Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 7864–7868, August 1993
Cell Biology

The SCL gene product is regulated by and differentially regulates cytokine responses during myeloid leukemic cell differentiation

(helix–loop–helix protein/M1 leukemia/oncostatin M/leukemia inhibitory factor/interleukin 6)

TAKAHIKO TANIGAWA*, NGAIRE ELWOOD*, DONALD METCALF*, DALE CARY*, ELISABETTA DELUCA*, NICOS A. NICOLA*, AND C. GLENN BEGLEY*†‡

*The Walter and Eliza Hall Institute of Medical Research and †Department of Diagnostic Haematology, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia

Contributed by Donald Metcalf, May 14, 1993

ABSTRACT **Differentiation induction in murine M1 leukemia cells by interleukin 6 (IL-6), leukemia inhibitory factor (LIF), and oncostatin M (OSM) is postulated to occur via a common receptor chain, gp130. In this study, growth factor-induced differentiation of M1 cells was accompanied by a late and persistent decrease in levels of mRNA and protein for a helix–loop–helix transcription factor, the SCL gene product. To evaluate whether reduced SCL expression was instrumental in monocyte differentiation, an SCL cDNA expression vector was introduced into M1 cells to obtain cell lines in which overexpression of SCL mRNA and protein was enforced. This resulted in a reduction in cells differentiating in response to LIF and OSM but not in response to IL-6. Scatchard analysis indicated that both parental and SCL-transfected cell lines exhibited similar receptor numbers and receptor affinities for LIF, OSM, and IL-6, suggesting that the differential responsiveness was not due to selective receptor down-modulation. Thus, these data implicate SCL in monocytic differentiation and provide evidence for differential receptor signaling pathways despite utilization of a common gp130 subunit by all three receptors.**

Proteins of the helix–loop–helix (HLH) family contain a common motif found in a variety of transcription factors with roles in the regulation of tissue-specific gene expression and developmental processes such as neurogenesis, myogenesis, germ-layer formation, and sex determination (1–3). The product of the SCL (*s*tem-cell *l*eukemia) gene is a member of this family (4, 5) and is implicated in proliferation and differentiation events in hemopoietic cells (6, 7). SCL (also known as Tal-1) (8, 9) is expressed in erythroid cells, mast cells, megakaryocytes, and aberrantly in certain T-cell lines (4, 8, 10–12). In addition, early myeloid cell lines such as M1, NFS60, FDCP1, and 32D also express SCL mRNA (12) and protein (N.E., C.G.B., and N.A.N., unpublished data), although SCL is not detected in populations of mature myeloid cells (11, 13). Recently, M1 murine leukemia cells (14) have been shown to differentiate in response to the growth factors interleukin 6 (IL-6), leukemia inhibitory factor (LIF), and oncostatin M (OSM) (15–17). These cells display specific binding for all three growth factors (18–20) and at least two receptor components are required for binding and growth factor signaling (18, 21–23). Despite differences in these receptor complexes, receptor signaling for IL-6, LIF, and OSM is postulated to occur via a common receptor chain (gp130) (24–27).

In the work reported here, we studied the behavior of SCL mRNA and protein during growth factor-induced differentiation of M1 leukemia cells. We then established M1/SCL cell lines in which overexpression of SCL mRNA and protein was enforced. Using these M1/SCL cell lines, we examined the effects of SCL overexpression on terminal differentiation induced by LIF, IL-6, and OSM.

MATERIALS AND METHODS

Enforced Expression of SCL in M1 Cells. An SCL retrovirus was constructed by introducing the entire murine SCL coding region (28) into the MPZen/Neo retrovirus (29) so that SCL expression was under control of the long terminal repeat promoter of myeloproliferative sarcoma virus. M1 cells were then infected by cocultivation with an SCL-retroviral "packaging" cell line (30). After 3 days of cocultivation, cells were cloned in agar by selection with the neomycin analogue G418 (400 μg/ml). On day 7, individual G418-resistant colonies were resuspended to establish four M1/SCL clonal cell lines. M1/SCL clonal cell lines were confirmed to express the retroviral (exogenous) SCL mRNA (see below). Three control cell lines were obtained by using the vector alone; clonal cell lines derived by G418 selection in agar (M1/neo cell lines) were examined in addition to parental M1 cells. All cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum. At various time points cells were prepared for morphological examination (using Cytospin preparations stained with May–Grunwald and Giemsa reagents and examining a minimum of 200 cells) and Northern analysis, with medium being changed every 3 days during the culture period.

Agar Cultures. Cells (300 per ml) were cultured with serial dilutions of purified recombinant mouse LIF, IL-6, or OSM in 35-mm Petri dishes using DMEM with a final concentration of 20% preselected bovine calf serum and 0.3% agar in a final volume of 1 ml. Cultures were incubated at 37°C in a fully humidified atmosphere of 10% CO_2 in air. Colonies (clones of >40 cells) were scored at ×35 magnifications with a dissection microscope after 7 days of incubation. Differentiated colonies were identified by their characteristic dispersed morphology (31).

Recloning experiments were performed by selecting consecutive colonies that had been cultured for 7 days in maximal concentrations of LIF. These colonies were resuspended and grown in 1-ml agar cultures containing LIF for a further 7 days.

Binding Studies on Intact Cells. Equilibrium binding studies at 0°C were performed for 4 hr and the data were analyzed by the method of Scatchard after correction for the bindable fraction and the specific radioactivity. LIF, IL-6, and OSM radiolabeling was performed as described (19, 32, 33).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LIF, leukemia inhibitory factor; IL-6, interleukin 6; OSM, oncostatin M; HLH, helix–loop–helix.

‡To whom reprint requests should be addressed at: The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia.

Northern Blotting. Poly(A)$^+$ RNA was isolated from cells as described (34). RNA samples were size-fractionated by electrophoresis in 0.8% agarose gels with 1× Mops buffer (20 mM Mops/1 mM EDTA/5 mM sodium acetate, pH 7.0) containing 0.22 M formaldehyde. RNA was transferred to nitrocellulose (Hybond C-extra, Amersham), baked at 80°C for 2 hr, and prehybridized at 42°C for >1 hr in 50% (vol/vol) formamide/4× Denhardt's solution (0.08% Ficoll/0.08% bovine serum albumin/0.08% polyvinylpyrrolidone)/5 mM EDTA/5× SSC (0.75 M NaCl/0.075 M sodium citrate, pH 7.0) containing denatured salmon sperm DNA at 100 μg/ml. ^{32}P-labeled probes were derived by random priming (Bresatec, Adelaide, Australia) and hybridization was performed overnight at 42°C by adding 1–5 × 10^6 cpm/ml to hybridization buffer. Filters were washed at 65°C in 0.2× SSC/0.1% SDS and exposed to film.

Western Immunoblots. A peptide corresponding to the carboxyl region of the predicted SCL protein was synthesized, conjugated to keyhole limpet hemacyanin, and used to immunize rabbits. Antiserum was titered by enzyme-linked immunosorbant assay using diluted peptide as primary antigen. To ensure that the antiserum detected the SCL protein Western blot analysis was initially established by using an SCL fusion protein expressed in *Escherichia coli* (N.E., unpublished work). To examine SCL protein, cells were suspended in 2% SDS/100 mM dithiothreitol/60 mM Tris, pH 6.8 and quick-frozen at −70°C prior to Western blot analysis. After SDS/polyacrylamide gel electrophoresis of cell extracts, proteins were transferred to nitrocellulose. The nitrocellulose filter was probed with antiserum and bound antibodies were detected by enhanced chemiluminescence (Amersham). Protein specificity was confirmed by peptide competition.

RESULTS

SCL Expression Following Induction of Differentiation in M1 Cells. The parental M1 cell line was cultured at 2 × 10^5 cells per ml in medium with LIF (4 ng/ml), IL-6 (25 ng/ml), or OSM (150 ng/ml) and subjected to morphological examination as well as Northern and Western analysis. Similar changes were observed in these parameters with all three growth factors.

There was no morphological evidence of differentiation after 1 day with growth factor and cells retained their immature, blast-like phenotype. However, by 2 days, 3 ± 2% (mean ± SD of five experiments) of the cells showed some evidence of monocytic differentiation with increased cytoplasm, vacuolation, and irregular nuclei. By day 3, 16 ± 4%, and by day 5, 91 ± 3%, of the cells (mean ± SD of four experiments) displayed a mature monocyte/macrophage phenotype regardless of the growth factor under study, and 100% of cells were differentiated by 7 days. During this time the total cell number increased ≈3-fold in LIF-, IL-6-, and OSM-stimulated cultures.

Fig. 1 shows Northern analysis of LIF-, IL-6-, and OSM-induced differentiation in the parental M1 cell line. The two SCL mRNA bands correspond to two different splicing products reported previously (28). By days 3–4 the level of SCL mRNA was markedly decreased in LIF-, IL-6-, or OSM-treated M1 cells and remained undetectable thereafter. Consistent with the morphological data, levels of mRNA for lysozyme, a known marker of monocyte/macrophage differentiation, increased by 3–4 days and continued to increase until day 7.

Fig. 2 shows Western analysis of LIF-induced monocytic differentiation of the parental M1 cell line. SCL protein of ≈49 kDa was evident in the untreated cells and specificity was confirmed by peptide competition. SCL protein decreased markedly at 4 days, although there was no change at earlier times, and was undetectable by 7 and 8 days. Similar

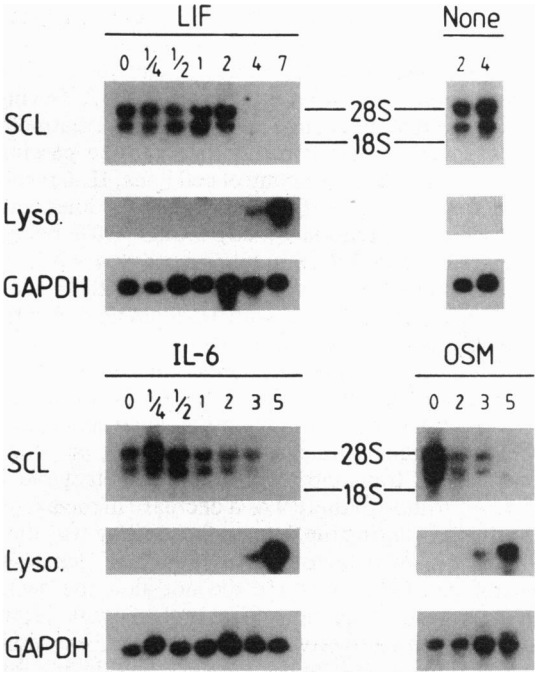

FIG. 1. Northern analysis of time course of LIF-, IL-6-, and OSM-induced myeloid differentiation of M1 parental cell line. Poly(A)$^+$ RNA was extracted from aliquots of cells at the times indicated. Approximately 3 μg was electrophoresed in each lane. Numbers above the lanes represent time (days). Filters were hybridized sequentially with probes specific for murine SCL, a 1.8-kb *Eco*RI cDNA fragment (28); murine lysozyme (Lyso.), a 780-bp *Eco*RI cDNA fragment (35); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a 1.2-kb *Pst* I cDNA fragment (36). Positions of 28S and 18S rRNAs are shown.

results were obtained with IL-6 and OSM; again SCL protein decreased at 3–4 days and was undetectable after 7 days (data not shown). These results indicated that the differentiation induced by LIF, IL-6, or OSM was accompanied by a marked decrease in SCL mRNA and protein, together with a rise in lysozyme mRNA and morphological changes of terminal differentiation.

Analysis of M1/SCL Cell Lines. M1 cells infected with the SCL retrovirus (M1/SCL cell lines) were cultured under the same conditions as the parental M1 cell line and subjected to morphological analysis. In contrast to the parental cells and the three M1/neo control cell lines, all four M1/SCL cell lines treated with LIF and OSM showed reduced monocytic

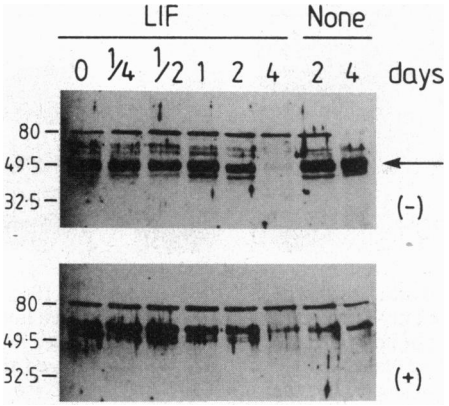

FIG. 2. Western analysis of time course of LIF-induced myeloid differentiation of M1 parental cell line. Time is indicated (days). Blots were analyzed with peptide competition (+) or without (−). Arrow indicates specific SCL protein band.

differentiation. By day 3 only 3 ± 1% (mean ± SD of three experiments) of the cells showed evidence of differentiation; the proportion was 42 ± 9% by day 5, and 76 ± 12% of the cells were terminally differentiated by day 7. During this period, total cell numbers increased ≈6-fold. Treatment with IL-6 gave a very different result. As with the parental M1 cells and the three M1/neo control cell lines, IL-6 resulted in ≈15% of cells from the four M1/SCL cell lines showing evidence of differentiation by day 3 and ≈90% being fully differentiated at day 5. Cell number increased ≈3-fold. Cultures containing mixtures of LIF or OSM with IL-6 did not alter the response observed with IL-6 alone, indicating no block to IL-6 action (data not shown).

A similar discrepancy among the M1/SCL cell lines in LIF, OSM, and IL-6 was observed with mRNA expression. Fig. 3 shows a typical Northern time-course analysis of one M1/SCL cell line after treatment with LIF, IL-6, or OSM. The exogenous SCL transcripts were readily detectable for at least 7 days. Although there was a decrease in the exogenous SCL transcripts during the 7 days, this decline was the same for all three growth factors as assessed by densitometric analysis of Northern blots and did not alter the decline in endogenous SCL transcripts (data not shown). However, regardless of growth factor, the level of SCL expression at day 7 remained significantly greater than in parental or control M1 cells (Fig. 1). In LIF- and OSM-treated cells, lysozyme mRNA was not detected until day 5, after which the level of this mRNA was markedly increased. In contrast, in IL-6-treated cells, lysozyme mRNA was clearly evident by day 3, the same time point as for parental M1 cells. Thus, consistent with the results of morphological examination, it appeared that enforced SCL overexpression reduced the terminal differentiation of M1 cells induced by LIF and OSM but not that induced by IL-6.

Influence of SCL Overexpression on Clonogenic Behavior of M1 Cells. Agar cultures were used to analyze the responsiveness of clonogenic M1/SCL cells to LIF, IL-6, and OSM (Fig. 4). In maximal concentrations of LIF, IL-6, and OSM, almost all colonies derived from control cell lines (three M1/neo cell lines and parental M1) displayed a differentiated phenotype. However, all four M1/SCL cell lines treated with similar concentrations of LIF or OSM showed only 20–40% differentiated colonies by day 7. Further, those colonies that did respond exhibited a reduction in quantitative responsiveness to LIF and OSM (Fig. 4). In contrast, M1/SCL cell lines exhibited essentially 100% differentiated colonies in response to IL-6 with a dose–response curve that was identical to that of control cell lines. Similar results were obtained for all

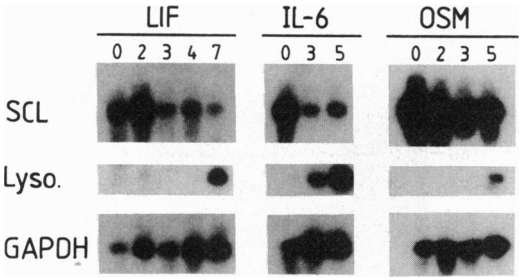

FIG. 3. Northern analysis of time course of LIF-, IL-6-, and OSM-induced myeloid differentiation of M1/SCL cell line. Approximately 3 μg of poly(A)$^+$ mRNA was electrophoresed in each lane. Numbers represent time (days). Filters were hybridized sequentially with the following probes: murine SCL; lysozyme (Lyso.); GAPDH (see Fig. 1 for description). The exposure shown for SCL in the OSM panel was deliberately selected to allow comparison with SCL mRNA levels in Fig. 1. The filter was hybridized and exposed under identical conditions (24 hr) and on the same day as the blots shown in Fig. 1. Shorter exposures (5 hr) are shown for LIF and IL-6.

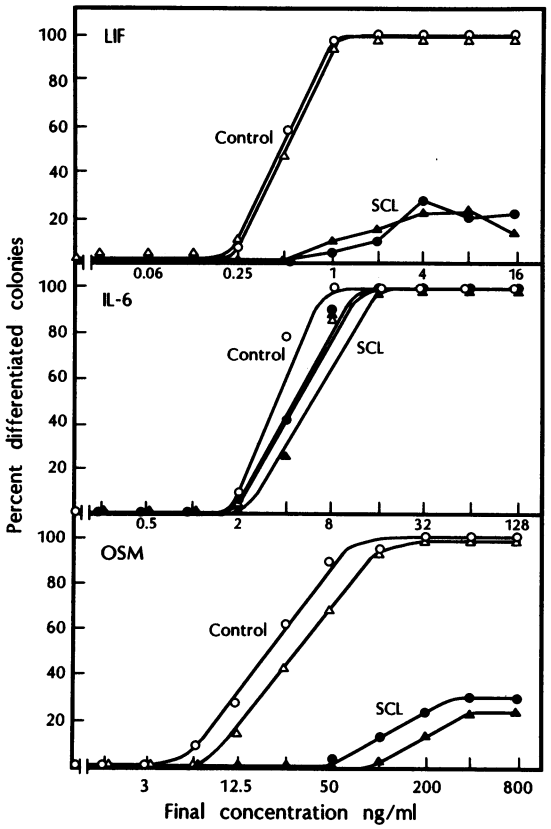

FIG. 4. Responsiveness of control and M1/SCL cell lines to LIF, IL-6, and OSM. Results for M1 parental cells (○), an M1/neo clonal cell line (△), and two M1/SCL clonal cell lines (clone S5, ●; clone S8, ▲) are shown. Similar results were obtained with all clonal cell lines. Note altered dose–response of M1/SCL clonal cell lines to LIF and OSM but not IL-6.

SCL-transfected and control cell lines. Thus the transfected SCL gene reduced LIF and OSM responsiveness while leaving IL-6 responsiveness unaltered.

In recloning experiments, colonies derived from control (M1/neo and parental M1) cell lines contained few or no clonogenic cells after 7 days in culture with LIF (0% clonogenic cells per colony for M1/neo cell line; ≈15% clonogenic cells per colony for parental M1 cells). In contrast, colonies derived from M1/SCL cell lines showed greater numbers of clonogenic cells after 1 week in LIF (≈70% clonogenic cells per colony for one M1/SCL cell line; 50% for another). Further, while the few secondary colonies from control cell lines were 100% differentiated, only 30% of secondary colonies from M1/SCL cell lines were differentiated (range, 0–60%). Thus although LIF was able to completely extinguish the M1 control cell lines in 1–2 weeks, this was not the case for the M1/SCL transfectants.

Enforced SCL Overexpression Does Not Alter LIF, OSM, or IL-6 Binding. Fig. 5 shows saturation binding isotherms and Scatchard analysis of the binding of ^{125}I-LIF, ^{125}I-OSM, and ^{125}I-IL-6 to M1 parental and M1/SCL cell lines. The numbers of LIF, OSM, and IL-6 binding sites on M1/SCL cell lines were the same as on parental and control cell lines. A single class of high-affinity LIF receptor was observed (≈340 sites per cell, K_d = 74 pM for parental M1 cells; ≈205 sites per cell, K_d = 92 pM for a typical M1/SCL cell line) (18, 19). Two classes of OSM receptor were observed on M1 parental and M1/SCL cell lines (≈240 high-affinity sites per cell, K_d = 2 nM for parental M1 cells; ≈343 high-affinity sites per cell, K_d = 2.5 nM for a typical M1/SCL cell line; ≈1100 low-affinity sites per cell, K_d = 32 nM for parental M1 cells; ≈1090 low-affinity sites per cell, K_d = 20 nM for a typical M1/SCL

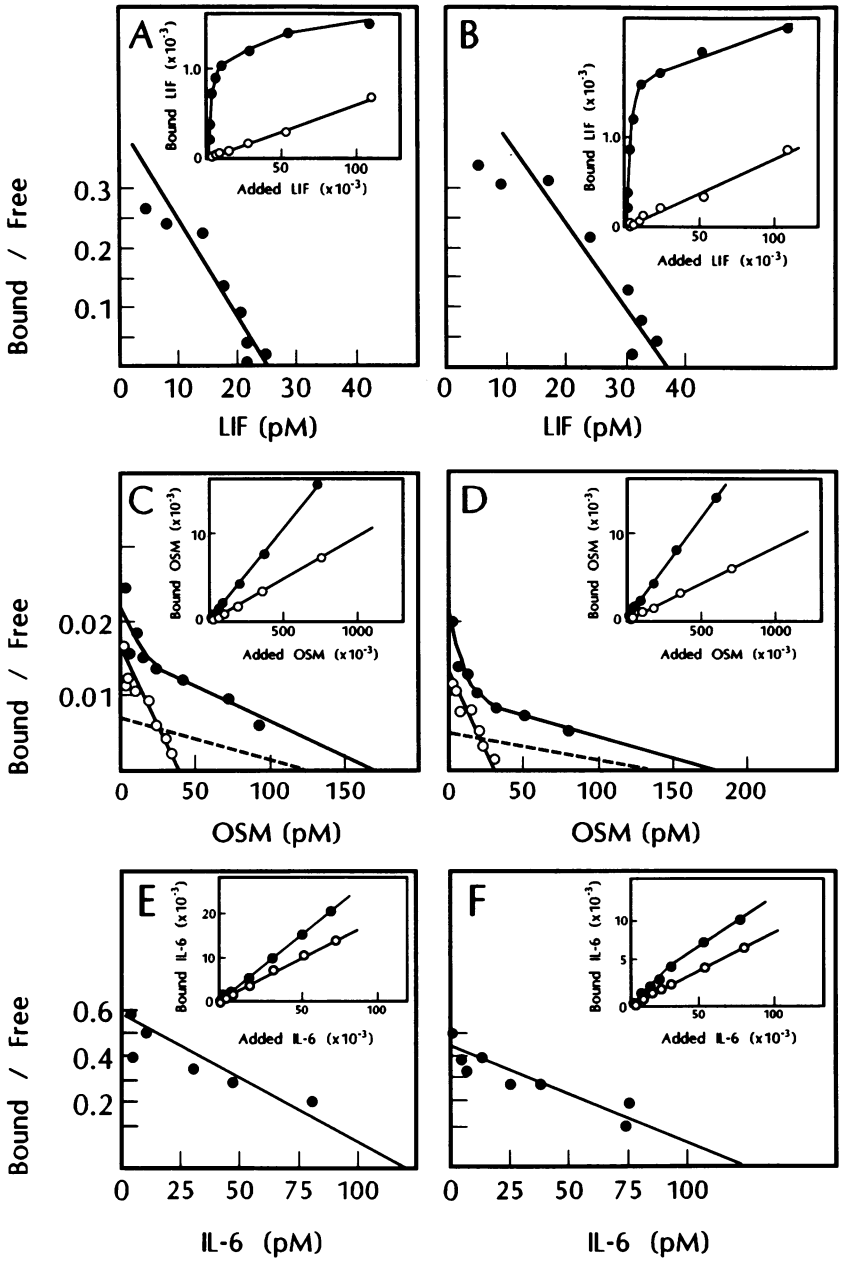

FIG. 5. Scatchard analysis of the binding of ^{125}I-LIF, ^{125}I-OSM, and ^{125}I-IL-6 to M1 control and M1/SCL cell line. Saturation binding isotherms are shown in *Insets*. Total binding (●) was determined in duplicate tubes and nonspecific binding (○) was that in the presence of at least 100-fold excess of unlabeled ligand. Specific binding (total minus nonspecific) was plotted in the Scatchard transformation. Broken lines indicate low-affinity OSM receptor. Open circles represent residual binding after a 20-min dissociation period and correspond to the high-affinity binding component. Results for M1 parental cell line (*A* and *E*), M1/SCL clone S5 (*B*), M1/neo clone N2 (*C*), M1/SCL clone S8 (*D*), and M1/SCL clone S2 (*F*) are shown. Similar results were obtained with all clonal cell lines examined.

cell line). ^{125}I-IL-6 binding on M1 and M1/SCL cells showed a single class of receptor (≈1200 sites per cell, K_d = 167 pM for parental M1 cells; ≈1600 sites per cell, K_d = 230 pM for a typical M1/SCL cell line). Therefore it appeared that overexpression of a transfected SCL gene did not affect LIF, OSM, or IL-6 receptor status.

DISCUSSION

In this report we have shown that monocytic differentiation of M1 leukemia cells induced by LIF, OSM, or IL-6 was accompanied by a marked decrease in SCL mRNA and protein (Figs. 1 and 2). Conversely, enforced overexpression of SCL resulted in reduced terminal differentiation and responsiveness of cells to LIF and OSM, but not to IL-6 (Figs. 3 and 4).

The HLH family of transcription factors is known to have an important role in differentiation events in a wide variety of tissues (1, 2), and the SCL protein has been similarly implicated in hemopoietic differentiation events (6, 7). In this study, additional support for this view was provided because SCL expression decreased during growth factor-induced monocytic differentiation in a manner similar to its decrease during chemically induced myeloid differentiation in K562 human erythroleukemia cells (13). This therefore parallels the normal pattern of SCL expression: SCL mRNA is detected in early myeloid "progenitor" cell populations but is not detected in mature monocyte/macrophage cell populations (11, 12, 37). In addition, these data link changes in expression (and regulation) of SCL with growth factor–receptor interactions occurring at the cell surface. This may at least in part by mediated by changes in phosphorylation status of SCL (38).

To determine whether the decrease in SCL expression might be necessary for the development of the monocytic phenotype, SCL was deliberately overexpressed in the leukemic cells. This resulted in reduced terminal myeloid differentiation and growth factor responsiveness of clonogenic cells. In this regard, SCL differed from c-*myc* (39), or c-*myb* (40), for which changes in expression occurred at earlier times and enforced expression abrogated terminal differentiation induced by growth factor. Thus it appeared that although the decrease in SCL occurred as a late consequence with all three growth factors, its enforced overexpression was directly able to (differentially) influence the ability of the cells

to undergo differentiation in response to LIF or OSM. It is unclear why not all clonogenic cells overexpressing SCL were refractory to differentiation induction, but this may have been due to variation in the level of SCL expression in individual cells.

These data also have implications for signaling through the LIF, OSM, and IL-6 receptor complexes. LIF, IL-6, and OSM share the ability to induce the differentiation of the murine myeloid leukemic cell line M1 and the human histiocytic leukemia U937 (20). This and other overlapping biological activities suggested that the signal transduction pathways for LIF, IL-6, and OSM may be interrelated, either at the level of receptors or through common intracellular signaling pathways. The membrane glycoprotein gp130 is recognized as essential for signal transduction by IL-6 (26). Although it lacks IL-6-binding activity, gp130 is able to generate a high-affinity IL-6 receptor complex when associated with the low-affinity IL-6 receptor and IL-6 (21). Similarly LIF binds to a low-affinity receptor chain which also then interacts gp130 (22). In addition, gp130 is able to bind OSM with low affinity, is essential for transducing signals for OSM, and interacts with the neurally restricted receptor for ciliary neurotropic factor (23, 41). Moreover, using M1 cells, Lord *et al.* (42) have reported that LIF and IL-6 utilize common intracellular signaling pathways, including tyrosine phosphorylation of a 160-kDa protein, to trigger an identical immediate early response upon induction of differentiation (42). However, the results presented in this paper suggest that at least in some situations, the receptor signaling pathways for LIF and OSM can be distinguished from that of IL-6. Because there was no detectable difference in receptor numbers or receptor affinity, it seems likely that the signaling pathways diverge beyond this point. Since both LIF and OSM receptors on M1 cells contain LIF receptor/gp130 heterodimers whereas IL-6 receptors contain gp130 with the low-affinity IL-6 receptor, it is probable that different signaling pathways are generated either by LIF receptor or by the heterodimer and that those pathways are acted upon either directly or indirectly by SCL. Therefore the M1/SCL cell lines described in this study may provide a valuable system for further dissecting the molecular mechanisms involved in the transduction of LIF, OSM, and IL-6 signaling.

We are indebted to Meredith Layton and Cathy Owczarek for recombinant human OSM and Richard Simpson for recombinant mouse IL-6. We thank Paula Gason and Carolyn Farley for expert technical assistance. The GP+E-86 packaging cell line was kindly provided by Dr. Arthur Bank (Columbia University, New York). This work was supported by grants from the Victorian Health Promotion Foundation, the Anti-Cancer Council of Victoria, the National Health and Medical Research Council, Canberra, Australian Medical Research and Development Corp., and U.S. National Institutes of Health Grant CA22556.

1. Murre, C., McCaw, P. S. & Baltimore, D. (1989) *Cell* **56,** 777–783.
2. Murre, C., McCaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Jan, Y. N., Cabrera, C. V., Buskin, J. N., Hauschka, S. D., Lassar, A. B., Weintraub, H. & Baltimore, D. (1989) *Cell* **58,** 537–544.
3. Thisse, B., Stoetzel, C., Gorostiza-Thisse, C. & Perrin-Schmitt, F. (1988) *EMBO J.* **7,** 2175–2183.
4. Begley, C. G., Aplan, P. D., Haynes, B., Waldmann, T. A. & Kirsch, I. R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 10128–10132.
5. Visvader, J. & Begley, C. G. (1991) *Trends Biochem. Sci.* **16,** 330–333.
6. Green, A. R., DeLuca, E. & Begley, C. G. (1991) *EMBO J.* **10,** 4153–4158.
7. Aplan, P. D., Nakahara, K., Orkin, S. H. & Kirsch, I. R. (1992) *EMBO J.* **11,** 4073–4081.
8. Chen, Q., Cheng, J.-T., Tsai, L.-H., Schneider, N., Buchanan, G., Carroll, A., Crist, W., Ozanne, B., Siciliano, M. J. & Baer, R. (1990) *EMBO J.* **9,** 415–424.
9. Bernard, O., Lecointe, N., Jonveaux, P., Souyri, M., Mauchauffe, M., Berger, R., Larsen, C. J. & Mathieu-Mahul, D. (1991) *Oncogene* **6,** 1477–1488.
10. Green, A. R., Salvaris, E. & Begley, C. G. (1991) *Oncogene* **6,** 475–479.
11. Visvader, J., Begley, C. G. & Adams, J. M. (1991) *Oncogene* **6,** 195–204.
12. Green, A. R., Lints, T., Visvader, J., Harvey, R. & Begley, C. G. (1992) *Oncogene* **7,** 653–660.
13. Green, A. R., Rockman, S., DeLuca, E. & Begley, C. G. (1993) *Exp. Hematol.* **21,** 525–531.
14. Ichikawa, Y. (1969) *J. Cell. Physiol.* **74,** 223–232.
15. Shabo, Y., Lotem, J., Rubinstein, M., Revel, M., Clark, S. C., Wolf, S. F., Kamen, R. & Sachs, L. (1988) *Blood* **72,** 2070–2073.
16. Hilton, D. J., Nicola, N. A., Gough, N. M. & Metcalf, D. (1988) *J. Biol. Chem.* **263,** 9238–9243.
17. Bruce, A. G., Hoggatt, I. H. & Rose, T. M. (1992) *J. Immunol.* **149,** 1271–1275.
18. Gearing, D. P. & Bruce, A. G. (1992) *New Biol.* **4,** 61–65.
19. Hilton, D. J., Nicola, N. A. & Metcalf, D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5971–5975.
20. Yamaguchi, M., Michishita, M., Hirayoshi, K., Yasukawa, K., Okuma, M. & Nagata, K. (1992) *J. Biol. Chem.* **267,** 22035–22042.
21. Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T. & Kishimoto, T. (1990) *Cell* **63,** 1149–1157.
22. Gearing, D. P., Thut, C. J., VandenBos, T., Gimpel, S. D., Delaney, P. B., King, J., Price, V., Cosman, D. & Beckmann, M. P. (1991) *EMBO J.* **10,** 2839–2848.
23. Rose, T. & Bruce, G. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8641–8645.
24. Miyajima, A., Hara, T. & Kitamura, T. (1992) *Trends Biochem. Sci.* **17,** 378–382.
25. Kishimoto, T., Akira, S. & Taga, T. (1992) *Science* **258,** 593–597.
26. Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T. & Kishimoto, T. (1989) *Cell* **58,** 573–581.
27. Gearing, D. P., Comeau, M. R., Friend, D. J., Gimpel, S. D., Thut, C. J., McGourty, J., Brasher, K. K., King, J. A., Gillis, S., Mosley, B., Ziegler, S. F. & Cosman, D. (1992) *Science* **255,** 1434–1437.
28. Begley, C. G., Visvader, J., Green, A. R., Aplan, P. D., Metcalf, D., Kirsch, I. R. & Gough, N. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 869–873.
29. Hariharan, I. K., Adams, J. M. & Cory, S. (1988) *Oncogene Res.* **3,** 387–399.
30. Markowitz, D., Goff, S. & Bank, A. (1988) *J. Virol.* **62,** 1120–1124.
31. Metcalf, D. (1989) *Leukemia* **3,** 349–355.
32. Nicola, N. A. & Metcalf, D. (1988) *Growth Factors* **1,** 29–39.
33. Nicola, N. & Cary, D. (1992) *Growth Factors* **6,** 119–129.
34. Gonda, T. J., Sheiness, D. K. & Bishop, J. M. (1982) *Mol. Cell. Biol.* **2,** 617–624.
35. Tso, J. Y., Sun, X. H., Kao, T., Reece, K. S. & Wu, R. (1985) *Nucleic Acids Res.* **13,** 2485–2502.
36. Cross, M., Mangelsdorf, I., Wedel, A. & Renkawity, R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6232–6236.
37. Mouthon, M.-A., Bernard, O., Mitjavila, M.-T., Romeo, P.-H., Vainchenkew, W. & Mathieu-Mahul, D. (1993) *Blood* **81,** 647–655.
38. Cheng, J.-T., Cobb, M. H. & Baer, R. (1993) *Mol. Cell. Biol.* **13,** 801–808.
39. Hoffman-Liebermann, B. & Liebermann, D. A. (1991) *Mol. Cell. Biol.* **11,** 2375–2381.
40. Selvakumaran, M., Liebermann, D. A. & Hoffman-Liebermann, B. (1992) *Mol. Cell. Biol.* **12,** 2493–2500.
41. Ip, N. Y., Hye, S. H., Boulton, T. G., Davis, S., Taga, T., Li, Y., Birren, S. J., Yasukawa, K., Kishimoto, T., Anderson, D. J., Stahl, N. & Yancopoulos, G. D. (1992) *Cell* **69,** 1121–1132.
42. Lord, K. A., Abdollahi, A., Thomas, S. M., DeMarco, M., Brugge, J. S., Hoffman-Lieberman, B. & Liebermann, D. A. (1991) *Mol. Cell. Biol.* **11,** 4371–4379.