Transforming Growth Factor \( \beta \) Selectively Inhibits Normal and Leukemic Human Bone Marrow Cell Growth In Vitro

By Garwin K. Sing, Jonathan R. Keller, Larry R. Ellingsworth, and Francis W. Ruscetti

The effects of transforming growth factor \( \beta_1 \) or \( \beta_2 \) (TGF-\( \beta_1 \) or -\( \beta_2 \)) on the in vitro proliferation and differentiation of normal and malignant human hematopoietic cells were studied. Both forms of TGF-\( \beta \) suppressed both the normal cellular proliferation and colony formation induced by recombinant human interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF). In the presence of GM-CSF or IL-3, optimal concentrations of TGF-\( \beta \) suppressed both normal and malignant hematopoietic cells. TGF-\( \beta \) significantly reduced colony formation induced by recombinant human interleukin-3 (IL-3) and granulocyte-macrophage colony (CFU-GM) progenitor cells by 90% to 100%, whereas granulocyte or monocyte cluster formation was not inhibited. In contrast, neither form of TGF-\( \beta \) had any effect on G-CSF-induced hematopoiesis. The suppressive action appeared to be mediated directly by TGF-\( \beta_1 \) since antiproliferative responses were also observed in accessory cell-depleted bone marrow cells. In contrast to normal bone marrow cells, both GM- and G-CSF-induced proliferation of cells from patients with chronic myelogenous leukemia were suppressed in a dose-dependent manner by TGF-\( \beta_1 \). Differential effects of TGF-\( \beta \) on the proliferation of established leukemia lines were also observed since most cell lines of myelomonocytic nature studied were strongly inhibited whereas erythroid cell lines were either insensitive or poorly inhibited by TGF-\( \beta \). These results suggest that TGF-\( \beta \) is an important modulator of human hematopoiesis that selectively regulates the growth of less mature hematopoietic cell populations with a high proliferative capacity as opposed to more differentiated cells, which are not affected by TGF-\( \beta \).

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TRANSFORMING growth factor \( \beta \) (TGF-\( \beta \)) is a member of a newly recognized family of polypeptide factors regulating cell growth and differentiation. The polypeptide is a 25-kilodalton disulfide-linked homodimer present in normal as well as transformed cells, with platelets being the major nonneoplastic storage site for the protein. Recent reports describe several functionally and structurally closely related forms of TGF-\( \beta \), including the identities of TGF-\( \beta_1 \) to cartilage-inducing factor A (CIF-A) and TGF-\( \beta_2 \) to cartilage-inducing factor B (CIF-B). Both TGF-\( \beta_1 \) and -\( \beta_2 \) share substantial amino acid homology (70%) and are equally potent in a variety of assay systems. Although TGF-\( \beta \) was originally identified by its ability to reversibly induce certain nonneoplastic cells to express a transformed phenotype and to undergo anchorage-independent growth, it was subsequently shown to have both growth-enhancing and growth-inhibitory properties, the predominant effect being dependent on the particular cell type and other growth factors present. It has also become apparent that TGF-\( \beta \) performs a more generalized function in such processes as embryogenesis, wound healing, bone resorption, and tissue stem cell proliferation. Moreover, TGF-\( \beta \) acts as an important immunomodulatory protein for cells of the immune system by counteracting the effects of interleukin-1 (IL-1) and inhibiting proliferation and Ig secretion of stimulated B cells, interleukin-2 (IL-2)-dependent T-cell proliferation, as well as natural killer cytotoxicity.

There are also certain lines of evidence to suggest that TGF-\( \beta \) may play a role in regulating hematopoiesis that are based on the fact that bone is a rich source of this polypeptide, being abundant in demineralized bone matrix, bone marrow, and fetal liver, which are the primary active sites for hematopoiesis. Recently, we and others have demonstrated that TGF-\( \beta \) inhibits the growth and colony formation of interleukin-3 (IL-3)-dependent normal and leukemic murine bone marrow cells in vitro. This work was therefore undertaken to investigate the effects of TGF-\( \beta_1 \) and -\( \beta_2 \) on the growth and differentiation of normal human hematopoietic cells and their leukemic counterparts.

MATERIALS AND METHODS

Cells
Bone marrow cells were obtained from healthy donors who had given informed consent. The mononuclear cells were isolated by centrifugation on Ficoll-Hypaque gradients, washed twice in phosphate-buffered saline, and suspended in Iscove’s modified Dulbecco’s medium (IMDM) containing 10% fetal calf serum (FCS) supplemented with penicillin (100 U/mL), streptomycin (100 \( \mu \)g/mL), and 3 mg/mL glutamine. In some experiments using partially enriched bone marrow cells, adherent cells were depleted as described elsewhere, while T cells were removed by E rosette formation. Cells purified in this manner contained less than 5% CD3+ cells as determined by immunofluorescent staining, while staining with nonspecific esterase showed the percentage of monocytic-macrophages to be depleted by 95%.

Leukemic cells were obtained from the peripheral blood of five patients with chronic myelogenous leukemia (CML) and nucleated, low-density cells were purified according to the methods described earlier. Cells from these patients contained more than 90% leukemic
cells as determined by morphology and by the presence of the Philadelphia chromosome. The leukemic cell lines used in this study were of the erythroid (OC-1, K562, and HEL), myeloid (KG-1 and HL60), and monocytic (U937 and THP-1) lineages, respectively. The cells were maintained in Falcon tissue culture flasks in RPMI 1640 (Advanced Biotechnologies, Silver Spring, MD) supplemented with 10% FCS, penicillin (100 U/mL), streptomycin (100 μg/mL), and 3 mg/mL glutamine. Cell viability was determined by examining cells in 0.4% trypan blue (GIBCO, Chagrin Falls, OH), while cell numbers were determined with a Coulter counter (Coulter Electronics, Hialeah, FL).

Reagents
Recombinant human granulocyte-macrophage colony-stimulating factor (rHuGM-CSF) and human IL-3 was obtained from Immunix Corp (Seattle) and recombinant human G-CSF from Amgen Corp (Thousand Oaks, CA). Stock solutions (10 μg/mL) were stored at 4°C and diluted in tissue culture medium containing 10% FCS for use. Human recombinant erythropoietin (Epo) was purchased from Amgen and was stored at -20°C before use. Bovine TGF-β1 (CIF-A) and TGF-β2 (CIF-B) were extracted from de-mineralized bone as described previously. The identity of these two bone-derived factors to platelet-derived TGF-β1 and -β2 has already been described. The purity of both preparations was established by analysis on sodium dodecyl sulfate–polyacrylamide gel electrophoresis and were free of endotoxin. Stock solutions were stored in 0.1 mmol/L HCl at -70°C at 1 mg/mL. For comparison, recombinant TGF-β1 and platelet-derived TGF-β2 were used in some experiments and were obtained from Oncogen Corp (Seattle) and R&D Systems (Minneapolis), respectively.

For antibody neutralization studies, affinity-purified rabbit neutralizing antibody to the N-terminus of TGF-β1 was used. This antibody blocks TGF-β1– but not TGF-β2–induced responses.

Colony Assays
**CFU-GEMM and BFU-E.** The colony assay for human CFU-GEMM and BFU-E was performed as described previously. Bone marrow cells were plated at 10³ in 35-mm Lux standard tissue culture dishes containing a 1-mL mixture of IMDM, 0.35% Sea-plaque agarose (FMC Bioproducts, Rockland, ME), 30% FCS, 1% detoxified bovine serum albumin (Sigma Chemical Co, St Louis), 2 U/mL Epo, 2 x 10⁻⁴ mol/L hemin, and a predetermined optimal concentration of rHuG-CSF (10 ng/mL) or IL-3 (10 U/mL). Dishes were incubated at 37°C in a humidified atmosphere flushed with 5% CO₂ in air. Colonies were scored with an inverted microscope after 14 days of incubation, and the colony- or cluster-bearing agarose layers were occasionally fixed with 3% glutaraldehyde in methanol and stained with benzidine and/or Jenner-Giemsa for morphologic analysis. For cytochemistry, fixed agarose layers were stained for alphanaphthyl acetate esterase or naphthol AS-D chloroacetate esterase according to routine hematologic methods.

**CFU-GM.** Colony (>50 cells/aggregate) and cluster (three to 50 cells/aggregate) formation of bone marrow cells was stimulated by a predetermined optimal dose of IL-3 or rHuGM- or G-CSF (10 ng/mL). Cells were plated at 10³ in 1 mL 0.35% agarose culture medium as described earlier except that Epo and hemin were omitted. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air and scored for colonies and/or clusters after 14 days of incubation.

**DNA Synthesis**
IL-3, GM- or G-CSF–treated cells were incubated with varying dilutions of TGF-β in multiwell tissue culture plates in a humidified atmosphere with 5% CO₂. Cells were cultured at 10⁴ cells/well in triplicate for a period of five days when maximum proliferation in the presence of CSF alone was obtained, and 1 μCi methyl ³H-thymidine (New England Nuclear, Boston; 6.7 Ci/mmol) was added to each well for the last 16 hours of culture. Cells were harvested with a Skatron cell harvester, and isotope uptake was determined by liquid scintillation counting. The effect of TGF-β on the proliferation of leukemic cell lines was assayed in a similar manner except that the cells grew in the absence of CSF, were harvested after 72 hours of culture, and were pulsed with ³H-thymidine for the last six hours of culture.

RESULTS
**Effects of TGF-β1 or -β2 on CSF-Induced Proliferation of Normal Human Bone Marrow Cells**
To determine whether TGF-β1 or β-2 could inhibit CSF-induced proliferation of hematopoietic progenitors, normal human bone marrow cells were cultured with GM- or G-CSF (10 ng/mL) and varying concentrations of TGF-β1 or -β2. The cells were cultured for five days and pulsed with ³H-thymidine. As shown in Fig 1, GM-CSF–induced cell proliferation was inhibited by both forms of TGF-β in a dose-dependent manner, with maximal inhibition occurring at concentrations greater than 1 ng/mL and an ED of approximately 0.2 to 0.3 ng/mL (8 to 12 pmol/L). Nevertheless, complete suppression of proliferation could not be obtained even at concentrations of up to 100 ng/mL, thus indicating the presence of a population of cells insensitive to the suppressive effects of TGF-β. Similar results were obtained with recombinant TGF-β1 or platelet-derived TGF-β2 (results not shown), thus demonstrating that the
biologic activities of TGF-β derived from different sources are identical.

When recombinant human IL-3 was used as the growth factor (10 U/mL), both forms of TGF-β suppressed bone marrow cell proliferation in a similar dose-dependent manner, with an ED$_{50}$ of approximately 0.2 ng/mL (8 pmol/L). Maximum inhibition was obtained with concentrations greater than 1 ng/mL, with TGF-β1 suppressing proliferation by 40% and TGF-β2 suppressing proliferation by 34%. Thus although the concentration of TGF-β required for half-maximal activity was similar for both forms, the degree of maximal suppression of GM-CSF- and IL-3-induced proliferation exerted by TGF-β1 was consistently 10% to 15% greater than that exerted by TGF-β2.

In contrast to GM-CSF and IL-3, G-CSF–driven proliferation was unaffected by either form of TGF-β at the physiologic concentrations shown to be inhibitory for GM-CSF–induced growth (Fig 1). Since hematopoietic cells responsive to stimulation by G-CSF are not necessarily responsive to the action of GM-CSF and since only 60% of GM-CSF–induced proliferation was inhibited, this suggests that not all progenitor cells in the bone marrow are equally sensitive to the antiproliferative effects of TGF-β.

**Effects of TGF-β1 or -β2 on Human Hematopoietic Colony Formation**

To study the effects of TGF-β1 or -β2 on the clonal proliferation and differentiation of hematopoietic cell progenitors, colony assays were used to determine the extent of inhibition of particular cell lineages. As shown in Fig 2, a dose-dependent inhibition of colony formation by CFU-GM, BFU-E, and CFU-GEMM was again seen for both forms of TGF-β. Interestingly, although colony formation by CFU-GM was almost completely inhibited by maximal concentrations of TGF-β1 or -β2, cluster formation was still observed at all concentrations of TGF-β used (Table 1). Direct microscopic examination of the cellular composition within the CFU-GM colonies or clusters from entire plates showed the presence of homogenous populations of either myelocytes and metamyelocytes or monocyte/macrophages, whereas neither mixed clusters nor colonies of granulocytes and monocyte/macrophages were seen, unlike the control cultures (Table 1). Furthermore, unlike day 14 CFU-GM, colony formation by day 7 CFU-GM, which are generally regarded to be more mature myeloid progenitors than are day 14 CFU-GM, were not significantly inhibited by TGF-β1 (Table 2). Thus multipotent or bipotent progenitor cells were sensitive to the suppressive effects of TGF-β1 or -β2 as opposed to the more differentiated unipotent progenitor cell, which was insensitive to TGF-β.

The addition of either form of TGF-β to cells cultured in the absence of CSF neither increased nor decreased the level of background colony formation, thus indicating that TGF-β alone exerted no effects on unstimulated cells (Tables 1 and 2). Furthermore, as in the proliferation studies, neither form of TGF-β had any effect on the development of G-CSF–induced colony formation (Table 1). Therefore, since there were no basic differences between the antiproliferative effects of TGF-β1 and -β2 other than slight dosage differ-
TGF-β INHIBITION OF HUMAN HEMATOPOIESIS

Table 1. Effect of TGF-β1 on the Colony Growth of CFU-GEMM, BFU-E, and CFU-GM From Normal Human Bone Marrow in Response to IL-3 or GM- or G-CSF

<table>
<thead>
<tr>
<th>CSF*</th>
<th>±TGF-β1†</th>
<th>CFU-GEMM</th>
<th>BFU-E</th>
<th>CFU-G</th>
<th>CFU-M</th>
<th>CFU-GM</th>
<th>Clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 ± 1</td>
<td>7 ± 1</td>
<td>5 ± 3</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>0</td>
<td>10.0</td>
<td>0</td>
<td>0</td>
<td>3 ± 1</td>
<td>7 ± 1</td>
<td>5 ± 3</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>GM</td>
<td>0</td>
<td>9 ± 3</td>
<td>43 ± 8</td>
<td>68 ± 22</td>
<td>20 ± 6</td>
<td>12 ± 5</td>
<td>75 ± 43</td>
</tr>
<tr>
<td>GM</td>
<td>0.1</td>
<td>4 ± 1</td>
<td>31 ± 3</td>
<td>64 ± 12</td>
<td>25 ± 9</td>
<td>6 ± 1</td>
<td>65 ± 12</td>
</tr>
<tr>
<td>GM</td>
<td>1.0</td>
<td>1 ± 1</td>
<td>3 ± 1</td>
<td>20 ± 8</td>
<td>2 ± 1</td>
<td>0</td>
<td>72 ± 19</td>
</tr>
<tr>
<td>GM</td>
<td>10.0</td>
<td>0</td>
<td>1 ± 1</td>
<td>2 ± 1</td>
<td>3 ± 2</td>
<td>0</td>
<td>76 ± 27</td>
</tr>
<tr>
<td>IL-3</td>
<td>0</td>
<td>8 ± 2</td>
<td>37 ± 7</td>
<td>9 ± 1</td>
<td>5 ± 1</td>
<td>1 ± 1</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>IL-3</td>
<td>0.1</td>
<td>2 ± 1</td>
<td>26 ± 4</td>
<td>2 ± 1</td>
<td>6 ± 1</td>
<td>1 ± 1</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>IL-3</td>
<td>1.0</td>
<td>0</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
<td>0</td>
<td>0</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>IL-3</td>
<td>10.0</td>
<td>0</td>
<td>5 ± 1</td>
<td>1 ± 1</td>
<td>0</td>
<td>0</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>35 ± 6</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
<td>29 ± 8</td>
</tr>
<tr>
<td>G</td>
<td>0.1</td>
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<td>51 ± 6</td>
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<td>31 ± 12</td>
</tr>
<tr>
<td>G</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>49 ± 7</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>23 ± 6</td>
</tr>
<tr>
<td>G</td>
<td>10.0</td>
<td>0</td>
<td>0</td>
<td>48 ± 5</td>
<td>4 ± 1</td>
<td>1 ± 1</td>
<td>37 ± 5</td>
</tr>
</tbody>
</table>

Results are expressed as the mean number of colonies ± SD per 10^5 cells of duplicate cultures from five experiments for GM- and G-CSF and two experiments for IL-3.

*10 ng/mL for GM-CSF and G-CSF and 10 U/mL IL-3.
†Concentration of TGF-β1 expressed as ng/mL.

Effects of Accessory Cell Depletion on TGF-β1 Activity.

To determine whether the suppressive influences of TGF-β1 were due to a direct effect on progenitor cells or whether it involved the recruitment of accessory cells, the effects of TGF-β1 were assessed on nonadherent low-density human bone marrow cells from which T lymphocytes and adherent monocytes and mature polymorphonuclear leukocytes were depleted. As shown in Table 3, suppression of colony formation was comparable to that obtained with unseparated nucleated bone marrow cells except that more progenitor cells were present per 10^5 cells plated due to the removal of accessory cells. Furthermore, the addition of anti-TGF-β1 antibody effectively neutralized the antiproliferative effects of TGF-β1 (Fig 3). Since we and others have shown that this antibody specifically blocks TGF-β1 but not TGF-β2 activity, this provides further evidence that the suppressive effect was specifically mediated by TGF-β1.

Effects of TGF-β1 on GM- or G-CSF–Induced Proliferation of CML

To test the effects of TGF-β on the proliferation of leukemic cells, cells from five patients with untreated CML were cultured with 10 ng/mL of GM- or G-CSF in the presence or absence of TGF-β1 as before. As shown in Fig 4, GM-CSF–induced proliferation of leukemic cells was inhibited by TGF-β1 in a dose-dependent manner, and cell proliferation was maximally inhibited by 45% at TGF-β1 concentrations of >5 ng/mL. It could be demonstrated that the cells responding to GM-CSF and TGF-β1 were predominantly leukemic myeloid progenitor cells and not peripheral blood cells since normal peripheral blood cells do not respond significantly to GM-CSF (results not shown) and since >90% of the GM-CSF–responsive cells contained the Philadelphia chromosome as determined by karyotype analysis (data not shown). In contrast to normal bone marrow cells, the proliferation of CML cells in response to G-CSF was also inhibited by TGF-β1 (Fig 4) in a dose-dependent fashion, with optimal concentrations of TGF-β1 suppressing proliferation by approximately 40%. Thus G-CSF–responsive leukemic cells differ from normal cells in their sensitivity to TGF-β.

The influences of TGF-β1 on GM-CSF–induced colony formation by CML cells were also studied in the colony assay. First, to determine the level of background colony formation, leukemic progenitor cells were cultured in the absence of GM-CSF with or without TGF-β1 and shown to form an average of 35 clusters irrespective of whether TGF-β1 was present (Table 4). The clusters consisted of myeloid or monocytic progenitor cells in various stages of

Table 2. Effect of TGF-β1 on the Growth of Day 7 and Day 14 CFU-GM in Response to GM-CSF

<table>
<thead>
<tr>
<th>GM-CSF*</th>
<th>TGF-β1†</th>
<th>Day 7 CFU-GM</th>
<th>Day 14 CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>15 ± 5</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>0</td>
<td>10.0</td>
<td>15 ± 5</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>25 ± 2</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>+</td>
<td>10.0</td>
<td>33 ± 7</td>
<td>46 ± 6</td>
</tr>
</tbody>
</table>

Results are expressed as the mean number of colonies ± SD per 10^5 cells of duplicate cultures from two experiments.

*GM-CSF, 10 ng/mL.
†TGF-β, 10 ng/mL.

Table 3. Influence of Accessory Cells on TGF-β1 Suppression

<table>
<thead>
<tr>
<th>Target Cells</th>
<th>±TGF-β1†</th>
<th>CFU-GEMM</th>
<th>BFU-E</th>
<th>CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light-density BMC</td>
<td>–</td>
<td>8 ± 3</td>
<td>27 ± 11</td>
<td>82 ± 30</td>
</tr>
<tr>
<td>Nonadherent and</td>
<td>–</td>
<td>8 ± 2</td>
<td>43 ± 15</td>
<td>107 ± 59</td>
</tr>
<tr>
<td>T-cell-depleted BMC</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>11 ± 9</td>
</tr>
</tbody>
</table>

Numbers of colonies derived from CFU-GEMM, BFU-E, or CFU-GM of duplicate cultures are expressed as the mean ± SD of two experiments.

Abbreviation: BMC, bone marrow cells.

*10 ng/mL.
differentiation, and differences between TGF-β1−treated
and control clusters were not seen. The addition of GM-CSF
stimulated the formation of large colonies containing >100
cells, and morphologic examination of these colonies demon-
strated mixed populations of early and late progenitor cells.
However, the addition of TGF-β1 resulted in a significant
reduction in colony number and size, with TGF-β1 at
concentrations of 2.5 ng/mL or more suppressing colony
formation by 40%, while the colonies that formed were
significantly smaller in size (less than 70 cells), were devoid
of early progenitor cells, and consisted either of myeloid or
monocytic cells at late stages of differentiation (Table 4).
Unlike controls, no mixed colonies containing monocytes and
granulocytes were seen following treatment with TGF-β.
Similar results were observed in cells from all five CML
patients, which suggests that TGF-β1 inhibits the develop-
ment of bipotent as opposed to unipotent colony-forming
progenitor cells.

Inhibition of Leukemic Cell Lines

To determine whether TGF-β1 showed any selectivity
with regard to lineage specificity, leukemic cells lines derived
from the myelocytic, monocytic, and erythrocytic series were
subjected to treatment with TGF-β1. A differential response
was seen in that sensitivity to inhibition by TGF-β1 was
predominantly displayed by cells of the myeloid-monocytic
series (Table 5). Of the three erythroid cell lines examined,
two were not suppressed by TGF-β1 (K562 and OC-1), while
the proliferation of HEL was inhibited by about 20%. However,
this line has also been reported to express characteris-
tics associated with nonerythroid lineages.31 In contrast,
the monocytic cell lines U937 and THP-1 were both strongly
inhibited by TGF-β1. With regard to the myeloid cell lines,
TGF-β1 strongly inhibited the proliferation of the myelo-
blast line KG-1, while the more differentiated promyelocyte
line HL60 was unaffected, again supporting the contention
that sensitivity to TGF-β1 is dependent on the cellular state
of differentiation.

DISCUSSION

It is widely believed that the control of hematopoiesis is
accomplished by appropriate combinations of positive and
negative influences. Although much information has accu-
ulated concerning positive regulators such as the CSFs,32,34

Table 5. Effects of TGF-β on the Proliferation of Myeloid
Leukemic Cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Effects of TGF-β1</th>
<th>ED50 (pmol/L)</th>
<th>Maximum Inhibition*</th>
<th>pmol/L TGF-β (Maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U937</td>
<td>I</td>
<td>24</td>
<td>60</td>
<td>400</td>
</tr>
<tr>
<td>THP-1</td>
<td>I</td>
<td>24</td>
<td>48</td>
<td>400</td>
</tr>
<tr>
<td>KG-1</td>
<td>I</td>
<td>14</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>HL60</td>
<td>NE</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>OC-1</td>
<td>NE</td>
<td>—</td>
<td>—</td>
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<tr>
<td>HEL</td>
<td>I</td>
<td>24</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>K562</td>
<td>NE</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Triplicate cultures of leukemic cells (10^6) were established in microtiter
plates with or without TGF-β added at various concentrations and incubated for 72 hours. Proliferation was determined by ³H-thymidine
uptake.

Abbreviations: I, inhibited; NE, no effect.

*Percent maximum inhibition is calculated as [(1 — test counts per minute)/control counts per minute] × 100.
the possible involvement of inhibitory factors that may limit hematopoiesis has remained a controversial issue, although some potential candidates have now been identified. These include cytokines such as tumor necrosis factor \(^{35,36}\) and the interferons \(^{37,38}\).

Much of the current interest in TGF-\(\beta\) research reflects its importance as a negative growth regulator for epithelial cells \(^{21-23}\) and for cells of mesenchymal origin such as lymphocytes \(^{21-23}\). Although TGF-\(\beta\) has been found in a wide variety of tissues, bone and platelets represent the main reservoirs of TGF-\(\beta\) in the body, which suggests that it may play an important role in the maintenance of homeostasis during hematopoiesis. The results presented here demonstrate that just as in the murine system, TGF-\(\beta1\) and -\(\beta2\) are potentially important regulators of hematopoietic progenitor cell growth in humans. Specifically, both TGF-\(\beta1\) and -\(\beta2\) inhibit GM-CSF- and IL-3-induced proliferation and colony growth in soft agar of normal bone marrow cells and their leukemic counterparts (Figs 1, 2, and 4; Tables 1, 2, and 4). Furthermore, there are several lines of evidence to suggest that TGF-\(\beta\) exerts a differential effect on hematopoiesis, being more suppressive for progenitor cells of earlier as opposed to later stages of differentiation. First, in the presence of GM-CSF or IL-3, TGF-\(\beta\) was found to be suppressive for early multipotential or bipotential colony-forming cells with a high proliferative capacity such as CFU-GEMM, BFU-E, or CFU-GM, whereas the formation of clusters of differentiated myeloid cells of single lineage were unaffected by TGF-\(\beta\) (Table 1). Second, whereas TGF-\(\beta1\) had no significant effect on the formation of day 7 colony formation by CFU-GM, the formation of colonies by day 14 CFU-GM was potently suppressed (Table 2). Since the former population is generally regarded as being composed of more mature progenitor cells than the latter is, this supports our contention that TGF-\(\beta1\) exerts its effects on immature as opposed to mature progenitor cells. Third, unlike normal progenitor cells cultured with GM-CSF, the proliferation and colony formation of G-CSF-responsive cells is insensitive to the suppressive influences of TGF-\(\beta\) (Fig 1, Table 1). Since G-CSF is distinguished by its ability to stimulate the development of mature granulocytic colonies in soft agar, it can be assumed that G-CSF-responsive cells are more differentiated than multipotential GM-CSF-responsive cells.

A similar pattern of responsiveness can be seen with CML cells cultured with GM-CSF and TGF-\(\beta\) (Table 4). While CML progenitors still formed small colonies or clusters despite the presence of TGF-\(\beta\), these were composed primarily of homogenous populations of myelocytes or monocytes. This is in contrast to the larger colonies that developed in the presence of GM-CSF alone, some of which were composed of a mixture of myelocytic and monocytic progenitors (Table 4), again suggesting that the suppressive influences of TGF-\(\beta\) are directed at early (bipotential) as opposed to late (unipotent) colony-forming cells. Among the myeloid leukemic cell lines, TGF-\(\beta\) was found to inhibit the proliferation of the myeloblastic cell line KG-1 but not of the more differentiated promyelocytic line HL60 (Table 5), again suggesting that sensitivity to TGF-\(\beta\) is related to the maturation state of the cell. Nevertheless, more conclusive evidence to support these data requires the demonstration that TGF-\(\beta\) suppresses the growth of primitive stem cells. Such experiments are currently being performed by using bone marrow enriched for My10 + cells, although we do have evidence to show that in the mouse system TGF-\(\beta\) inhibits the development of the high proliferative potential colony-forming cell, which is the earliest measurable progenitor cell at this time (J.R. Keller, personal communication).

The data presented in this report clearly demonstrate that TGF-\(\beta\) is a potent inhibitor of hematopoietic cell growth. The specificity of this reaction is clearly demonstrated by the fact that TGF-\(\beta1\)-induced suppression is abrogated by the addition of antibodies specifically raised to TGF-\(\beta1\) but not -\(\beta2\) (Fig 3) and that similar results were obtained when purified TGF-\(\beta\) derived from different sources was used. Nevertheless, there is a possibility that the suppressive influences are indirect and are mediated via accessory cells. However, bone marrow cells depleted of adherent and lymphocytic accessory cells displayed the same pattern of suppression as unseparated cells following treatment with TGF-\(\beta\) (Table 3). This would argue against the involvement of accessory cells in mediating suppression.

There is evidence to suggest that TGF-\(\beta\) acts as a negative regulator of cell growth via a paracrine or autocrine mechanism in breast cancer, \(^{39}\) which raises the possibility that escape from normal growth control and subsequent development of neoplasia could result from a decreased production of TGF-\(\beta\) or the reduced expression of receptors for the negative regulator. In the case of CML cells and most of the myeloid leukemic cell lines examined, our studies would suggest that escape from negative regulation does not involve a lack of sensitivity to TGF-\(\beta\) since these cells were equally suppressed by TGF-\(\beta\) as normal progenitor cells (Fig 4, Table 4). However, escape from negative regulation might arise from an inability to process TGF-\(\beta\) into an active form since it has been demonstrated that freshly secreted TGF-\(\beta\) is inactive and exists in a latent form unless activated through enzymatic or nonenzymatic pathways. \(^{1}\) Nevertheless, cells of the erythroid lineage do show a differential responsiveness to TGF-\(\beta\) in that BFU-E formation by normal bone marrow progenitors is strongly inhibited by TGF-\(\beta\), whereas most erythroblastic cells are unaffected (Table 5). We are currently investigating whether this lack of response lies at the level of receptor expression since all cell types responding to TGF-\(\beta\) examined thus far have demonstrable receptors for TGF-\(\beta\) on their surfaces. \(^{7}\)

The results presented in this report correlate with those described by previous workers for the mouse system \(^{24-26}\), although Ohta et al\(^{26}\) consistently failed to detect suppressive activity by TGF-\(\beta2\) even at concentrations of up to 1 nmol/L; neither were significant receptors for TGF-\(\beta2\) seen on the cell surface. The reason for this discrepancy is not known since we have detected similar suppressive activities for both forms of TGF-\(\beta\) irrespective of their source, and we have also found similar activities in the mouse system. \(^{26}\) It is possible that this discrepancy is due to the source of TGF-\(\beta2\) used by Ohta et al or to differences in the cells used for the study.

In conclusion, we have demonstrated that TGF-\(\beta\) is a differential rather than a general regulator of human hema-
topoiesis that inhibits the growth of early as opposed to late progenitor cells. However, the physiologic significance of these observations remains unclear. If TGF-β were to limit the differentiation and proliferation of committed progenitor cells, this could provide a homeostatic mechanism whereby the production of fully differentiated hematopoietic cells is controlled while the pool of primitive multipotential stem cells is maintained.

It is not known why TGF-β inhibits the proliferation of CML cells but not normal bone marrow cells in response to G-CSF, although it may merely be a reflection of the leukemic nature of the former population. Nevertheless, this observation raises the possibility that combinations of G-CSF and TGF-β might be used following various forms of cancer therapy whereby the population of normal hematopoietic cells is preferentially expanded by G-CSF, provided G-CSF does not play a role in early hematopoietic cell development.

Finally, it is also possible that TGF-β could protect normal bone marrow progenitor cells from the toxic effects of chemotherapeutic agents during treatment of nonhematologic tumors. Such potential therapeutic applications of TGF-β are under investigation.

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