Ciliary Neurotropic Factor, Interleukin 11, Leukemia Inhibitory Factor, and Oncostatin M Are Growth Factors for Human Myeloma Cell Lines Using the Interleukin 6 Signal Transducer GP130

By Xue-Guang Zhang,[*][‡] Jong-Jiang Gu,[*][‡] Zhao-Yang Lu,[*][‡] Kiyoshi Yasukawa,[§] George D. Yancopoulos,[‖] Katherine Turner,[¶] Mohammed Shoyab,[**] Tetsuya Taga,[‡‡] Tadamitsu Kishimoto,[¶¶] Régis Bataille,[**] and Bernard Klein[*]

From the [*]Institute for Molecular Genetics, CNRS BP5051, 34033 Montpellier Cedex 1, France; the [‡]Immunology Research Unit, Suzhou Medical College, Suzhou, 215007, People's Republic of China; the [§]Biotechnology Research Laboratory, Tosoh Corporation, Kanagawa 252, Japan; [‖]Regeneron Pharmaceuticals, Inc., Tarrytown, New York 10591; the [¶]Genetics Institute, Cambridge, Massachusetts 02140; the [**]Fred Hutchinson Cancer Research Center, Seattle, Washington 98104-2092; the [‡‡]Institute for Molecular and Cellular Biology, Osaka University; the [¶¶]Department of Medicine III, Osaka University Medical School, Osaka 565, Japan; and the [**]Laboratory for Immunological and Hematological Oncogenesis, Institute of Biology, 44035 Nantes Cedex 01, France.

Summary

Interleukin 6 (IL-6) is a major growth factor for tumor plasma cells involved in human multiple myeloma (MM). In particular, human myeloma cell lines (HMCL), whose growth is completely dependent on addition of exogenous IL-6, can be obtained reproducibly from every patient with terminal disease. Four cytokines, ciliary neurotropic factor (CNTF), IL-11, leukemia inhibitory factor (LIF), and oncostatin M (OM), use the same transducer chain (signal transducer gp130) as IL-6 and share numerous biological activities with this IL. We found that these four cytokines stimulated proliferation and supported the long-term growth of two out of four IL-6–dependent HMCL obtained in our laboratory. Half-maximal proliferation was obtained with cytokine concentrations ranging from 0.4 to 1.2 ng/ml for IL-11, LIF, and OM. CNTF worked at high concentrations only (90 ng/ml), but addition of soluble CNTF receptor increased sensitivity to CNTF 30-fold. The growth-promoting effect of these four cytokines was abrogated by anti-gp130 antibodies, contrary to results for anti-IL-6 receptor or anti-IL-6 antibodies. No detectable changes in the morphology and phenotype were found when myeloma cells were cultured with one of these four cytokines instead of IL-6. Concordant with their IL-6–dependent growth, the four HMCL expressed membrane IL-6R and gp130 detected by FACS® analysis. LIF-binding chain gene (LIFR) was expressed only in the two HMCL responsive to LIF and OM.

Multiple myeloma (MM) is a B cell neoplasia affecting the late stages of B cell differentiation (plasma cells). We and others have shown that IL-6 is a major growth factor for tumor plasma cells in vitro and that treatments with anti-IL-6 antibodies could result in blockage of tumor proliferation in vivo (1–3). In addition, we have been able to obtain human myeloma cell lines (HMCL) reproducibly, whose growth is completely dependent on addition of exogenous IL-6 (4 and Zhang, X.-G., unpublished results).

As IL-11, leukemia inhibitory factor (LIF), and oncostatin M (OM) share similar biological effects with IL-6 as inducers of acute-phase proteins, stimulators of thrombopoiesis, and as competence factor for hematopoietic cells (5–8), we investigated whether they could support the growth of human myeloma cells. The overlapping biological activities of these cytokines is to be explained by their use of multicomponent receptors, including a common transducer gp130 unit (9–13). gp130 was first identified as an IL-6 signal transducer (14). IL-6 binds to its specific receptor (IL-6R), with a low affinity of about 10^{-9} M (15). IL-6R has no transducing activity, but the complex formed by IL-6 and IL-6R binds to gp130 (14), resulting in the formation of a high affinity IL-6 receptor, dimerization, phosphorylation, and activation of the transducer gp130 (14, 16). LIF was initially shown to bind with

low affinity to a receptor subunit (LIFRβ) related to gp130 (17). The transducer gp130 was subsequently found to convert LIFRβ into a high affinity binding site for OM as well as LIF (9). Finally, the IL-11 receptor is presently unidentified but was recently shown to include the transducer gp130 (12). Another cytokine, ciliary neurotropic factor (CNTF), also uses the transducer gp130. It has a more restricted spectrum of biological activity than IL-6, LIF, or OM, possibly because of restricted expression of the CNTF binding protein (termed CNTFRα) found mainly in neuronal tissues (18). CNTFRα binds CNTF with low affinity (18). This receptor has no cytoplasmic tail and is linked to the cell membrane through glycosyl phosphatidylinositol. Addition of CNTFRα to the LIFRβ/gp130 complex has been shown to convert a functional LIF or OM receptor into a functional CNTF receptor (19). CNTFRα and IL-6R share strong homologies (15, 18), and a unique feature is that their soluble forms have an agonist activity, whereas all known soluble cytokine receptors have an antagonist activity. When bound to their respective ligands, they can activate the transducer complex (14, 20, 21).

As noted above, the common use of the IL-6 signal transducer gp130 by CNTF, IL-11, LIF, and OM may account for their overlapping biological effects with IL-6. An immediate consequence could be that these four cytokines, like IL-6, are also involved in numerous diseases: infectious, autoimmune, and cancer diseases, and particularly human MM (6).

We report here that cytokines CNTF, IL-11, LIF, and OM are potent growth factors for human myeloma cells. Their myeloma cell growth factor activity is inhibited by anti-gp130 antibodies unlike anti-IL-6 or anti-IL-6R antibodies.

Materials and Methods

HMCL. The four HMCL (XG-1, XG-2, XG-4, XG-6) were obtained by culturing freshly explanted myeloma cells from patients with terminal disease with a combination of IL-6 and GM-CSF (4 and Zhang, X.-G., unpublished results). These HMCL have a plasma cell phenotype and the same immunoglobulin gene rearrangements as patients' tumor cells, and their growth is completely dependent on addition of IL-6. Their characteristics are reported elsewhere (4 and Zhang, X.-G., manuscript in preparation). HMCL were free of mycoplasma contamination and were routinely cultured in RPMI 1640 supplemented with 5% FCS and 1 ng/ml of recombinant (r)IL-6.

HMCL Proliferation Assay. To investigate their responsiveness to various cytokines, XG cells were washed once with culture medium, incubated for 5 h at 37°C in culture medium alone, and washed again twice. Cells were then cultured at various concentrations in 96-well flat-bottomed microplates for 2 or 5 d with either culture medium alone or graded concentrations of various cytokines. BE-8 anti-IL-6, BR-6 anti-IL-6R, and GPX7 and GPZ35 anti-gp 130 mAbs (22) were added at the beginning of the cultures (final concentration 10 μg/ml). A mouse purified IgG1 (BL-5) was used as control antibody. 0.5 μCi of tritiated thymidine (25 Ci/mM; CEA, Saclay, France) was added for the last 8 h of culture, and tritiated thymidine incorporation was determined as reported elsewhere (23).

Phenotypic Analysis. The phenotype of the XG lines was studied using mAbs to various human antigens and an anti–mouse Ig antibody coupled with fluorescein isothiocyanate (Immunotech, Marseilles, France). Fluorescence analysis was performed with a flow cytometer (ATC 3000/RCA 3000; Bruker, Wissembourg, France). Antibodies to CD19, CD21, CD23, CD28, CD32, CD38, CD40, and CD56 antigens were purchased from Immunotech. The characteristics of MT18 anti-IL-6R and GPX7 and GPZ35 anti-gp130 were previously reported (22, 24). Cytoplasmic immunoglobulin heavy and light chains were detected by specific antibodies coupled with fluorescein (Kallestadt, Austin, TX).

Detection of LIFRβ Expression by PCR. Aliquots of XG cells (50 × 10⁶ cells), cultured with 1 ng/ml of rIL-6, were harvested during the exponential growth phase and frozen in nitrogen for RNA studies. cDNA was synthesized from 1 μg total RNA from each cell line. 35 cycles of PCR (45 s at 94°C, 45 s at 65°C, 90 s at 72°C) followed by 3-min extension at 72°C were carried out using primers corresponding to nucleotides 155–175 and 1501–1525 of LIFRβ sequence (17). This gives a 1.37-kb product. 15 μl of each 25-μl PCR reaction were electrophoresed on 1.5% agarose gel in TAE buffer and transferred onto Hybond filters (Amersham, Les Ulis, France). Filters were hybridized with a LIFRβ cDNA probe labeled with [³²P]dCTP by a random priming method (Boehringer Mannheim, Meylan, France) and were exposed to Kodak XAR5 films at −70°C for 2 h.

Immunoglobulin Production. Immunoglobulin production was measured in culture supernatants of XG cells using an ELISA and anti–human heavy and light immunoglobulin chains purchased from Immunotech. Supernatants were prepared by culturing 5 × 10⁵ cells/ml for 2 d with the various cytokines.

Reagents. Purified rIL-6 was provided by D. Stinchcomb (Synergen, Boulder, CO), rOM by M. Shoyab (Bristol Myers Squibb, Seattle, WA), rCNTF and and rCNTFRα by Dr. G. D. Yancopoulos (Regeneron, Tarrytown, NY), rLIF by A. Godard (INSERM U211, Nantes, France), and neutralizing BR-6 anti-IL-6R and B-E8 anti-IL-6 mAb by Dr. J. Wijdenes (Innotherapie, Besançon, France).
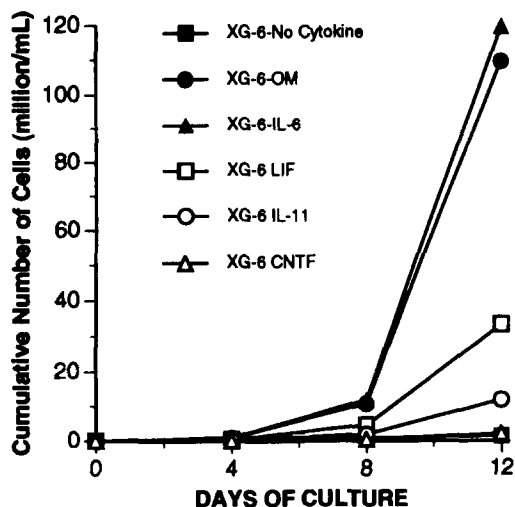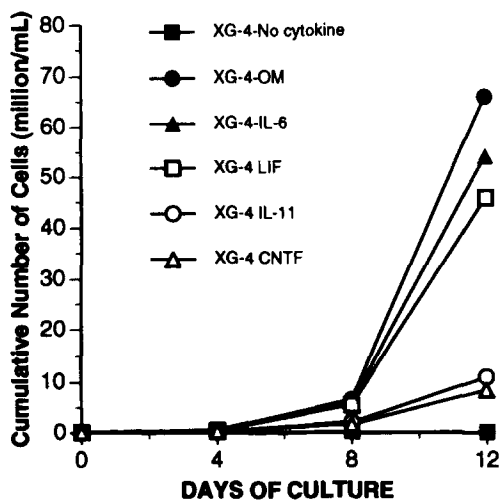
Results

CNTF, IL-11, LIF, and OM Are Growth Factors for Human Myeloma Cells. Results are shown in Figs. 1 and 2. XG-1, XG-2, XG-4, and XG-6 HMCL could not proliferate and grow without addition of an exogenous cytokine, and high proliferation was induced by rIL-6. The four cytokines (rCNTF, rIL-11, rLIF, and rOM) also induced significant proliferation of XG-4 and XG-6 myeloma cells.

Proliferation of XG-1 and XG-2 cells was supported by rIL-6 alone. Half-maximal proliferation of XG-4 and XG-6 myeloma cells was induced by 100 and 70 pg/ml of rIL-6, 800, and 1200 pg/ml of rIL-11, 324 and 470 pg/ml of rLIF, and 440 and 410 pg/ml of rOM, respectively. Only XG-4 cells were significantly stimulated by large concentrations of rCNTF (half-maximal proliferation induced by 90 ng/ml of rCNTF). XG-6 cells were only marginally stimulated, and XG-1 and XG-2 cells were not stimulated by rCNTF (Fig. 1).

rIL-11, rLIF, or rOM supported the long-term growth of XG-4 and XG-6 (>6 mo), unlike XG-1 and XG-2 HMCL (Fig. 2), making it possible to obtain XG-4 or XG-6 subclones whose growth was strictly dependent on addition of the initial cytokine. XG-4, but not XG-6 HMCL, could be grown with rCNTF alone (Fig. 2).
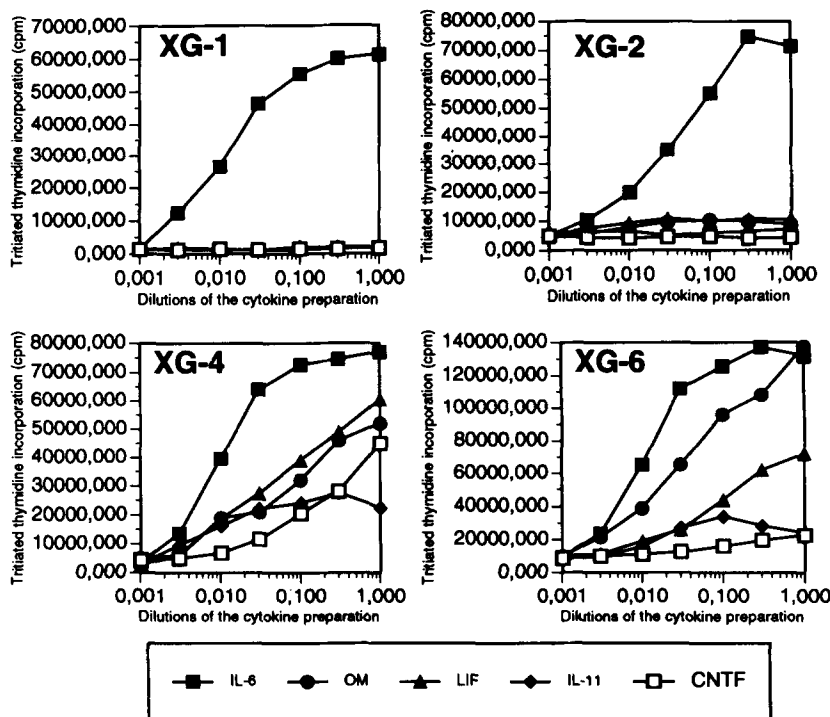
Figure 1. Proliferative response of human myeloma cell lines to IL-6, CNTF, IL-11, LIF, and OM. XG-cells were cultured at a concentration of 10^5 cells/ml for 5 d with graded dilutions of a cytokine preparation containing either 1 ng/ml of rIL-6, 810 ng/ml of rCNTF, 100 ng/ml of rIL-11, 10 ng/ml of rLIF, or 10 ng/ml of rOM. Proliferation was assayed using tritiated thymidine incorporation, and results are mean values determined on sextuplate culture wells.

No changes in the morphology, phenotype or immunoglobulin secretion of XG-4 and XG-6 myeloma cells were found when cultured for more than 4 wk with IL-11, LIF, or OM instead of IL-6 (results not shown).

Myeloma-Cell Growth Factor Activity of CNTF, IL-11, LIF, and OM Was Mediated Through IL-6 Signaling gp130 Transducer. Proliferation of XG-4 and XG-6 cells induced by rIL-6, rCNTF, rIL-11, rLIF, or rOM was completely inhibited by anti-gp130 mAb (Fig. 3). Anti-IL-6R or anti-IL-6 mAb completely inhibited rIL-6-induced proliferation and did not affect growth induced by rCNTF, rIL-11, rLIF, or rOM (Fig. 3).

Soluble CNTFRα Potentiated the CNTF Response of HMCL. As noted above, rCNTF induced a proliferative response in XG-4 cells at high concentration only (half-maximal proliferation obtained with 90 ng/ml). Addition of soluble CNTFRα increased the sensitivity of XG-4 cells 30-fold, half-maximal proliferation being obtained with 3 ng/ml of rCNTF (Fig. 4). Again, the potentiating effect of sCNTFRα was completely abrogated by anti-gp130 mAb (Fig. 4).

Expression of LIFRβ Gene by XG Cells. The four HMCL expressed membrane IL-6R and the transducer gp130, which could be detected by a specific mAb and FACS® analysis (Becton Dickinson & Co., Mountain View, CA). LIFRβ

Figure 2. Long-term growth of human myeloma cell lines stimulated by IL-6, CNTF, IL-11, LIF, or OM. XG-4 or XG-6 cells were cultured at a cell concentration of 10^5 cells/ml with either no exogenous cytokine, or 1 ng/ml of rIL-6, 300 ng/ml of rCNTF, 10 ng/ml of rIL-11, 10 ng/ml of rLIF, or 10 ng/ml of rOM. For each culture group, XG cells were diluted every 4 d at a cell concentration of 10^5 cells/ml in fresh culture medium containing the initial cytokine concentration. Results are the cumulative numbers of cells generated in each culture group.
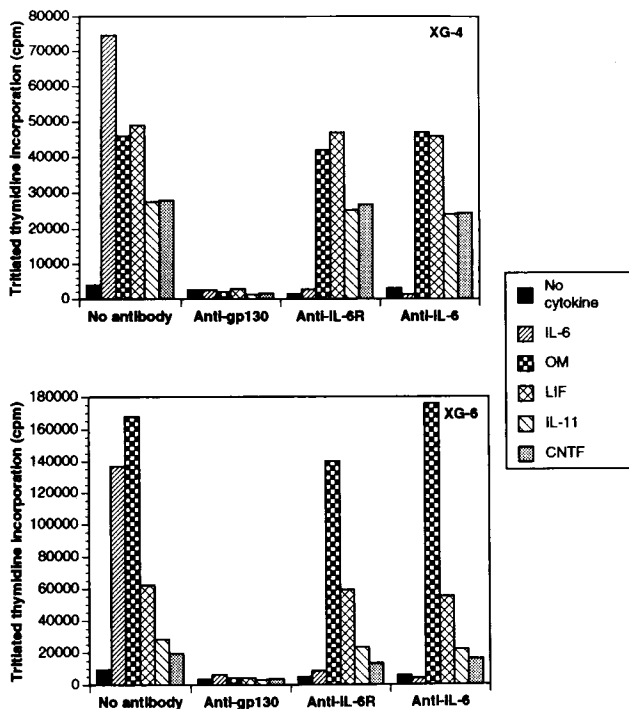
Figure 3. Growth factor activity of CNTF, IL-11, LIF, and OM is abrogated by anti-gp130 antibodies. XG-4 and XG-6 cells were cultured at a concentration of 10^5 cells/ml for 5 d with either no cytokine, 300 pg/ml of rIL-6, 300 ng/ml of rCNTF, 10 ng/ml of rIL-11, 10 ng/ml of rLIF, or 10 ng/ml of rOM. For each cytokine, culture groups containing either a control antibody, 10 μg/ml of GPX7 and GPZ35 anti-gp130 mAb, 10 μg/ml of BR-6 anti-IL-6R mAb, or 10 μg/ml of B-E8 anti-IL-6 mAb were prepared. Proliferation was assayed by tritiated thymidine incorporation. Results are mean values determined on sextuplate culture wells.

mRNA was detected by PCR amplification in XG-4 and XG-6 cells but not in XG-1 and XG-2 cells (Fig. 5).

Discussion

We and others have shown that IL-6 is a major growth factor for the proliferation of tumor plasma cells in human
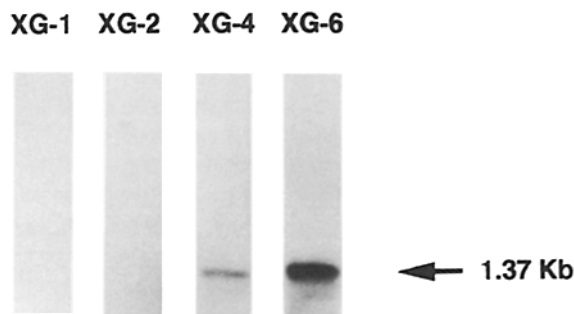

Figure 5. Detection of LIFRβ gene expression in XG cells by PCR. cDNA was synthesized from 1 μg total RNA from each XG cell line. 35 cycles of PCR (45 s at 94°C, 45 s at 60°C, and 90 s at 72°C) were carried out using primers corresponding to nucleotides 155–175 and 1501–1525 of LIFRβ sequence. The 1.37-kb product of PCR was electrophoresed in 1.5% agarose, transferred onto filters, and hybridized with a LIFRβ cDNA probe labeled with ^{32}P.

multiple myeloma (1, 2). In particular, for every patient with terminal disease, IL-6 enables human myeloma cell lines to be obtained whose proliferation is completely dependent on addition of exogenous IL-6 (4 and Zhang, X.-G., unpublished results).

In this work, we showed that IL-11, OM, and LIF were growth factors similar to IL-6 for two of the four HMCL studied. These cytokines allowed us to obtain subclones whose growth is completely dependent on addition of IL-11, LIF, or OM. The four HMCL expressed membrane IL-6R and the transducer gp130 which could be detected by a specific mAb and FACS® analysis. The sensitivity to LIF or OM was found to correlate with the presence of LIFRβ mRNA which was detected in XG-4 and XG-6 but not in XG-1 and XG-2 cells. It is likely that these two HMCL also express an IL-11-binding protein not presently identified.

XG-4 and XG-6 cells were stimulated only at high concentrations of CNTF (half-maximal proliferation induced by 90 ng/ml). This biological effect can be explained by a possible low affinity interaction of CNTF with the heterodimer
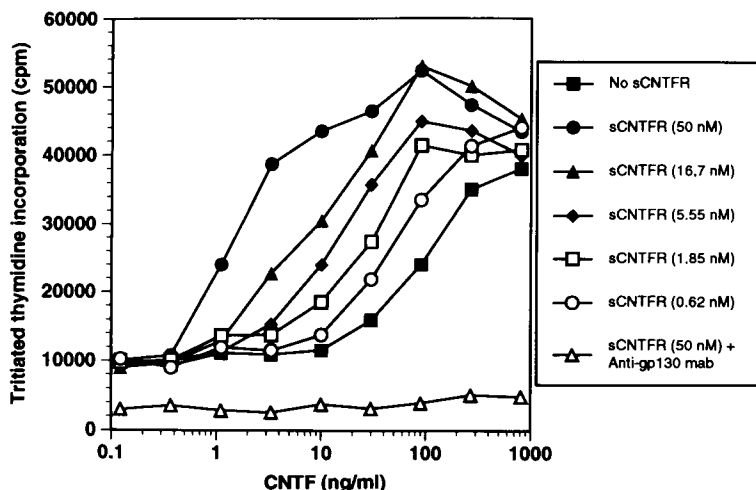
Figure 4. Potentialization of CNTF response by soluble CNTF receptor. 10^5 XG-4 cells/ml were cultured for 5 d with various CNTF concentrations and various concentrations of sCNTFRα (0–50 nM). In one culture group, 10 μg/ml of the GPX7 and GPZ35 anti-gp130 mAb were added. Proliferation was assayed using tritiated thymidine incorporation. Results are mean values determined on sextuplate culture wells.

LIFRβ and gp130 in the absence of CNTFRα, as previously reported (20). This was confirmed by the 30-fold increase in the sensitivity of myeloma cells to CNTF in the presence of soluble CNTFRα. Soluble IL-6R can also potentiate the response of cells to IL-6 (14, 21). Thus, soluble forms of these receptors bound to their ligand actually behave like cytokines that are able to activate their transducer complex. This concept is supported by the fact that IL-12 is a heterodimer composed of one chain homologous to IL-6 and another chain homologous to IL-6R (25). Our previous finding that the presence of high concentration of soluble IL-6R in patients with MM is associated with poor prognosis might in part be explained by this concept (26). Soluble forms of LIFRβ and gp130 have also been found in the serum of mice or humans. These soluble forms have an antagonist activity (27, 28).

Thus, we showed that mechanisms previously demonstrated with transfected cells are operational in freshly obtained tumor cell lines (9–21). In a recent study, Nishimoto et al. (29) demonstrated that LIF and OM are also growth factors for freshly explanted myeloma cells. In the case of multiple my-eloma, we previously showed that IL-6 overproduction in vivo was associated with poor prognosis (30) and that treatment with anti-IL-6 antibodies could result in complete blockage of tumor proliferation in vivo (3). It is now essential to determine whether CNTF, IL-11, LIF, OM, soluble IL-6R, or soluble CNTFRα is produced in tumor proliferation sites in patients with MM. In particular, it is important to find out whether resistance to therapy in patients treated with anti-IL-6 antibodies is associated with production of one of these cytokines in vivo (3).

The fact that the 5 cytokines using the same gp130 transducer are myeloma cell growth factors suggests that the concept of multiple myeloma as an IL-6–related disease should be extended to that of a gp130-dependent disease. Kaposi sarcoma, another IL-6-related disease, is also related to OM (31). Thus, agents able to block the activation of gp130 without killing cells expressing it (i.e., almost every cell in the body) should be extremely useful for treatment of the numerous diseases initially considered to be IL-6 related.

References

1. Kawano, M., T. Hirano, T. Matsuda, T. Taga, Y. Horii, K. Iwato, H. Asaoku, B. Tang, O. Tanabe, H. Tanaka, et al. 1988. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. *Nature (Lond.)*. 332:83.

2. Klein, B., and R. Bataille. 1992. Cytokine network in human multiple myeloma. *Hematol. Oncol. Clin. N. Am.* 6:273.

3. Klein, B., J. Wijdenes, X.-G. Zhang, M. Jourdan, J.-M. Boiron, J. Brochier, J. Liautard, M. Merlin, C. Clement, B. Morel-Fournier, et al. 1991. Murine anti-interleukin-6 monoclonal antibody therapy for a patient with plasma cell leukemia. *Blood.* 78:1198.

4. Klein, B., X.-G. Zhang, M. Jourdan, J.-M. Boiron, M. Portier, Z.-Y. Lu, J. Wijdenes, J. Brochier, and R. Bataille. 1990. Interleukin-6 is the central tumor growth factor in vitro and in vivo in multiple myeloma. *Eur. Cytokine Net.* 1:193.

5. Kishimoto, T., S. Akira, and T. Taga. 1992. Interleukin-6 and its receptor - a paradigm for cytokines. *Science (Wash. DC)*. 258:5082.

6. Hirano, T., S. Akira, T. Taga, and T. Kishimoto. 1990. Biological and clinical aspects of interleukin 6. *Immunol. Today.* 11:443.

7. Hilton, D.J. 1992. LIF: lots of interesting functions. *TIBS (Trends Biochem. Sci.).* 5:72.

8. Yan, Y.C., and T.G. Yin. 1992. Interleukin-11 and its receptor. *Biofactors.* 4:1.

9. Gearing, D.P., M.R. Comeau, D.J. Friend, S.D. Gimpel, C.J. Thut, J. McGourthy, K.K. Brasher, J.A. King, S. Gillis, B. Mosley, et al. 1992. The IL-6 signal transducer, gp130: An oncostatin M receptor and affinity converter for the LIF receptor. *Science (Wash. DC)*. 255:1434.

10. Ip, N.Y., S.H. Nye, T.G. Boulton, S. Davis, T. Taga, Y. Li, S.J. Birren, K. Yasukawa, T. Kishimoto, D.J. Anderson, et al. 1992. CNTF and LIF act on neuronal cells via shared signaling pathways that involve the IL-6 signal transducing receptor component gp130. *Cell.* 69:1121.

11. Taga, T., M. Narazaki, K. Yasukawa, T. Saito, D. Miki, M. Hamaguchi, S. Davis, M. Shoyab, G.D. Yancopoulos, and T. Kishimoto. 1992. Functional inhibition of hematopoietic and neurotrophic cytokines by blocking the interleukin 6 signal transducer gp130. *Proc. Natl. Acad. Sci. USA.* 89:10998.

12. Yin, T., T. Taga, M.L. Tsang, K. Yasukawa, T. Kishimoto, and Y.C. Yang. 1993. Involvement of IL-6 signal transducer gp130 in IL-11-mediated signal transduction. *J. Immunol.* 151: 2555.

13. Liu, J., B. Modrell, A. Aruffo, J.S. Marken, T. Taga, K. Yasukawa, M. Murakami, T. Kishimoto, and M. Shoyab. 1992. Interleukin-6 signal transducer gp130 mediates oncostatin M signaling. *J. Biol. Chem.* 267:16763.

14. Taga, T., M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano, and T. Kishimoto. 1989. Interleukin-6

triggers the association of its receptor with a possible signal transducer, gp130. *Cell.* 58:573.

15. Yamasaki, K., T. Taga, Y. Hirata, H. Yawata, Y. Kawanishi, B. Seed, T. Taniguchi, T. Hirano, and T. Kishimoto. 1988. Cloning and expression of the human interleukin-6 (BSF-2/IFNβ2) receptor. *Science (Wash. DC).* 242:825.

16. Murakami, M., M. Hibi, N. Nakagawa, T. Nakagawa, K. Yasukawa, K. Yamanishi, T. Taga, and T. Kishimoto. 1993. IL-6-induced homodimerization of gp130 and associated activation of a tyrosine kinase. *Science (Wash. DC).* 260:1805.

17. Gearing, D.P., C.J. Thut, T. Vandenbos, S.D. Gimpel, P.B. Delaney, J. King, V. Price, D. Cosman, and M.P. Beckman. 1991. Leukemia inhibitory factor receptor is structurally related to the IL-6 signal transducer, gp130. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2839.

18. Davis, S., T.H. Aldrich, S.M. Valenzuela, V. Wong, M.E. Furth, S.P. Squinto, and G.D. Yancopoulos. 1991. The receptor for ciliary neurotrophic factor. *Science (Wash. DC).* 253:59.

19. Davis, S., T.H. Aldrich, N. Stahl, L. Pan, T. Taga, T. Kishimoto, N.Y. Ip, and G.D. Yancopoulos. 1993. LIFRβ and gp130 as heterodimerizing signal transducers of the tripartite CNTF receptor. *Science (Wash. DC).* 260:1805.

20. Davis, S., T.H. Aldrich, N.Y. Ip, N. Stahl, S. Schere, T. Farruggella, P.S. DiStefano, R. Curtis, N. Panayotatos, H. Gascan, et al. 1993. Released form of CNTF receptor α. Component as a soluble mediator of CNTF responses. *Science (Wash. DC).* 259:1736.

21. Yasukawa, K., K. Futatsugi, T. Saito, H. Yawata, M. Narazaki, H. Susuki, T. Taga, and T. Kishimoto. 1992. Association of recombinant soluble IL-6-signal transducer, gp130, with a complex of IL-6 and soluble IL-6 receptor, and establishment of an ELISA for soluble gp130. *Immunol. Lett.* 31:123.

22. Saito, T., T. Taga, D. Miki, K. Futatsugi, H. Yawata, H.T. Kishimoto, and K. Yasukawa. 1993. Preparation of monoclonal antibodies against the IL-6 signal transducer, gp130, that can inhibit IL-6-mediated functions. *J. Immunol. Methods.* 163:217.

23. Jourdan, M., X.G. Zhang, M. Portier, J.M. Boiron, R. Bataille, and B. Klein. 1991. IFN-α induces autocrine production of

IL-6 in myeloma cell lines. *J. Immunol.* 147:4402.

24. Hirata, Y., T. Taga, M. Hibi, N. Nakano, T. Hirano, and T. Kishimoto. 1989. Characterization of IL-6 receptor expression by monoclonal and polyclonal antibodies. *J. Immunol.* 143:2900.

25. Gearing, D.P., and D. Cosman. 1990. Homology of the p40 subunit of natural killer cell stimulatory factor (NKSF) with the extracellular domain of the interleukin-6 receptor. *Cell.* 66:9.

26. Gaillard, J.P., R. Bataille, H. Brailly, C. Zubert, K. Yasukawa, M. Attal, N. Maruo, T. Taga, T. Kishimoto, and B. Klein. 1993. Increased and highly stable levels of functional soluble interleukin-6 receptor in sera of patients with monoclonal gammopathy. *Eur. J. Immunol.* 23:820.

27. Layton, M.J., B.A. Cross, D. Metcalf, L.D. Ward, R.J. Simpson, and N.A. Nicola. 1992. A major binding protein for leukemia inhibitory factor in normal mouse serum: identification as a soluble form of the cellular receptor. *Proc. Natl. Acad. Sci. USA.* 89:8616.

28. Narazaki, M., K. Yasukawa, T. Saito, J. Ohsugi, H. Fukui, Y. Koishihara, G.D. Yancopoulos, T. Taga, and T. Kishimoto. 1993. Soluble forms of the interleukin-6 signal transducing receptor component gp130 in human serum possessing a potential to inhibit signals through membrane anchored gp130. *Blood.* 82:1120.

29. Nishimoto, N., A. Ogata, Y. Shima, K. Yoshizaki, and T. Kishimoto. 1993. Anti-gp130 antibody inhibited the oncostatin M, leukemia inhibitory factor and interleukin-6 dependent growth of human plasmacytoma cells. 5th International Conference on Human Leukocyte Differentiation Antigens. *Tissue Antigens.* 42:334.

30. Bataille, R., M. Jourdan, X.G. Zhang, and B. Klein. 1989. Serum level of interleukin 6, a potent myeloma cell growth factor, as a reflection of disease severity in plasma cell dyscrasias. *J. Clin. Invest.* 84:2008.

31. Miles, S.A., O. Martinez-Maza, A. Rezai, L. Magpantay, T. Kishimoto, S. Nakamura, S.F. Radka, and P.S. Linsley. 1992. Oncostatin M as a potent to suppress mitogen for AIDS-kaposi's sarcoma-derived cells. *Science (Wash. DC).* 255:1432.