Growth Response of Acute Myeloblastic Leukemia Cells to Recombinant Human Thrombopoietin

By Itaru Matsumura, Yuzuru Kanakura, Takashi Kato, Hirokazu Ikeda, Jun Ishikawa, Yoko Horikawa, Koji Hashimoto, Yasuhiro Moriyama, Tohru Tsujimura, Tetsuo Nishiura, Hiroshi Miyazaki, and Yuji Matsuzawa

Thrombopoietin (TPO) is a newly identified hematopoietic growth factor that stimulates both megakaryopoiesis and thrombopoiesis through its interaction with a specific cell surface receptor encoded by the c-mpl proto-oncogene. In an effort to investigate the effect of TPO on human myeloid leukemia cells, the expression of c-mpl and the proliferative response to recombinant human (rh) TPO were investigated in a series of patients with acute myeloblastic leukemia (AML). Of 50 cases of AML, the c-mpl mRNA was detectable by means of Northern blot analysis in 26 cases, and the in vitro treatment with rhTPO led to proliferation of AML cells in 22 cases. The c-mpl expression and proliferative response to rhTPO was observed in all subtypes of AML and did not correlate with French-American-British classification, whereas all cases of M7-type AML cells expressed c-mpl and proliferated in response to rhTPO. Furthermore, rhTPO-induced proliferation of AML cells was augmented with the addition of interleukin-3 (IL-3), IL-6, stem cell factor, or granulocyte-macrophage colony-stimulating factor. These results suggested that c-mpl may be functional in terms of supporting proliferation of various types of AML cells and that TPO may contribute, at least in part, to abnormal growth of the cells, especially in combination with other hematopoietic growth factors.

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The c-mpl PROTO-ONCOGENE was originally identified as the cellular homologue of the oncogene v-mpl transduced in the myeloproliferative leukemia retrovirus that induces a broad spectrum of mammalian leukemias. The c-mpl proto-oncogene encodes a transmembrane receptor that is a member of hematopoietin receptor superfamily with high sequence similarity to the receptors for erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF). It has been reported that c-mpl is expressed exclusively on hematopoietic tissues, particularly on CD34+ hematopoietic stem cells, megakaryocytes, and platelets, although a low level of c-mpl expression is also detectable on endothelial cells. The treatment of CD34+ cells with antisense oligodeoxynucleotides to c-mpl mRNA has been shown to result in selective inhibition of in vitro megakaryocytic colony formation without affecting the growth of erythroid or granulomacrophage colonies. These results suggested that putative c-mpl ligand may play a crucial role in megakaryopoiesis.

Recently, several groups of investigators have purified and cloned the mpl ligand that has NH2-terminal domain homologous to EPO and COOH-terminal domain unrelated to any known protein. The in vitro and in vivo studies have shown that the mpl ligand has activity in stimulating both megakaryopoiesis and platelet production. Also, Kato et al have recently purified a novel thrombopoietic factor from the plasma of irradiated rats by measuring its activity on in vitro megakaryocyte production. They have subsequently cloned the genes for rat and human thrombopoietic factors and have shown them to have sequence identity to the mpl ligand. Thus, the mpl ligand is now shown to be the long-sought platelet growth factor, thrombopoietin (TPO) itself.

The proliferation and maturation of normal hematopoietic cells are positively or negatively regulated by a variety of hematopoietic growth factors. In addition to normal hematopoietic cells, proliferation of acute myeloblastic leukemia (AML) cells in vitro can be stimulated in most cases by one or more hematopoietic growth factors such as interleukin-3 (IL-3), IL-6, stem cell factor (SCF), and granulocyte-macrophage colony-stimulating factor (GM-CSF). Furthermore, a number of studies have suggested that disregulated expression of hematopoietic growth factors and/or their receptors could be involved in some aspect of excessive proliferation and aberrant differentiation of leukemia cells. It is therefore important to clarify the role of hematopoietic growth factors and their receptors in growth of AML cells not only for a better understanding of mechanisms underlying abnormal growth of AML cells but also for evaluating therapeutic procedure of the factors in patients with AML. However, in the case of c-mpl, the functional role of c-mpl in AML cells is not known, although c-mpl expression has previously been reported to be detected in about half the number of patients with AML. In this study, we therefore examined the expression of c-mpl in a series of AML cases and investigated the effects of recombinant human TPO (rhTPO) on proliferation of AML cells with or without combination with other hematopoietic growth factors.

MATERIALS AND METHODS

Hematopoietic growth factors. Highly purified rhSCF was provided by Dr Kristzina M. Zsebo (Amgen, Thousand Oaks, CA). rhTPO, rhGM-CSF, rhIL-3, rhIL-6, and rhEPO were gifts from Kirin Brewery Co Ltd (Tokyo, Japan). Molecular cloning and expression of human TPO cDNA was described previously. The rhIL-2, with a specific activity of 4.0 × 10^7 U/mg protein, was a gift of Takeda Chemical Industries, Ltd (Osaka, Japan).

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Cell lines. M07E, a human GM-CSF-, SCF-, IL-2-, and IL-3-dependent cell line, was obtained from Dr Steven Clark (Genetics Institute, Cambridge, MA) and was originally established by Avanzi et al. from the peripheral blood of an infant with an acute megakaryocytic leukemia. TF-1, a human GM-CSF- and IL-3-dependent cell line that was established from the peripheral blood of a patient with erythroleukemia, was kindly provided by Dr T. Kitanura (DNAX Research Institute, Palo Alto, CA). M07E and TF-1 were cultured in RPMI 1640 medium (Nakarai Tesque, Kyoto, Japan) supplemented with 10% fetal calf serum (FCS; Flow, North Ryde, Australia) and 10 ng/mL of rhGM-CSF at 37°C. HMC-1, a human mast cell line established by Butterfield et al. was cultured in RPMI 1640 medium supplemented with 10% FCS. KU812-F (a human chronic myelogenous leukemia cell line), HEL (a human erythroleukemia cell line), K562 (a human chronic myelogenous leukemia cell line), THP-1 (a human monocytic leukemia cell line), KG1 (a human myeloblastic leukemia cell line), HL-60 (a human promyelocytic leukemia cell line), and U937 (a human monocytic leukemia cell line) were obtained from the Japanese Cancer Research Bank (Tokyo, Japan) and were adapted to grow and maintained in RPMI 1640 supplemented with 10% FCS.

Patients and leukemic cells. Peripheral blood or bone marrow samples were obtained from 50 patients with AML and 5 patients with acute lymphoblastic leukemia (ALL). All samples were obtained after informed consent was given. The diagnosis of leukemia was made by morphologic, cytochemical, and immunologic analyses, and each case was classified according to French-American-British (FAB) criteria. Three cases were classified as FAB M0, 6 as M1, 9 as M2, 6 as M3, 8 as M4, 10 as M5, 4 as M6, and 4 as M7. Mononuclear cells were isolated with Ficoll-Hyphaque (Nycomed Pharma AS, Oslo, Norway) density gradient centrifugation and cryopreserved in 10% dimethylsulfoxide in the vapor phase of liquid nitrogen until use. Samples used in this study contained greater than 90% leukemia cells and had a viability greater than 90% after thawing.

Cell proliferation assay. The frozen cells were thawed, washed, and suspended in RPMI 1640 medium supplemented with 10% FCS. The cells of each cell line were washed and suspended in serum-free Cos medium (Cosmo Bio, Tokyo, Japan). To quantitate the proliferation of cells, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma, St Louis, MO) rapid colorimetric assay was used as previously reported. In brief, triplicate aliquots of cells (3.0 × 10^4 cells) resuspended in 100 μL RPMI 1640 supplemented with 10% FCS or Cos medium were cultured in 96-well flat-bottom microtiter plates for 72 hours at 37°C in the presence or absence of the growth factor(s). In some experiments, cells were cultured with various concentrations of rhTPO (0 to 100 ng/mL) but in most experiments cells were treated with 20 ng/mL rhTPO, because this concentration of rhTPO induced almost maximal proliferation of human leukemia cells. The concentrations of other growth factor were as follows: rhSCF, 100 ng/mL; rhIL-2, 50 ng/mL; rhIL-3, 10 ng/mL; rhIL-6, 50 ng/mL; rhGM-CSF, 10 ng/mL; and rhEPO, 10 U/mL. RPMI (10 μL of 5 mg/mL solution in PBS) was added for the final 4 hours of culture, and 100 μL of acid isopropanol (0.04 N HCl in isopropanol) was added to all wells and mixed. The optical density (OD) was then measured on the Microelisa plate reader (Corona Electric, Ibaragi, Japan) with a test wavelength of 540 nm and a reference wavelength of 620 nm. This assay was found to give equivalent results obtained by β-thymidine incorporation or cell enumeration as described previously.

Detection of c-mpl mRNA by Northern blot and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Total cellular RNA was isolated with guanidine isocyanate procedure. For RT-PCR, 2.5 μg of total cellular RNA was reverse-transcribed at 37°C for 60 minutes in a final volume of 50 μL with 200 U of Moloney leukemia virus reverse transcriptase (GIBCO BRL, Gaithersburg, MD), 2 μL of oligo dT primers (2 μmol/L), 10 mmol/L dithiothreitol, 50 U of RNAase (Toyobo, Osaka, Japan), and 1 mmol/L of dNTP mix using the reaction buffer provided by the manufacturer. The cDNA product (1.5 μL) was resuspended in a total volume of 15 μL containing 0.375 U of Taq DNA polymerase (Promega, Madison, WI), 2 mmol/L MgCl₂, 200 μmol/L dNTP mix, 15 pmol of forward and reverse primers, and 1× reaction buffer provided by the manufacturer. To amplify the fragment included by both forms of c-mpl, the samples were denatured for 3 minutes at 94°C, followed by 30 cycles of amplification (94°C for 30 seconds for denaturation; 50°C for 30 seconds for annealing; and 72°C for 90 seconds for extension). Aliquots (10 μL) were resolved on 1.5% agarose gels containing ethidium bromide to examine the existence and size of PCR products. PCR fragments were subcloned into Blue Script II SK (+) (Stratagene, San Diego), and sequence analysis was performed by dideoxynucleotide terminal method to confirm that the correct sequence of c-mpl were amplified. For Northern blot analysis, equal amounts of RNA (15 μg) were size-fractionated by electrophoresis through 1.0% formaldehyde agarose gels. After blotting to the nylon membranes, the filters were prehybridized and then hybridized with random ^32P-labeled probe in rapid hybridization buffer (American, Tokyo, Japan) for 3 hours at 65°C. The probe was 170 bp of Sph I-Nco I fragment correspond- ing to the c-mpl transcript in AML cells was expressed predominantly in intensity of c-mpl transcripts, the expression levels of c-mpl in AML cells were generally lower than those in TF-1 cells. The ratio of the K-form transcript was noted that, despite a considerable patient-to-patient variation in intensity of c-mpl transcripts, the expression levels of c-mpl in AML cells were generally lower than those in TF-1, M07E, and HEL cells with the exception of some AML cases.

With regard to FAB type, mRNA of c-mpl were present in 2 of 3 M0 cases, 3 of 6 M1 cases, 7 of 9 M2 cases, 2 of
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6 M3 cases, 3 of 8 M4 cases, 2 of 10 M5 cases, 3 of 4 M6 cases, and 4 of 4 M7 cases (Table 2). By contrast, c-mpl was not detected in any of ALL cases (Table 2). Thus, c-mpl transcripts were not limited to the specific types of AML, and there was no significant correlation between c-mpl expression and FAB classification. However, c-mpl expression was observed with high frequency in M7 type.

In addition to Northern blot analysis, we examined the expression of c-mpl using RT-PCR. Despite little message detected by Northern blot analysis in a substantial fraction of AML cases, RT-PCR analysis showed that both forms of c-mpl transcripts were noticeable in all cases of AML (data not shown). Furthermore, both forms of transcripts were amplified in KU812-F and HMC-1 cells, but not in KG-1, HL-60, K562, THP-1, or U937 cells (data not shown).

Proliferative response of human leukemia cell lines to rhTPO. To determine whether rhTPO induced proliferation of human leukemia cells, we first examined the dose-dependent effects of rhTPO on two human leukemia cell lines, M07E and TF-1, both of which proliferate in response to other hematopoietic growth factors such as GM-CSF, IL-3, and SCF. The cells were cultured in the various concentrations of rhTPO for 3 days, followed by measurement of cell proliferation with the MTT colorimetric method. Both M07E and TF-1 cells showed a dose-dependent proliferation in response to rhTPO over the range of 1 to 100 ng/mL, and almost maximal proliferation was observed at 20 ng/mL of rhTPO (Fig 2A). Furthermore, when M07E and TF-1 cells were cultured with rhTPO (20 ng/mL) in combination with rhIL-2 (50 ng/mL), rhIL-3 (10 ng/mL), rhIL-6 (50 ng/mL), rhSCF (100 ng/mL), rhGM-CSF (10 ng/mL), or rhEPO (10 U/mL), the growth factor-dependent proliferation of the cells was augmented by the addition of rhTPO (Fig 2B); especially in TF-1 cells, rhTPO by itself has a minimal effect on proliferation of the cells, but the growth induced by rhIL-3, rhSCF, rhGM-CSF, or rhEPO was markedly augmented by rhTPO.

Effects of rhTPO on proliferation of AML cells. We next examined the effects of rhTPO on in vitro proliferation of AML cells. Of 50 AML cases tested, 22 cases showed proliferative response to rhTPO at a concentration of 20 ng/mL (Tables 1 and 2). The proliferation of AML cells was induced in 19 of 26 c-mpl-positive cases, whereas rhTPO-induced proliferation was also observed in 3 of 24 AML cases in which c-mpl mRNA was barely detectable by Northern blot analysis (Table 2). With respect to FAB type, the proliferative response to rhTPO were observed not only in M7 (megakaryocytic) type but also in other types of AML. rhTPO induced proliferation of AML cells in 2 of 3 M0 cases, 1 of 6 M1 cases, 5 of 9 M2 cases, 1 of 6 M3 cases, 2 of 8 M4 cases, 5 of 10 M5 cases, 2 of 4 M6 cases, and 4 of 4 M7 cases (Tables 1 and 2).

To further compare the effect of rhTPO with that of other hematopoietic growth factors, AML cells were cultured with rhIL-3, rhIL-6, rhSCF, or rhGM-CSF, all of which had been shown to promote the growth of AML cells. As shown in Tables 1 and 2, rhIL-3, rhIL-6, rhSCF, and rhGM-CSF induced proliferation of AML cells in 41, 22, 28, and 37 of 50 cases, respectively. The proliferative responses to those hematopoietic growth factors varied considerably from case to case, but in most of AML cases except M0 and M7 types rhIL-3 or rhGM-CSF had a more potent activity in stimulating proliferation of AML cells than rhTPO. In addition, it was noted that proliferation of AML cells in the rhTPO-responsive cases could be promoted by one or more different hematopoietic factors (Table 1). As was the case for hematopoietic cell lines, furthermore, rhTPO augmented the rhIL-3-, rhIL-6-, rhSCF-, or rhGM-CSF-induced proliferation of AML cells in a substantial fraction of cases.

DISCUSSION

During the past decade, a large number of hematopoietic growth factor receptors have been isolated and characterized at molecular and functional levels. Among these receptors, c-mpl has been thought to play a role in megakaryopoiesis based on its restricted expression on cells of megakaryocytic lineage as well as hematopoietic stem cells, and also on the selective inhibition of megakaryocyte colony formation by the treatment with c-mpl antisense oligonucleotide. Recent protein purification and gene cloning of the mpl ligand, TPO, provide direct evidence that the interaction between c-mpl and TPO is indispensable for normal megakaryopoiesis and thrombopoiesis. However, the role of c-mpl
Table 1. Expression of c-mpl mRNA in AML and ALL Cells and Their Proliferative Responses to Hematopoietic Growth Factors

<table>
<thead>
<tr>
<th>Case No.</th>
<th>FAB*</th>
<th>Age/Sex</th>
<th>Source</th>
<th>c-mpl mRNA</th>
<th>Proliferative Responses to Growth Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>50/M</td>
<td>PB (+)</td>
<td>37</td>
<td>965</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M0</td>
<td>60/F</td>
<td>BM (-)</td>
<td>15</td>
<td>150</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M0</td>
<td>55/M</td>
<td>PB (+)</td>
<td>10</td>
<td>450</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M1</td>
<td>81/M</td>
<td>PB (+)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M1</td>
<td>53/M</td>
<td>PB (+)</td>
<td>15</td>
<td>150</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M1</td>
<td>42/F</td>
<td>BM (+)</td>
<td>15</td>
<td>150</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M1</td>
<td>75/M</td>
<td>BM (-)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M1</td>
<td>65/M</td>
<td>BM (-)</td>
<td>15</td>
<td>150</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M1</td>
<td>176/F</td>
<td>BM (-)</td>
<td>15</td>
<td>150</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>23/M</td>
<td>BM (+)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>49/N</td>
<td>BM (-)</td>
<td>15</td>
<td>150</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>37/F</td>
<td>PB (+)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>43/F</td>
<td>PB (+)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>57/F</td>
<td>BM (+)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>36/F</td>
<td>BM (+)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>55/M</td>
<td>PB (+)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>64/M</td>
<td>BM (-)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>76/F</td>
<td>BM (-)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
<tr>
<td>M2</td>
<td>76/M</td>
<td>BM (-)</td>
<td>15</td>
<td>200</td>
<td>TPO - IL-3 - SCF - GM-CSF - IL-3 + TPO - IL-6 + TPO - SCF + TPO - GM-CSF + TPO</td>
</tr>
</tbody>
</table>

Abbreviations: BM, bone marrow; PB, peripheral blood.

* Types of leukemia were determined by clinical, morphologic, and cytochemical criteria according to FAB classification.

† (-), no detection; (+), faint expression; (++) intensive expression of P form c-mpl mRNA.

‡ The proliferative responses were measured by an MTT colorimetric assay. The results are shown as the mean (±SD) of replicate cultures. Standard deviation in most of experiments was less than 10% of the mean values.

§ Significant additive effect of TPO (P < .01) when compared with control value by t-test.

|| Significant additive effect of TPO (P < .01) when compared with each growth factor.
Table 2. Summary of c-mpl Expression and Proliferative Responses to Hematopoietic Growth Factors in a Total of 50 AML Cases

<table>
<thead>
<tr>
<th>FAB Class</th>
<th>No. of Cases Screened</th>
<th>c-mpl mRNA No. of Cases</th>
<th>No. of Proliferative Cases to Each Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>3 (+)</td>
<td>2 2 2 1 1 1</td>
<td>TPO IL-3 IL-6 SCF GM-CSF</td>
</tr>
<tr>
<td>M1</td>
<td>6 (+)</td>
<td>3 1 2 1 1 2</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>9 (+)</td>
<td>7 5 5 4 6 6</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>6 (+)</td>
<td>2 1 2 2 0 2</td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>8 (+)</td>
<td>3 2 2 1 1 2</td>
<td></td>
</tr>
<tr>
<td>M5</td>
<td>10 (+)</td>
<td>2 2 2 0 1 2</td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>4 (+)</td>
<td>3 2 3 2 3 3</td>
<td></td>
</tr>
<tr>
<td>M7</td>
<td>4 (+)</td>
<td>1 0 1 0 1 1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50 (+)</td>
<td>26 19 22 12 17 21</td>
<td></td>
</tr>
</tbody>
</table>

- Number of the cases with (+) or without (-) the expression of c-mpl.

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In this study, we found that the c-mpl proto-oncogene was detectable by Northern blot analysis in 26 of 50 AML cases but not in any ALL cases tested. The amount of transcripts varied from case to case, and there was no significant correlation between the amount and expression of c-mpl and FAB classification. This observation was largely consistent with the previous findings by Vigon et al., but in our study c-mpl was found to be detectable in each subtype of AML and all of M7 cases showed positive signal for c-mpl transcript. When AML cells were cultured in the presence of rhTPO, proliferation of AML cells was stimulated with rhTPO alone in 19 of 26 AML cases that expressed c-mpl. Despite only a limited number of cases, furthermore, rhTPO induced proliferation of AML cells in which c-mpl mRNA was detectable by RT-PCR but not by Northern blot analysis. Proliferative response to rhTPO was observed not only in all of M7 cases but also in a substantial fraction of AML cases other than M7. These results suggested that c-mpl may be functional in terms of supporting proliferation of various types of AML cases.

In addition to TPO, a number of hematopoietic growth factors such as IL-3, IL-6, SCF, and GM-CSF were shown to stimulate AML cells as well as megakaryocytes and were suggested to be involved in the growth of myeloid leukemia cells. As compared with rhTPO, proliferation of AML cells was stimulated with significantly higher frequency by rhIL-3 or rhGM-CSF, with slightly higher frequency by rhSCF, and with similar frequency by rhIL-6. The proliferative responses of AML cells to each of these hematopoietic growth factors were neither correlated with c-mpl expression nor with TPO responsiveness. However, the rhIL-3, rhGM-
that action of TPO will be restricted to megakaryocytes and platelets. However, in the case of AML, TPO had a proliferative potential on a wide variety of AML cases other than cases of megakaryocytic (M7) type. In addition, a detailed understanding of TPO effect on other cell types, including very immature hematopoietic stem cells, awaits further studies. A clear and decisive information concerning the effect of TPO on various types of normal and abnormal hematopoietic cells will be necessary to construct novel therapeutic strategies for the treatment of thrombocytopenic patients with TPO.

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