Selective Regulation of the Activity of Different Hematopoietic Regulatory Proteins by Transforming Growth Factor β1 in Normal and Leukemic Myeloid Cells

By Joseph Lotem and Leo Sachs

The viability of normal bone marrow myeloid precursor cells induced by interleukin-6 (IL-6) or IL-1α and the ability of IL-6 and IL-1α to induce the formation of colonies of granulocytes, macrophages, or megakaryocytes in densely seeded bone marrow cultures was suppressed by transforming growth factor-β1 (TGF-β1). Induction of normal bone marrow colony formation by IL-3 was much less sensitive to TGF-β1, and there was little or no effect of TGF-β1 on colony formation induced by macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage CSF (GM-CSF). In different clones of myeloid leukemic cells, TGF-β1 suppressed differentiation induced with IL-6, IL-1α, or lipopolysaccharide (LPS), but did not suppress differentiation induced with IL-3 or GM-CSF. The effect of TGF-β1 on differentiation of the leukemic cells can be dissociated from its effect on cell growth. TGF-β1 suppressed the production of IL-6 in normal bone marrow cells cultured with IL-1α and the production of IL-6 and GM-CSF in leukemic cells cultured with IL-1α or LPS. The suppression of IL-6 production can explain the suppression by TGF-β1 of the effects of IL-1α and LPS that are mediated by IL-6. TGF-β1 also suppressed differentiation in clones of myeloid leukemic cells induced with differentiation factor/leukemia inhibitory factor and tumor necrosis factor. In different leukemic clones TGF-β1 suppressed or enhanced induction of differentiation with dexamethasone. The results show that TGF-β1 can selectively control the activity of different molecular regulators of normal and leukemic hematopoiesis.

TRANSFORMING growth factor-β (TGF-β) was originally described as a factor that stimulated the anchorage-independent growth in agar of anchorage-dependent fibroblasts.[1,2] There are now several proteins that belong to the TGF-β family,[3-8] and TGF-β1 can have stimulatory or inhibitory effects on cell growth and differentiation in various cell types.[3-6] In the present experiments we study the effect of TGF-β1 on the activity of different hematopoietic regulatory proteins to determine the possible selective regulation by TGF-β1 of the activity of the hematopoietic proteins interleukin-1α (IL-1α), IL-6, IL-3, macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage CSF (GM-CSF), differentiation factor/leukemia inhibitory factor (D factor/LIF), and tumor necrosis factor (TNF). We analyze the activity of hematopoietic proteins on normal myeloid cells and their development to colonies of granulocytes, macrophages, or megakaryocytes, and on different clones of myeloid leukemic cells. These leukemic cells include clones that can be induced to differentiate to macrophages and/or granulocytes with IL-6, which we had previously called macrophage and granulocyte inducer-type 2 (MGI-2),[9-15] IL-1,[13-17] GM-CSF or IL-3,[12,14,15,18-20] D-factor/LIF,[13,21,22] or TNF.[23,24] We have also used leukemic clones that can be induced to differentiate with other compounds, such as the glucocorticosteroid dexamethasone,[25,26] the phorbol ester tumor promoter 12-0-tetradecanoyl-phorbol-13-acetate (TPA),[27,28] bacterial lipopolysaccharide (LPS),[29,30] or dimethylsulfoxide (DMSO).[31]

The present results indicate that TGF-β1 can selectively control the activity of different hematopoietic regulators on normal and leukemic myeloid cells, that the effect of TGF-β1 on differentiation can be dissociated from its effect on growth, and that TGF-β1 can suppress production of the hematopoietic regulatory proteins IL-6 and GM-CSF.

MATERIALS AND METHODS

Cells and cell culture. Different clones of myeloid leukemic cells isolated from the M1 myeloid leukemia cell line derived from an SL mouse with myeloid leukemia[32] were used. Clones 3 and 11 were isolated in Rehovot, Israel,[33] and clone T22 was isolated in Saitama, Japan[34] and obtained from Dr M. Hozumi. All three clones derived from the M1 cell line can be induced to differentiate with IL-6, IL-1, or dexamethasone; clone 11 can also be induced to differentiate with LPS and clone T22 with D-factor/LIF.[11-17,21,22,25,26,29,30,34] In addition, in some experiments we used a subclone of clone 11 (11R-IL-1), which is also inducible with IL-6 or LPS but resistant to induction of differentiation with IL-1.[17] Clone 7-M12 was isolated from a myeloid leukemia in an x-irradiated SJL/J mouse[35]; it can be induced to differentiate to mature macrophages with GM-CSF or IL-3.[14,18-20] Clone 6 was isolated from another x-ray–induced myeloid leukemic SJL/J mouse[35] and can be induced to partially differentiate with dexamethasone.[36] The human leukemic HL-60 cells can be induced to differentiate to macrophages by TNF[23,24] and to granulocytes with DMSO.[31] All the clones were grown in Dulbecco's modified Eagle's medium (DMEM) (H-21; GIBCO, Grand Island, NY) with 10% heat-inactivated (56°C, 30 minutes) horse serum (GIBCO) and multiplied in suspension as myeloblasts to promyelocytes. Normal myeloid precursor cells were enriched from the bone marrow of LPS-resistant C3H/HeJ mice by two cycles of removal of C3 rosette-forming cells as previously described,[37] and consisted of 90% ± 3% myeloid precursor cells up to the promyelocyte stage, 3% ± 1% myelocytes and metamyelocytes, and 2% ± 1% each of some other cell types (eosinophils, lymphocytes, and nucleated erythroid cells). These other cell types did not survive in the cultures.

Hematopoietic cell regulators. Pure recombinant mouse IL-6[38] (rIL-6) was obtained from Dr J. Van Snick; recombinant human D-factor/LIF (COS-1 supernatant)[39] (rD-factor/LIF) from Dr S.C. Clark; pure recombinant human IL-1α[40] (rIL-1α) from Dr P.

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Lomedico; pure recombinant mouse TNF[41] (rTNF) from Drs W. Fiers and J. Tavernier; pure recombinant mouse GM-CSF[42] (rGM-CSF) from Dr S. Gillis; antiserum to mouse GM-CSF from Dr J.F. De Lamarter[43]; and pure recombinant mouse IL-3[44] (rIL-3) from Dr P. Vassalli. Mouse M-CSF was prepared from L929 fibroblasts. Bacterial LPS (*Escherichia coli*) (Difco Lab, Detroit, MI), dexamethasone (Sigma Chemical Co, St Louis, MO), the tumor-promoting phorbol ester TPA (Sigma), and DMSO (Merck, Darmstadt, West Germany) were also used. Pure human platelet TGF-β1[5] was obtained from Dr M.B. Sporn or from British Biotechnology Ltd (Oxford, England), and results with both sources were the same. TGF-α (British Biotechnology) was also used in some experiments.

Assays for induction of differentiation and for IL-6 activity. Different clones of myeloid leukemic cells were seeded at 2.5×10^4 to 1.5×10^5 cells/mL without or with the materials to be assayed, and were analyzed for differentiation-associated properties 4 days later. The percent of Fc and C3 rosette-forming cells was determined using sheep erythrocytes coated with antibody or antibody and complement, respectively, as described.[25] Production of lysozyme by these cells was measured using the turbidometric method with *Micrococcus lysodeikticus* as described.[45] The cells were also analyzed for morphologic differentiation on cytocentrifuge smears stained with May-Grünwald Giemsa. They were scored as blasts (myeloblasts and promyelocytes), intermediate stages (myelocytes and monocyte-like cells), and mature macrophages or granulocytes by counting 500 cells. To assay for IL-6 activity, clone 11 cells were seeded at 7.5×10^4/mL with the material to be tested together with 5 μg/mL polymyxin B (Sigma) to neutralize possible contamination with LPS,[20] and production of lysozyme was measured after 4 days. When IL-1 was present in the material to be assayed for IL-6 activity, a subclone of clone 11 cells resistant to IL-1 (clone 11R-IL-1)[17] was used. To ensure that the lysozyme-inducing activity is due to IL-6, the activity was assayed without or with 2 μg/mL monoclonal antimouse IL-6 antibody (IgG fraction).[30]

Assay for induction of granulocyte, macrophage, and megakaryocyte colonies in agar. Bone marrow cells were collected from the femurs of 2- to 3-month-old LPS-resistant C3H/HeJ mice, and seeded at sparse or dense seeding levels (5×10^4 or 1×10^6 nucleated cells/35-mm Petri dish, respectively) in 0.8 mL of 0.33% agar on top of a 2.5 mL harder agar base (0.5%)[46] that contained rIL-1α, rIL-6, rIL-3, rGM-CSF, or M-CSF. Both layers contained DMEM and 20% heat-inactivated horse serum. Colonies of granulocytes or macrophages with 50 or more cells per colony and colonies of megakaryocytes with three or more giant cells per colony[47] were scored after 7 days of incubation at 37°C. To identify GM-CSF activity in conditioned media, 5×10^4 normal bone marrow cells were seeded in agar as described previously without and with 0.2% antiserum against mouse GM-CSF.[43]

All the experiments were performed at least three times, and the reproducibility was generally up to ±20% of the mean values.

RESULTS

Effect of TGF-β1 on viability and colony formation by normal bone marrow myeloid precursor cells. The effect of TGF-β1 was tested on an enriched population of normal myeloid precursor cells from bone marrow cultured in suspension, and on colony formation by unfractionated normal bone marrow cells in agar. The bone marrow cells were seeded for colony formation at sparse or dense seeding levels (5×10^4 or 1×10^6 nucleated cells/35-mm Petri dish, respectively). The sparse seeding level was used for rIL-3, rGM-CSF, and M-CSF. The dense seeding level was used for rIL-6 and rIL-1α because sparsely seeded bone marrow cultures did not give colonies with rIL-6[12,47] or rIL-1α. When TGF-β1 was added with other proteins, they were always added together at the beginning of the experiment. At 1 ng/mL, TGF-β1 by itself did not maintain the viability of the myeloid precursor cells and strongly suppressed the ability of rIL-6 and rIL-1α to maintain cell viability (Fig 1A). TGF-β1 at 1 ng/mL also strongly suppressed the formation of granulocyte, macrophage, and megakaryocyte colonies induced with rIL-6 or rIL-1α (Table 1). In contrast, 1 ng/mL TGF-β1 did not suppress cell viability or colony formation induced by rIL-3, rGM-CSF, or M-CSF (Fig 1B, Table 2). At 10 ng/mL TGF-β1, there was some inhibitory effect on granulocyte or macrophage colony formation induced by rIL-3, and little or no effect on colonies induced by M-CSF or rGM-CSF (Table 2).

The results indicate that the response of normal myeloid precursors and their colony-forming cells to IL-6 or IL-1 is very sensitive to TGF-β1, that the response of these cells to IL-3 is less sensitive, and that the response to M-CSF or GM-CSF is the most resistant to TGF-β1.

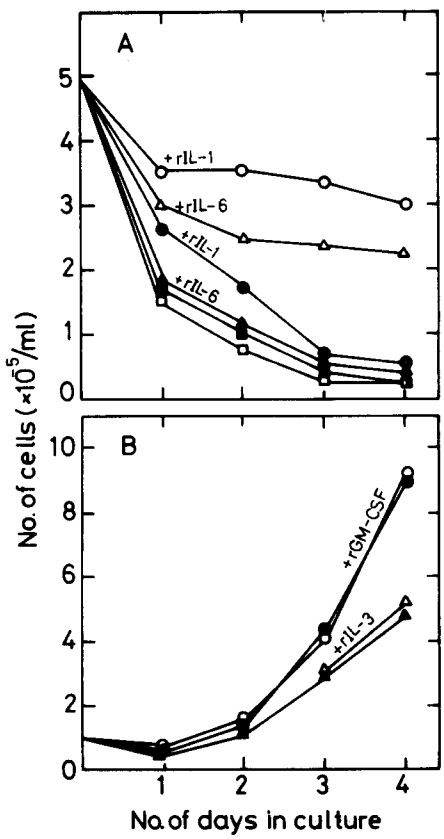

Fig 1. Effect of TGF-β1 on viability and growth of normal myeloid precursor cells in mass culture. Normal myeloid precursor cells isolated from mouse bone marrow[37] were seeded at 5×10^5 cells/mL (A) or at 1×10^5 cells/mL (B) with different hematopoietic regulatory proteins without or with 1 ng/mL TGF-β1, and cell number was determined after 1 to 4 days. rIL-6, rGM-CSF, and rIL-3 were added at 5 ng/mL, and rIL-1α at 10 ng/mL. Open symbols, without TGF-β1; closed symbols, with TGF-β1.

Table 1. Regulation by TGF-β1 of Granulocyte, Macrophage, and Megakaryocyte Colony Formation From Normal Bone Marrow Cells Incubated With IL-6 or IL-1α

Inducer Added*	TGF-β1 Added†	No. of Granulocyte or Macrophage Colonies‡	No. of Megakaryocyte Colonies		
		>50 Cells	3-4 Cells	5-7 Cells	>8 Cells
None	–	1 ± 1	63 ± 5	10 ± 2	1 ± 1
	+	0	2 ± 1	0	0
rIL-6	–	15 ± 2	178 ± 25	66 ± 10	23 ± 3
	+	1 ± 1	8 ± 2	3 ± 1	0
rIL-1α	–	40 ± 6	89 ± 10	37 ± 5	8 ± 2
	+	3 ± 1	12 ± 2	1 ± 1	0

*Bone marrow cells were seeded in agar at 1 × 10^6 cells per 35-mm Petri dish either without or with 5 ng/mL rIL-6 or 10 ng/mL rIL-1α. Colonies were counted after 7 days. The cells were densely seeded because sparsely seeded bone marrow cultures (5 × 10^4 cells per 35-mm Petri dish, as used in Table 2) did not give rise to colonies with rIL-6[12,47] or rIL-1α. The formation of megakaryocyte colonies in these densely seeded cultures without adding an inducer was due to the endogenous production of IL-6.[47]

†TGF-β1, 1 ng/mL, was added.

‡More than 90% of the colonies that developed with rIL-6 or rIL-1α were granulocyte colonies.

Effect of TGF-β1 on differentiation and growth of myeloid leukemic cells. Different clones of myeloid leukemic cells can be induced to differentiate to mature macrophages and/or granulocytes with one or more of the hematopoietic regulatory proteins IL-6, IL-1, GM-CSF, or IL-3, and with other compounds such as TNF, D-factor/LIF, LPS, or dexamethasone. We have determined to what extent the differentiation response of these leukemic cells is affected by TGF-β1, and whether the pattern of sensitivity to TGF-β1 of the response of leukemic cells to different inducers is similar to that found with normal myeloid precursor cells.

TGF-β1, 1 ng/mL, by itself induced clone 11 leukemic cells to weakly adhere to the tissue-culture Petri dishes (15% ± 5% of the cells) and to undergo some morphologic change to more differentiated cells (12% ± 2% intermediate

Table 2. Effect of TGF-β1 on Granulocyte or Macrophage Colony Formation From Bone Marrow Cells and Growth of Enriched Myeloid Precursor Cells in Mass Culture Incubated With IL-3, M-CSF, or GM-CSF

Inducer Added*	TGF-β1 Added (ng/mL)†	No. of Granulocyte or Macrophage Colonies >50 Cells	No. of Cells in Mass Culture (× 10^{-5}/mL)‡
rIL-3	–	84 ± 10	5.3 ± 0.8
	+ (1)	113 ± 15	5.1 ± 0.6
	+ (10)	15 ± 2	2.2 ± 0.3
M-CSF	–	68 ± 8	3.8 ± 0.4
	+ (1)	65 ± 6	3.7 ± 0.6
	+ (10)	59 ± 4	1.4 ± 0.2
rGM-CSF	–	73 ± 6	9.5 ± 1.3
	+ (1)	81 ± 8	9.4 ± 1.2
	+ (10)	98 ± 10	6.8 ± 1.5

*Bone marrow cells were seeded in agar at 5 × 10^4 cells per 35-mm Petri dish either without or with: 5 ng/mL rIL-6; 10 ng/mL rIL-1α; 5 ng/mL rGM-CSF; 5 ng/mL rIL-3; or M-CSF (40% conditioned medium from L929 fibroblasts).

†TGF-β1 was added at 1 ng/mL or 10 ng/mL as indicated in the parenthesis.

‡Enriched myeloid precursors were seeded at 1 × 10^5 cells/mL and the number of cells counted after 4 days in culture.

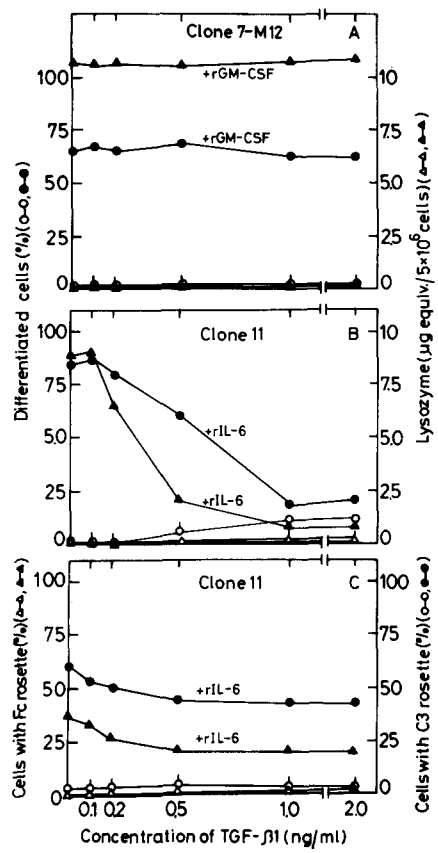

Fig 2. Effect of TGF-β1 on differentiation of myeloid leukemic cells induced to differentiate with rGM-CSF or rIL-6. Clones 7-M12 and 11 myeloid leukemic cells were seeded with different concentrations of TGF-β1 without (△—△ and ○—○) or with (▲—▲ and ●—●) 10 ng/mL rGM-CSF (clone 7-M12) or 2 ng/mL mouse rIL-6 (clone 11). Differentiation-associated properties were determined after 4 days. Differentiated cells include both intermediate stages and mature macrophages. The differentiated cells in clone 11 incubated with TGF-β1 alone were only intermediate stages and no mature cells. Similar results were obtained with 1 or 10 ng/mL TGF-β1.

stages but no mature cells) (Fig 2B). However, there was no induction of the differentiation markers Fc or C3 rosettes or lysozyme (Fig 2). When added together with other compounds that induce differentiation, 1 ng/mL TGF-β1 suppressed the induction of differentiation in clone 11 cells with rIL-6 (Fig 2, B and C) and in clone 3 cells with rIL-1α (Table 3). The later-appearing markers such as lysozyme and morphologically differentiated cells were suppressed to a higher degree than the earlier markers Fc and C3 rosettes (Fig 2). In addition, TGF-β1 did not suppress IL-6–induced cell adherence to the Petri dish, which is another early marker induced in clone 11 cells by IL-6. TGF-β1 also suppressed the induction of differentiation in clone 11 with LPS and dexamethasone; in clone T22 with rD-factor/LIF; and in HL-60 cells with rTNF, and to a lesser extent with DMSO (Table 3). In contrast to this inhibition of differentiation, TGF-β1 enhanced the differentiation of clone 6 cells by dexamethasone (Table 4). There was no effect on differentiation in any of these leukemic clones with 1 to 10 ng/mL TGF-α. The results with clone 7-M12 cells have shown that TGF-β1 by itself (1 to 10 ng/mL) did not induce differentiation in this clone and that it did not affect the induction of differentiation with rGM-CSF or rIL-3 (Fig 2A, Table 4). However, TGF-β1 suppressed differentiation in clone 7-M12 cells incubated with a low concentration of rGM-CSF together with the tumor-promoting phorbol ester TPA, which enhances differentiation by GM-CSF[27] (Table 4). This indicates that the inability of TGF-β1 to suppress differentiation in clone 7-M12 with GM-CSF was not due to a specific property of this clone, but rather to the nature of the signal produced by the inducer.

Clone 11 leukemic cells incubated with TGF-β1 showed a concentration-dependent inhibition of cell growth, and the concentration required to reduce cell number by 50% (EC_{50}) was about 1 to 1.5 ng/mL (40 to 60 pmol/L) (Fig 3). Similar results were obtained with two other clones derived from the M1 cell line (clones 3 and T22), and with clone 7-M12 in which TGF-β1 inhibited cell growth with an EC_{50} of about 15 pmol/L (Fig 3). In contrast, there was no inhibition of the growth of clone 6 cells by TGF-β1, even at 10 ng/mL (400 pmol/L). When leukemic cells were induced to differentiate with different inducers (Fig 3, Table 3), the total number of cells increased during the 4 days of the experiment, although to a lower degree than in cells that had not been induced to differentiate, except for clone 7-M12, which showed about the same total number of cells after 4 days in culture with or without GM-CSF (Fig 3) or IL-3. In the experiments on the effect of TGF-β1 on differentiating cells, TGF-β1 was always added together with the inducer of differentiation at the beginning of the experiment. In cultures with rGM-CSF or rIL-6, even 10 ng/mL TGF-β1 showed no decrease in cell number in clones 7-M12 and 11, respectively (Fig 3). Similarly, TGF-β1 at 1 ng/mL did not reduce the number of cells in cultures containing other inducers of cell differentiation (Table 3). This indicates that differentiating myeloid leukemic cells are less susceptible to the growth inhibitory effect of TGF-β1 compared with undifferentiated myeloid leukemic cells during the exponential growth phase. The results also indicate (Fig 3, Table 3) that in all cases where TGF-β1 suppressed differentiation, this was not associated with the resumption of cell growth to the level found in cells that had not been induced to differentiate. None of the clones tested showed inhibition of cell growth in the absence or presence of a differentiation inducer by up to 10 ng/mL TGF-α.

The present results indicate that, as with normal myeloid cells, there is a selective regulation by TGF-β1 of the activity of different hematopoietic regulatory proteins on myeloid

Table 3. Inhibition by TGF-β1 of Differentiation of Myeloid Leukemic Cells Induced by Various Inducers

Clone	Inducer Added*	TGF-β1 Added†	No. of Cells ($\times 10^{-5}$/mL)	Cell Types (%)		
				Blasts	Intermediate Stages	Mature Macrophages (M) or Granulocytes (G)
11	None	–	22.5 ± 2.3	98 ± 3	2 ± 1	0
	Dexamethasone	–	3.6 ± 0.6	42 ± 6	55 ± 5	3 ± 1 (M)
		+	3.0 ± 0.5	86 ± 4	14 ± 2	0
	LPS	–	5.0 ± 0.6	38 ± 5	37 ± 6	25 ± 3 (M)
		+	4.8 ± 0.7	88 ± 7	12 ± 3	0
3	None	–	16.3 ± 2.0	99 ± 1	1 ± 1	0
	rIL-1α	–	4.5 ± 0.6	51 ± 6	37 ± 5	12 ± 4 (M)
		+	3.8 ± 0.5	88 ± 3	12 ± 2	0
T22	None	–	9.5 ± 0.6	99 ± 1	1 ± 1	0
	rD-factor/LIF	–	2.5 ± 0.3	46 ± 4	32 ± 8	22 ± 4 (M)
		+	2.5 ± 0.4	93 ± 5	7 ± 2	0
HL-60	None	–	10.8 ± 2.0	91 ± 8	9 ± 1	0
	rTNF	–	7.5 ± 0.5	10 ± 3	35 ± 6	55 ± 7 (M)
		+	7.0 ± 0.6	60 ± 6	30 ± 3	10 ± 3 (M)
	DMSO	–	4.3 ± 0.6	0	68 ± 10	32 ± 5 (G)
		+	4.5 ± 0.6	11 ± 2	73 ± 10	16 ± 3 (G)

*Cells were seeded at 1 to 1.5 × 10^5 cells/mL and cultured for 4 days either without or with 40 ng/mL (10^{-7} mol/L) dexamethasone; 100 ng/mL bacterial LPS; 10 ng/mL rIL-1α; 10 ng/mL rTNF; or 1.4% (vol/vol) DMSO.

†TGF-β1 was added at 1 ng/mL together with the inducers of differentiation to clones 11, T22, and HL-60 and at 0.5 ng/mL to clone 3, which showed toxicity at 1 ng/mL.

Table 4. Effect of TGF-β1 on Differentiation of Myeloid Leukemic Clones 7-M12 and 6

Clone	Inducer Added* Type	Amount ng/mL	TGF-β1 Added†	Differentiation-Associated Properties: Rosettes (%) Fc	Rosettes (%) C3	Lysozyme (μg equiv/ 5 × 10^6 cells)	Cell Types (%) Blasts	Intermediate Stages	Mature Macrophages
7-M12	None	—	−	ND	ND	0.1 ± 0.1	98 ± 3	2 ± 1	0
	rIL-3	10	−			2.0 ± 0.3	68 ± 5	27 ± 4	5 ± 2
			+			2.0 ± 0.3	70 ± 2	26 ± 5	4 ± 1
	rGM-CSF	1	−			0.9 ± 0.2	97 ± 3	3 ± 1	0
			+			0.7 ± 0.1	98 ± 2	2 ± 1	0
	TPA	100	−			0.4 ± 0.1	95 ± 4	5 ± 2	0
			+			0.3 ± 0.1	97 ± 3	3 ± 1	0
	rGM-CSF + TPA	1 + 100	−			8.3 ± 0.6	52 ± 8	38 ± 6	10 ± 3
			+			1.1 ± 0.4	98 ± 2	2 ± 1	0
6	None	—	−	10 ± 2	6 ± 1	0.1 ± 0.1	100	0	0
			+	13 ± 2	7 ± 1	0.1 ± 0.1	100	0	0
	Dexamethasone	40	−	20 ± 3	8 ± 2	0.1 ± 0.1	99 ± 1	1 ± 1	0
			+	31 ± 4	20 ± 4	0.2 ± 0.1	75 ± 3	22 ± 4	3 ± 1

Abbreviation: ND, not done.

*Cells were seeded at 3 × 10^4 cells/mL and cultured for 4 days either without or with 10 ng/mL rIL-3; 1 ng/mL rGM-CSF; 100 ng/mL TPA; 1 ng/mL rGM-CSF together with 100 ng/mL TPA; or with 40 ng/mL dexamethasone.

†TGF-β1 was added at 1 ng/mL and similar results were obtained with 10 ng/mL.

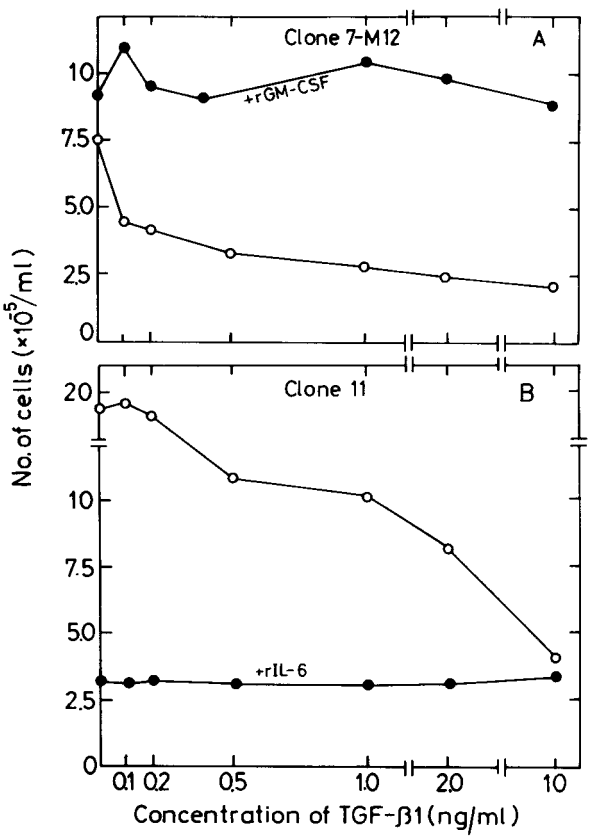

Fig 3. Effect of TGF-β1 on the growth of myeloid leukemic cells incubated with and without hematopoietic regulatory proteins. Clone 7-M12 and 11 myeloid leukemic cells were seeded at 2.5 × 10^4 or 7.5 × 10^4 cells/mL, respectively, with different concentrations of TGF-β1 without (○—○ or with (●—●) 10 ng/mL rGM-CSF (clone 7-M12) or 2 ng/mL rIL-6 (clone 11), and the number of cells determined after 4 days.

leukemic cells, the growth inhibitory effect of TGF-β1 can be dissociated from its effect on cell differentiation (clones 7-M12 and 6), TGF-β1 can differentially affect differentiation in the same clone by different inducers (clone 7-M12 with GM-CSF with and without TPA), and that TGF-β1 can exert opposite effects on differentiation in different leukemic clones even when treated with the same inducer (dexamethasone in clones 6 *v* clone 11).

Suppression by TGF-β1 of LPS and IL-1–induced production of IL-6 and GM-CSF. Clone 11 can be induced to differentiate with LPS and clone 3 with IL-1α, and this is mediated by the production of IL-6 by the cells.[16,17,29,30] Incubation of clone 11 with LPS and TGF-β1 suppressed production of IL-6 (Table 5). The production of IL-6 by normal bone marrow cells incubated with rIL-1α was also suppressed by TGF-β1 (Table 5). This suppression of IL-6 production can explain the suppression by TGF-β1 of the effects of LPS and IL-1α that are mediated with IL-6. Induction of differentiation of clone 11 cells by IL-6 or LPS and of clone 3 with IL-1α is also associated with induction of GM-CSF production,[17,48-50] and this was also suppressed by TGF-β1 (Table 5). These results indicate that the induced production of both IL-6 and GM-CSF can be suppressed by TGF-β1.

DISCUSSION

The present results indicate that TGF-β1 can selectively control the activity of different hematopoietic regulators on normal and leukemic myeloid cells. The properties studied include suppression of cell viability and colony formation by normal bone marrow myeloid precursor cells, and suppression of cell growth and differentiation in different clones of myeloid leukemic cells. In one of the leukemic clones, TGF-β1 enhanced the effect of dexamethasone on induction of differentiation. The results also indicate that the growth-inhibiting effect of TGF-β1 can be dissociated from its effect

Table 5. TGF-β1 Inhibits LPS– and IL-1α–Induced Production of IL-6 and GM-CSF

Cells	Material Added*	IL-6 Activity† (μg lysozyme/5 × 10^6 cells)		GM-CSF Activity‡ (no. of granulocyte or macrophage colonies)	
		– Anti–IL-6 Antibody	+ Anti–IL-6 Antibody	– Anti–GM-CSF Antibody	+ Anti–GM-CSF Antibody
Clone 11	rIL-6	ND	ND	43 ± 5	0
	rIL-6 + TGF-β1	ND	ND	1 ± 1	0
	LPS	12.5 ± 1.5	0.2 ± 0.1	82 ± 10	0
	LPS + TGF-β1	0.2 ± 0.1	0.2 ± 0.1	1 ± 1	0
Clone 3	rIL-1α	6.2 ± 0.8	0.1 ± 0.1	27 ± 5	0
	rIL-1α + TGF-β1	0.3 ± 0.1	0.1 ± 0.1	0	0
Normal bone marrow cells	rIL-1α	2.2 ± 0.5	0.2 ± 0.1	0	0
	rIL-1α + TGF-β1	0.3 ± 0.1	0.2 ± 0.1	0	0

*Clone 11 and clone 3 cells were seeded at 5 × 10^5 cells/mL with 2 ng/mL mouse rIL-6, 100 ng/mL LPS, or 10 ng/mL rIL-1α, respectively, and normal bone marrow cells were seeded at 2 × 10^6 cells/mL with 10 ng/mL rIL-1α, without or with 0.5 ng/mL TGF-β1 for clone 3 or 1 ng/mL TGF-β1 for clone 11 or normal bone marrow cells. The conditioned medium was collected after 48 hours. There was no detectable IL-6 in cells cultured for 48 hours without adding an inducer.

†IL-6 activity was assayed on clone 11 R-IL-1 cells incubated for 4 days with 10% of the above conditioned media and 5 μg/mL polymyxin B was added to neutralize the residual LPS in the medium. This concentration of polymyxin B can completely neutralize 200 ng/mL LPS.[20] The residual TGF-β1 in the conditioned medium added (0.1 ng/mL) was not sufficient to inhibit differentiation in clone 11 or clone 11 R-IL-1 cells by IL-6 (Fig 2). The antimouse IL-6 antibody (monoclonal)[30] was added at 2 μg/mL. ND, not done because of input of IL-6.

‡GM-CSF activity was assayed on normal bone marrow cells by induction of granulocyte or macrophage colonies in the absence or presence of 0.2% rabbit antiserum against mouse GM-CSF.[43] The number of colonies induced by 10% of the conditioned media per 5 × 10^4 normal bone marrow cells was counted after 7 days.

on cell differentiation. The formation of normal granulocyte or macrophage colonies with IL-3, GM-CSF, or M-CSF, and induction of differentiation in a clone of leukemic cells with IL-3 or GM-CSF, were not suppressed by a low concentration of TGF-β1 that suppressed these properties and the formation of normal megakaryocyte colonies with IL-6 or IL-1α. In agreement with our results, it has also been shown that TGF-β1 can inhibit IL-1–induced cell cycling in long-term cultures of hematopoietic progenitor cells.[51] It is interesting that although TGF-β1 suppressed GM-CSF– or IL-3–induced colony formation by what have been defined as primitive progenitor cells,[51-55] TGF-β1 in the present and other experiments[51-55] showed much lower or no suppression of IL-3–, GM-CSF–, or M-CSF–induced colony formation by what may be later progenitor cells.

The present results suggest that there may be differences in the intracellular signals induced by IL-6 and IL-1 compared with those induced by IL-3, GM-CSF, and M-CSF, which show differential sensitivity to TGF-β1. The fact that both IL-6 and IL-1 support the viability of the enriched myeloid precursor cell population used in our studies but do not induce the strong multiplication induced by GM-CSF, IL-3, or M-CSF, supports this suggestion. Our results are also compatible with other studies showing inducer-dependent inhibitory effects of TGF-β1 on multiplication of fibroblasts, and opposite effects of TGF-β1 as seen by inhibition or induction of differentiation in other cell types.[3-6] Our experiments have shown that although induction of differentiation can be associated with a decreased cell growth, suppression of this differentiation by TGF-β1 was not associated with the resumption of cell growth to the level found in cells that had not been induced to differentiate. In contrast, suppression of IL-1– or IL-6–induced differentiation in clone 11 cells by monoclonal antibody to IL-6[17,30] or by the addition of 0.5 mmol/L 2-methoxy benzamide (data not shown) abolishes the differentiation-associated inhibition of cell growth. Therefore, the results suggest that the differentiating cells retain some sensitivity to the growth-inhibiting effect of TGF-β1.

Previous experiments have shown that clone 11 cells treated with LPS produce IL-6, and this mediates the differentiation induced by LPS.[29,30] We have also shown that the megakaryocyte colony-inducing activity of IL-1α[47] and the differentiation-inducing activity of IL-1α on myeloid leukemic cells[17] are indirect and are mediated by the production of IL-6. We have now found that the production of IL-6 in leukemic clone 11 cells induced with LPS, and in normal bone marrow cells and leukemic cells induced with IL-1α, was suppressed by TGF-β1. This can explain the suppression by TGF-β1 of differentiation induced with LPS and IL-1α and suppression of the induction of megakaryocyte colonies with IL-1α. Therefore, these results and suppression of the activity of IL-6 by TGF-β1 indicate that TGF-β1 can suppress both the production of IL-6 and responsiveness to IL-6. In addition, TGF-β1 also suppressed production of GM-CSF in leukemic cells induced to differentiate with IL-6, IL-1, or LPS so that production of both IL-6 and GM-CSF are sensitive to TGF-β1.

It has been reported that certain human myeloid leukemic cells produce TGF-β.[56] It will be interesting to determine to what extent the production of TGF-β by leukemic cells interferes with the ability of differentiation-inducing compounds to induce differentiation in myeloid leukemic cells from patients. It will also be interesting to determine the role of secreted TGF-β during leukemogenesis on the selective suppression of normal bone marrow hematopoiesis.

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