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Inhibitory Action of Transforming Growth Factor-β on Induction of Differentiation of Myeloid Leukemia Cells

Junko Okabe-Kado,[1] Yoshio Honma, Moriaki Hayashi and Motoo Hozumi

Department of Chemotherapy, Saitama Cancer Center Research Institute, Ina-machi, Saitama 362

The effect of transforming growth factor-β (TGF-β) on induction of differentiation of mouse myeloid leukemia M1 cells was investigated. TGF-β1 induced adherence of M1 cells to plastic dishes and inhibited their proliferation. However, it did not induce differentiation-associated properties, such as phagocytic activity, lysozyme activity or morphological maturation. TGF-β1 also caused dose-dependent inhibition of dexamethasone-induced differentiation of M1 cells. The inhibitory activity of TGF-β1 was 20 times that of TGF-β2 on M1 cells. These results suggest that TGF-β1 inhibits proliferation and dexamethasone-induced differentiation of M1 cells by interacting with receptors that can distinguish between TGF-β1 and TGF-β2. TGF-β1 had a much lower inhibitory effect on the growth of a variant M1 cell clone, which was resistant to differentiation inducers, and it did not induce adherence of the resistant M1 cells.

Key words: Transforming growth factor-β — Myeloid leukemia cells — Differentiation inhibitor

Transforming growth factors (TGF-βs) are polypeptides that act hormonally in controlling the proliferation and differentiation of many types of cells.[1,2] The immunohistochemical demonstration of high levels of TGS-β in the bone marrow and hematopoietic progenitors of fetal liver[3] suggests that TGF-β may be involved in regulation of hematopoiesis. In fact, TGF-β has recently been shown to inhibit the proliferation of i) murine factor-dependent hematopoietic progenitor cells in response to interleukin-3 (IL-3) or granulocyte-macrophage colony-stimulating factor (GM-CSF),[4] ii) murine CSF-1-dependent macrophage progenitor cells,[5] iii) human erythroid (CFU-E and BFU-E), multilineage (CFU-GEMM) colony-forming cells,[6] and iv) mouse megakaryocyte (CFU-MK) and granulocyte-macrophage (CFU-GM) colony-forming cells.[7] These findings suggest that TGF-β may be involved in negative regulation of hematopoietic cells. On the other hand, TGF-β also possesses multiple and complex regulatory functions that influence cell differentiation.[1] In the present work, we investigated the effects of TGF-β on proliferation and induction of differentiation of mouse myeloid leukemia M1 cells.

MATERIALS AND METHODS

Chemicals Highly purified porcine TGF-β1 and TGF-β2 were purchased from R & D Systems (Minneapolis, MN). Human platelet TGF-β1 was purchased from Collaborative Research (Bedford, MA). Dexamethasone was obtained from Sigma Chemical Co.

[1] To whom correspondence should be addressed.

Cell line and cell culture M1 cells were cultured in suspension in Eagle's minimum essential medium with twice the normal concentrations of amino acids and vitamins, and supplemented with 10% heat-inactivated calf serum.[8]

Assay of properties of differentiated cells Phagocytosis, lysozyme activity, and adherence to plastic dishes were assayed as reported previously.[8,9] The percentages of cells that were morphologically similar to macrophages were determined in May-Gruenwald-Giemsa-stained smears.

RESULTS

Effect of TGF-β on growth of M1 cells M1 clone S-2 cells can be induced to differentiate by various differentiation inducers. Clone R-1 cells are resistant to differentiation inducers and are much more leukemogenic than S-2 cells.[8] In liquid culture, TGF-β1 inhibited the growth of S-2 cells (Fig. 1A), but had a much lower inhibitory effect on the growth of R-1 cells (Fig. 1B and C). Under similar conditions, TGF-β2 did not inhibit the growth of S-2 cells (data not shown).

Effect of TGF-β1 on differentiation-associated properties of M1 cells TGF-β1 induced adherence of S-2 cells to plastic dishes (Fig. 2). However, it induced scarcely any phagocytosis, lysozyme activity or morphological change (Table I). It did not induce adherence of R-1 cells to plastic dishes even at higher concentrations or on longer treatment (Fig. 2). These results suggest that TGF-β1 induces the adherence of S-2 cells to plastic dishes without inducing differentiation of M1 cells.

Effect of TGF-β1 on induction of differentiation of M1 cells Dexamethasone, an inducer of differentiation of

M1 cells, enhanced all the differentiation-associated properties of the cells tested (Table I). TGF-β1 caused dose-dependent inhibition of dexamethasone-induced phagocytic activity of M1 clone S-2 cells (Fig. 3), and it also inhibited all the other differentiation-associated properties except adherence (Table I). TGF-β1 had 20 times more effect than TGF-β2 on S-2 cells (Fig. 3A and B). These results suggest that TGF-β1 inhibits proliferation and induction of differentiation of S-2 cells by interacting with receptors that can distinguish between TGF-β1 and TGF-β2. TGF-β1 also inhibited the induction of phagocytic activity by other differentiation inducers of M1 cells, such as 1α,25-dihydroxyvitamin D_3 and conditioned medium from mouse peritoneum (Table II). These results suggest that TGF-β1 may have a direct

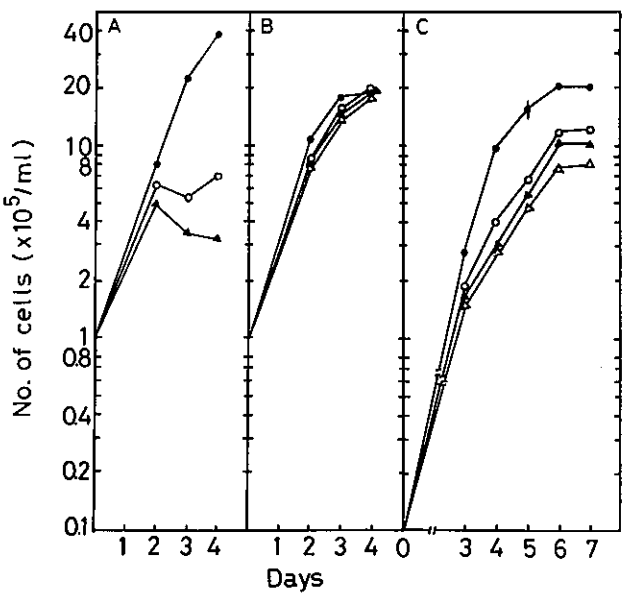

Fig. 1. Effects of TGF-β1 on growth of M1 clone S-2 (A) and R-1 (B and C) cells. No addition (●), 0.5 ng/ml (○), 2.5 ng/ml (▲), and 10 ng/ml (△) of porcine TGF-β1.

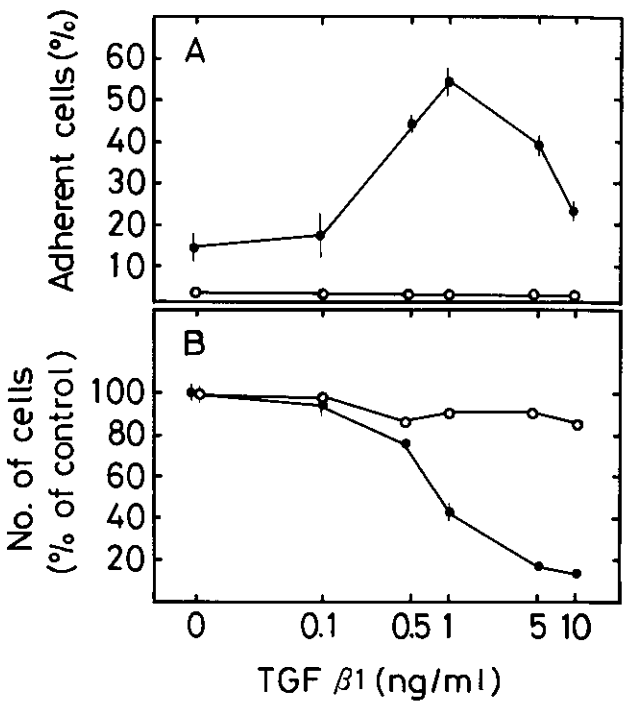

Fig. 2. Induction of adherence to plastic dishes of M1 clone S-2 cells by TGF-β1. M1 clone S-2 (●) and R-1 (○) cells (1×10^5/ml) were incubated with porcine TGF-β1 for 4 days, and then the adherence (A) and the number of cells (B) were determined. Bars, SE (n=3).

Table I. Differentiation-associated Properties of TGF-β-treated M1 Cells

	Dexamethasone	TGF-β1	
		−	+
Adherence (%)	−	7.4±0.3	64.5±1.0
	+	48.8±3.7	58.7±2.4
Phagocytosis (%)	−	6.5±3.7	3.5±3.8
	+	65.5±7.1	7.3±2.8
Lysozyme activity (μg/mg protein)	−	0.22	0.28
	+	7.74±0.16	2.12±0.29
Morphological change (macrophage %)	−	0	0
	+	26.3±11.8	6.5±2.6

Values are means for duplicate determinations or mean±SE for 4 assays. Adherence and phagocytosis were assayed after treatment for 2 days and lysozyme activity and morphological changes were measured after treatment for 4 days.

Table II. Effect of TGF-β1 on Induction of Differentiation of M1 Cells by Various Inducers of Differentiation

Inducer	TGF-β1	No. of cells ($\times 10^5$/ml)	Phagocytic activity (%)
Dexamethasone	−	15.8±1.1	73.0±4.5
($1\times10^{-8}M$)	+	11.9±1.1	18.3±7.5
1α,25-Dihydroxy-	−	15.1±2.7	42.8±6.3
vitamin D_3 (20 ng/ml)	+	6.8±0.8	11.5±4.8
Conditioned medium[a)]	−	10.7±0.3	55.1±7.9
(20%)	+	8.7±1.1	36.5±3.1

a) Conditioned medium from the peritoneum of syngeneic SL mice was prepared as reported previously[9] and used as a protein inducer for differentiation of M1 cells.

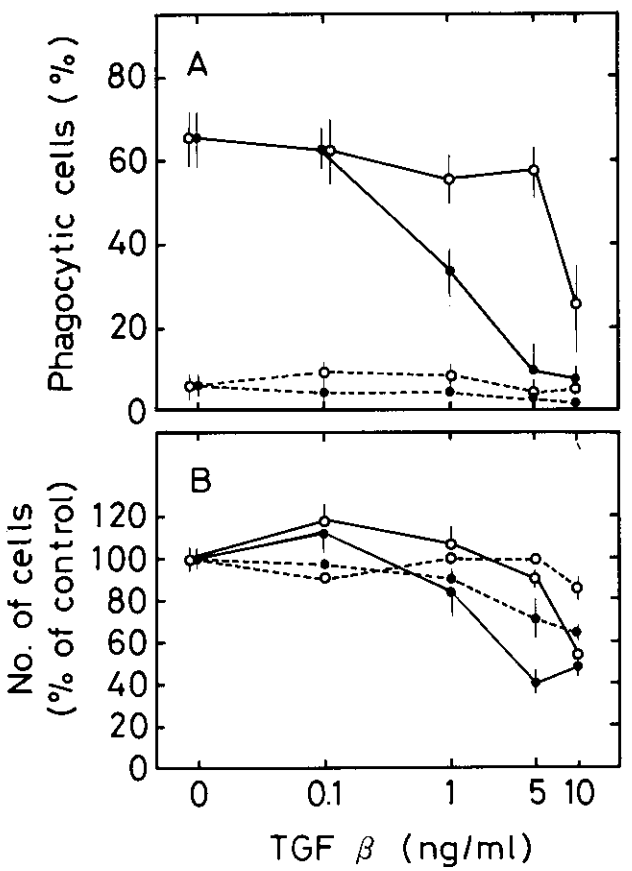

Fig. 3. Inhibition of dexamethasone-induced phagocytic activity by TGF-β1. M1 clone S-2 cells (2×10^5/ml) were incubated with porcine TGF-β1 (●) or TGF-β2 (○) in the presence (solid line) or absence (broken line) of dexamethasone (1×10^{-8} *M*) for 2 days. Then phagocytic activity (A) and the number of cells (B) were assayed. The numbers of cells in control culture with and without dexamethasone were 14.0±0.2(SE)$\times10^5$/ml and 13.6±0.8(SE)$\times10^5$/ml, respectively. Bars, SE (n=4).

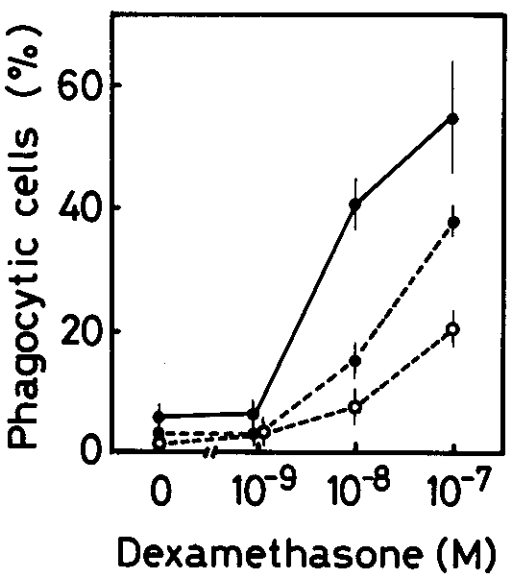

Fig. 4. Sensitivity to dexamethasone of TGF-β-treated Ml cells. Ml clone S-2 cells (2×10^5/ml) were cultured with (broken line) or without (solid line) porcine TGF-β1 (5 ng/ml) for 1 day. The nonadherent cells (○) were separated from adherent cells (●) and each fraction was washed with TGF-β-free medium and incubated with dexamethasone for 1 day more. Bars, SE (n=4).

effect on M1 cells rather than acting on differentiation inducers.

As TGF-β1-treated S-2 cells became adherent to plastic dishes, we examined the sensitivities of adherent and nonadherent S-2 cells to dexamethasone. About 50% of the S-2 cells treated with TGF-β1 (5 ng/ml) for 1 day adhered to plastic dishes. Under these conditions, the inhibitory effect of TGF-β1 on proliferation of the S-2 cells was insignificant. But on treatment with TGF-β1,

both the adherent and the nonadherent S-2 cells became less sensitive to dexamethasone (Fig. 4). Therefore, it is unlikely that adherence itself reduced the sensitivity of the cells to dexamethasone.

DISCUSSION

TGF-β has multifunctional properties because it either stimulates or inhibits the proliferation and differentiation of many types of cells.[1,2] Recently the effects of TGF-β1 on hematopoiesis have been studied.[10–12] TGF-β1 selectively inhibits early hematopoietic progenitor growth and differentiation but not more mature progenitors. TGF-β1 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. Thus TGF-β1 functions as a selective regulator of differentiating normal hematopoietic cells, and suppresses myeloid leukemic cell growth.[10–12] The present results demonstrated that TGF-β1 inhibited the proliferation of differentiation-inducible (S-2) M1 cells but did not inhibit that of undifferentiating (R-1) M1 cells in liquid culture. There was no detectable difference between the differentiation-inducible M1 cells and undifferentiating M1 cells in their tumor-related surface antigen, cell morphology, *in vitro* proliferation rate, or agglutinabilities with several lectins.[9] However, the R-1 cells were much more leukemogenic than S-2 cells, and the survival time of syngeneic mice inoculated with R-1 cells was less than that of mice inoculated with the S-2 cells.[8,9] Studies are required on whether the significant difference between the sensitivity to TGF-β1 of R-1 cells and S-2 cells is related to that between the sensitivity to differentiation inducers, and that between the leukemogenicity of R-1 cells and S-2 cells.

We previously reported that the nondifferentiating (R-1) M1 cells produce a protein factor(s) that inhibits induction of differentiation of M1 cells.[8] This inhibitory protein factor (I-factor) was shown to be closely associated with resistance of the leukemic cells to differentiation inducers. Recently, we purified the I-factor (Mr 68,000, PI 8.8–9.0) from conditioned medium of R-1 cells.[9] As we found in this work that TGF-β1 inhibits the induction of differentiation of M1 cells, we are now examining the relationship of TGF-β1 to the I-factor.

TGF-β1 could induce adhesion of S-2 cells but not of R-1 cells to plastic tissue culture dishes (Fig. 2). TGF-β strongly stimulates the expression of fibronectin, various types of collagen, and matrix proteoglycans in many types of cells.[13] Furthermore, TGF-β can control the level and topography of cell adhesion receptors.[13] Therefore, the adhesion of S-2 cells to plastic dishes induced by TGF-β1 may be mediated by the expression of some matrix components and/or cells adhesion receptors, and S-2 cells should be useful in studies on the biochemical events required for induction of adhesion by TGF-β.

Under the conditions in which TGF-β1 induced adherence of S-2 cells, it did not induce other differentiation-associated properties (Table I). Therefore, TGF-β1 is not an inducer of differentiation of M1 cells, although it stimulates the differentiation of some other cells.[14]

Despite their structural differences, TGF-β1 and TGF-β2 are equally potent in inhibiting epithelial cell proliferation and adipogenic differentiation.[4] However, in the presence of an inducer of differentiation, TGF-β1 inhibited induction of differentiation of S-2 cells, whereas TGF-β2 did not (Fig. 3). These findings suggest that TGF-β1 acts on S-2 cells, like normal hematopoietic progenitor cells,[4] by interacting with receptors that can distinguish between TGF-β1 and TGF-β2. It will be interesting to analyze the receptors for TGF-β on differentiation-inducible leukemic S-2 cells and non-differentiating leukemic R-1 cells. As the expressions of fibronectin and type 1 collagen increase during inhibition of differentiation of preadipocytes and myoblasts by TGF-β,[2] it will also be of interest to examine whether the expression of matrix components is involved in the mechanisms of inhibition by TGF-β1 of differentiation of M1 cells.

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