the hIL-6R and hLIFR did not result in high-affinity binding of hIL-6 or hLIF; (ii) neither the low-affinity hIL-6R nor hIL6R-B10G complex could bind hLIF directly; (iii) human LIF and hOSM competed with each other for binding to the hLIFR-B10G complex; and (iv) the hLIFR did not bind hIL-6 (*1*) or hOSM (*4*).

Both slide autoradiography and direct binding analyses demonstrated that hOSM bound with low affinity to the protein encoded by B10G in the absence of the hLIF receptor (K_d = 7.7 × 10^{-9} M; Fig. 2). The affinity of binding of hOSM to COS-7 cells expressing B10G was similar to that of low-affinity hOSM binding to H2981 human lung carcinoma cells (K_d = 8.6 × 10^{-9} M) (*4*), suggesting that gp130 may also be a component of the OSM-specific receptor on H2981 cells. Weak binding of hLIF to COS-7 cells expressing B10G was observed by the slide-autoradiography method (the number of grains counted was approximately one fiftieth of that after binding of hOSM) (*14*) but the affinity of this interaction was not measurable by Scatchard analysis (Fig. 1). Human IL-6 did not bind to B10G expressed on COS-7 cells.

To confirm the apparent relationship between the LIF-OSM and IL-6 receptor systems we used a variant of the murine IL-6–dependent line, B9, that was transfected with the low-affinity hLIF receptor (B9-hLIFR) (*4*). This cell line has high-affinity hLIF receptors with mixed subunits that derive from the transfected low-affinity hLIF receptor and an endogenous murine affinity-converting subunit (presumably murine gp130); these high-affinity receptors bind hOSM (*4*). Incubation of B9-hLIFR cells with excess mIL-6 reduced the number of high-affinity hLIF binding sites but had no effect on low-affinity hLIF binding sites (Fig. 3A). We therefore studied the effect of mIL-6 on binding of hOSM to the high-affinity LIF receptors. Incubation with excess hLIF completely blocked hOSM binding (*4*), but mIL-6 competed less effectively for hOSM binding sites, and the affinity of hOSM binding was unchanged (Fig. 3B). Similarly, we studied the effect of excess hLIF or hOSM on the binding of mIL-6 to endogenous binding sites. In untransfected B9 cells, mIL-6 binding was not influenced by excess hLIF or hOSM (Fig. 3D), presumably reflecting the much higher affinity of mIL-6 for the IL-6 receptor–gp130 complex than hLIF or hOSM for murine gp130. However, the binding of mIL-6 to B9-LIFR cells was partially competed by hLIF or hOSM (Fig. 3C). The high-affinity mIL-6 receptors may be converted to a low-affinity form by the sequestration of gp130 molecules into hLIF receptor complexes in the presence of excess hLIF or hOSM. Although we observed a reduction in high-affinity IL-6 binding sites, we could not measure the small number (~500 sites per cell; Fig. 3) of low-affinity binding sites presumably generated (expected K_d = 5 × 10^{-9} M) (*7*).

Similar interactions between receptor subunits have been proposed for the low-affinity granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3, and IL-5 receptors and their high-affinity converter, KH97 (*15*) (Fig. 4). However, gp130 appears to be both a signal transducer and a low-affinity OSM receptor, whereas KH97 has not been reported to bind any cytokine on its own.

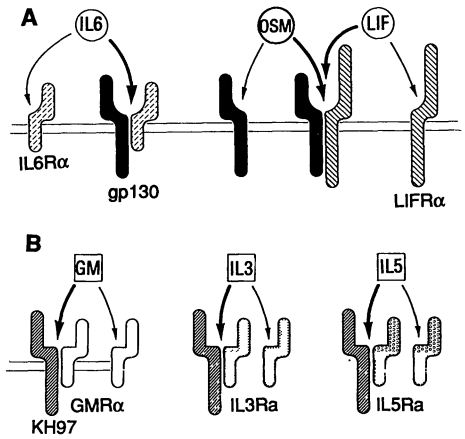

Fig. 4. Comparison of the IL-6, OSM, and LIF receptor system (**A**) to the GM-CSF, IL-3, and IL-5 receptor system (**B**). High-affinity binding is represented by broad arrows and low-affinity binding by thin arrows.

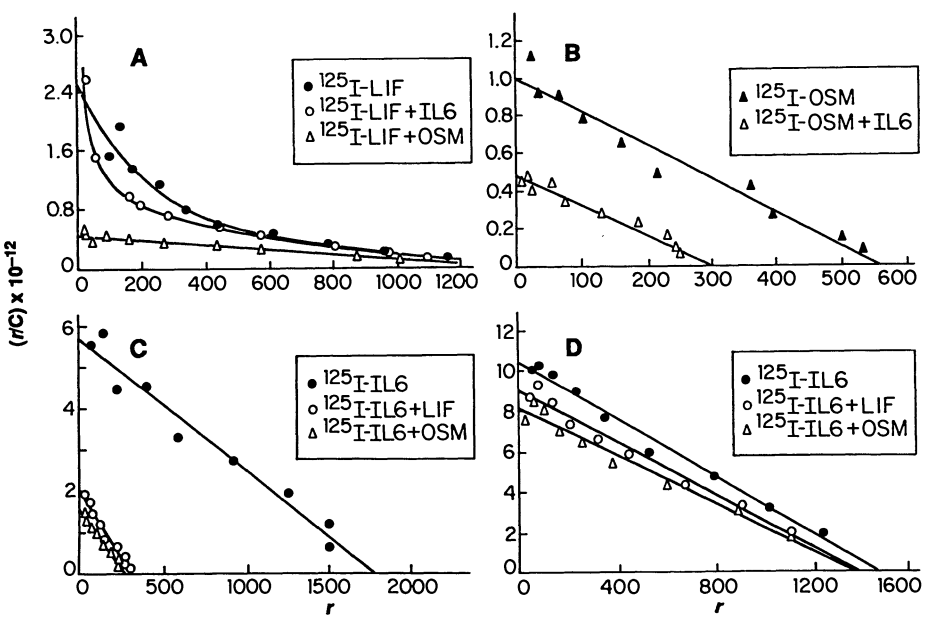

Fig. 3. Competition of binding of hLIF, hOSM, and mIL-6. Binding to B9-hLIFR cells (**A** through **C**) and B9 cells (**D**). (A) ^{125}I-labeled hLIF binding in the absence or presence of unlabeled hOSM or mIL-6. (B) ^{125}I-labeled hOSM binding in the absence or presence of mIL-6. Under the same conditions hLIF completely competes the hOSM binding (*4*). (C and D) ^{125}I-labeled mIL-6 binding in the absence or presence of hLIF or hOSM. Neither hLIF nor hOSM binds to untransfected B9 cells (*4*). Experimental conditions were as described (*22*). Calculated equilibrium dissociation constants and site numbers (±SD) for each ^{125}I-labeled ligand (*) were as follows. (A) *LIF + no competitor, K_{d1} = 1.1 ± 1.0 × 10^{-10} M, $r_{max\,1}$ = 224 ± 75, K_{d2} = 2.8 ± 1.0 × 10^{-9} M, $r_{max\,2}$ = 1200 ± 60; *LIF + IL-6, K_{d1} = 1.3 ± 1.2 × 10^{-11} M, $r_{max\,1}$ = 34 ± 18, K_{d2} = 1.6 ± 0.2 × 10^{-9} M, $r_{max\,2}$ = 1250 ± 40; *LIF + OSM, K_d = 3.1 ± 0.4 × 10^{-9} M, r_{max} = 1390 ± 140. (B) *OSM + no competitor, K_d = 5.9 ± 0.8 × 10^{-10} M, r_{max} = 570 ± 70; *OSM + IL-6, K_d = 6.2 ± 0.3 × 10^{-10} M, $r_{max\,2}$ = 300 ± 35. LIF competed all *OSM binding (*4*). (C) *IL-6 + no competitor, K_d = 3.1 ± 0.5 × 10^{-10} M, $r_{max\,1}$ = 1780 ± 65; *IL-6 + LIF, K_d = 1.6 ± 0.2 × 10^{-10} M, r_{max} = 330 ± 10; *IL-6 + OSM, K_d = 1.8 ± 0.2 × 10^{-10} M, r_{max} = 290 ± 25. (D) *IL-6 + no competitor, K_d = 1.4 ± 0.1 × 10^{-10} M, $r_{max\,1}$ = 1460 ± 100; *IL-6 + LIF, K_d = 1.6 ± 0.2 × 10^{-10} M, r_{max} = 1400 ± 130; *IL-6 + OSM, K_d = 1.7 ± 0.2 × 10^{-10} M, r_{max} = 1380 ± 50.

Reported homologies between the IL-6 receptor, ciliary neurotrophic factor receptor (*16*), and the natural killer cell stimulatory factor (*17*) indicate possible interactions of these molecules with gp130. By contrast, the G-CSF receptor, which is structurally similar to both gp130 and the LIF receptor, binds G-CSF and transduces a signal (as a homodimer) independently of gp130 (*18*). Thus, gp130 as well as KH97 in a separate but parallel system might be focal points of signal transduction for a variety of cytokines, providing an explanation for the functional redundancy of those cytokines.

REFERENCES AND NOTES

1. D. P. Gearing *et al.*, *EMBO J.* **10**, 2839 (1991).
2. D. P. Gearing, *Ann. N.Y. Acad. Sci.* **628**, 9 (1991); D. J. Hilton and N. M. Gough, *J. Cell. Biochem.* **46**, 21 (1991).
3. Y. Yamamoto-Yamaguchi, M. Tomida, M. Hozumi, *Exp. Cell. Res.* **164**, 97 (1986); D. J. Hilton, N. A. Nicola, D. Metcalf, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5971 (1988); *J. Cell. Physiol.* **146**, 207 (1991); S. B. Rodan, G. Wesolowski, D. J. Hilton, *Endocrinology* **127**, 1602 (1990); M. Tomida *et al.*, *FEBS Lett.* **268**, 261 (1990).
4. D. P. Gearing and A. G. Bruce, *New Biol.* **4**, 61 (1992).
5. D. Cosman *et al.*, *Trends Biochem. Sci.* **15**, 265 (1990).
6. T. Taga *et al.*, *Cell* **58**, 573 (1989).
7. M. Hibi *et al.*, *ibid.* **63**, 1149 (1990).
8. R. Fukunaga *et al.*, *ibid.* **61**, 341 (1990); A. Larsen *et al.*, *J. Exp. Med.* **172**, 1559 (1990).
9. T. M. Rose and A. G. Bruce, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8641 (1991).
10. The hLIFR cDNA (50 ng) was mixed with 1 μg of each pool of 600 to 800 clones from the placental cDNA library and then transfected into COS-7 cells and assayed for receptor binding as described (*1*).
11. K. Yamasaki *et al.*, *Science* **241**, 825 (1988).
12. T. Taga, Y. Kawanashi, R. Hardy, *J. Exp. Med.* **166**, 967 (1987); H. Baumann, H. Isseroff, J. Latimer, *J. Biol. Chem.* **263**, 17390 (1988).
13. M. Hatakeyama *et al.*, *Science* **244**, 551 (1989); T. Honjo, *Curr. Biol.* **1**, 201 (1991).
14. D. P. Gearing, D. J. Friend, M. R. Comeau, B. Mosley, unpublished observations.
15. K. Hayashida *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9655 (1990); T. Kitamura, N. Sato, K.-I. Arai, *Cell* **66**, 1165 (1991); J. Tavernier *et al.*, *ibid.*, p. 1175; R. Devos *et al.*, *EMBO J.* **10**, 2133 (1991).
16. S. Davis *et al.*, *Science* **253**, 59 (1991).
17. D. P. Gearing and D. Cosman, *Cell* **66**, 175 (1991).
18. R. Fukunaga, E. Ishizaka-Ikeda, N. Nagata, *J. Biol. Chem.* **265**, 14008 (1990); R. Fukunaga, E. Ishizaka-Ikeda, C. X. Pan, *EMBO J.* **10**, 2855 (1991); S. F. Ziegler *et al.*, *New Biol.* **3**, 1242 (1991).
19. After DEAE-dextran–mediated transfection (*1*), COS-7 cells were replated on fresh dishes on day 1 and removed from the dishes with nonenzymatic cell-dissociation solution (Sigma) on day 2. Binding experiments were performed essentially as described (*4*). Cells were incubated at 1.6×10^6 cells per milliliter and the appropriate concentration of ^{125}I-ligand in a total volume of 150 μl at 25°C for 2 hours. A 200-fold molar excess of unlabeled ligand was used to determine nonspecific binding. At the end of the incubation, duplicate 60-μl portions were removed, and bound and free ^{125}I-labeled ligands were separated on phthalate oil columns as described (*4*). Scatchard analysis of binding isotherms (*20*) was done with the program RS/1 (Bolt, Beranek and Newman, Boston, MA). The hLIF and hOSM were expressed in yeast, purified, and ^{125}I-labeled with Enzymobeads (Bio-Rad) to 2×10^{15} and 5×10^{15} cpm/mmol, respectively (*1*). The cDNA clone encoding hOSM was isolated from a human T cell cDNA library with an oligonucleotide based on the published sequence (*21*). Purified hIL-6 (H. Sassenfeld, Immunex) was ^{125}I-labeled to a specific activity of 10^{15} cpm/mmol.
20. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
21. N. Malik *et al.*, *Mol. Cell. Biol.* **9**, 2847 (1989).
22. Binding assays were done essentially as above (*19*) except that cells were incubated at 1.6×10^7 cells/ml. A 200-fold molar excess of unlabeled ligand was used to determine nonspecific binding and competition assays were done with unlabeled ligands at a final concentration of 2 μg/ml. Murine IL-6 (R+D Systems, Minneapolis, MN) was ^{125}I-labeled to 4×10^{15} cpm/mmol. The number of low-affinity hLIF receptors expressed on these B9-hLIFR cells is lower than that described (*4*) although the number of high-affinity LIF and OSM binding sites has remained approximately constant. During the course of the transfection and selection procedure the B9-hLIFR cells (and control cells) lost their requirement for IL-6 (*4*).

23. We are indebted to T. Sato, K. Brasel, J. Slack, and U. Martin for technical assistance; to D. A. Anderson for the human T cell cDNA library; and to L. Park, S. Dower, B. Thoma, and J. Sims for critical comments on the manuscript.

4 December 1991; accepted 13 February 1992

Inhibition of Development of Kaposi's Sarcoma–Related Lesions by a Bacterial Cell Wall Complex

SHUJI NAKAMURA, SHINSAKU SAKURADA, S. ZAKI SALAHUDDIN, YASUAKI OSADA, NORIKO G. TANAKA, NORITSUGU SAKAMOTO, MASAYASU SEKIGUCHI, ROBERT C. GALLO*

In vitro and in vivo model systems for the study of human immunodeficiency virus (HIV)–associated Kaposi's sarcoma (KS) were used to evaluate compounds for their potential as therapeutic agents. A sulfated polysaccharide–peptidoglycan compound (SP-PG) produced by bacteria controlled the in vitro growth of acquired immunodeficiency syndrome (AIDS)–associated, KS-derived spindle-shaped cells (AIDS-KS cells) at noncytotoxic concentrations. Angiogenesis induced by AIDS-KS cells in the chicken chorioallantoic membrane assay was blocked by SP-PG, which also inhibited the vascular hyperpermeability response and the angiogenesis associated with the induction of KS-like lesions that develop after subcutaneous inoculation of AIDS-KS cells into nude mice. Suramin, pentosan polysulfate, and interferon α, which are currently in use for therapy of KS, were either less effective than SP-PG or much more cytotoxic, or both.

KAPOSI'S SARCOMA (KS) IS CHARACTERIZED by microvascular proliferation (angiogenesis) in the initial stage of lesion development that is soon followed by the presence of proliferating spindle cells, edema, and infiltration by multiple cell types (*1*). We developed in vitro systems for the long-term culture of AIDS-KS cells and in vivo systems that simulate the formation of KS-related lesions (*2, 3*). AIDS-KS cells induce vascularization on chicken chorioallantoic membranes (CAM), and when transplanted into nude mice they induce vascular hyperpermeability and resultant edema (*4*), angiogenesis, and the development of KS-like lesions of murine origin (*3*).

Kaposi's sarcoma is currently treated with cytotoxic agents such as vinblastine, bleomycin (*5, 6*), and suramin (*7*), or with cytokines like interferon α (IFN-α) (*8*). Both of these forms of therapy may effect multiple cell types and many cell functions. An angiostatic compound, pentosan polysulfate (*9, 10*), has also been used, but many potential angiostatic compounds (*11–16*) still remain to be clinically tested. One of these is SP-PG, a naturally occurring sulfated polysaccharide–peptidoglycan produced by a specific species of the bacterium *Arthrobacter* sp. (strain AT-25, Daiichi Pharmaceutical). Although SP-PG has not yet been purified to homogeneity, the most active fraction has a molecular size of 30 kD (*17*) and inhibits the growth of subcutaneously inoculated solid tumors (which require angiogenesis for their growth), while not affecting growth of ascites tumor cells of the same origin (*18*). Although subcutaneous tumor growth is inhibited by SP-PG, in vitro growth of these tumor cells is not inhibited; however, SP-PG does inhibit growth of vascular endothelial cells in vitro (*19*).

The AIDS-KS cells were more sensitive to SP-PG than normal endothelial cells

S. Nakamura and S. Z. Salahuddin, Department of Internal Medicine, University of Southern California, Los Angeles, CA 90033, and IMMAT Huntington Memorial Hospital, Los Angeles, CA 91105.
S. Sakurada and R. C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
Y. Osada, N. G. Tanaka, N. Sakamoto, M. Sekiguchi, Daiichi Pharmaceutical Co., Ltd., Tokyo, 103, Japan.

*To whom correspondence should be addressed.