Jpn. J. Cancer Res. 83, 499–504, May 1992

Suppressive Effect of LD78 on the Proliferation of Human Hemopoietic Progenitors

Hiroko Shiozaki,[1] Toshio Ide,[1,2] Junji Nakao,[2] Takayuki Imamura,[2] Mitsuru Nakamura,[1] Kazunori Shimada,[3] Yasusada Miura[1] and Toshio Suda[1,4]

[1]*Division of Hematology, Department of Medicine, Jichi Medical School, Minamikawachi-machi, Tochigi-ken 329-04,* [2]*Laboratory of Molecular Genetics, Department of Research and Development, The Chemo-Sero-Therapeutic Research Institute, Kyokushi-mura, Kikuchi-gun, Kumamoto-ken 861-15 and* [3]*Reserach Institute for Microbial Diseases, Osaka University, Yamadaoka Suita-shi, Osaka-fu 565*

LD78 is a cDNA newly isolated from human stimulated tonsillar lymphocytes. The expression of LD78 is related to inflammatory responses and its structure has a homology with macrophage inflammatory protein 1-α, which is known to have an inhibitory effect on murine CFU-S. Using a colony assay technique, we examined the effects of LD78 on human hemopoietic progenitors. The addition of doses of 100 ng/ml or more of LD78 suppressed the colony formation of KMT-2, a factor-dependent myelomonocytic cell line established from cord blood cells; this suppressive activity was neutralized by the addition of antibody against LD78. The same doses of LD78 suppressed the formation of neutrophil, macrophage, and megakaryocytic colonies which were supported by human interleukin-3 and erythropoietin; however, LD78 did not affect colony formation by either non-phagocytic mononuclear cells or sorted $CD34^+$ cells. The conditioned medium of KMT-2 cells or peripheral blood mononuclear cells cultured with LD78 suppressed colony formation by $CD34^+$ cells. From these findings, it is suggested that LD78 affects phagocytic cells and induces factors that are inhibitory for hemopoiesis. We consider LD78 to be a new cytokine that plays an inhibitory role in hemopoiesis.

Key words: LD78 — Hemopoietic progenitor — CD34

LD78 is one of a newly isolated superfamily of inducible proteins that are related to inflammatory responses, wound healing, and tumorigenesis.[1] LD78 has 92 amino acids including a putative signal sequence of 22 amino acids. It is encoded by a cDNA named pLD78. It was isolated from a library constructed of poly(A)$^+$ RNAs of human tonsillar lymphocytes stimulated by the tumor promoter, phorbol 12-myristate 13-acetate (PMA), and by phytohemagglutinin (PHA). The 5′ flanking regions of this gene were found to have homology with the corresponding regions of human interleukin-2 (IL-2) and γ interferon (γIFN) genes.[2,3] Moreover, its structure has 75% homology to that of macrophage inflammatory protein-1α (MIP-1α), which has been reported to inhibit the proliferation of murine CFU-S.[4] Based on the analyzed amino acid residues of pLD78 cDNA, LD78 has been classified as a member of the superfamily of β-thromboglobulin,[5] suggesting that LD78 encodes a novel cytokine, with an as-yet-unknown functional role. Using a clonal culture system, we examined the effects of recombinant LD78 on the differentiation and proliferation of human hemopoietic progenitors and LD78 target cells.

MATERIALS AND METHODS

Cell line KMT-2 cells are a human myelomonocytic cell line established from umbilical cord blood.[6] The growth of these cells is dependent on human interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) and is responsive to IL-6, but not to IL-1α, IL-2, IL-4, IL-5, or erythropoietin.

Human bone marrow cells Bone marrow samples were obtained from normal healthy volunteers who had given their informed consent. Bone marrow mononuclear cells were separated by Ficoll-Conray (IBL Co. Gunma) density centrifugation; the interface cells were harvested and washed twice in Iscove's modified Dulbecco's medium (IMDM, GIBCO, Grand Island, NY). To prepare the non-phagocytic fraction, iron-laden cells were removed by the use of a magnet.[7]

Clone-sorting A sorting gate, based on the light scattering properties of CD34 bright cells, was established as described previously.[8] Cells were sorted from whole $CD34^+$ cells and pooled using FACStar plus and an automated cell deposition unit (Becton Dickinson Immunocytometry Systems, Inc., Mountain View, CA). Dead cells stained with 7-AAMD were gated out by FACS at the time of cell sorting.

Yeast-derived LD78 polypeptide (yLD78) Recombinant LD78 polypeptide was isolated from the culture supernatant of yeast that harbored an expression plasmid carrying LD78 cDNA. The LD78 cDNA was inserted into the yeast expression vector, pYG100, in which a foreign gene was epressed under the control of glyceraldehyde-3-phosphate dehydrogenase promotor. The resulting plasmid (pYG-LD78) was used to transform the yeast *S. cerevisiae*. The yLD78 expressed in yeast was secreted

[4] To whom correspondence should be addressed.

into the culture medium by its own secretion signal peptide. The yLD78 was then purified from the culture supernatant by ultrafiltration, precipitation of yLD78 in the presence of 100 m*M* sodium acetate, and separation by Mono Q (Pharmacia) column chromatography. Finally, the purified yLD78 was passed through a polymixin column to remove endotoxin. To determine the purity of yLD78, protein samples were electrophoresed in 0.1% sodium dodecyl sulfate (SDS)-20% polyacrylamide gel. Gel was stained with Coomassie Brilliant Blue R-250. Electrophoretic transfer of proteins to a polyvinylidene difluoride filter was performed by TRANS-BLOT SD (BIOi-RAD Laboratories, Richmond, CA). Immunoblot analysis was performed essentially as described by Robbs *et al.*,[9] except for the incubation buffer, which contained 0.15 *M* NaCl.[10]

Antibody against LD78 Anti-LD78 serum was prepared from a goat which had been subcutaneously immunized three times with 2 mg of purified rLD78 with Freund's complete adjuvant. The antiserum was purified by using an Affigel Protein A MAPS-II kit (Japan BIO-RAD Laboratories Inc., Tokyo). Antibody activity against contaminating yeast antigens was removed by adsorption to yeast cell extracts.

Other hemopoietic factors Recombinant human erythropoietin (Epo) with a specific activity of 80,000 U/ml was provided by Snow Brand Co., Ishibashi, Tochigi. Recombinant human interleukin-3 with a specific activity of 21,900 U/ml was prepared by the Genetics Institute, Cambridge, MA.

Clonal cell culture Cultures of 2×10^3/ml KMT-2 cells, 1×10^5 or 5×10^4/ml of normal bone marrow cells and 500 sorted $CD34^+$ cells were prepared in 35-mm non-tissue culture dishes (Falcon, Oxnard, CA) containing methylcellulose (Aldrich Chemical Co., Milwaukee, WI) in IMDM that contained 30% human platelet-poor plasma, 1% deionized bovine serum albumin (Sigma Chemical Co., St. Louis, MO), 5×10^{-5} mol/liter 2-mercaptoethanol (Sigma), 2 U/ml rEpo, 2 ng/ml rIL-3, and an appropriate amount of LD78. The cultures were incubated for 15 days at 37°C in a humidified atmosphere of 5% CO_2 and 5% O_2. On days 7 and 14 of culture, colony formation was counted and classified by using an inverted microscope. To confirm colony type, each colony was lifted with a 3-μl Eppendorf micropipette in medium containing 10% fetal calf serum. The samples were spun in a centrifuge (Cytospin; Shandon Southern, Sewickley, PA) and stained with May-Grünwald-Giemsa.

Northern blot analysis RNA was extracted from KMT-2 cells cultured with or without rLD78 by the guanidinium thiocyanate/LiCl method.[11] Hybridization and washing were performed as described elsewhere.[12] Transforming growth factor β (TGFβ) probe cocktail (British Bio-technology Ltd., BPR78), tumor necrosis factor α (TNFα) cDNA (provided by Dr. M. Akashi), and the synthetic oligonucleotides of human leukemia inhibitory factor (LIF) were labeled with [^{32}P]phosphates and were used as probes. Five micrograms of poly(A)$^+$ RNA, denatured at 65°C in the presence of formamide and formaldehyde, was subjected to electrophoresis on a vertical 1.2% agarose gel in the presence of formaldehyde, and was then blotted to a nylon membrane filter with 10× SSC. Blots were prehybridized for 4 h at 42°C in 5×SSPE, 50% formamide, and salmon sperm DNA (50 μg/ml), and were then hybridized with ^{32}P-labeled probes under the same conditions for 14 h. The filter was then washed at 45°C with 0.2× SSC and 0.1% SDS.

RESULTS

Recombinant LD78 polypeptide was isolated from the culture supernatant of yeast that harbored an expression plasmid carrying LD78 cDNA. To determine the purity of yLD78, we performed SDS-polyacrylamide gel electrophoresis and immunoblot analysis. As shown in Fig. 1, we did not observe any other band except LD78. Contaminating endotoxin concentration was less than 1 ng per 100 μg of LD78 polypeptide.

We first examined the effects of LD78 on KMT-2 cells. Colony formation by KMT-2 cells was not observed in the presence of LD78 alone. The colony formation of KMT-2 cells supported by IL-3 was inhibited by LD78 in a dose-dependent manner (Table I). The number of

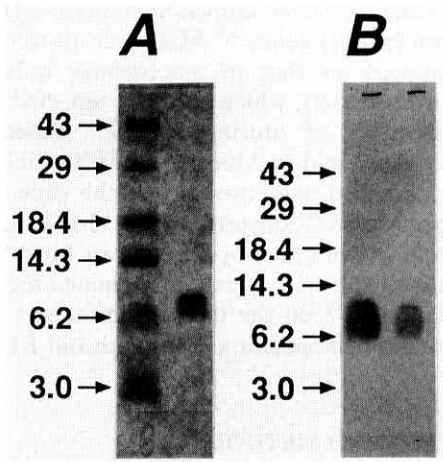

Fig. 1. SDS-polyacrylamide gel and immunoblot analysis of purified yLD78α. A: CBB staining; left lane, molecular weight markers (BRL) right lane, 3 μg of purified yLD78. B: Immunoblot analysis; left lane, 500 ng of purified yLD78 right lane, 100 ng of purified yLD78.

colonies formed by these KMT-2 cells was decreased by half by the addition of 0.1–1.0 μg/ml of LD78. To test the specificity of the suppressive effect of yLD78, we added goat anti-LD78 antibody to culture dishes which contained LD78 and IL-3. Anti-LD78 antibody neutralized the suppressive effect on colony formation (Table II). The addition of 20 μg/ml of anti-LD78 antibody had no significant effect on the colony formation of KMT-2 cells in the presence of IL-3. We observed the colony growth of KMT-2 sequentially every 3 days until 18 days. The suppressive effect of LD78 on colony formation was maximum on days 6 to 9, and this effect diminished after day 15 (Fig. 2).

To examine the effects of LD78 on human hemopoietic progenitors, we prepared two fractions of human bone marrow cells: mononuclear cells and non-phagocytic mononuclear cells. LD78 alone did not support any colony formation of human bone marrow cells. We then observed the effects of various concentrations (0–1,000 ng/ml) of LD78 on colony formation supported by rEpo and rIL-3. In these cases, the suppressive effects of LD78 tended to be dose-dependent. LD78 suppressed granulocyte-macrophage (GM) and megakaryocyte (Meg) colony formation of bone marrow mononuclear cells at doses higher than 100 ng/ml (Tables III and IV). However, LD 78 did not affect the growth of erythroid colony-forming cells (CFU-E), erythroid burst-forming cells (BFU-E), or mixed colony-forming cells (data not shown). When non-phagocytic mononuclear cells were

Table I. Day-8 Colony Formation by KMT-2 Cells

	Exp. 1	Exp. 2
IL-3	125±4	109±6
IL-3+LD78 (1 ng/ml)	120±3 (−4.0)	82±0 (−24.8)*
IL-3+LD78 (10 ng/ml)	115±5 (−8.0)	78±5 (−28.4)*
IL-3+LD78 (100 ng/ml)	84±10 (−32.8)	68±3 (−37.6)*
IL-3+LD78 (100 ng/ml)	57±5 (−54.4)***	47±1 (−56.9)***

Number of KMT-2 cell colonies formed by 2×10^3 cells per dish in the presence of IL-3 and IL-3 plus different concentrations of LD78.
* Student's t test against IL-3 alone; * $P<0.05$, *** $P<0.01$.

Table II. Day-8 Colony Formation by KMT-2 Cells

IL-3	431±19
IL-3+LD78	285±25 (−33.9)*
IL-3+LD78+Ab (0.2 μg/ml)	321±61 (−25.5)
IL-3+LD78+Ab (2.0 μg/ml)	381±51 (−11.6)
IL-3+LD78+Ab (20 μg/ml)	448±24 (+3.9)
IL-3+Ab (20 μg/ml)	410±3 (−1.0)

Number of KMT-2 cell colonies formed by 2×10^3 cells per dish in the presence of IL-3 and 100 ng/ml LD78, plus different concentrations of anti-LD78 antibody.
* $P<0.05$.

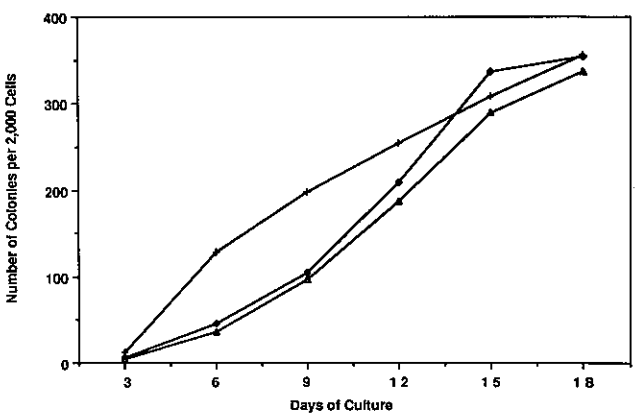

Fig. 2. Time course of colony formation by KMT-2 cells in the presence of rLD78. Cells were plated at a density of 2×10^3 cells/ml with 2 ng/ml of rhIL-3 in the absence (+) or presence of rLD78 (1.0 μg/ml; ◆) and (0.1 μg/ml; △). This is a representative result of three repeated experiments.

Table III. Granulocyte Macrophage Colony Formation by Human Bone Marrow Cells

	Exp. 1	Exp. 2
IL-3+Epo	88±2.5	103±3.5
IL-3+Epo+LD78 (10 ng/ml)	70±6.5 (−20.5)	105±3.5 (+1.9)
IL-3+Epo+LD78 (100 ng/ml)	51±2.0 (−42.0)***	81±4.5 (−21.4)
IL-3+Epo+LD78 (500 ng/ml)	41±6.5 (−53.4)*	62±12.5 (−39.8)
IL-3+Epo+LD78 (1000 ng/ml)	38±4.0 (−56.8)***	50±5.0 (−51.5)**

Number of GM colonies formed by 1×10^5 human bone marrow cells per dish in the presence of IL-3, Epo, and different concentrations of LD78. Student's t test against IL-3+Epo; * $P<0.05$, ** $P<0.02$, *** $P<0.01$.

Table IV. Megakaryocyte Colony Formation by Human Bone Marrow Cells

	Exp. 1	Exp. 2
IL-3 + Epo	26 ± 6.0	18 ± 1.0
IL-3 + Epo + LD78 (10 ng/ml)	24 ± 0.0 (−7.6)	16 ± 1.0 (−11.1)
IL-3 + Epo + LD78 (100 ng/ml)	13 ± 1.5 (−50.0)	11 ± 0.0 (−38.9)**
IL-3 + Epo + LD78 (500 ng/ml)	9 ± 3.5 (−65.4)	9 ± 1.0 (−50.0)**
IL-3 + Epo + LD78 (1000 ng/ml)	8 ± 0.0 (−69.2)**	5 ± 0.0 (−72.2)***

Number of Meg colonies formed by 1×10^5 human bone marrow cells per dish in the presence of IL-3, Epo, and different concentrations of LD78.
** $P < 0.02$, *** $P < 0.01$.

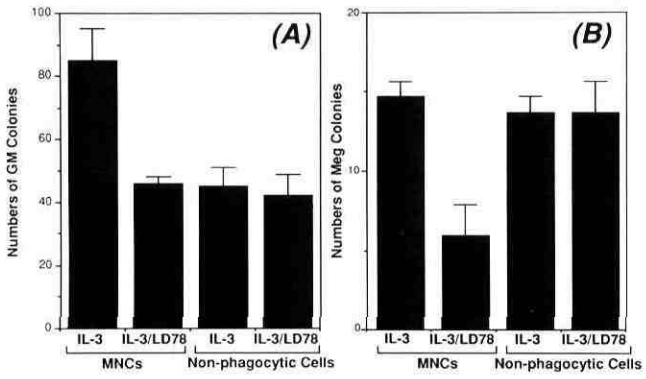

Fig. 3. Number of GM colonies (A) and Meg colonies (B) formed by 1×10^5 mononuclear cells and non-phagocytic cells in the presence of IL-3 alone or IL-3 and LD78 (100 ng/ml). The data represent the results of three repeated experiments.

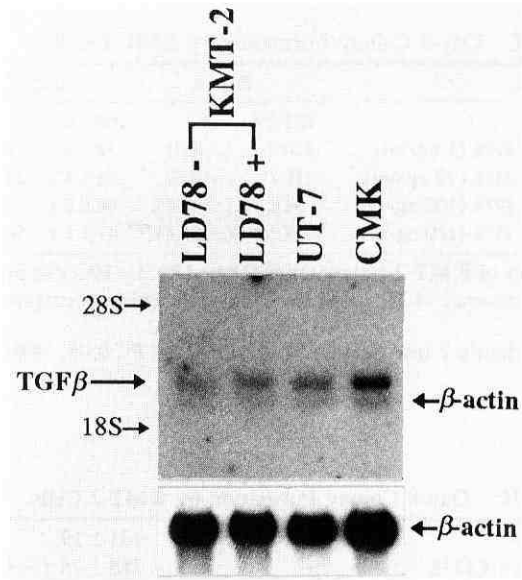

Fig. 4. Northern blot analysis of KMT-2 cells cultured with or without rLD78. Poly(A)$^+$ RNAs (5 μg) of KMT-2, UT-7, and CMK cells were electrophoretically separated, transferred to a nylon membrane, and hybridized with ^{32}P-labeled TGFβ or β-actin cDNA. The migration of 18S and 28S ribosomal RNA is indicated. UT-7[17] and CMK[18] are megakaryocytic cell lines.

used as target cells, neither 100 ng/ml nor 1,000 ng/ml dose of LD78 had any suppressive effect on GM and Meg colony formation (Figs. 3 A and B). To confirm this, we examined the effects of LD78 on CD34-positive cells collected by FACS; we found that LD78 had no effect on the colony growth of CD34-positive cells. To clarify the mechanism of the suppressive effect of LD78 on hemopoietic progenitors, we prepared a conditioned medium of KMT-2 and peripheral mononuclear cells. These cells were cultured in the presence or absence of 1,000 ng/ml LD78 for 3 days and each supernatant was harvested after centrifugation and filtered through a Millipore membrane. We found that 10% LD78-primed supernatant suppressed colony formation, while 10% LD78-non-primed supernatant did not (Table V).

We attempted to identify the molecules induced in KMT-2 cells by LD78 stimulation. Northern blot analysis showed that expression of TGFβ mRNA was not changed by LD78 treatment (Fig. 4). We also tested the expression of LIF and TNFα mRNA; however, we were unable to find any significant difference between the mRNA expressions of untreated and LD78-treated KMT-2 cells.

DISCUSSION

LD78 is a novel inducible protein whose expression relates to inflammatory responses.[1] Using yeast JN8906 and a plasmid insertion technique, we formed recombinant LD78. Another inducible protein, MIP, has recently been reported[13–16]; it has three subtypes of different amino acid residues, MIP-1α, MIP-1β, and MIP-2. MIP-1α has been shown to have a suppressive effect on murine CFU-S, while MIP-1β and MIP-2 were not as

Table V. Colony Formation by Human CD34-positive Cells

	Exp. 1	Exp. 2
PHA-LCM+Epo	173±10	140±6
PHA-LCM+Epo+LD78[a]	160±1 (−7.5)	129±20 (−7.9)
PHA-LCM+Epo+PB-CM[b]	169±14 (−2.3)	141±10 (+0.7)
PHA-LCM+Epo+PB-LD-CM[c]	138±12 (−20.2)	106±5 (−24.3)**
PHA-LCM+Epo+KMT-2-CM[d]	156±3 (−9.8)	125±15 (−10.7)
PHA-LCM+Epo+KMT-2-LD-CM[e]	115±9 (−33.5)***	69±10 (−50.7)*

Numbers of colonies formed by 500 CD34-positive cells per dish.
a) One μg/ml LD78 was added directly.
b, c) Five % conditioned medium of peripheral blood mononuclear cells (PB-CM) or these cells cultured with 1 μg/ml LD78 (PB-LD-CM).
d, e) Five % conditioned medium of KMT-2 cells (KMT-2-CM) or these cells cultured with 1 μg/ml LD78 (KMT-2-CM). Final concentrations of LD78 in PB-LD-CM and KMT-2-LD-CM were 50 ng/ml.
Student's *t* test against PHA-LCM+Epo; * $P<0.05$, ** $P<0.02$, *** $P<0.01$.

effective as MIP-1α. It has been shown that LD78 has 75% homology to MIP-1α. Broxmeyer *et al.* reported that purified recombinant MIP-1 and natural MIP-1 suppressed human CFU-GM in the presence of rGM-CSF plus IL-3 and that one type of MIP suppressed BFU-E supported by Epo.[4] The effects of MIP-1 on Meg colony formation were not examined in their study. We speculated that LD78 had the same suppressive effect on human hemopoietic progenitors as MIP-1α had on such murine cells. Here, we report the effects of the human rLD78 on the differentiation and proliferation of human hemopoietic progenitors.

LD78 alone did not support and colony formation in human bone marrow cells; however LD78 suppressed colony formation in GM, Meg, and KMT-2 cells supported by IL-3. The suppressive effect of LD78 on colony formation did not appear to be cytotoxic, because this effect was at a maximum on days 6 to 9 of culture and it diminished after 12–15 days. Since the effect of LD78 was neutralized by the anti-LD78 antibody, it appears that the suppressive effect was LD78-specific.

However, LD78 had no suppressive effect on the colony growth of non-phagocytic or partially purified progenitor cells (CD34-positive cells). From these findings, we hypothesized that accessory cells such as monocytes or macrophages were necessary for the suppressive effect of LD78 on GM and Meg colony formation. The supernatants of LD78-primed KMT-2 or peripheral blood mononuclear cells showed an inhibitory effect on the colony growth of CD34-positive cells. We presume that LD78 acts on macrophages to induce inhibitory molecules against hemopoietic progenitors since, (i) LD78 does not have a suppressive effect on non-phagocytic cells, (ii) KMT-2 cells have myelomonocytic characteristics, in that they are HLA-DR, CD13, CD33, MCS-2, and LeuM1-positive, and (iii) receptors have been demonstrated on myelomonocytic cells (Y. Yamamura *et al.*, unpublished observation).

To identify the molecules induced by myelomonocytic cells, we examined the mRNA levels of TGFβ, LIF and TNFα in KMT-2 cells with or without LD78 treatment. Especially, we have observed that TGFβ suppresses GM colony formation partially (unpublished data). However, we did not find enhanced expression of such genes after this treatment. If this system is effective for identifying LD78-induced gene(s), the possibility remains that an unknown factor(s) is released from monocytes or macrophages by LD78. A receptor assay of LD78 would also be required for the understanding of its biological effect.

Moreover, since LD78 had no effect on erythroid colony formation, it is suggested that LD78 acts on GM or Meg committed progenitors rather than on pluripotent stem cells. We consider LD78 to be a new factor that has inhibitory effects on hemopoietic progenitors.

ACKNOWLEDGMENTS

We wish to thank Ms. Motoko Yoshida for preparing the manuscript. This work was partially supported by grants from the Ministry of Education, Science and Culture of Japan.

(Received September 28, 1991/Accepted February 3, 1992)

REFERENCES

1) Obaru, K., Fukuda, M., Naeda, S. and Shimada, K. A cDNA clone used to study mRNA inducible in human tonsillar lymphocytes by a tumor promoter. *J. Biochem.*, **99**, 885–894 (1986).
2) Yamamura, Y., Hattori, T., Obaru, K., Sakai, K., Asou, N., Takatsuki, K., Ohmoto, Y., Nomiyama, H. and Shimada, K. Synthesis of a novel cytokine and its gene (LD78) expression in hematopoietic fresh tumor cells and cells lines. *J. Clin. Invest.*, **84**, 1707–1712 (1989).
3) Nakao, M., Nomiyama, H. and Shimada, K. Structure of human genes coding for cytokine LD 78 and their expression. *Mol. Cell. Biol.*, **10**, 3646–3658 (1990).
4) Broxmeyer, H. E., Sherry, B., Lu. L., Cooper, S., Oh, K., Olson, P. T., Kwon, B. S. and Cerami, A. Enhancing and suppressing effects of recombinant murine macrophage inflammatory protein on colony formation *in vitro* by bone marrow myeloid progenitor cells. *Blood*, **76**, 1110–1116 (1990).
5) Obaru, K., Hattori, T., Yamamura, Y., Takatsuki, K., Nomiyama, H., Maeda, S. and Shimada, K. A cDNA clone inducible in human tonsillar lymphocytes by a tumor promoter codes for a novel protein of the β-thromboglobulin superfamily. *Mol. Immunol.*, **26**, 423–426 (1989).
6) Tamura, S., Sugawara, M., Tanaka,H., Tezuka, E., Nihira, S., Miyamoto, C., Suda, T. and Ohta, Y. A new hematopoietic cell line, KMT-2, having human interleukin-3 receptors. *Blood*, **76**, 501–507 (1990).
7) Suda, T., Yamaguchi, Y., Suda, J., Miura, Y., Okano, A. and Akiyama, Y. Effect of interleukin 6 (IL-6) on the differentiation and proliferation of murine and human hemopoietic progenitors. *Exp. Hematol.*, **16**, 891–895 (1988).
8) Ema, H., Suda, T., Miura, Y. and Nakauchi, H. Colony formation of clonesorted human hemopoietic progenitors. *Blood*, **10**, 1941–1946 (1990).
9) Robbs, M., Nichols, J. C., Whoriskey, S. K. and Murphy, J. R. Isolation of hybridoma cell lines and characterization of monoclonal antibodies against cholera endotoxin and its subunits. *Infect. Immun.*, **38**, 267–272 (1982).
10) Zucker, D. and Murphy, J. R. Monoclonal antibody analysis of diphtheria toxin-I. Localization of epitopes and neutralization of cytotoxicity. *Mol. Immunol.*, **24**, 785–793 (1984).
11) Auffray, C. and Rougeon, F. Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *Eur. J. Biochem.*, **107**, 303–310 (1980).
12) Maniatis, T., Fritsch, E. F. and Sambrook, J. "Molecular Cloning, A Laboratory Manual" (1982). Cold Spring Harbor Laboratory, New York.
13) Davatelis, G., Olson, P. T., Wolpe, S. D., Hermsen, K., Luedke, C., Gallegos, C., Coit, D., Merryweather, J. and Cerami, A. Cloning and characterization of a cDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokinetic properties. *J. Exp. Med.*, **167**, 1939–1944 (1988).
14) Wolpe, S. D., Davatelis, G., Sherry, B., Beutler, B., Hesse, D. G., Nguyen, H. T., Moldawer, L. L., Nathan, C. F., Lowry, S. F. and Cerami, A. Macrophage secrete a novel heparin-binding protein with inflammatory and neutrophill chemokinetic properties. *J. Exp. Med.*, **167**, 570–581 (1988).
15) Davatelis, G., Wolpe, S. D., Sherry, B., Dayer, J. M., Chicheportiche, R. and Cerami, A. Macrophage inflammatory protein-1: a prostaglandin-independent endogenous pyrogen. *Science*, **243**, 1066–1068 (1989).
16) Graham, G. J., Wright, E. G., Hewick, R., Wolpe, S. D., Wikie, N. M., Donaldson, D., Lorimore, S. and Pragnell, I. B. Identification and characterization of an inhibitor of haemopoietic stem cell proliferation. *Nature*, **344**, 442–444 (1990).
17) Komatsu, N., Nakauchi, H., Miwa, A., Ishihara, T., Eguchi, M., Moroi, M., Okada, M., Sato, Y., Wada, H., Yawata, Y., Suda, T. and Miura, Y. Establishment and characterization of a human leukemic cell line with megakaryocytic features: dependency on granulocyte-macrophage colony-stimulating factor, interleukin 3, or erythropoietin for growth and survival. *Cancer Res.*, **51**, 341–348 (1991).
18) Sato, T., Fuse, A., Eguchi, M., Hayashi, Y., Sugita, K., Nakazawa, S., Minato, K., Shima, Y., Komori, I., Sunami, S., Okimoto, Y. and Nakajima, H. Establishment and characterization of a megakaryoblastic cell line (CMK) from a Down's syndrome patient with acute megakaryoblastic leukemia. *Br. J. Haematol.*, **72**, 184–190 (1989).