Transforming Growth Factor β Is a Potent Inhibitor of Interleukin 1 (IL-1) Receptor Expression: Proposed Mechanism of Inhibition of IL-1 Action

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Summary

Transforming growth factor β (TGF-β) acts as a potent inhibitor of the growth and functions of lymphoid and hemopoietic progenitor cells. Cell proliferation depends not only on the presence of growth factors, but also on the development of specific receptor-signal transducing complexes. We therefore investigated whether the inhibitory actions of TGF-β could be mediated by inhibition of growth factor receptors. TGF-β inhibited the constitutive level of interleukin 1 receptor (IL-1R) expression on several murine lymphoid and myeloid progenitor cell lines, as well as IL-1R expression induced by interleukin 3 (IL-3) on normal murine and human bone marrow cells. Furthermore, treatment of bone marrow progenitor cells with TGF-β concomitantly inhibited the ability of IL-1 to promote high proliferative potential (HPP) colony formation as well as blocked IL-1–induced IL-2 production by EL-4 6.1 cells. These findings provide the first evidence that the inhibitory action of TGF-β on the growth and functional activities of hematopoietic and T cells is associated with a reduction in the cell surface receptor expression for IL-1.

Transforming growth factor β (TGF-β) is a highly conserved polypeptide, originally characterized by its ability to induce the anchorage-independent growth of NRK 49F fibroblasts in soft agar (1). Since then, TGF-β has been shown to be multifunctional in its influence on cell growth. While TGF-β acts as a growth factor for mesenchymal cells, osteoclasts, and Schwann cells, it is also a potent inhibitory signal for a variety of other growth factor–driven biological functions (2). We have shown that TGF-β is a selective negative regulator of CSF-driven growth of both murine and human immature hemopoietic cells (3–4). In particular, TGF-β inhibits the growth of primitive bone marrow progenitors such as the high proliferative potential colony-forming cells (HPP-CFC),[1] which develop in response to hemopoietin-1 (IL-1) plus CSFs (5). TGF-β also affects other IL-1-driven biological functions, including collagenase production by synovial cells, thymocyte and lymphocyte proliferation, as well as chondrocyte activation (2, 6–8).

TGF-β may inhibit growth factors such as IL-1 at the level of receptor expression. In this context, high affinity IL-1R have been detected on a variety of responsive cells, including fibroblasts, keratinocytes, T cells, B cells, monocytes, and neutrophils (9–12). Based on studies of cells transfected with a cDNA encoding for IL-1R, it has been suggested that the density of IL-1R can regulate IL-1 action (13). We therefore tested the effect of TGF-β on the level of IL-1R expressed on murine lymphoid and myeloid progenitor cell lines. We evaluated the effect of TGF-β on IL-1R present on several IL-3–dependent early myeloid progenitor cell lines. Although IL-1R were not significantly detectable on freshly isolated normal murine and human bone marrow cells, treatment with IL-3 resulted in a significant upregulation of receptor expression. Treatment of these IL-3–treated normal hemopoietic progenitor cells, myeloid cell lines, as well as a T cell line with physiological concentration of TGF-β markedly reduced the number of IL-1Rs and concomitantly resulted in a loss of responsiveness to IL-1. These findings suggest that the inhibi-

[1] *Abbreviations used in this paper:* DSS, disuccinimidyl suberate; 5-FU, 5-fluorouracil; HPP-CFC, high proliferative potential colony-forming cells.

tory action of TGF-β may be based on a reduction in cell surface IL-1R expression.

Materials and Methods

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

Cytokines. Bovine TGF-β was purified to homogeneity using a previously described procedure (14). Murine rIL-3 was obtained from the supernatant of COS-7 cells transfected with IL-3 cDNA using a previously described procedure (15). This supernatant does not contain other CSF activities as measured on factor-dependent cell lines and the endotoxin level was found to be <0.038 ng/ml. Human IL-3 was obtained from Immunex Corp. (Seattle, WA) and human rIL-1α was provided by P. Lomedico from Hoffmann-La Roche, Nutley, NJ.

Cell Lines. The murine IL-3-dependent myeloid hemopoietic cell lines FDC-P1, 32D-c123, and 32D-c113 were derived from long-term bone marrow cultures (16, 17). NFS-60, NFS-58, and DA-3 were derived from the preleukemic spleens from mice infected with murine leukemia viruses (18). These cell lines have the characteristics of immature hemopoietic progenitors as determined by morphology, cytochemistry, and cell surface marker expression. The murine myeloid cell line FW311 was derived from fetal liver and the murine myeloid cell line AC-2 was provided by J. Garland. The murine cell line EL-4 6.1, a variant subline of EL-4 thymoma cells (19), was provided by H.R. MacDonald (Ludwig Institute for Cancer Research, Basel, Switzerland). The murine T cell line LBRM-33-1A4 was purchased from American Type Culture Collection (Rockville, MD).

Preparation of Iodinated IL-1α. Human rIL-1α was labeled with ^{125}I using chloramine-T reagent as described previously (20). The radiolabeled IL-1α had a specific activity that ranged from 1 to 3 $\times$ 10^{15} cpm/mmol. There was no significant loss of biological activity of the radiolabeled IL-1α as measured using the thymocyte co-mitogenic activity assay.

Receptor Binding Assay. After the different treatments, the cell suspension was washed once with cold medium and the cell pellet was treated for 1 min on ice with 50 mM glycine-HCl (pH 3) to remove possible endogenous IL-1. Such treatment removes >95% of the bound ^{125}I-IL-1α. Subsequently, the cells were washed twice with binding medium (RPMI 1% BSA supplemented with 0.1% sodium azide and 10 mM Hepes) and incubated at 4°C with 500 pM ^{125}I-labeled human IL-1α in a final volume of 0.2 ml. After 2–3 h incubation at 4°C, cell-bound radioactivity was separated from unbound ^{125}I-IL-1α by centrifugation of the sample through a mixture of 1.5:1 (vol/vol) dibutyl phthalate/bis(2-ethylhexyl)-phthalate (Eastman Kodak Co., Rochester, NY). Nonspecific binding was determined by incubating the cells with labeled IL-1α in the presence of 50-fold excess of unlabeled ligand.

Affinity Cross-linking. After a 1-h incubation with ^{125}I-IL-1 at 4°C, the cells were washed once in cross-linking buffer (PBS, 10 mM $MgCl_2$, pH 8.3) and incubated for 30 min at 4°C with constant rotation in 1 ml of cross-linking buffer containing 100 μg disuccinimidyl suberate (DSS; Pierce Chemical Co., Rockford, IL). Receptor-bound ligands were cross-linked using 100 μg/ml DSS and rotated at 4°C for 30 min. After two washes in TRIS buffer, 5 $\times$ 10^6 cells were solubilized for 30 min at 4°C in lysis buffer (20 mmol/L Tris-HCl, pH 7.4, 50 mmol/liter NaCl, 0.5% NP-40, 0.5% sodium deoxycholate, 4 mmol/liter iodoacetic acid, 5 mmol/liter sodium pyrophosphate, 5 mmol/liter sodium fluoride and 1 mmol/liter PMSF). Lysates were centrifuged at 10,000 *g* for 10 min to remove nuclei and other debris. The protein content of each sample was determined using a commercially available kit (BioRad Protein Assay; BioRad Laboratories, Richmond, CA), and samples containing equal amounts of proteins were run under reducing condition on 7–15% SDS-PAGE. Affinity labeled protein was detected by autoradiography of the dried gels.

HPP Colony Formation. The bone marrow cells were aspirated from the femur of BALB/c mice injected 5 d previously with 5-fluorouracil (5-FU; LyphoMed Inc., Rosemont, IL) at 150 mg/kg and plated into a double-layer agar culture system as previously described (21). After 14 d of incubation, HPP-CFC that represent colonies >0.5 mm in diameter were scored using a dissecting microscope.

IL-2 Production and Assay. EL-4 6.1 cells were cultured at 2 $\times$ 10^5 cells/ml for 24 h with or without various agents. IL-2 activity in the supernatants was quantitated by their ability to support the growth of the IL-2–dependent murine CTLL-2 cells as previously described (22). Units of IL-2 were analyzed using the PROBIT program.

Results

Dose- and Time-dependent Effect of TGF-β on NFS-60 Cells. Using ^{125}I-IL-1α, we found that three of the seven IL-3–dependent myeloid progenitor cell lines tested, namely NFS-60, NFS-58, and AC-2, were positive for IL-1R expression (ratio of specific/nonspecific binding, >1) (Table 1). We used the IL-1R$^+$ cell line NFS-60 to study the effect of TGF-β on IL-1R expression in detail. When NFS-60 cells maintained in IL-3 were treated for 24 h with increasing concentrations of TGF-β, we observed a dose-dependent reduction in IL-1R

Table 1. *IL-1R Expression on Murine Hemopoietic Progenitor Cell Lines*

Cell lines*	Specific binding‡	Ratio
	cpm	
NFS-60	1,202 ± 203	4.2
NFS-58	1,653 ± 263	3.8
AC-2	1,605 ± 47	4.8
DA-3	153 ± 7	<1
FW311	<50	<1
FDC-P1	<50	<1
32D-c113	<50	<1
32D-c123	198 ± 23	<1

* Factor-dependent cell lines were maintained in RPMI medium containing 20% WEHI-3 conditioned medium.

‡ The specific binding was determined by subtracting the number of counts per minute bound in the presence of 50-fold excess IL-1α. Each point represent the mean ± SEM of two or more determinations. In our assay, the ratio of specific/nonspecific binding >1 was statistically shown to reflect the presence of a detectable and measurable number of receptors.

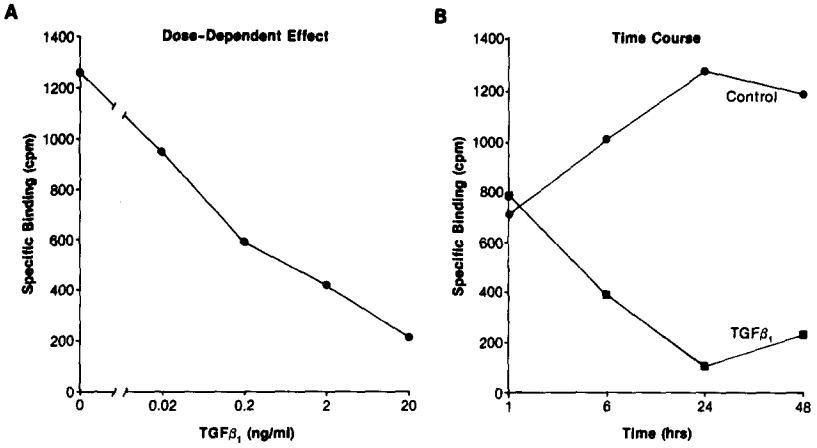

Figure 1. Kinetics and dose-dependent effect of TGF-β on IL-1R expression. NFS-60 cells were incubated 24 h with or without increasing concentrations of TGF-β (*A*) or with a constant dose of TGF-β (20 ng/ml) for different periods of time (*B*). At the end of the incubation, the samples were processed as described in the Materials and Methods. Each point represents the mean of duplicate determination.

expression with an ED_{50} of 0.2 ng/ml (10^{-11} M) and a maximal effect at 20 ng/ml (Fig. 1 *A*), concentrations that are within the physiological range (2). Kinetic studies revealed that >50% inhibition of TGF-β action occurred within 6 h and maximal effect (>90%) was observed at 24 h (Fig. 1 *B*). The inhibitory effect of TGF-β was not due to a direct competition of TGF-β for IL-1 binding sites on the cell surface. As much as 250 ng/ml (10^{-8} M) of unlabeled TGF-β in our binding assay did not complete with the binding of radiolabeled IL-1α (data not shown). Moreover, high cell viability was maintained as determined by trypan blue exclusion.

Selective Effect of TGF-β on Cell Surface Protein Expression. To determine whether the inhibition of IL-1R expression by TGF-β reflects a general decrease in cell surface protein, we tested the effect of TGF-β on a variety of murine myeloid cell surface antigens. Data from cytofluorometric analysis indicated that the addition of TGF-β to NFS-60 cells under culture conditions resulting in maximal inhibition of IL-1R (24 h, 20 ng/ml) did not affect the expression of any of the five cell surface antigens tested (data not shown). These included Thy-1.2 (T cells and progenitor cells), Ly-5 (pan leukocyte), Mac-1 (granulocytes and macrophages), RBC-8C5 (granulocytes), and LY-17 (Fc receptor).

Equilibrium Binding of ^{125}I-IL-1α to NFS-60 Cells. To determine whether the reduction in IL-1 binding was due to a decrease in the number of IL-1R, NFS-60 cells maintained in IL-3 were incubated for 24 h with or without TGF-β. The cells were washed, treated with glycine-HCl, and then incubated at 4°C with increasing concentrations of ^{125}I-IL-1α. A plot of specific counts bound, as a function of radio ligand concentration (Fig. 2 *A*), indicated that binding was dose dependent and saturable. Pretreatment with TGF-β almost completely abolished the subsequent specific binding of labeled IL-1 without any noticeable effect on the nonspecific binding component (Fig. 2 *B*). Data from Scatchard analysis (Fig. 2 *C*) revealed one class of IL-1R on NFS-60 cells with an average of 400 receptors per cell and an affinity of $1.0 \pm 0.2 \times 10^{-10}$ M. Treatment with TGF-β decreased the number of IL-1R to <50 per cell without any significant reduction in affinity ($K_d = 1.3 \pm .4 \times 10^{-10}$ M). The decrease in the number of receptors per cell was consistently observed in all experiments ($n = 6$). These results demonstrated that the inhibition of ^{125}I-IL-1 binding to TGF-β–treated NFS-60 cells is related to a net decrease in IL-1R number rather than reduced receptor affinity.

Contribution of Endogenous IL-1 Production in the Inhibition of IL-1R Expression by TGF-β. Since TGF-β can induce the expression of IL-1 mRNA in human monocytes (23), the inhibition of ^{125}I-IL-1α binding on TGF-β–treated progenitor cells might be due to the occupancy and internalization of the receptor by endogenously produced IL-1. To assess this possibility, we tested the ability of the conditioned medium of NFS-60 cells, treated with TGF-β for 24 h, to compete with ^{125}I-IL-1α in an IL-1 receptor assay. Using LBRM-33-1A4 as a cellular source of high affinity IL-1R, we could not detect any competitive binding by TGF-β–treated NFS-60 cells supernatants down to a receptor detection limit of 60 pM IL-1 (Fig. 3). These data suggested that TGF-β was not inducing the production of IL-1 by the myeloid cells. LPS, a potent IL-1 inducer, also failed to induce the production of detectable IL-1 binding activity by NFS-60 cells. Additionally, TGF-β did not change the level or rate of IL-1R internalization by labeled IL-1α (data not shown).

Affinity Cross-linking of ^{125}I-IL-1α to Control and TGF-β–treated Cells. Affinity labeling of the hemopoietic progenitor cell lines NFS-60 and AC-2, which were maintained in IL-3, with ^{125}I-IL-1 followed by cross-linking revealed one sharp band of ~65–70 kD (Fig. 4). This band was ablated by addition of 50-fold excess of unlabeled IL-1, and thus represents an IL-1 binding protein on NFS-60 and AC-2 cells. Pretreatment of the cells for 24 h with TGF-β resulted in a marked inhibition of the 65–70-kD IL-1 binding protein on NFS-60 and AC-2 cells (Fig. 4).

Induction of IL-1R Expression on Murine and Human Bone Marrow Cells by IL-3. Using ^{125}I-IL-1α, we found that normal unstimulated murine and human bone marrow cells do not show significant binding of radiolabeled IL-1. However, 24–48 h incubation with IL-3, a T lymphocyte derived growth factor for hemopoietic progenitor cells (24), resulted in a significant increase in IL-1 binding (Table 2).

Cross-linking data indicated that murine bone marrow cells displayed a faint but identical band of 65–70 kD, whose in-

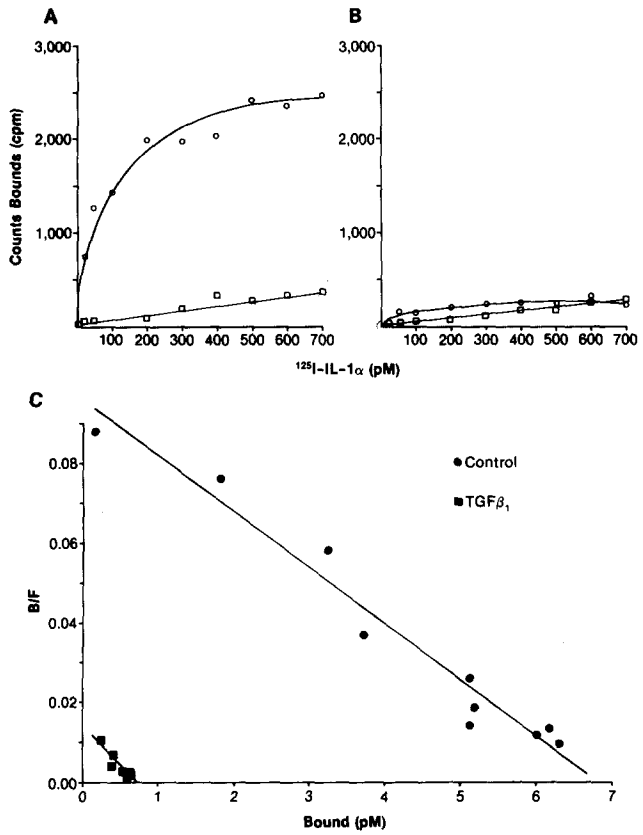

Figure 2. Comparison of ^{125}I-IL-1α dose-response binding to control and TGF-β–treated NFS-60. The cells were incubated 24 h in (*A*) the absence or (*B*) presence of 20 ng/ml TGF-β. At the end of the incubation, the cells were processed as described in the Materials and Methods. ^{125}I-IL-1α binding was determined by adding various concentrations of labeled IL-1 alone (*open circle*) or in the presence of 50-fold excess of unlabeled IL-1α (*open square*). (*C*) Scatchard analysis of equilibrium binding of ^{125}I-IL-1 to control or TGF-β–treated cells. Each point is derived from the mean of duplicate determinations from which nonspecific binding was subtracted. Scatchard data were analyzed using the LIGAND program.

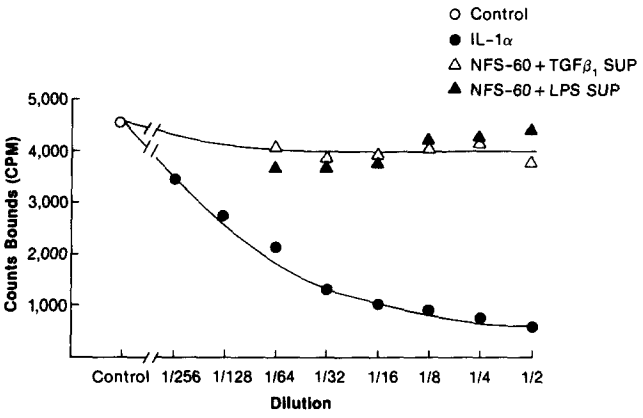

Figure 3. Role of endogenous IL-1 in TGF-β inhibition of IL-1 receptor. NFS-60 cells were incubated for 24 h in the presence or absence of TGF-β (20 ng/ml) or LPS (2 μg/ml) and then the supernatants were tested for IL-1 competitive activity in a radioreceptor assay using LBRM-33-1A4 cells.

tensity was markedly increased when the bone marrow cells were treated for 24 h with IL-3. The same autoradiogram revealed an additional inhibitable 180-kD band on IL-3–treated bone marrow cells, but the significance of this component is not clear. The 65–70 kD band was also detected on bone marrow cells treated with 5-FU, an anticycling drug that is cytotoxic for actively cycling progenitors but does not affect the slowly cycling immature progenitors. Pretreatment with TGF-β for 24 h inhibited IL-1 binding capacity of 5-FU–treated bone marrow cells and prevented the increase in IL-1 binding induced by IL-3 on normal bone marrow cells. These data support the hypothesis that TGF-β inhibits IL-1R expression on early normal bone marrow progenitor cells.

Effect of TGF-β on IL-1R Expression by a T Cell Line. To determine whether the effect of TGF-β on progenitor cells could be applied to other cell types, we performed similar cross-linking experiments using EL-4, a murine thymoma cell line. As previously reported, labeled IL-1 binds predominantly to a 78–85-kD protein on this cell type (Fig. 4) (25, 26). Twenty-four hours of pretreatment with TGF-β also inhibited the majority of IL-1 binding protein on the T cell line (Fig. 4).

Biological Effect of TGF-β on IL-1–induced Biological Activities. We next evaluated the relationship of the inhibition of IL-1R expression on bone marrow and EL-4 cells to the biological effects of IL-1. First, using the soft agar colony formation assay and IL-3 as a co-stimulator, we observed that the addition of TGF-β abrogated the effect of IL-1 on HPP-CFC colony formation (Table 2). These results show that TGF-β inhibits the effect of IL-1 on the growth of progenitor enriched 5-FU–treated murine bone marrow cells along with the reduction of IL-1R expression. We also evaluated the effect of TGF-β on IL-1–induced IL-2 production in the murine cell line, EL-4 6.1 C10. As already reported, this T cell line produces a detectable amount of IL-2 when stimulated with IL-1 alone (27). Our data indicates that pretreatment with TGF-β for 8–12 h inhibited the ability of IL-1 alone or in combination with calcium ionophore to induce IL-2 production by EL-4 6.1 cells (Table 3).

Discussion

Several laboratories have demonstrated that TGF-β negatively regulates the growth of murine and human hematopoietic cells in vitro and in vivo (3–5, 28–30). In addition, we have shown that TGF-β selectively inhibits the growth of primitive hematopoietic progenitors (CFU, granulocytes [G], erythroid [E], megakaryocytes [M], macrophages [M] [CFU-GEMM] and HPP), while more committed progenitors (CFU-G, CFU-M, and CFU-E) are insensitive (3, 4). IL-1 is known to be biochemically identical to hemopoietin-1 and has positive effects on hemopoiesis in promoting the growth and survival of early progenitor cells (31–32). Since HPP progenitor cells require IL-1 and CSFs to proliferate and differentiate in vitro and are inhibited by TGF-β (5), we asked whether one potential mechanism of TGF-β action might be at the level of IL-1 receptor expression. Using cross-linking technology we found that IL-1R expression is markedly inhibited on IL-3–treated and 5-FU–treated bone marrow cells.

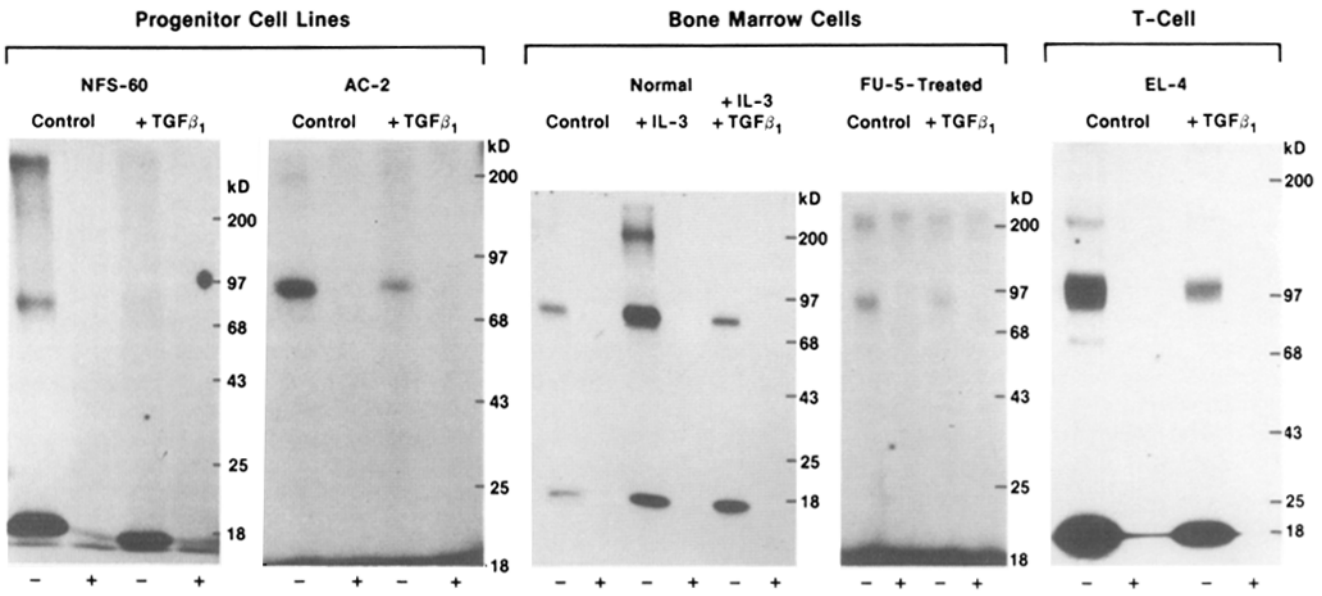

Figure 4. Affinity labeling of IL-1R on control and TGF-β-treated cells. IL-1α binding proteins were detected as described in Materials and Methods. For this assay, the cells were incubated with 2.5 nM of ^{125}I-labeled IL-1α alone (−) or in the presence (+) of 125 nM IL-1α.

However, the expression of IL-1R in normal and 5-FU-treated bone marrow is not sufficient to allow accurate equilibrium binding studies. Thus, we identified several IL-1R$^+$ murine hematopoietic progenitor cell lines representative of immature hematopoietic cells, blocked in differentiation. Treatment of these progenitor cell lines with TGF-β markedly inhibited IL-1R expression without a general inhibition of cell surface protein expression, suggesting that one mechanism of growth inhibition might be related to the decrease in the number of cell surface IL-1R.

Since IL-1 has been reported to inhibit the expression of receptors for IL-1 through the internalization of the receptor-ligand complex (33), we tested whether induction of IL-1 by TGF-β was responsible for the reduction in IL-1R. The supernatant from TGF-β–treated NFS-60 cells failed to compete for radiolabeled IL-1 in binding assays. Thus, in contrast to data reported for mature monocytes (23), the early myeloid cell lines do not produce IL-1 or "IL-1–like" binding activities in response to TGF-β. These data suggest that TGF-β acts directly to inhibit the expression of IL-1R. Whether TGF-β inhibits IL-1R expression at the level of transcription will be investigated using a cDNA probe for IL-1R (25).

Two different classes of antigenically and biochemically distinct IL-1 receptors have been identified by different laboratories (34–35). By affinity cross-linking, the molecular weight of the TCR (EL-4) is 87 kD, whereas the B cell receptor (70Z/3 cells) is 66 kD (35). The presence of a B cell–type IL-1R has also been reported on a mature bone marrow–derived granulocytic population (33). In our experiments, affinity labeling and cross-linking showed that TGF-β inhibited a 65–70-kD IL-1α binding protein on freshly aspirated progenitor cells (5-FU–treated) and on the progenitor cell lines. Based on the molecular weight of the IL-1R present on progenitor cells, our data suggest that these progenitor cells expressed the B cell–type receptor. In addition, TGF-β also inhibited the 80–85-kD protein expressed on T cells. Thus, TGF-β inhibits both classes of IL-1R.

On normal bone marrow cells, IL-3 treatment upregulates two different molecular mass IL-1 binding proteins (65–70 kD, 180 kD), both of which are inhibitable when TGF-β is present in the assay. These results indicate that TGF-β can inhibit the induction by IL-3 of IL-1R in vitro. Recent data from our laboratory indicate that TGF-β can also similarly inhibit IL-1R expression induced in vivo on bone marrow cells (manuscript in preparation), which supports the pro-

Table 2. *IL-1R Expression on Murine and Human Hemopoietic Bone Marrow Cells*

Cell source*	Factor	Specific binding‡	Ratio
		cpm	
Murine bone marrow cells	none	153 ± 17	<1
Human bone marrow cells	none	<50	<1
Murine bone marrow cells	IL-3	1,102 ± 92	2.3
Human bone marrow cells	IL-3	529 ± 16	2.5

* Murine bone marrow cells were obtained as described in Material and Methods. The cells were incubated 24 h with or without murine IL-1 used at 20 ng/ml. Human bone marrow cells were obtained from healthy donors and the mononuclear cells were isolated by centrifugation on Ficoll-Hypaque gradient. The cells were incubated 48 h in the presence or absence of 10 ng/ml human IL-3.
‡ The specific binding and ratio was determined as described at the legend of Table 1.

Table 3. *Effect of TGF-β on IL-1 Activity*

Types of assay	Stimulators	Without TGF-β	With TGF-β
HPP-type colony formation*		*Colonies/5 × 10⁴ bone marrow cells*	
	Medium	0	0
	IL-1 2 ng	0	0
	IL-1 20 ng	0	0
	IL-3	2 ± 0.7	0
	IL-1 2 ng + IL-3	12.5 ± 1.1	0
	IL-1 20 ng + IL-3	17.5 ± 0.4	0
IL-1 production‡		*U/ml*	
	Medium	0	0
	IL-1	81	0
	Ionophore	0	0
	IL-1 + Ionophore	107	0

* The HPP colony formation assay was performed as described in Material and Methods. For this assay, 20 ng/ml of TGF-β was added to the cultures, human recombinant IL-1α was used at the indicated doses and murine recombinant IL-3 was used at 20 ng/ml.
‡ The IL-2 production assay was performed as previously described (21) using EL-4 6.1 C10 cells, an IL-1-responsive murine thyoma cell line. For this assay, human IL-1α was used at 30 pg/ml, and calcium ionophore (A23187) was used at 5×10^{-6} M. Units of IL-2 were analyzed using the PROBIT program.

posed role for TGF-β as a negative regulator of hemopoietic cell growth in vivo. In this regard, the inhibition of IL-1–dependent hematopoiesis by TGF-β may ultimately result in a depletion of macrophages and other marrow-derived inflammatory cells, since we find (unpublished observation) that the bone marrow of TGF-β–treated mice becomes hypoplastic.

It has been postulated that the mechanism regulating IL-1 responsiveness is related to IL-1R expression. This is supported by experiments showing that IL-1 induction of prostaglandins and CSFs by CHO cells that express low numbers of IL-1R is increased by transfection with cDNA encoding for IL-1R (13). Additionally, treatment of fibroblasts with platelet-derived growth factor increased the number of IL-1R together with the capacity of the cells to proliferate in response to IL-1 (36). The results presented here demonstrate that TGF-β inhibits HPP colony formation together with an inhibition of IL-1R expression. Since HPP cells also require IL-3 and CSF-1 in addition to IL-1 for continued growth, it is possible that the growth inhibitory effect of TGF-β may also involve inhibition of receptors for the CSFs. In addition, it is possible that the downregulation of IL-1R occurs secondarily to inhibition of cell growth/colony formation. However, we consider this unlikely since 50% of the IL-1R downmodulation occurs within 6–8 h, reaching a maximum at 24 h which precedes any observed effect on cell growth (usually detectable within 3–5 d). In fact, recent data from our laboratory indicate that expression of CSF receptors was also inhibited by TGF-β (manuscript in preparation). Thus the diminution of cell surface IL-1R on progenitor cells represents a model for the mechanism of growth inhibition by TGF-β that could possibly be extended to other cell surface receptors.

IL-2 production by the T cell line EL-4 6.1 induced by IL-1 alone is inhibited by pretreatment with TGF-β, which argues for a direct relationship between IL-1R inhibition and T cell function. Thus, TGF-β may block IL-1–driven T cell activation, by inhibiting cell surface IL-1R expression. Our findings that TGF-β inhibits IL-1R expression on T cells provides a basis for the proposed immunosuppressive role for this cytokine (37) that can block IL-1–driven T cell–dependent B cell reactions and thymocyte proliferation.

TGF-β has been proposed to be a pro-inflammatory agent, based on its ability to chemoattract peripheral monocytes and to induce IL-1 mRNA expression (23). On the other hand, TGF-β reduces the production of H_2O_2 by already activated macrophages (38). Even though injections of TGF-β induce a mononuclear cell infiltrate and edema, TGF-β, unlike IL-1, does not induce any erythema as in classical inflammation. Our data can reconcile these apparently contradictory observations since the inhibition of IL-1R expression by TGF-β renders the cells unresponsive to IL-1 thus providing a mechanism by which TGF-β minimizes inflammatory responses and promotes the repair process.

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