Transforming Growth Factor-β1 Induces Apoptosis Independently of p53 and Selectively Reduces Expression of Bcl-2 in Multipotent Hematopoietic Cells*

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The precise role of TGF-β1 in hematopoiesis has been further complicated by the demonstration that TGF-β1 can also induce apoptosis of both normal and leukemic progenitors (19–22). The addition of TGF-β1 to primitive hematopoietic progenitors has been shown to inhibit the survival-promoting effects of several cytokines including stem cell factor and Flt3-L (21, 22). These effects could be significant; one study has already indicated that removal of endogenous TGF-β1 from cultures could be of benefit in ex vivo expansion protocols to improve the number of cells with long term bone marrow reconstitution activity (16). Although there are now substantial data on the actions of TGF-β1 on primitive hematopoietic cells, the molecular mechanisms that mediate these effects have remained elusive. While some groups have correlated the growth-regulatory effects of TGF-β1 with changes in receptor expression for survival/growth-stimulatory factors such as the colony-stimulating factors (23–25), others have shown that this mechanism cannot always account for the activities of this cytokine (26, 27). Postreceptor mechanisms must therefore also be involved in mediating the effects of TGF-β1 on hematopoietic cells.

It is becoming increasingly clear that many survival- and death-inducing stimuli mediate their effects, at least in part, through the modulation of Bcl-2 family members (28). This family comprises proteins that can antagonize or promote cell survival. The ratio of homodimers and heterodimers within the Bcl-2 family has been suggested to determine cell survival in many cell systems. For example, homodimers of the proapoptotic family member Bax can induce apoptosis, while the formation of Bax/Bcl-2 heterodimers promote survival (29, 30).
Positive regulators of cell survival such as interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor have been shown to exert their effects by up-regulating the expression of the antiapoptotic proteins Bcl-2 and Bcl-xL, respectively (31–33). There is now evidence that modulation of Bcl-2 members can also play a part in the proapoptotic activities of TGF-β1 in a variety of different cell types including some leukemic cells (34–39).

In the present study, the IL-3-dependent and multipotent FDCP-Mix cell line was utilized as a model to study the growth-inhibitory and proapoptotic effects of TGF-β1 on normal, primitive hematopoietic progenitors. FDCP-Mix cells are karyotypically normal and have previously been shown to be responsive to the growth inhibitory effects of MIP-1α (40). We report that TGF-β1 is able to reversibly inhibit the growth of FDCP-Mix cells cultured in high concentrations IL-3 that promote proliferation, while in lower IL-3 concentrations TGF-β1 induces apoptosis independent of p53, and this is inhibited by Bcl-2 expression.

**EXPERIMENTAL PROCEDURES**

**Maintenance of FDCP-Mix Cells in Culture**—p53<sup>−/−</sup> FDCP-Mix cells were generated using long term marrow cultures derived from p53<sup>−/−</sup> mice as described previously (41). FDCP-Mix cells expressing Bcl-2 were generated as described by Fairbairn et al. (42). The multipotent FDCP-Mix, Bcl-2-transfected, and p53<sup>−/−</sup> FDCP-Mix cells were maintained in Fischer’s medium supplemented with preselected batches of horse serum (HS; 20%, v/v) and 5% IL-3-conditioned medium (43). Puromycin (1 mg/ml) was also included in the Bcl-2 FDCP-Mix cell cultures. In these culture conditions, the FDCP-Mix cultures maintain a primitive blast cell phenotype. The cells were subcultured twice a week to a cell concentration of 6–8 × 10<sup>4</sup> cells/ml and maintained at

**Fig. 1. TGF-β1 reversibly inhibits IL-3-stimulated proliferation of FDCP-Mix cells.** The effect of TGF-β1 on IL-3-stimulated colony formation (A) and IL-3-stimulated [3H]thymidine incorporation (B) was determined. To test whether TGF-β1-mediated growth inhibition was reversible, cells were incubated with IL-3 (10 ng/ml) in the presence or absence of a range of TGF-β1 concentrations for 48 h, washed free of growth factors, and plated out in soft gel colony-forming assays in the presence of IL-3 alone (C). Data shown are the mean values ± S.E. of representative experiments performed at least three times, with triplicates in each experiment.
free of cytokines and cultured in the presence of 0.01 ng/ml IL-3. Plates were incubated at 37 °C in a humidified incubator with 5% CO₂.

The number of viable, apoptotic, and dead cells was determined using the annexin V, propidium iodide (PI)-based assay and analyzed using a FacsVantage flow cytometer after 24, 48, and 72 h. The data presented are the mean of four individual experiments performed three times, with triplicates in each experiment.

TABLE I

| Time (h) | IL-3 ng/ml | TGF-β1 µM | Viable, no staining | PI-negative and annexin-positive | Annexin- and PI-positive |
|---------|------------|-----------|----------------------|----------------------------------|--------------------------|
| 24      | 0.01       | 0         | 74.3 ± 3.7           | 17.7 ± 3.1                       | 7.9 ± 0.6                |
|         | 100        | 0         | 60.3 ± 4.2           | 27.0 ± 3.4                       | 12.5 ± 0.9               |
|         | 100        | 0         | 94.4 ± 0.6           | 4.2 ± 0.4                        | 1.3 ± 0.1                |
| 48      | 0.01       | 0         | 94.6 ± 0.7           | 3.9 ± 0.5                        | 1.3 ± 0.2                |
|         | 100        | 0         | 53.9 ± 3.4           | 32.9 ± 2.5                       | 12.9 ± 2.4               |
|         | 100        | 100       | 20.0 ± 6.6           | 58.9 ± 6.7                       | 20.8 ± 4.5               |
| 72      | 0.01       | 0         | 93.2 ± 0.5           | 4.4 ± 0.1                        | 2.3 ± 0.4                |
|         | 100        | 0         | 92.9 ± 1.3           | 4.9 ± 0.8                        | 2.1 ± 0.4                |
|         | 100        | 100       | 6.8 ± 2.6            | 6.7 ± 2.6                        | 22.9 ± 6.8               |
|         | 0          | 87.9 ± 2.5 | 6.6 ± 1.8           | 5.0 ± 0.9                        | 1.9 ± 0.2                |
|         | 100        | 100       | 92.8 ± 0.8           |                                   |                          |

37 °C in 5% CO₂ in air.

Colony-forming Assays—Cells in logarithmic phase were washed free of growth factors and plated out (2 × 10⁵ cells/plate) in triplicate in Iscove's modified Dulbecco's medium (IMDM), 20% (v/v) HS, 10% (v/v) bovine serum albumin, 0.1% (v/v) TGF-β1 added at 1% (v/v) agar, and the appropriate concentration of TGF-β1 added at 1% (v/v). Plates were incubated at 37 °C in a humidified incubator with 5% CO₂. After 7 days, plates were removed, and the number of colonies was scored. Colonies were defined as aggregates of more than 50 cells.

³H/Thymidine Incorporation—The rate of cell proliferation was determined by measuring DNA synthesis using the incorporation of [³H]thymidine into chromosomal DNA as described previously (44).

Reversibility of TGF-β1 Growth Inhibition—Cells were washed free of cytokines and seeded (1.5 × 10⁶ cells/ml) in IMDM supplemented with 10% (v/v) HS, 10 ng/ml recombinant murine IL-3 (R&D Systems, Abingdon, UK), and the appropriate concentration of TGF-β1 (R&D Systems). Cells were incubated for 48 h at 37 °C in a humidified incubator with 5% CO₂ washed thoroughly, and then plated out in soft gel colony-forming assays.

Measurement of Cell Viability and Apoptosis—Cells were washed and resuspended (1.5 × 10⁶ cells/ml) in IMDM supplemented with 10% (v/v) HS and the appropriate concentration of recombinant murine IL-3 and TGF-β1. After 24, 48, and 72 h, samples were taken, and viability was determined by trypan blue exclusion. (Our previous work has shown that trypan blue exclusion gives similar results to acridine orange staining.) Apoptotic assays were performed using the annexin V, propidium iodide-based assay (R&D Systems) and analyzed using a FacsVantage flow cytometer (Becton Dickinson Co., Mountain View, CA).

Western Blotting—Western blotting was carried out as described previously (45). Correct protein loading is ensured by measurement of protein concentration, and even transfer was assessed by Ponceau S staining of filters. Antibodies used were polyclonal murine Bcl-2, murine Bax, and human Bcl-2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Polyclonal antibodies for murine Bad were obtained from Transduction Laboratories, Inc. (Lexington, KY).

RESULTS

TGF-β1 Inhibits the Growth of FDCP-Mix Cells—We have previously shown that FDCP-Mix cells are susceptible to growth suppression by the growth inhibitor MIP-1α (40). To establish whether FDCP-Mix cells were a good model system for studying TGF-β1 actions on primitive hematopoietic cells, the responses of this cell line to TGF-β1 were assessed. We first examined the effects of TGF-β1 on IL-3-stimulated colony formation. TGF-β1 alone was unable to induce colony formation.
(data not shown). Concentrations of TGF-β1 of 50 pM or higher were shown to cause a major reduction in colony number compared with control cells treated with IL-3 alone (Fig. 1A). Maximal inhibition of colony formation occurred at TGF-β1 concentrations above 75 pM. At this dose, TGF-β1 decreased colony number by approximately 70%.

As colony-forming assays were performed over a 7-day period, to assess the kinetics of TGF-β1 growth inhibition we cultured FDCP-Mix cells in IL-3 for 24, 48, and 72 h in the presence or absence of TGF-β1 and then performed [3H]thymidine incorporation assays. TGF-β1 had relatively little effect in the presence of IL-3 (10 ng/ml) after 24 h, but beyond 48 h there was a marked inhibition of proliferation at TGF-β1 concentrations above 15 pM (Fig. 1B). Flow cytometric analysis of cell cycle status revealed that TGF-β1 did not induce growth arrest at any phase of the cell cycle. The proportion of cells in each phase of the cell cycle was similar for cells cultured in the presence or absence of TGF-β1 even after 72 h (percentage of cells in S/G2/M without TGF-β1 was 21.9 ± 1.4%; with TGF-β1, it was 20.8 ± 2.1%). TGF-β1 therefore appears to be slowing down progression of cells through all phases of the cell cycle. We next investigated the reversibility of TGF-β1-induced growth suppression. Following 48-h treatment with IL-3 (10 ng/ml) and various concentrations of TGF-β1, cells were washed free of cytokines and then plated out in soft gel colony-forming assays (Fig. 1C). Cells pretreated with TGF-β1 were able to form similar numbers of colonies as controls. TGF-β1 does not therefore affect the viability of clonogenic cells.

**TGF-β1-induced Apoptosis**—While the viable cells remaining after TGF-β1 treatment are still clonogenic, it still remains possible that TGF-β1 can selectively kill a subset of nonclonogenic cells present in FDCP-Mix cultures. We tested this possibility using first viable cell counts and then the apoptotic-specific annexin V assay. TGF-β1 induced no loss of cells in the presence of 10 ng/ml IL-3 (Fig. 2D); similarly, there was no increase in the percentage of cells staining for the apoptotic and dead cell-specific markers annexin V and propidium iodide, respectively (Table I). Thus, in the concentration of IL-3 employed in colony forming and [3H]thymidine incorporation assays, TGF-β1-induced growth inhibition was not a result of reduced viability.

Previous studies on primitive hematopoietic progenitors have shown that TGF-β1 can antagonize the survival-promoting effects of cytokines such as stem cell factor and Flt3-L (21, 22). To determine whether TGF-β1 could also inhibit IL-3-stimulated survival, FDCP-Mix cells were cultured in a range of IL-3 concentrations (0–10 ng/ml) in the presence or absence of TGF-β1 (0–100 pM), and then the cell viability and the number of apoptotic cells were assayed. In concentrations of IL-3 that promoted survival with little detectable proliferation (0.01 and 0.1 ng/ml), TGF-β1 (≥50 pM) induced a marked decrease in cell viability (Fig. 2A and B). These effects became more prominent over time, with the most significant effects on viability seen after 72 h. Annexin V assays performed under the same conditions confirmed that this reduction in viability was a result of TGF-β1-induced apoptosis. After 72 h in the presence of 0.01 ng/ml IL-3, only 7% of cells treated with 100 pM TGF-β1 were still viable compared with 44% of untreated cells (Table I).

**TGF-β1-induced Apoptosis Is Independent of p53**—Having established that the FDCP-Mix cell line is a good model for studying both the antiproliferative and proapoptotic actions of TGF-β1 on primitive progenitors, we next attempted to discern the mechanisms by which these effects occur. Studies on non-hematopoietic cells have implicated the tumor suppressor p53 in the growth-suppressive effects of TGF-β1 (46–49). The detection of a TGF-β1-resistant subpopulation in high proliferative potential colony-forming cells (HPP-CFC) isolated from p53-deficient mice (but not wild type littermates) has suggested that p53 may play a role in TGF-β1 growth-inhibitory pathways in hematopoietic cells (50). In order to test this, we utilized a multipotent and IL-3-dependent FDCP-Mix cell line isolated from long term bone marrow cultures derived from p53null mice (p53null FDCP-Mix). TGF-β1 reversibly suppressed IL-3-stimulated proliferation of p53null FDCP-Mix cells in a dose-dependent manner as determined by soft gel colony-forming assays (Fig. 3A) and [3H]thymidine incorporation assays (Fig. 3B). Since p53 has been shown to mediate the effects of many apoptotic stimuli in hematopoietic cells, we also examined the effects of TGF-β1 on p53null FDCP-Mix viability. While apoptosis induced by IL-3 withdrawal was delayed in p53null FDCP-Mix cells compared with FDCP-Mix cells expressing wild type p53, no significant differences in their responses to TGF-β1 could be detected. This delay in apoptosis, in p53null...
A alone (Fig. 4, the presence of IL-3 concentrations that promoted survival or apoptosis in primitive myeloid progenitors.

absolutely necessary for FDCP-Mix survival. Data therefore demonstrate that high levels of Bcl-2 are not absolutely required for TGF-β1-induced effects on primitive myeloid cell survival can be inhibited by down-regulation of Bcl-2, we used retroviral technology to generate clonal cell populations of FDCP-Mix that express human Bcl-2 (Fig. 6A). These FDCP-Mix Bcl-2 cell lines were able to undergo normal normal myeloid differentiation when cultured in appropriate conditions (42). Overexpression of Bcl-2 did not affect the sensitivity of FDCP-Mix cells to the growth-inhibitory effects of TGF-β1. IL-3-stimulated colony formation (Fig. 6B) and [3H]thymidine incorporation (Fig. 6C) of FDCP-Mix Bcl-2 cells were reversibly suppressed by TGF-β1 to a similar degree as parental cells. Trypan blue and annexin V assays demonstrated that these Bcl-2-expressing cell lines were more resistant than parental cells to apoptosis induced by IL-3 deprivation. When we expressed human Bcl-2 in FDCP-Mix cells, we observed a delay in apoptosis of the cells cultured in the absence or in low concentrations of IL-3 (Fig. 7A, Table II). However, when we examined the effects of TGF-β1 on FDCP-Mix-Bcl2 cells cultured in low concentrations of IL-3, there was a complete abrogation of TGF-β1-induced apoptosis (Fig. 7, A and B). TGF-β1-treated FDCP-Mix Bcl-2 cells underwent apoptosis at a similar rate as untreated cells in all concentrations of IL-3 tested (Fig. 7, A–D). Thus, it appears that TGF-β1-mediated effects on primitive myeloid cell survival can be inhibited by ectopic expression of Bcl-2 protein. This effect, in the presence of low concentrations of IL-3, has a major impact on cellular survival.

DISCUSSION

TGF-β1 is a pleiotrophic cytokine that can regulate the proliferation, development, and functional activity of a wide range of cell types including all of the hematopoietic cell lineages. Depending on the stage of differentiation and the presence of other cytokines, TGF-β1 can either suppress or enhance hematopoietic cell growth (8–11). In general, the growth-inhibitory effects of TGF-β1 have been shown to be restricted to more primitive hematopoietic cell populations including the most primitive LTRCs (4–6). As well as suppressing the growth of primitive hematopoietic cells, TGF-β1 can also regulate their survival. Treatment of primitive hematopoietic progenitors with TGF-β1 has been shown to antagonize the survival effects of several hematopoietic growth factors, resulting in their ap-
optosis (21). Although the biological effects of TGF-β1 on primitive hematopoietic cells are now well characterized, limited numbers of primary cells and a lack of appropriate models have meant that the molecular mechanisms underlying these effects are still poorly defined. It is anticipated that a clearer understanding of such mechanisms will be of benefit in the development of new strategies to facilitate in vitro manipulation of stem cells.

TGF-β1 potently and reversibly suppressed the growth of the FDCP-Mix stem cells cultured in high concentrations of IL-3. These growth-suppressive effects, however, were only apparent after incubation periods greater than 24 h. The need for such prolonged exposure times with TGF-β1 has previously been reported by other groups and suggests that growth inhibition could be mediated, at least in part, at the level of gene expression (4, 23, 54). Interestingly, TGF-β1 did not induce a G1/S arrest in FDCP-Mix cells as has been observed in other cell systems (55–57), and neither was any other phase of the cell cycle specifically arrested. The observed antiproliferative effects of TGF-β1 on FDCP-Mix cells may therefore be explained by delayed progression of cells through all phases of the cell cycle. This general slowing down of the cell cycle by TGF-β1 has previously been noted in other hematopoietic systems and would suggest that TGF-β1 can act at multiple sites within the cell cycle (58, 59).

Jacobsen et al. (21) have shown that TGF-β1 can induce apoptosis of primitive Lin− Sca-1+ hematopoietic progenitors cultured in the presence of single cytokines such as stem cell factor and IL-6, which promote survival but have little effect on mitogenesis. Similarly, in lower concentrations of IL-3 that supported only survival, TGF-β1 induced apoptosis of FDCP-Mix cells. In common with the growth-suppressive effects of TGF-β1 on FDCP-Mix cells, significant levels of apoptosis were not detected until cells had been incubated with TGF-β1 for greater than 24 h. Transient incubations of primitive hematopoietic progenitors with TGF-β1 have also been reported to be less effective in counteracting the survival effects of viability-promoting cytokines (21). The addition of TGF-β1-neutralizing antibodies to suspension cultures (either in the presence or absence of other cytokines) has been shown to enhance the survival of primitive hematopoietic cells including the very primitive LTRCs (16, 21). It has been proposed therefore that
removal of endogenous TGF-β1 from ex vivo cultures could be used to improve the numbers of LTRCs. Such improvements are likely to be of benefit in clinical applications such as gene therapy, where stem cell numbers are often limiting and cell cycling is required for gene transfer. While endogenous TGF-β1 can clearly influence the viability of primitive progenitors cultured in vitro, it remains unclear whether these proapoptotic effects play any significant role in vivo, where cytokine profiles are likely to be more complex.

Resistance to TGF-β1-mediated growth inhibition is a common feature of many malignancies of lymphoid and epithelial origin and may contribute to their aberrant proliferation (60). Analysis of some malignant cells has correlated this insensitivity to TGF-β1 with the expression of mutant forms of p53 (46, 47). This and the demonstration that overexpression of mutant p53 can confer partial resistance to TGF-β1 have suggested that p53 may be involved in TGF-β1 growth-inhibitory pathways (48, 49). A role for p53 in the growth-inhibitory actions of TGF-β1 on primitive hematopoietic cells was also recently suggested following the detection of a TGF-β1-resistant subpopulation in HPP-CFC (very primitive progenitors) isolated from p53-deficient but not wild type mice (50). In contrast to the data obtained from Sasaki et al. (50), we found that FDCP-Mix p53 wild-type cells displayed similar growth responses to TGF-β1 as FDCP-Mix cells expressing wild type p53. As seen in other cell types, FDCP-Mix cells lacking p53 died more slowly than normal FDCP-Mix cells in the absence of IL-3 (51). In contrast, p53 deficiency had no significant effect on the ability of TGF-β1 to induce apoptosis. Thus, in FDCP-Mix cells there is no absolute requirement for p53 in TGF-β1 growth inhibitory and apoptotic pathways. The reason for the differential sensitivities of FDCP-Mix p53 wild-type cells and the HPP-CFC subpopulation detected by Sasaki et al. may be that they represent distinct subsets of primitive hematopoietic progenitors.

Recent studies on leukemic cell lines have suggested that the proapoptotic effects of TGF-β1 may be mediated in part through the modulation of Bcl-2 family members (37–39). In this study, we have shown that TGF-β1-induced apoptosis of FDCP-Mix cells is associated with a down-regulation of Bcl-2 but not Bcl-xL expression. TGF-β1 reduced protein levels of Bcl-2 in FDCP-Mix cells after 24 h and inhibited proliferation. Transfected FDCP-Mix cells expressing high levels of Bcl-2 were found to be resistant to the proapoptotic but not the growth-suppressive effects of TGF-β1. It has already been shown that Bcl-2 can affect cell cycle entry (61); as such, the decrease in Bcl-2 levels observed upon the addition of TGF-β1 are unlikely to be involved in the TGF-β1-mediated growth inhibition. We therefore conclude that the proapoptotic effects of TGF-β1 on primitive hematopoietic cells can be abrogated by an increase in cellular Bcl-2 protein levels. While others have shown that IL-3 and other survival-promoting cytokines can increase the levels of antiapoptotic proteins (31–33, 62, 63), we did not find any differences in Bcl-2 or Bcl-xL levels between FDCP-Mix cells that had been cultured in low or high IL-3 concentrations. Furthermore, we found that TGF-β1 could decrease Bcl-2 protein levels in FDCP-Mix cells in the presence of high concentrations of IL-3. IL-3-stimulated survival of FDCP-Mix cells therefore does not appear to be mediated through changes in Bcl-2 or Bcl-xL expression. Motyl et al. (38) have shown that TGF-β1-induced apoptosis of leukemia cells was accompanied with an increase in Bax levels as well as down-regulation of Bcl-2. Protein levels of Bax, however, were unaltered by TGF-β1 in FDCP-Mix cells; so was the expression of another proapoptotic member protein, Bad.

During the past 5 years, it has become evident that regulation of Bcl-2 family members can also occur at a post-translational level. Bcl-2 is phosphorylated on serine residues in response to a variety of stimuli including IL-3 (64–66). Depending on the extracellular stimulus, this phosphorylation can either enhance (67) or attenuate Bcl-2 survival function (64, 68, 69). The proapoptotic member Bad is also phosphoryl-

![FIG. 6. FDCP-Mix cells expressing high levels of Bcl-2 are still sensitive to the growth-inhibitory effects of TGF-β1.](image)
TGF-β1 induces apoptosis and reduces Bcl-2 expression.

Fig. 7. TGF-β1-induced apoptosis is blocked by high levels of Bcl-2. The effect of Bcl-2 on TGF-β1-mediated cell death of FDCP-Mix cells was assessed using the FDCP-Mix Bcl-2 (clone 15) cell line. Cell viability was assessed using trypan blue cell counts. FDCP-Mix Bcl-2 cells were washed free of cytokines and cultured in the presence of 0.01 (A), 0.1 (B), 1 (C), or 10 ng/ml IL-3 (D) and a range of TGF-β1 concentrations. The percentage of dead cells after 24, 48, and 72 h was determined using trypan blue. Data are the mean values ± S.E. of representative experiments performed three times, with triplicates in each experiment.

Table II

| Time | IL-3 | TGF-β1 | Viable, no staining | PI-negative and annexin-positive | Annexin- and PI-positive |
|------|------|--------|---------------------|----------------------------------|--------------------------|
|      | ng/ml | μM     | %                   | %                                | %                        |
| 24   | 0.01 | 0      | 83.8 ± 2.1          | 10.3 ± 1.5                       | 5.8 ± 0.5                |
|      | 100  |        | 82.8 ± 2.1          | 10.7 ± 1.6                       | 6.3 ± 0.4                |
|      | 0    | 100    | 95.3 ± 0.8          | 2.7 ± 0.5                        | 1.7 ± 0.1                |
| 48   | 0.01 | 0      | 95.4 ± 0.6          | 2.6 ± 0.2                        | 1.7 ± 0.1                |
|      | 100  |        | 59.7 ± 12.3         | 26.2 ± 6.8                       | 13.8 ± 5.3               |
|      | 0    | 100    | 51.0 ± 14.8         | 23.4 ± 5.5                       | 15.2 ± 9.0               |
| 72   | 0.01 | 0      | 96.0 ± 1.0          | 2.2 ± 0.4                        | 1.7 ± 0.6                |
|      | 100  |        | 96.1 ± 0.8          | 2.1 ± 0.2                        | 1.6 ± 0.5                |
|      | 0    | 100    | 51.0 ± 5.8          | 32.2 ± 0.9                       | 16.5 ± 5.4               |
|      | 0    | 100    | 48.9 ± 7.9          | 33.2 ± 2.4                       | 17.3 ± 5.4               |

This article discusses the role of TGF-β1 in regulating apoptosis and Bcl-2 expression, highlighting a causal link between TGF-β-induced decreases in Bcl-2 and induction of apoptosis. The addition of IL-3 will stimulate signaling events that promote survival independent of changes in Bcl-2 levels. Our data reveal one way in which primitive cells, which are not actively proliferating, can be induced to undergo apoptosis by a key regulator of hematopoiesis. This mechanism is via modulation of one member of the Bcl-2 family, Bcl-2 itself. However, it may still be possible that TGF-β1 also regulates Bad, Bax, or Bcl-xL activity and that of Bcl-2, through processes such as phosphorylation and subcellular localization. Studies are now under way to test this possibility.

References
1. Brumley, H. E. (1996) in Blood Cell Biochemistry (Whetton, A. D., and Gordon, J., eds) pp. 121–150, Plenum Press, New York
2. Jetten, A. M., Shirley, J. E., and Stinner, G. (1986) Exp. Cell Res. 167, 539–549
3. Kurokowa, M., Lynch, K., and Podeszwa, D. K. (1987) Biochem. Cell Biol. Commun. 142, 775–782
4. Keller, J. R., Mantel, C., Sing, G. K., Ellingsworth, L. R., Ruscetti, S. K., and
