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Transforming Growth Factor-β1 Enhances the Suppression of Human Hematopoiesis by Tumor Necrosis Factor-α or Recombinant Interferon-α

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The effects of transforming growth factor-β1 (TGF-β1) on human hematopoiesis were evaluated in combination with two other regulatory cytokines, namely, recombinant human tumor necrosis factor-α (TNF-α) and recombinant human interferon-α (rIFN-α). Combinations of TNF-α and TGF-β1 resulted in a synergistic suppression of colony formation by erythroid progenitor cells (BFU-E) and an additive suppression of granulocyte-macrophage (CFU-GM) and multipotential (CFU-GEMM) progenitor cells. In addition, TGF-β1 synergized with rIFN-α to suppress CFU-GM formation, while the combined suppressive effects of both cytokines on CFU-GEMM and BFU-E were additive. When TGF-β1 was tested with TNF-α or IFN-α on granulocyte/macrophage colony-stimulating factor (GM-CSF)-stimulated bone marrow cells in a 5-day proliferation assay, the antiproliferative effects of TGF-β1 and TNF-α were additive, while those with TGF-β1 and rIFN-α were synergistic. A similar pattern was seen in the suppression of the myeloblastic cell line KG-1 where TGF-β1 in combination with TNF-α resulted in an additive suppression while inhibition by TGF-β1 and IFN-α was synergistic.

These results demonstrate for the first time the cooperative effects between TGF-β and TNF-α and IFN-α in the suppression of hematopoietic cell growth, raising the possibility that TGF-β might be used in concert with TNF-α or IFN-α in the treatment of various myeloproliferative disorders.

Key words: TGF-β, TNF-α, IFN-α, hematopoiesis, synergy

Transforming growth factor-β (TGF-β) is a 25-kd disulfide-linked homodimer produced by a variety of normal and transformed cells [1,2]. It has also been found

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to be synthesized and released by a variety of hematopoietic cells, including platelets [3], T cells [4], macrophages [5], and bone matrix [6] and can both stimulate and inhibit cell proliferation, depending largely on the cell type and the nature of other cytokines present [7,8]. More recently, it has also been shown to be a potent inhibitor of hematopoiesis [9–11], selectively suppressing the growth of early bi- or multipotent progenitor cells as opposed to later committed progenitor cells [10,11]. Amongst other monokines also shown to regulate hematopoiesis in vitro are tumor necrosis factor-α (TNF-α) and interferon-α (IFN-α), both of which suppress colony formation in vitro by human bone marrow granulocyte-macrophage (CFU-GM), erythroid (BFU-E), and multipotential (CFU-GEMM) progenitor cells [12–14]. In addition, both TNF-α and IFN-α synergize with IFN-γ in mediating the anti-proliferative effect [15,16].

In the present study, we demonstrate an enhancement of the antiproliferative effect of TNF-α and IFN-α by TGF-β1 on the growth of human hematopoietic cell progenitors in vitro which can either be additive or synergistic.

MATERIALS AND METHODS

Cells and Materials

Bone marrow cells were obtained from healthy donors who had given informed consent. Nonadherent light-density cells were isolated and purified as described previously [17,18]. The cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% heat-inactivated fetal calf serum (FCS) (Hyclone, Logan, UT) supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), and 3 mg/ml glutamine. The myeloblastic cell line KG-1 was maintained in tissue culture flasks in RPMI 1640 (Advanced Biotechnologies, Silver Spring, MD) supplemented with FCS, antibiotics, and glutamine as for IMDM.

Recombinant human granulocyte/macrophage colony-stimulating factor (GM-CSF) was a gift from Immunex Corp. (Seattle, WA). Bovine TGF-β1 was obtained from Collagen Corp. (Palo Alto, CA), rIFN-α from Hoffman LaRoche (Nutley, NJ), and rTNF-α from Dai Nippon (Osaka, Japan). Erythropoietin (Epo) was purchased from AMGEN (Thousand Oaks, CA).

Colony Assays

The colony assay for human CFU-GEMM, BFU-E, and CFU-GM was performed as described previously [19]. Bone marrow cells were plated at 10^5 cells in 35-mm LUX standard tissue culture dishes containing a 1 ml mixture of IMDM, 0.35% Seaplaque Agarose (FMC Bioproducts, Rockland, ME), 30% FCS, 1% detoxified bovine serum albumin (Sigma Chemical Co., St Louis, MO), 2 U/ml Epo, 2×10^{-4} M hemin (Sigma), and a predetermined optimal concentration of GM-CSF (10 ng/ml). Dishes were incubated at 37°C in a humidified atmosphere flushed with 5% $C0_2$ in air. Colonies (>50 cells) were scored with an inverted microscope after 14 days of incubation.

DNA Synthesis

Cellular proliferation (10^5 cells/100 μl) was assayed after 5 days in suspension culture by ^{3}H-thymidine incorporation (New England Nuclear, Boston, MA; 6.7 Ci/mmol) as described previously [10].

RESULTS

We have previously shown the optimal antiproliferative effects of TGF-β1 on normal hematopoiesis to be at 10 ng/ml (400 pM) [11]. A suboptimal concentration of TGF-β1 (0.1 ng/ml) was therefore selected for assessing its suppressive effects in combination with varying concentrations of TNF-α or IFN-α on normal human bone marrow colony formation. The addition of TGF-β1 enhanced TNF-α-mediated suppression of CFU-GEMM, BFU-E, and CFU-GM colony formation (Fig. 1). A synergistic response was seen in the inhibition of BFU-E colonies at low concentrations of TNF-α (0.1–1 U/ml), with the degree of enhancement ranging from 4 to 7 times the expected additive response. For example, formation of BFU-E was inhibited by approximately 35% with 0.1 ng/ml TGF-β1 and 1 U/ml TNF-α, which individually inhibited colony formation by 2% and 3%, respectively. The predicted additive effect would be 5%, while the actual measured response was seven times higher. While IFN-α at optimal doses of 100 U/ml suppressed CFU-GEMM and BFU-E by >90% (Fig. 2A,B) combinations of TGF-β1 with suboptimal concentrations of IFN-α augmented suppression to levels attained with 100 U/ml IFN-α alone. Furthermore, a synergistic response was observed in the suppression of CFU-GM with the degree of enhancement being 2.9–5.3 times the expected additive response (Fig. 2C). For instance, CFU-GM formation was inhibited approximately 53% by 0.1 ng/ml TGF-β1 in combination with 10 U/ml of IFN-α, whereas the degree of inhibition by either cytokine alone was 2% and 16%, respectively, giving a predicted additive effect of 18%.

The effects of mixed cytokines on GM-CSF-induced proliferation were also studied in suspension cultures. As demonstrated in Figures 3 and 4, the anti-proliferative activity of TNF-α or IFN-α at 0.1–100 U/ml was significantly enhanced by the addition of 0.1–1 ng/ml TGF-β1. To show the nature of these cooperative interactions, both sets of data were plotted in an isobologram in which concentrations of these cytokines, which individually or in combination inhibited growth by 40% were plotted in Figures 3B and 4B. In this graphic analysis marked departure of the line connecting the experimental points below the diagonal is indicative of synergistic action [20]. The results of this analysis indicated that the combined antiproliferative effects of TNF-α and TGF-β1 were additive whereas those of TGF-β1 with IFN-α were synergistic.

To further delineate the nature of the interaction between TGF-β1 and other regulatory cytokines, combinations of TGF-β1 and TNF-α or IFN-α were tested on the myeloblast cell line KG-1. As shown in Figures 5 and 6, the interaction between TGF-β1 and TNF-α was additive, while that between TGF-β1 and IFN-α was synergistic, with combinations of the latter giving an augmented suppression ranging from 1.6 to 2.1 times the additive response.

DISCUSSION

It has been well established that TNF-α and the different classes of interferons exert suppressive effects on myeloproliferation either individually or synergistically in combination [12–16]. The suppressive effects of TGF-β1 on hematopoietic cell growth has only recently been demonstrated [9–11]. To our knowledge, this is the first report demonstrating the augmentation of TNF-α-or IFN-α-mediated suppression

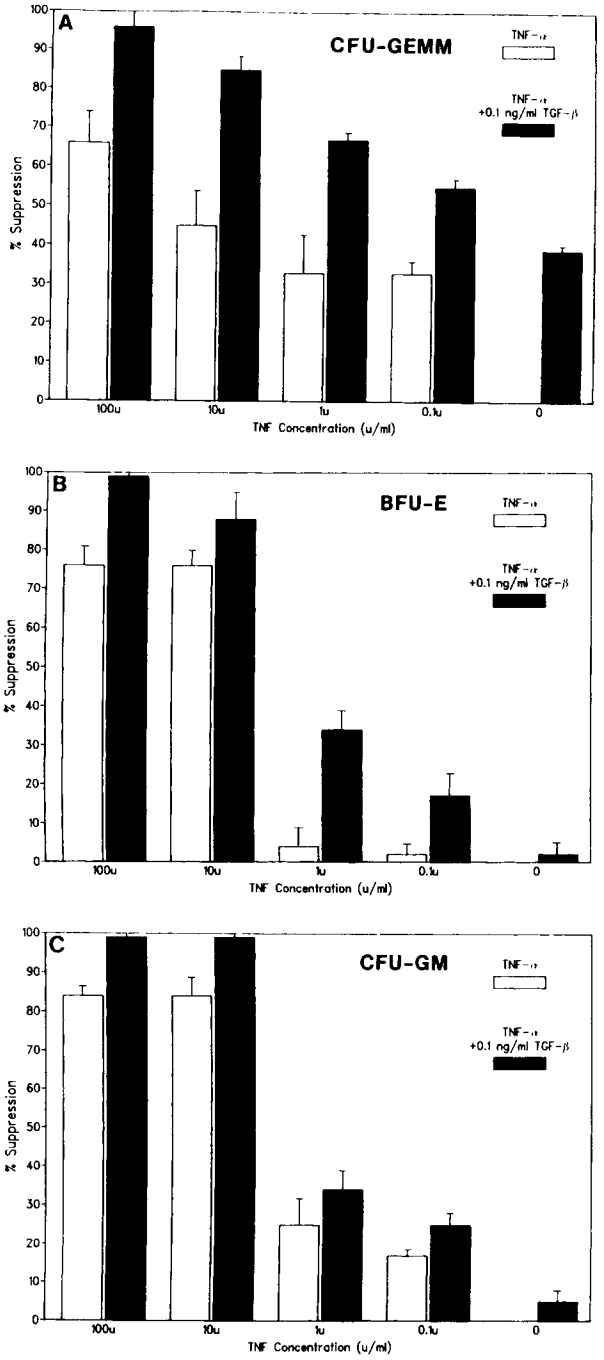


Fig. 1. Synergistic inhibition of A) CFU-GEMM, B) BFU-E, and C) CFU-GM colony formation by 0.1 ng/ml TGF-β1 and varying concentrations of TNF-α. Percentage suppression is expressed as mean ± SEM of four experiments. Control colonies per 10^5 nonadherent bone marrow cells were 9 ± 3, 31 ± 5, and 70 ± 19 for CFU-GEMM, BFU-E, and CFU-GM, respectively.

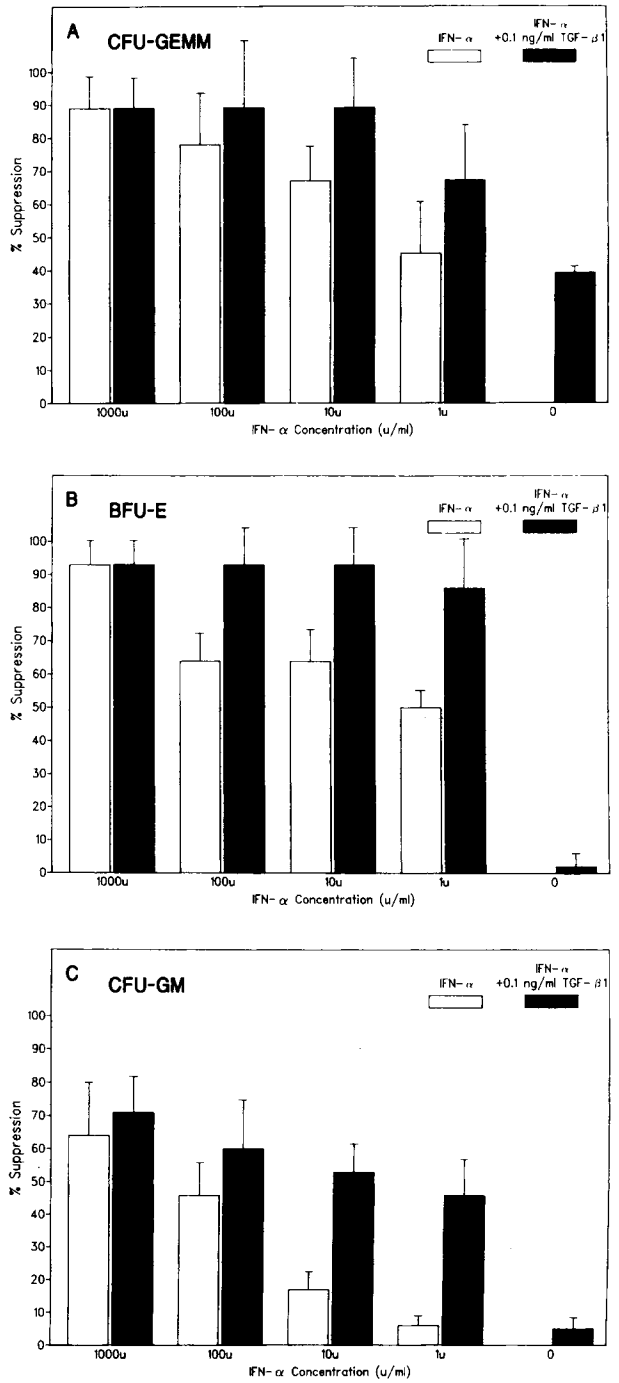


Fig. 2. Synergistic inhibition of **A)** CFU-GEMM, **B)** BFU-E, and **C)** CFU-GM colony formation by 0.1 ng/ml TGF-β1 and varying concentrations of IFN-α. Percentage suppression is expressed as mean ± SEM of four experiments. Control colonies per 10^5 nonadherent bone marrow cells were 9 ± 3, 31 ± 5, and 70 ± 19 for CFU-GEMM, BFU-E, and CFU-GM, respectively.

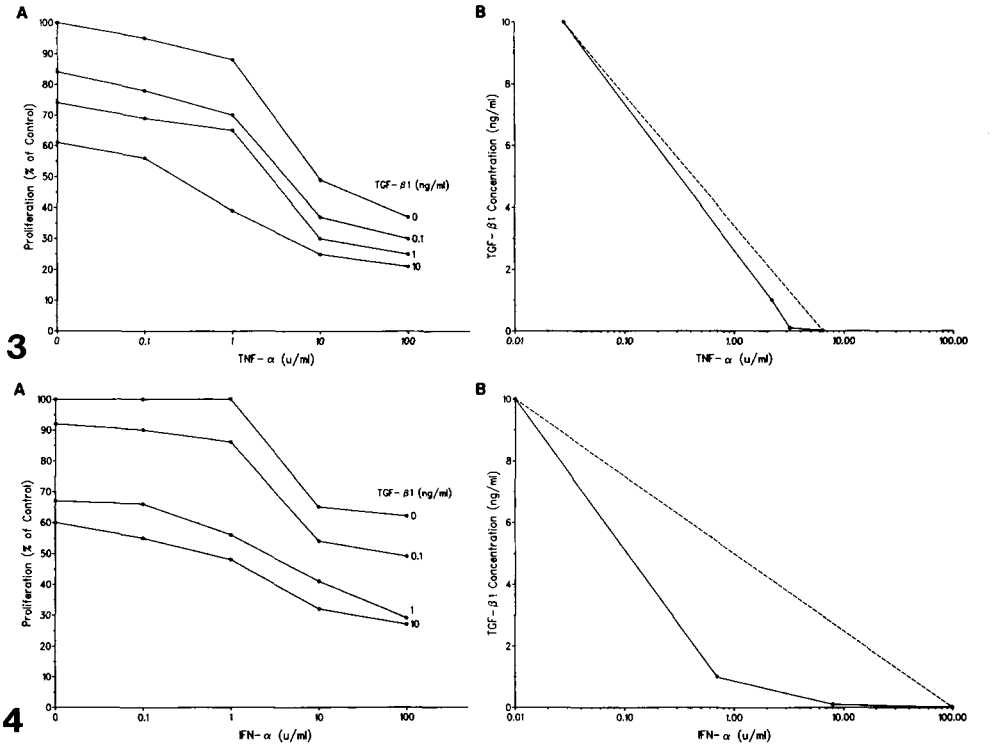
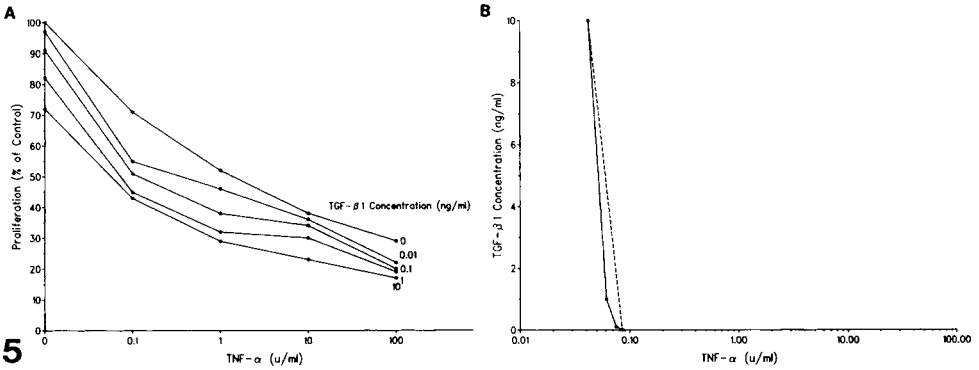


Fig. 3. Antiproliferative activity of TGF-β1 and TNF-α on GM-CSF-induced bone marrow cell proliferation. Cells (10^5) seeded in microtiter plates were cultured with the TGF-β1 and TNF-α concentrations indicated. **A:** The ^{3}H-thymidine incorporation relative to untreated control cultures is shown, expressed as a percentage. **B:** Isobologram of the antiproliferative effect of TGF-β1 and TNF-α, which was plotted as described in Results.

Fig. 4. Antiproliferative activity of TGF-β1 and IFN-α combinations on GM-CSF-induced proliferation of bone marrow cells. **A:** Cells seeded in microtiter plates were treated for 5 days with the TGF-β1 and IFN-α concentrations indicated. The ^{3}H-thymidine incorporation relative to untreated control cultures is shown. **B:** Isobologram of the antiproliferative effect of TGF-β1 and IFN-α.

of bone marrow progenitor cell proliferation by TGF-β1. While combinations of TGF-β1 and TNF-α were additive in the suppression of CFU-GEMM and CFU-GM colony formation (Fig. 1) synergistic interactions were observed in the suppression of BFU-E colony formation. Combinations of both cytokines increased the antiproliferative activity by 4–7 times the expected additive response. When combinations of TGF-β1 and TNF-α were added to GM-CSF-stimulated bone marrow cells in a 5-day proliferation assay, the pattern of suppression induced by both factors was additive, as depicted in the isobologram (Fig. 3).

Furthermore, synergistic responses were generated between TGF-β1 and IFN-α in appropriate dose combinations for the suppression of CFU-GM colony formation, whereas the combined suppressive effects on colony formation by CFU-GEMM and BFU-E were strictly additive (Fig. 2); and the overall suppression of bone marrow cell proliferation in suspension assays demonstrated a synergistic response (Fig. 4). Similar results have been reported by Brown et al. [21] where TGF-β1 was shown to synergize with low concentrations of IFN-γ in suppressing the proliferation



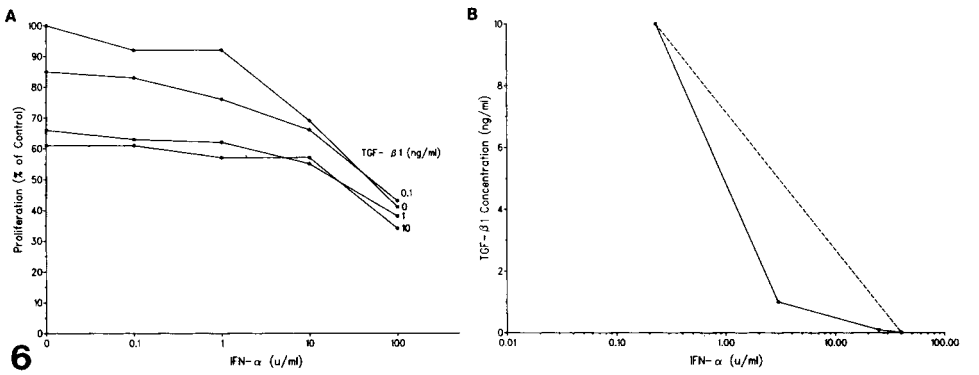


Fig. 5. Antiproliferative activity of TGF-β1 and TNF-α combinations on KG-1 proliferation. **A:** Cells (2×10^4) were seeded in microtiter plates and treated for 3 days with the TGF-β1 and TNF-α concentrations indicated. **B:** Isobologram of the antiproliferative effect of TGF-β1 and TNF-α.

Fig. 6. Antiproliferative activity of TGF-β1 and IFN-α combinations on KG-1 proliferation. **A:** Cells (2×10^4) were seeded in microtiter plates and treated for 3 days with the TGF-β1 and IFN-α concentrations indicated. **B:** Isobologram of the antiproliferative effect of TGF-β1 and IFN-α.

of human melanoma cells, whereas a strict additive response was seen with higher concentrations of IFN-γ.

It could be argued that the augmented suppression induced by TGF-β1 might occur via an indirect effect, such as the possible stimulation of bone-marrow derived monocytes to produce additional amounts of TNF-α or IFN. Indeed, it has recently been reported that prostaglandin E synergizes with IFN in the suppression of CFU-GM, although this synergistic effect was found to be due to the induction of TNF-α by marrow-adherent cells [22]. However, since the experiments in this present study were performed on adherent cell-depleted populations of normal human bone marrow, it is unlikely that the stimulation of monocyte-derived cytokines played a significant role in this augmented suppression. However, in the case of normal bone marrow, it is also not known whether the combined suppressive effects of TGF-β1 and TNF-α or IFN-α were exerted on similar or different cell populations, since bone marrow consists of a heterogenous population of hematopoietic cells. Nevertheless, all three cytokines have been demonstrated to exert similar potent suppressive effects on CFU-GEMM, BFU-E, and CFU-GM [9–14], suggesting substantial overlap amongst the various susceptible cell types. In addition, at least with the leukemic cell line KG-1,

which represents a homogenous population of myeloid precursor cells, it can be demonstrated that TGF-β1 augments the antiproliferative effects of TNF-α or IFN-α (Figs. 5, 6).

Both TNF-α and IFN-α are currently the subject of extensive phase I and phase II clinical trials, and while the former has yielded disappointing results to date, the latter has been shown to be highly active against hairy cell leukemia [23–25] and has demonstrated antitumor responses in patients with chronic granulocytic leukemia [26], cutaneous T cell lymphomas [27], and multiple myeloma [28]. However, as with most biological response modifiers, both agents induce host toxicity when administered frequently at high doses [29]. Based on the results presented in this work, it is possible that TGF-β1 might be used in combination with TNF-α or IFN-α in the immunotherapy of certain myeloproliferative disorders at doses giving minimum toxicity with maximum therapeutic effect.

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