

TRANSFORMING GROWTH FACTOR  $\beta$ 1 SELECTIVELY  
REGULATES EARLY MURINE HEMATOPOIETIC  
PROGENITORS AND INHIBITS THE GROWTH OF  
IL-3-DEPENDENT MYELOID LEUKEMIA CELL LINES

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The identification of stimulatory factors for hematopoietic cells has greatly enhanced our understanding of the regulation of hematopoiesis (1, 2). While these factors augment cellular proliferation and differentiation, less is known about those factors that might be involved in either negative growth regulation or the regulation of homeostasis of hematopoiesis. A new family of polypeptide growth factors that regulate cell growth and differentiation has recently been identified which includes transforming growth factor type  $\beta$  (TGF- $\beta$ ),<sup>1</sup> inhibin, Müllerian inhibitory substance, and decapentaplegic gene complex (3). TGF- $\beta$  activity was originally identified in the supernatants of transformed fibroblasts by its ability to induce the anchorage-independent growth (transformed phenotype) of NRK 49F fibroblasts in soft agar (4, 5). TGF- $\beta$  was initially purified to homogeneity from human platelets (6) and was subsequently found to be produced by many normal tissues (7). TGF- $\beta$  is a 25-kD disulfide-linked homodimeric protein whose sequence is highly conserved, with a single amino acid difference between mouse and man (8). Recently, a second form of TGF- $\beta$  was identified in bovine bone called CIF-B (9), now known as TGF- $\beta$ 2, that shares significant homology to the first type of TGF- $\beta$ , now known as TGF- $\beta$ 1 (10). TGF- $\beta$ 1 has been shown to be multifunctional in its influence on cellular proliferation by also acting as a growth inhibitor of many different cell types including hepatocytes, keratinocytes and embryo fibroblasts (7, 11).

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<sup>1</sup> *Abbreviations used in this paper:* Epo, erythropoietin; GEMM, granulocyte, erythroid, megakaryocyte, and macrophage colonies; GM-CSF, granulocyte/macrophage colony-stimulating factors; TGF-B, transforming growth factor B.

In cells of the immune system, TGF- $\beta$ 1 has been shown to have antiproliferative effects on both IL-2-dependent T lymphocyte proliferation (12) as well as IL-1-mediated thymocyte proliferation (13). In other immunocompetent cells, TGF- $\beta$ 1 suppresses the secretion of Ig by B cells (14) and blocks the cytotoxic activity of NK cells (15).

Immunohistochemical studies using antibodies to the NH<sub>2</sub> terminus of TGF- $\beta$ 1 demonstrated that TGF- $\beta$ 1 is locally produced by cells in centers of active hematopoiesis (fetal liver and bone marrow) and lymphopoiesis (Hassall's corpuscles) (16). In addition, the relative yield of TGF- $\beta$ 1 from normal tissue varies 100-fold, with the presence of large amounts in cortical bone and human platelets (9, 17), suggesting that the biological significance of TGF- $\beta$ 1 in vivo may be in tissue repair and bone formation (7, 18).

Since TGF- $\beta$  is produced in local areas of hematopoiesis, it may play a role in regulating hematopoietic cell growth and differentiation. Thus, experiments were designed to examine the effects of TGF- $\beta$ 1 on normal murine hematopoietic progenitors as well as their leukemic counterparts including both IL-3-dependent and -independent myeloid leukemic cells.

## Materials and Methods

*Animals.* BALB/c mice were obtained from the animal production area of the NCI-Frederick Cancer Research Facility (Frederick, MD).

*Growth Factors.* Murine IL-3 was purified to homogeneity from WEHI-3 cell supernatants using previously described procedures (19). Purified recombinant murine granulocyte/macrophage colony-stimulating factor (GM-CSF) was supplied by Immunex Corporation, Seattle, WA. Purified human recombinant granulocyte (G)-CSF was supplied by Lawrence Souza, Amgen Corp. Purified murine recombinant IL-4 (IL-4/BSF-1) was purchased from Genzyme Corp., Boston, MA. Human urinary erythropoietin (Epo) was purchased from Amgen Corp. Murine CSF-1 was obtained from the conditioned media of L929 cells. Bovine TGF- $\beta$ 1 was purified to homogeneity using previously described procedures (9). Antiserum to TGF- $\beta$ 1 has been previously characterized (16) and was used as described in the figure legends.

*Cell Lines.* The cell lines FDC-P1 (20), 32D-cl23 and B6SutA (21) were derived from long-term in vitro mouse bone marrow cultures, while NFS-60 and DA-3 (22) were derived from the preleukemic spleen from mice infected with murine leukemia viruses. These lines represent phenotypically early hematopoietic progenitors by morphology, cytochemistry, and surface markers. The other cell lines were maintained in RPMI 1640 in 10% FCS and included DS19 (23), TP3 (24), P388D1, J774A.1, WEHI-3B, and P815 obtained from the American Type Culture Collection, Rockville, MD.

*Bone Marrow Proliferation Assay.* Bone marrow was aspirated from the femurs of BALB/c mice with RPMI 1640, then washed twice and resuspended in RPMI 1640 containing 10% FCS and seeded into 96-well microtiter plates (Costar, Cambridge, MA) at  $10^5$  cells in 100  $\mu$ l of medium. Proliferation was assessed after 72 h of incubation at 37°C in 5% CO<sub>2</sub> with an overnight pulse of 1  $\mu$ Ci [<sup>3</sup>H]TdR (6.7 Ci/mmol) (New England Nuclear, Boston, MA). Cell cultures were harvested with a multiple sample harvester onto glass fiber filters. Individual filters were placed in 2 ml of PCS scintillation fluid (Amersham Corp., Arlington Heights, IL) and radioactivity was assessed by liquid scintillation counting.

*Cell Proliferation Assays.* IL-3-dependent or -independent cell lines used in proliferation assays were washed twice in serum-free RPMI 1640 and plated into 96-well microtiter plates at a density of  $10^4$  cells in 100  $\mu$ l of RPMI 1640 containing 5% FCS, unless otherwise indicated. Cultures were incubated 48 h at 37°C, 5% CO<sub>2</sub> with a 6-h pulse of [<sup>3</sup>H]TdR and harvested as described above for bone marrow.

*Splenocyte Proliferation Assay.* Spleen cells from normal or phenylhydrazine hydrochloride treated mice, and splenocytes from mice infected with the polycythemia-inducing strain of

the spleen focus forming virus (SFFV) were obtained as previously described (25). Cells were cultured in the presence or absence of Epo at 37°C at a concentration of  $4 \times 10^6$  cells/ml in 0.1 ml of RPMI 1640, 10% FCS, 0.1 mM 2-ME for 24 h with a 2-h pulse of [<sup>3</sup>H]TdR, and they were harvested as described above for the bone marrow.

*Soft Agar Colony Formation.* A modification of the method of Stanley et al. (26) was used to measure colony formation of bone marrow cells. Briefly, BALB/c bone marrow cells were suspended in 1 ml RPMI 1640, 10% FCS, 0.1 mM 2-ME in 0.3% Seaplaque agarose (Rockland, ME) with a source of CSF and incubated in 35-mm Lux petri dishes (Miles Laboratories Inc., Naperville, IL) at 37°C in 5% CO<sub>2</sub> and scored for colony growth (CFU) at the indicated times. For GM colony numbers, all colonies >50 cells are counted. For the growth of multipotential colonies, granulocyte, erythroid, megakaryocyte, and macrophage (CFU-GEMM), cultures were additionally supplemented with hemin and Epo. In the CFU-GEMM assay, only the colonies that contain all four lineages are counted, even though other colonies are present. For the formation of erythroid colonies, CFU-E, cells were cultured for 2 d in a plasma clot system (27) in the presence of 0.5 U/ml Epo and the clots were stained with benzidine and examined microscopically for benzidine-positive colonies.

## Results

*Effect of TGF-β1 on Factor-induced Murine Bone Marrow Proliferation.* Normal bone marrow cells are known to proliferate in the presence of a number of growth factors. IL-3 promotes the differentiation of multipotent stem cells as well as more lineage-restricted hematopoietic progenitors, while other growth factors such as G-CSF, GM-CSF, and Epo are known to act upon cells later in the various hematopoietic lineages and exhibit relatively restricted lineage specificity (1, 2). To test the effects of TGF-β1 on the proliferation of bone marrow cells in the presence of various factors, freshly aspirated cells were used in a standard proliferation assay. As shown in Fig. 1, IL-3-driven proliferation of bone marrow cells was inhibited by TGF-β1 in a dose-dependent manner with an ED-50 of 0.05 ng/ml (2 pM). The same effect was observed whether the assay was performed with a low dose of IL-3 (5 U/ml) or saturating amounts of IL-3 (50 U/ml). Kinetic experiments demonstrated that maximum inhibition was observed after the cells had been incubated for 48 h with TGF-β1. The antiproliferative effects were not due to cytotoxicity since TGF-β1 did not affect the viability of cultured bone marrow cells (data not shown).

In contrast, TGF-β1 showed no effects on G-CSF- or GM-CSF-driven proliferation in the presence of increasing concentrations of TGF-β1 (Fig. 1). Thus, TGF-β1 showed a differential effect on CSF-induced normal bone marrow proliferation. In addition, not all of the bone marrow proliferation stimulated by IL-3 is inhibitable by TGF-β1.

*Effect of TGF-β1 on Murine Hematopoietic Colony Formation.* Under conditions of limiting cell number, soft agar assays provide a convenient means to measure the clonal proliferation as well as the differentiation potential of hematopoietic progenitors. Therefore, soft agar colony assays were used to determine the extent of TGF-β1 inhibition within particular cell lineages. TGF-β1 suppressed IL-3-induced CFU-GM colony formation using 10 ng/ml TGF-β1, a concentration that maximally inhibited [<sup>3</sup>H]TdR incorporation in the bone marrow proliferation assay (Table I). While overall colony formation was consistently inhibited by TGF-β, small clusters (<20 cells) and a few colonies were also consistently observed. This effect was observed when 5 U/ml or 50 U/ml of IL-3 was used. TGF-β1 inhibition of IL-3-induced CFU-GM colonies occurred in a dose-dependent manner with an ED-50 of 5–10 pM (Fig. 2).

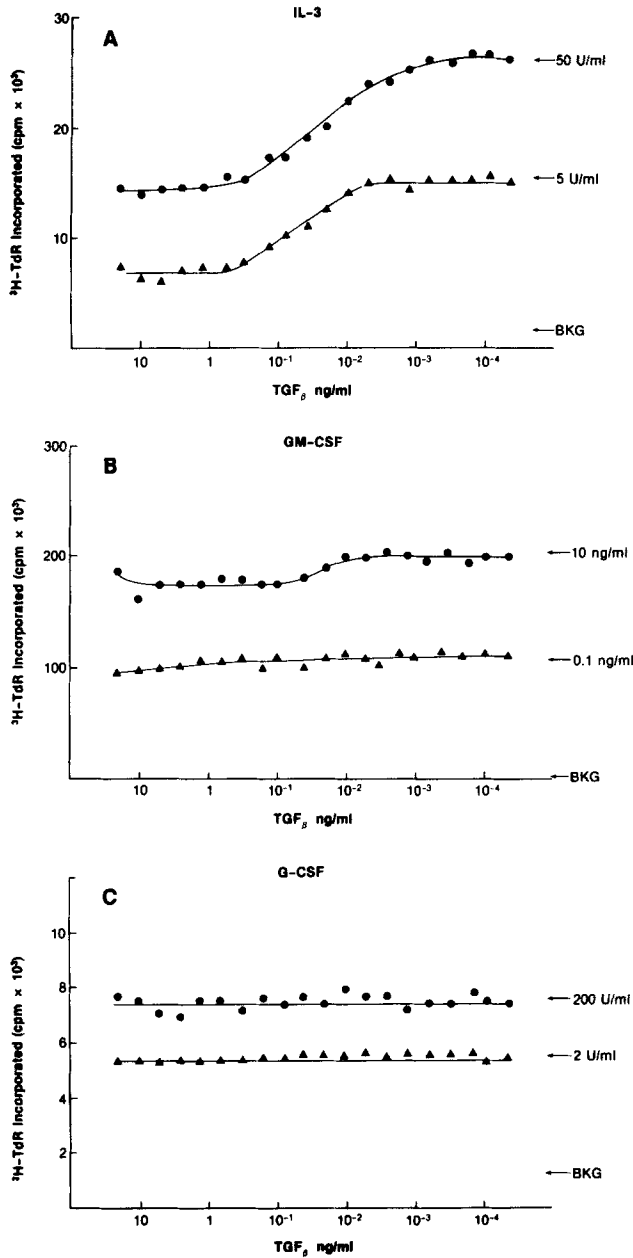


FIGURE 1 Dose response of TGF- $\beta$ 1 on factor-induced normal bone marrow proliferation. Murine bone marrow harvested and plated as described in Materials and Methods and TGF- $\beta$ 1 was used at a concentration of 10 ng/ml and diluted at the indicated concentrations into culture medium containing a constant amount of IL-3, 50 U/ml ( $\bullet$ ) or 5 U/ml IL-3 ( $\blacktriangle$ ) in panel A, and 10 ng/ml GM-CSF ( $\bullet$ ) or 0.1 ng/ml GM-CSF ( $\blacktriangle$ ) in panel B, and 200 U/ml G-CSF ( $\bullet$ ) or 2 U/ml G-CSF ( $\blacktriangle$ ) panel C. Arrows on each panel indicate factor-induced proliferation in the absence of TGF- $\beta$ 1 or background proliferation (BKG).

In agreement with proliferation data, TGF- $\beta$ 1 had no inhibitory effect on G-CSF- or GM-CSF-promoted hematopoietic cell colony formation, although an increased colony number was obtained in some experiments with GM-CSF (Table I). When adherent bone marrow cells were removed by absorbance to plastic petri dishes before plating in the assay, increased colony formation observed with GM-CSF was decreased (data not shown).

TABLE I  
Effect of TGF- $\beta$ 1 on Hematopoietic Colony Formation in Soft Agar

Stimulator	Number of colonies*	
	+ TGF- $\beta$ 1 <sup>†</sup>	- TGF- $\beta$ 1
None	0	0
IL-3 (50 U/ml)	5 $\pm$ 3	52 $\pm$ 5
IL-3 (5 U/ml)	0	6 $\pm$ 3
GM-CSF (1 ng/ml)	387 $\pm$ 11	113 $\pm$ 12
GM-CSF (0.1 ng/ml)	56 $\pm$ 4	27 $\pm$ 12
G-CSF (100 U/ml)	20 $\pm$ 4	25 $\pm$ 4

\* Colonies were scored after six to eight days incubation. Means  $\pm$  SEM are representative of three separate experiments.

<sup>†</sup> Cultures were supplemented with 10 ng/ml TGF- $\beta$ 1.

Thus, TGF- $\beta$ 1 differentially regulates colony formation in that it is a potent inhibitor of IL-3-induced colony formation but not of GM-CSF- or G-CSF-promoted colony formation.

*Effect of TGF- $\beta$ 1 on Multipotential Colony Formation.* IL-3 promotes the proliferation and differentiation in soft agar of many hematopoietic lineages from bone marrow and in the presence of Epo can induce the proliferation and differentiation of one of the earliest in vitro colonies, CFU-GEMM, a colony consisting of four hematopoietic lineages (1, 2). Colony assays to measure CFU-GEMM formation were set up to determine if TGF- $\beta$ 1 had an effect on the formation of these earliest hematopoietic colonies. In the absence of TGF- $\beta$ 1, between five and eight CFU-GEMM colonies were scored in three separate experiments, while in the presence of TGF- $\beta$ 1 no CFU-GEMM colonies were detected (Table II). This result is also depicted in the transmission photomicrograph in Fig. 3, where in the absence of TGF- $\beta$ 1 (panel A) a multilineage colony with cell types from the four lineages is observed, while in the presence of TGF- $\beta$ 1 only small clusters of differentiated granulocytes or macrophages were obtained. Therefore, TGF- $\beta$ 1 blocks the proliferation and differentiation of early hematopoietic progenitors (CFU-GEMM) while allowing clusters (<20 cells) of more differentiated progeny to develop, which explains why IL-3-induced bone marrow proliferation is not completely inhibited by TGF- $\beta$ 1.

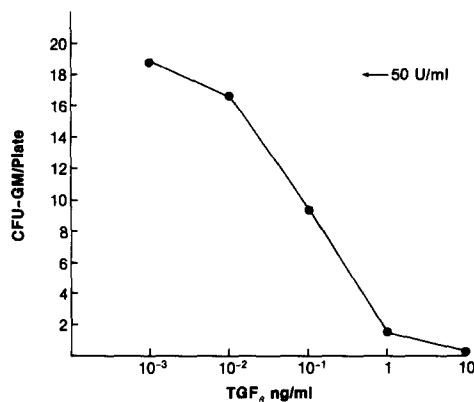


FIGURE 2. Dose response of TGF- $\beta$ 1 inhibition of IL-3-induced bone marrow cell soft agar colony formation. Bone marrow cells were plated into soft agar and assayed according to the procedures in the Materials and Methods and TGF- $\beta$ 1 was diluted at the indicated concentrations into medium containing a constant amount of IL-3, 50 U/ml (●). Arrow indicates the IL-3-induced colony formation in the absence of TGF- $\beta$ 1.

TABLE II  
*Effect of TGF- $\beta$ 1 on IL-3-dependent Multipotential Hematopoietic Colony Formation*

Stimulator	TGF- $\beta$ 1*	CFU-GEMM <sup>†</sup>
Epo (2 U/ml)	-	0
IL-3 (50 U/ml) + Epo (2 U/ml)	-	5-8
IL-3 (50 U/ml) + Epo (2 U/ml)	+	0

\* Cultures were grown in the presence (+) or absence (-) of 1 ng/ml TGF- $\beta$ 1.

<sup>†</sup> Colony formation was scored after 10 d. These data are representative of three separate experiments.

*Effect of TGF- $\beta$ 1 on Erythroid Development.* Two factors are needed to promote the growth of erythroid development in CFU-GEMM colonies, namely IL-3, which affects the growth and differentiation of early progenitor cells, and Epo, which stimulates the proliferation and terminal differentiation of late erythroid progenitors. Since the data on CFU-GEMM formation indicated that TGF- $\beta$ 1 could inhibit the proliferation and differentiation of early erythroid progenitor cells, we were interested in whether or not it would also inhibit the Epo-driven later stages of erythroid cell growth. As shown in Fig. 4, Epo promotes the proliferation of spleen cells from phenylhydrazine-treated mice (90% are erythroid precursors) in a dose-dependent manner. In the presence of saturating amounts of Epo, TGF- $\beta$ 1 had no inhibitory effect when tested over a wide range of concentrations. In addition, TGF- $\beta$ 1 had no effect on Epo-induced late erythroid colony formation (CFU-E) (Table III). Therefore, TGF- $\beta$ 1 appears to suppress the proliferation and differentiation of early uncommitted hematopoietic progenitor cells but is inactive on later precursor cells that are committed to a particular lineage.

*Effect of TGF- $\beta$ 1 on Factor-dependent and -independent Myeloid Leukemic Cell Lines.* Since the data suggest that TGF- $\beta$ 1 might play a role in regulating the growth and differentiation of early but not late hematopoietic progenitor populations, we examined a variety of factor-dependent progenitor cell lines representing leukemic clonal populations or early myeloid cells blocked in early stages of differentiation based on their phenotype, function, and morphology (22). As shown in Fig. 5, TGF- $\beta$ 1 inhibits IL-3-induced proliferation of the NFS-60 cell line using either 0.5 U/ml or 50 U/ml of IL-3. Data from several experiments demonstrate that the inhibition is dose-dependent with an ED-50 of 5-15 pM. Using a polyclonal antiserum to TGF- $\beta$ 1, the inhibitory effects of TGF- $\beta$ 1 on IL-3-driven proliferation were effectively neutralized in a dose-dependent manner, while normal rabbit serum showed no effect (Fig. 6), demonstrating the action of TGF- $\beta$ 1 could be specifically neutralized. In addition, GM-CSF-induced and G-CSF-induced proliferation of NFS-60 cells is also inhibited by TGF- $\beta$ 1 in a dose-dependent manner (panels B and C) with ED-50s of 5-15 pM. NFS-60 cells also provide a convenient means to test two other growth factors, CSF-1 and BSF-1/IL-4. As shown in Fig. 7, the proliferation of NFS-60 cells in the presence of saturating amounts of each factor was inhibited by TGF- $\beta$ 1 in dose-dependent manner with ED-50s of 5-10 pM for CSF-1 and IL-4.

Inhibition of proliferation in the presence of TGF- $\beta$ 1 was consistently observed among other factor dependent cell lines regardless of their derivation with similar results (Table IV). In addition to the factor-dependent cell lines, a factor-independent

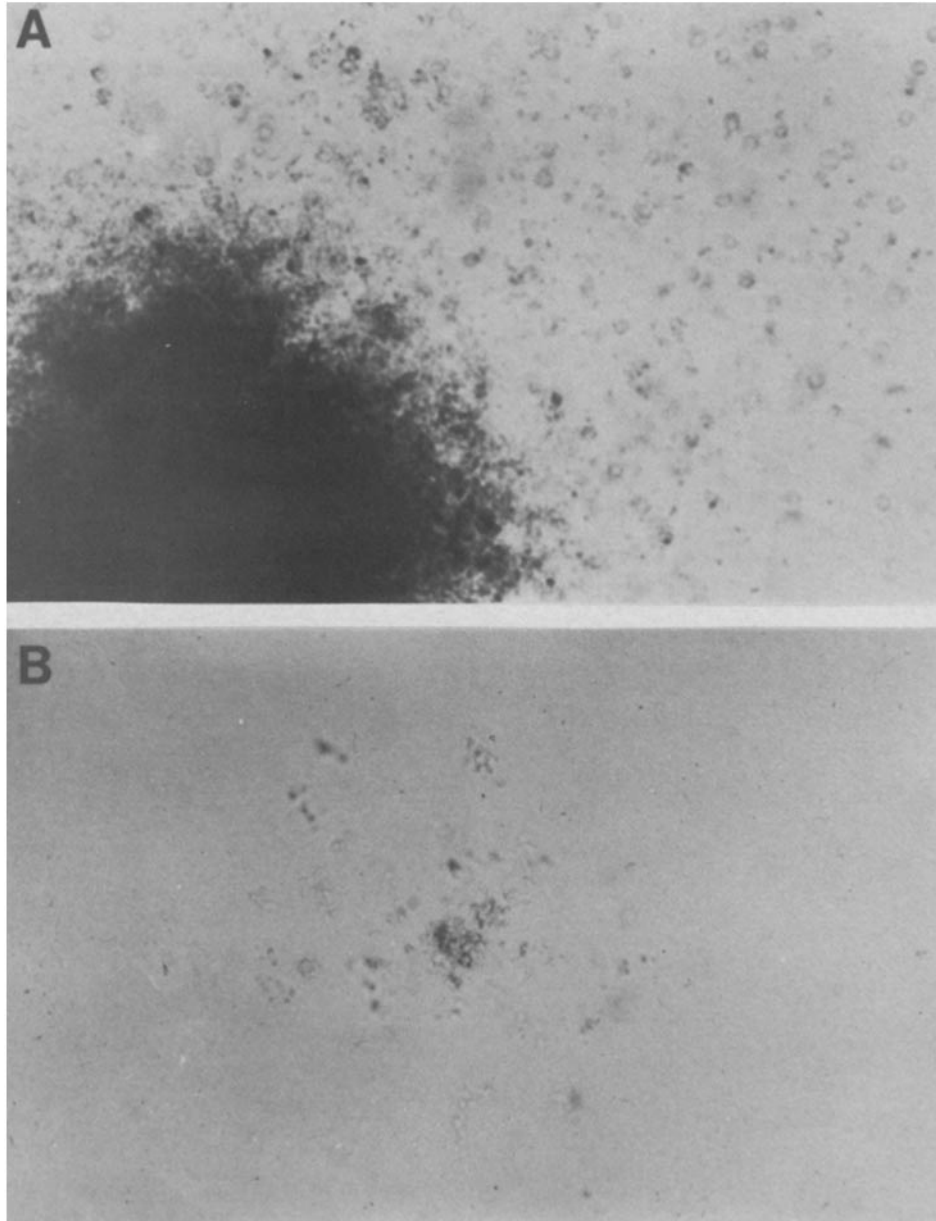


FIGURE 3. Transmission photomicrographs of IL-3-induced multipotential (CFU-GEMM) bone marrow colonies in soft agar in the absence (A) or presence (B) of TGF- $\beta$ 1.

myelomonocytic cell line, WEHI-3B, was also inhibited in a dose-dependent manner by TGF- $\beta$ 1. TGF- $\beta$ 1 did not induce the differentiation of those lines that were inhibited. In contrast, growth of factor independent cell lines of erythroid, macrophage and mast cell lineages, blocked late in their respective lineages, were not inhibited by TGF- $\beta$ 1.

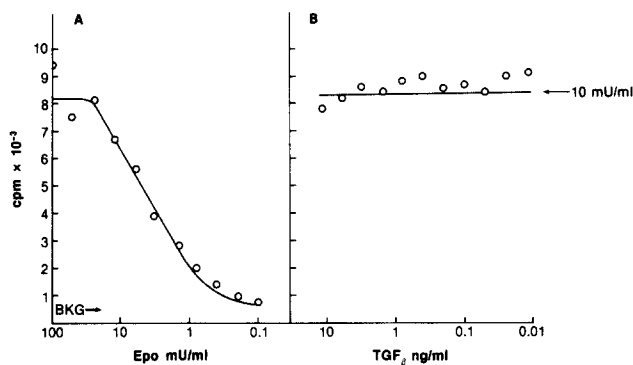


FIGURE 4. Effect of TGF- $\beta$ 1 on Epo-induced proliferation of splenocytes from phenylhydrazine treated mice. Splenocytes were obtained and assayed by the procedures in the Materials and Methods. Epo was diluted at the indicated concentrations into culture medium (A) or TGF- $\beta$ 1 was diluted at the indicated concentrations into medium containing a constant amount of Epo, 100 U/ml (B). Arrows indicate Epo-induced proliferation in the absence of TGF- $\beta$ 1 or background proliferation (BKG).

### Discussion

The production of blood cells is regulated by cells which produce stimulatory and inhibitory molecules, as well as those cells that can respond to these factors (1, 2). Evidence presented here suggests that TGF- $\beta$ 1 may serve a critical regulatory role in murine hematopoiesis in that it is a potent negative regulator of early but not late hematopoietic growth and differentiation. Specifically, TGF- $\beta$ 1 inhibits early IL-3-induced bone marrow progenitor cell proliferation and colony growth in soft agar, but has no suppressive influence on the proliferation and differentiation induced by other later acting hematopoietic growth factors, G-CSF, GM-CSF, and Epo. In addition, while colony formation is significantly inhibited in cultures containing IL-3 and TGF- $\beta$ 1, clusters and small colonies of differentiated myeloid cells of one lineage were consistently seen. This is consistent with bone marrow proliferation data that show some IL-3-induced proliferation occurs in the presence of TGF- $\beta$ 1. Also, TGF- $\beta$ 1 selectively blocks the growth and differentiation of early multipotential progenitor cells (IL-3-regulated CFU-GEMM), a component of which is representative of early erythroid differentiation, while it has no effect on the late stages of erythroid differentiation (Epo-regulated CFU-E). Thus, the inhibitory effect of TGF- $\beta$ 1 on hematopoietic cells appears to be a function of the state of cellular differentiation,

TABLE III  
*Effect of TGF- $\beta$ 1 on Erythropoietin-induced Colony Formation*

Cell source	Number of CFU-E*	
	+ TGF- $\beta$ 1 <sup>†</sup>	- TGF- $\beta$ 1
Normal splenocytes <sup>§</sup>	64	60
Phenylhydrazine splenocytes <sup>§</sup>	1,684	1,635
FVP splenocytes <sup>§</sup>	2,298	2,340

\* Erythroid colonies were scored after 48 h according to the assay described in the procedures.

<sup>†</sup> Cultures were supplemented with 1 ng/ml TGF- $\beta$ 1.

<sup>§</sup> Normal, phenylhydrazine-treated, and Friend virus-infected splenocytes were obtained as described in the Materials and Methods. These data are representative of three separate experiments.



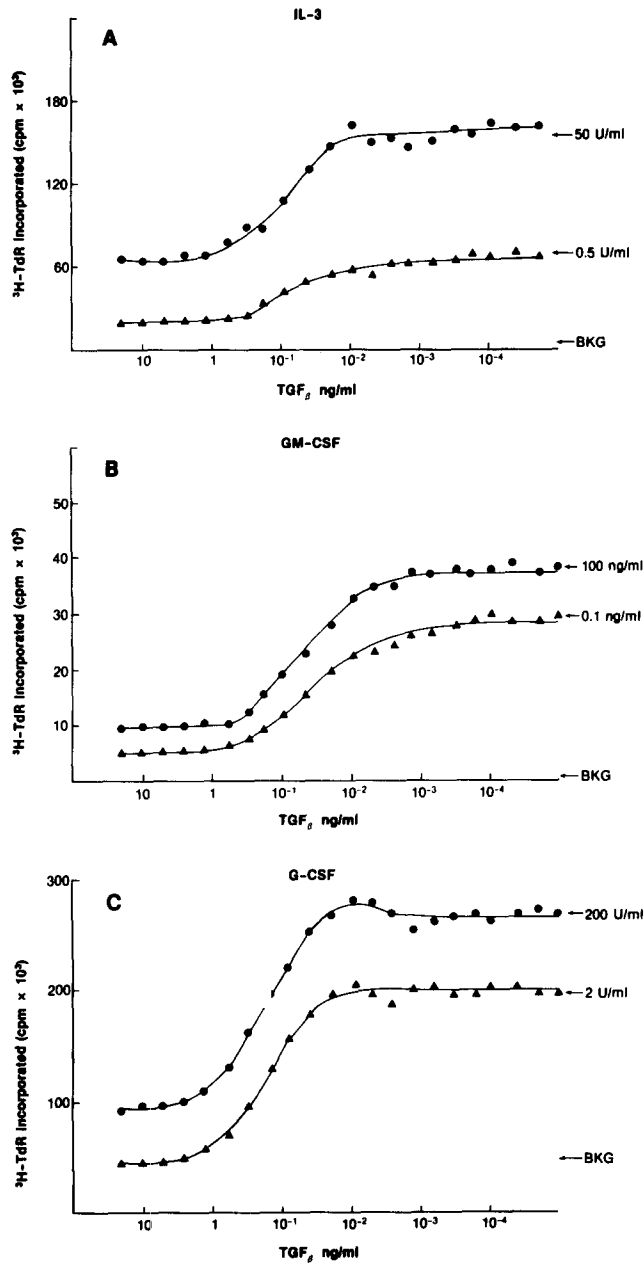


FIGURE 5. Dose response of TGF- $\beta$ 1 inhibition on factor-induced NSF-60 cell proliferation. NFS-60 cells were seeded into microtiter wells at a density of  $10^5$  cells/ml and harvested according to the procedures outlined in the Materials and Methods. TGF- $\beta$ 1 was diluted at the indicated concentrations into medium containing a constant amount of IL-3, 50 U/ml ( $\bullet$ ), or .5 U/ml IL-3 ( $\blacktriangle$ ) in panel A; and 100 ng/ml GM-CSF ( $\bullet$ ) or 0.1 ng/ml GM-CSF ( $\blacktriangle$ ) in panel B; and 200 U/ml G-CSF ( $\bullet$ ) or 2 U/ml G-CSF ( $\blacktriangle$ ) in panel C. Arrows indicate IL-3-, GM-CSF-, and G-CSF-induced proliferation in the absence of TGF- $\beta$ 1 or background proliferation (BKG).

such that, as erythroid and myeloid cells undergo maturation there is a point at which they become insensitive to the inhibitory effect of TGF- $\beta$ 1. The resistance to the inhibitory action of TGF- $\beta$ 1 could be a result of a change in the affinity or number of TGF- $\beta$ 1 receptors. Therefore, experiments have been initiated to determine both the biological effect as well as the receptor status on purified populations of more differentiated hematopoietic progenitors.

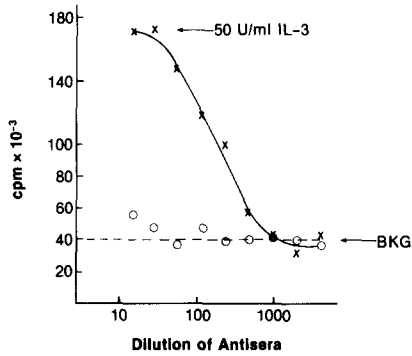


FIGURE 6. Neutralization by anti-TGF- $\beta$ 1 of the TGF- $\beta$ 1-induced inhibition of NFS-60 cell proliferation. Normal rabbit serum (O) or polyclonal anti-TGF- $\beta$ 1 rabbit serum (X) were incubated with 10 ng/ml TGF- $\beta$ 1 at the indicated dilutions in 50  $\mu$ l media at 37°C. After 1 h of incubation, NFS-60 cells were added to a final concentration of  $10^5$ /ml in 50 U/ml IL-3 and cells were harvested as described in the Materials and Methods. Arrows indicate IL-3-induced proliferation in the absence of TGF- $\beta$ 1, or background proliferation (BKG).

While the soft agar colony assay measures the clonal growth and differentiation of hematopoietic progenitors, it is not possible under these circumstances to rule out that the action of an exogenous factor might be due to the indirect effects on other subpopulations. However, since receptors for TGF- $\beta$ 1 are present on all cell types (7), to conclusively prove that TGF- $\beta$ 1 is acting directly, one would need a pure population of progenitor cells. A reasonable approach would be to examine clonal hematopoietic growth factor cell lines. The results with NSF-60 cells, which are clonal as demonstrated by the presence of a rearranged *c-myc* locus in all cells (28), suggests that TGF- $\beta$ 1 can act directly on hematopoietic cell progenitors.

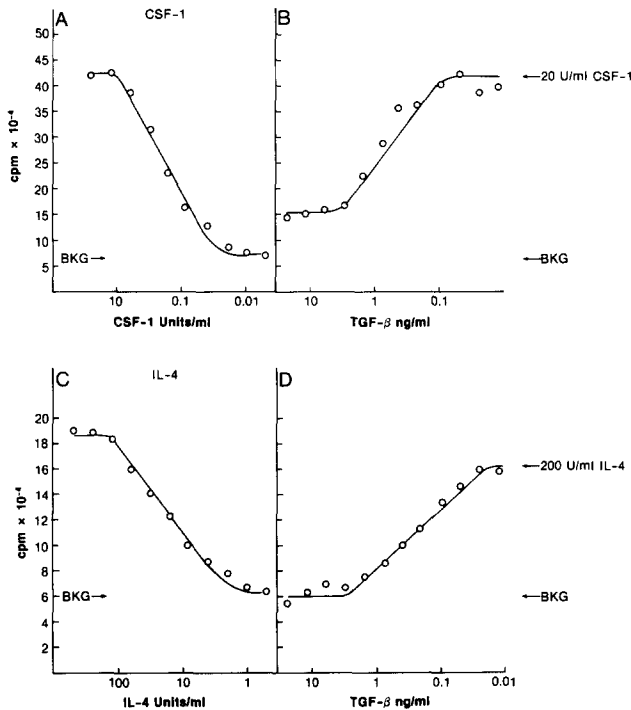


FIGURE 7. Dose response of TGF- $\beta$ 1 on CSF-1 and IL-4-induced proliferation of NFS-60 cells. NFS-60 cells were plated into microtiter wells at a density of  $10^5$ /ml and harvested according to procedures in the Materials and Methods. CSF-1 (A) and IL-4 (C) were diluted at the indicated concentrations into culture medium, or TGF- $\beta$ 1 was diluted at the indicated concentrations into medium containing a constant amount of CSF-1, 20 U/ml (B), and IL-4 200 U/ml (D). Arrows indicate CSF-1- and IL-4-induced proliferation in the absence TGF- $\beta$ 1 or background proliferation (BKG).

TABLE IV  
*Effect of TGF- $\beta$ 1 on Leukemic Cell Lines*

Cell line*	Lineage	IL-3 dependence	TGF- $\beta$ 1 percent inhibition <sup>†</sup>
NFS-60	Myeloid	+	60-70
DA-3	Myeloid	+	80-90
32D-c123	Myeloid	+	70-80
FDC-P1	Myeloid	+	80-90
B <sub>6</sub> SutA	Myeloid	+	65-75
P388D1	Macrophage	-	0
J774A.1	Macrophage	-	0
WEHI-3	Myelomonocytic	-	80-90
TP-3	Erythroid	-	0
DS19	Erythroid	-	0
P815	Mast cell	-	0

\* Cell proliferation assays were performed according to the procedures in the Materials and Methods.

<sup>†</sup> TGF- $\beta$ 1 was diluted into cell proliferation assays to generate a dose response curve starting at a concentration of 40 ng/ml. The range represents the maximum percent inhibition obtained from the dose-response curves from three separate experiments.

Nonetheless, experiments have been designed to support the direct action of TGF- $\beta$ 1 by testing IL-3-induced Thy-1<sup>+</sup> murine progenitors obtained by FACS (29).

Since both murine and human leukemic cells have been shown to maintain growth factor responsiveness in vitro, a loss of negative growth control might contribute to a preleukemic state by permitting the unrestrained growth of factor-dependent leukemic clones. Such a process might result, for example, in the out-growth of IL-3-dependent myeloid leukemic progenitor cells. Our results indicate, however, that the growth of IL-3-dependent leukemias in vitro is inhibited by TGF- $\beta$ 1 and that this effect is seen regardless of the growth factor used to signal proliferation. It is possible that additional events (abrogation of factor dependence) may be required to escape negative regulation. Alternatively, others (7, 11, 30, 31) found that the human melanoma cell line A549 produces an inactive or latent form of TGF- $\beta$ 1, while it is potently inhibited by exogenous TGF- $\beta$ 1, thus leading to the proposal that a lesion in TGF- $\beta$ 1 activation might have contributed to the evolution of this cancer. Our laboratory has recently found that while IL-3-dependent myeloid leukemic cells express messenger RNA for TGF- $\beta$ 1 (manuscript in preparation), no TGF- $\beta$ 1 bioactivity in the supernatant has been detected. Thus, experiments are underway to determine if the TGF- $\beta$ 1 protein is produced and whether it is secreted in a latent or inactive form which might result in the breakdown of negative autocrine growth control.

In summary, TGF- $\beta$ 1 may serve a critical regulatory role in murine hematopoiesis in that it is a potent negative regulator of early but not late hematopoietic differentiation and proliferation. Thus, in normal cell populations, TGF- $\beta$ 1 is a differential regulator of growth factor-promoted hematopoietic differentiation. In addition, TGF- $\beta$ 1 inhibits the growth of differentiated-arrested factor-dependent and -independent leukemic myelomonocytic cells but not those leukemias blocked late in their lineage.

The capacity to selectively inhibit early marrow cell and leukemic cell prolifera-

tion could have clinical applications. A variety of hematopoietic tumors may be responsive in vivo to TGF- $\beta$ 1 inhibition, which could be a useful adjunct to cytoreductive combination chemotherapy. In addition, if TGF- $\beta$ 1 growth-arrested marrow stem cells are less sensitive to the toxic effects of chemotherapeutic agents, TGF- $\beta$ 1 may be a valuable component of cancer treatment programs that have dose-limiting myelotoxicity. Experiments to evaluate these potential therapeutic applications of TGF- $\beta$ 1 are underway.

### Summary

Transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) has been shown to be associated with active centers of hematopoiesis and lymphopoiesis in the developing fetus. Therefore, the effects of TGF- $\beta$ 1 on mouse hematopoiesis were studied. TGF- $\beta$ 1 is a potent inhibitor of IL-3-induced bone marrow proliferation, but it does not inhibit the proliferation induced by granulocyte/macrophage, colony-stimulating factor (CSF), granulocyte CSF, and erythropoietin (Epo). TGF- $\beta$ 1 also inhibits IL-3-induced multipotential colony formation of bone marrow cells in soft agar, which includes early erythroid differentiation, while Epo-induced terminal differentiation is unaffected. In addition, IL-3-induced granulocyte/macrophage colonies were inhibited; however, small clusters of differentiated myeloid cells were consistently seen in cultures containing IL-3 and TGF- $\beta$ 1. Thus, TGF- $\beta$ 1 selectively inhibits early hematopoietic progenitor growth and differentiation but not more mature progenitors. TGF- $\beta$ 1 is also a potent inhibitor of IL-3-dependent and -independent myelomonocytic leukemic cell growth, while the more mature erythroid and macrophage leukemias are insensitive. Therefore, TGF- $\beta$ 1 functions as a selective regulator of differentiating normal hematopoietic cells, and suppresses myeloid leukemic cell growth.

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