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Transforming Growth Factor-Beta Induces Hemoglobin Synthesis in a Human Erythroleukemia Cell Line

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Summary: We have examined the effects of $TGF_{\beta 1}$ and $TGF_{\beta 2}$ on the HEL human erythroleukemia cell line. It was observed that $TGF_{\beta 1}$ and 2 induced hemoglobin synthesis in these cells without causing a significant negative effect on cell proliferation. The cell surface markers glycophorin A and transferrin receptor that are associated with erythroid differentiation were also increased. This cell line may provide a model system in which to study the regulation of globin gene expression by a physiological growth factor known to act on hemopoietic cells.

Transforming growth factor-beta (TGF_{β}) is a ubiquitous polypeptide that can regulate growth and differentiation in both normal and neoplastic cells by autocrine or paracrine mechanisms (1). The protein is a 25-KDa disulfide linked dimer and exists in several distinct forms with $TGF_{\beta 1}$ and $TGF_{\beta 2}$ being the most abundant (2). TGF_{β} is expressed and released by many different cells, including those of the hematopoietic system, and is stored in both bone and platelets (1). TGF_{β} has been termed multifunctional because it can be either a positive or a negative regulator of cell proliferation and has other diverse regulatory roles (3). Among the cells negatively regulated by TGF_{β} are cells of the immune and hematopoietic systems (4,5). TGF_{β} has been shown to inhibit *in vitro* colony formation of erythroid, multipotential and granulocytic-macrophage progenitor cells (4,5). This inhibitory effect was

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Abbreviation: TGFß, transforming growth factor beta.

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lost as these early cells differentiated (5). These observations were suggestive that the effects of TGF_β may be differentiation stage specific (5).

In this report we have described that both $TGF_{\beta 1}$ and $TGF_{\beta 2}$ induced hemoglobin synthesis in a HEL human erythroleukemia cell line without significant alteration of cell proliferation. Hemoglobin production was demonstrated by both the presence of benzidine positive cells and an increase in α globin RNA following exposure to TGF_β. The cell surface markers associated with erythroid differentiation, glycophorin A and transferrin receptor were also increased following TGF_β exposure.

MATERIALS AND METHODS

Cells Culture. The HEL cell lines were kindly provided by Drs. Papayannopoulous and Ablashi. The cells were grown in RPMI 1640 Medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (GIBCO), 100 u/ml of penicillin and 100 µg/ml of streptomycin (GIBCO). Highly purified human $TGF_{\beta 1}$ and porcine $TGF_{\beta 2}$ (R&D Systems, Inc., Minneapolis, MN) were added to HEL cells freshly seeded at 3×10^5 cell/ml. Hemoglobin induction was determined by benzidine staining as described by Parker et al. (6). A minimum of 200 cells were counted. These results were consistent in at least 10 separate experiments. For cell growth determination, cells were seeded in duplicate at the concentration of 3×10^5 cells/ml and were counted daily using an hemacytometer. Cell viability was assessed by trypan blue exclusion.

Northern Blot Analysis. At the designated time points, total cellular RNA was isolated by the guanidine thiocyanate - cesium chloride method as previously described (7). Twenty micro-grams of total cellular RNA was electrophoresed through 1% agarose/formaldehyde gels and transferred to nylon membrane (Micro Separation, Inc., Westboro, MA). The blots were hybridized to oligo-labeled (Pharmacia, Piscataway, NY) cDNA probes. The cDNA probes for α and β globin was obtained from the American Type Culture Collection (Rockville, MD). β actin was purchased from Oncor (Gaithersburg, MD).

Cell Surface Analysis. Cells at days 3 and 5 were incubated with monoclonal antibodies directed either against glycophorin A (AMAC, Inc., Westbrook, MI) or the transferrin receptor (Becton-Dickinson, Mountain View, CA) for 30-40 min on ice. The cells were washed with PBS and then incubated with goat anti-mouse Ig for 30 min on ice. They were subsequently washed and analyzed by flow cytometry (FACSCAN, Becton-Dickinson). The fluorescence was measured on a log scale.

RESULTS AND DISCUSSION

Only one HEL cell line was induced to produce hemoglobin by both $TGF_{\beta 1}$ and $TGF_{\beta 2}$ and this particular cell line was designated as HEL-T. The other HEL cell line did not produce hemoglobin either in response to hemin, a known inducer of hemoglobin, or to TGF_β. At the $TGF_{\beta 1}$ concentration of 1 ng/ml, approximately 20%-25% of the HEL-T cells contained hemoglobin by day 3 as indicated by benzidine positivity and, by day 5, approximately 45%-55% of the cells were benzidine positive (Figure 1a). There was approximately a 3-fold increase in hemoglobin in TGF_β-treated cells by day 5 (0.00 vs 0.045 mg/ml) as determined by spectrophotometric analysis. The presence of hemoglobin was visually observed by the presence of a red cell pellet in the TGF_β-treated

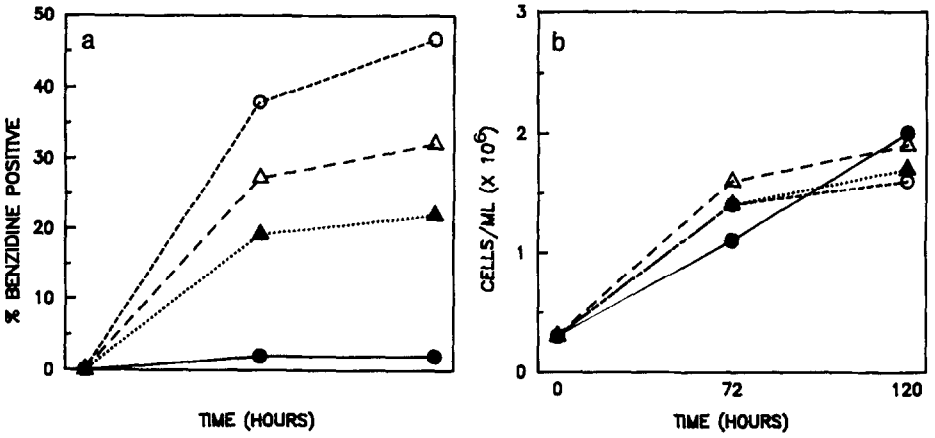

Figure 1.(a). Hemoglobin induction by $TGF_{\beta 1}$ and 2 as determined by benzidine staining (• Control, O $TGF_{\beta 1}$ 1ng/ml, ▲ $TGF_{\beta 2}$ 1ng/ml, △ $TGF_{\beta 2}$ 3ng/ml). (b). The effect of $TGF_{\beta 1}$ and $TGF_{\beta 2}$ on HEL-T cell growth. (• Control, O $TGF_{\beta 1}$ 1ng/ml, △ $TGF_{\beta 2}$ 1ng/ml, ▲ $TGF_{\beta 2}$ 3ng/ml)

cells and was demonstrated by both cellulose acetate and citrate agar electrophoresis (data not shown). $TGF_{\beta 1}$ at concentrations as high as 3ng/ml did not significantly induce a higher increase in benzidine positivity, and conversely $TGF_{\beta 1}$ at the concentration of 0.1ng/ml did not significantly induce hemoglobin production. The ability of $TGF_{\beta 2}$ to induce hemoglobin accumulation was less than that of $TGF_{\beta 1}$ and was dose dependent. At 3 ng/ml of $TGF_{\beta 2}$, approximately 35% of the cells were benzidine positive by day 5 (Figure 1a). Whereas after a 5 day exposure to 1ng/ml of $TGF_{\beta 2}$, approximately 23% of the cells were benzidine positive (Figure 1a). In contrast to earlier findings of Sing et al. (5), in which it was observed that $TGF_{\beta 1}$ inhibited HEL cell growth by approximately 20%, we found that various concentrations, $TGF_{\beta 1}$ and β_2 usually had no consistent significant inhibitory effect on proliferation of the HEL-T cells but rather had a modest growth enhancement effect on the cells (Figure 1b). Although the reason for this apparent discrepancy is not known, it is possible that the proliferation of different HEL clones could be differentially altered by TGF_{β}. Also it may be possible that the different sources of TGF_{β} could have played a role in the conflicting data. We used purified human $TGF_{\beta 1}$ and Sing et al. (5) used purified bovine $TGF_{\beta 1}$. However, it was stated by Sing et al. (5) that they have observed similar results in some experiments using different sources of $TGF_{\beta 1}$. In our experiments it was also observed that HEL-T cells treated with either $TGF_{\beta 1}$ or β_2 had an increase in thymidine incorporation on day 3 (data not shown). Cell viability was unaffected by either $TGF_{\beta 1}$ or β_2 as determined by trypan blue exclusion. HEL-T cells had to be exposed continuously to TGF_{β} for 48 hrs before significant hemoglobin production could be observed (data not shown).

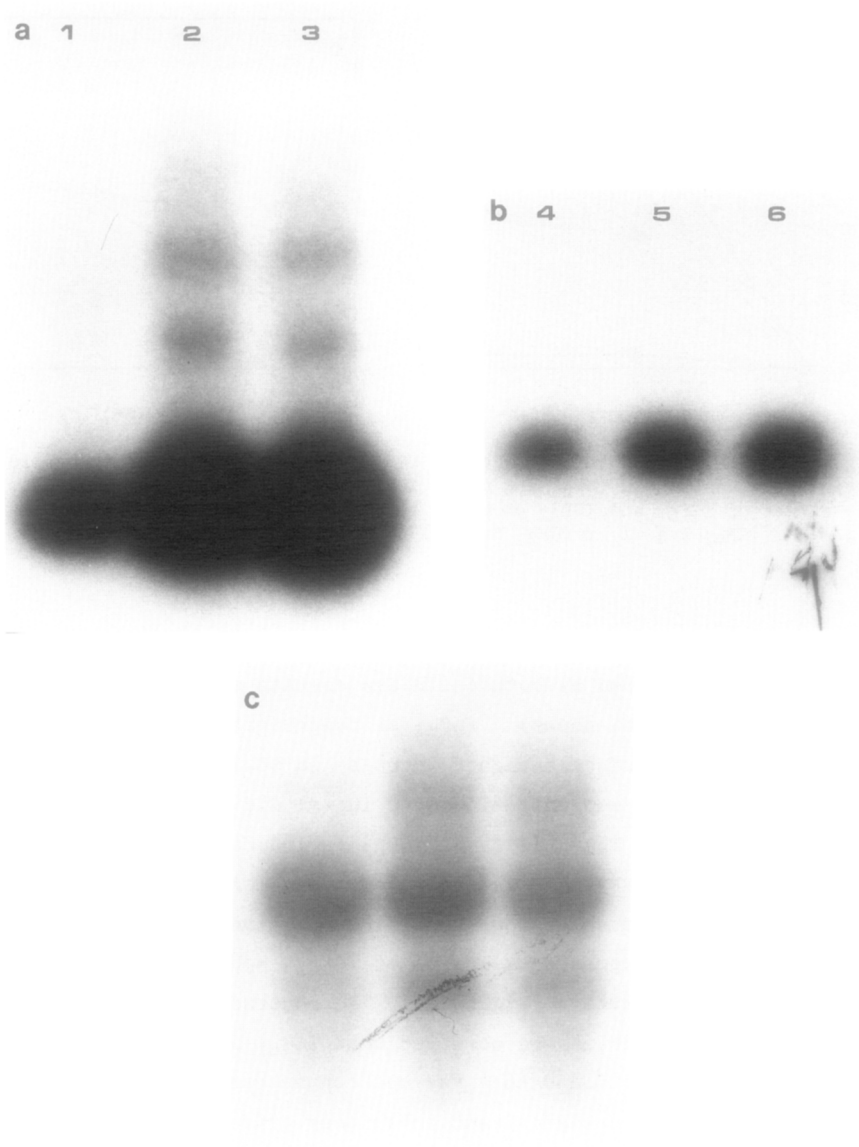

Figure 2. Northern blot analysis. a) α globin RNA expression of (1) untreated HEL-T cells (2) cells treated with 1 ng/ml of $TGF_{\beta 1}$ for 3 days and (3) for 5 days. b) ß globin RNA expression of (4) untreated HEL-T cells (5) cells treated with 1 ng/ml of $TGF_{\beta 1}$ for 3 days and (6) for 5 days. c) reprobe of (a) with ß actin.

HEL cells have been reported to constitutively express globin chain mRNA (8). Although α-globin RNA was expressed in the HEL-T cell line, a significant increase in the level of α globin RNA was seen after treatment with 1ng/ml of $TGF_{\beta 1}$ (Figure 2a). The level of α-globin RNA was maximally induced by day 3 (Figure 2a). Cells treated for 5 days with $TGF_{\beta 1}$ showed no further increase in the RNA level of α globin. The ß-globin RNA was also

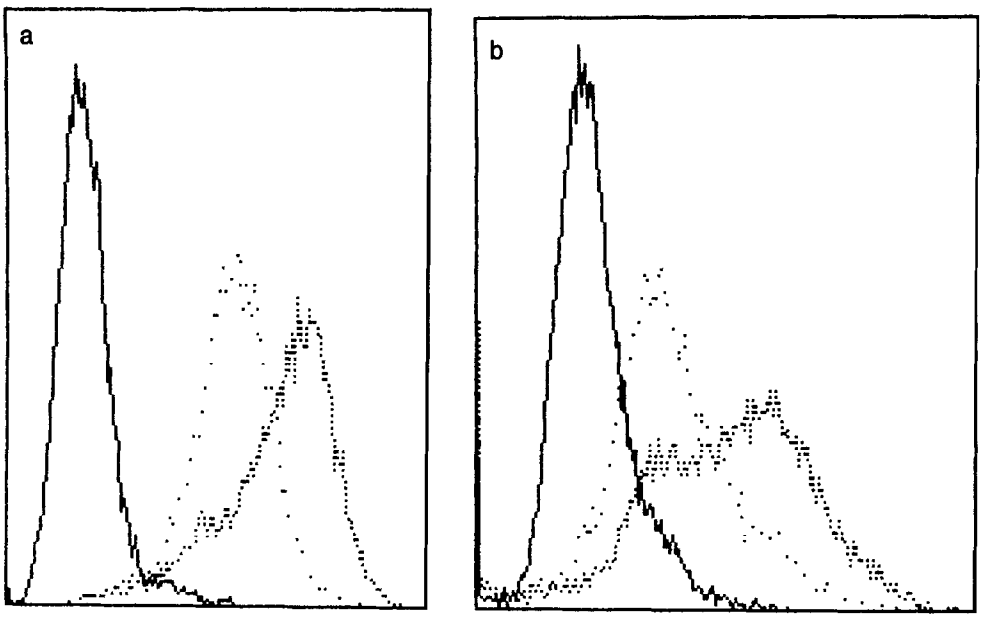

Figure 3. Cell surface analysis of HEL-T cells. (a) Glycophorin A expression on untreated (• • • • • •) and 1 ng/ml of $TGF_{\beta 1}$-treated cells (•••••••). (b) Transferrin receptor expression on untreated (• • • • •) and 1 ng/ml of $TGF_{\beta 1}$-treated cells (•••••••). In both panels, the isotype matched negative control is represented as (————).

constitutively expressed (8), but treatment with $TGF_{\beta 1}$ only modestly induced the RNA levels of ß globin (Figure 2b).

Earlier reports have demonstrated that glycophorin was constitutively expressed on the cell surface of the HEL cells (9). The HEL-T cells likewise expressed glycophorin A, and expression increased markedly after treatment with $TGF_{\beta 1}$ (Figure 3a) and $TGF_{\beta 2}$. The expression of transferrin receptors also increased after exposure to $TGF_{\beta 1}$ (Figure 3b) and $TGF_{\beta 2}$. The expression of both glycophorin A and transferrin receptor were maximally expressed by day 5. $TGF_{\beta 1}$ caused no consistent significant decrease in the anti-leukocyte surface marker, Hle-1 (CD 45).

Previous studies by Papayannopoulou et al. (9) have shown that the HEL erythroleukemia cell line expressed several erythroid-specific surface markers as well as megakaryocytic markers. These HEL cells constitutively expressed low RNA levels of globin chains and hemoglobin production could be induced or increased by various agents such as hemin and σ-aminolevulinic acid (9). Papayannopoulou et al. (9) also noted that different clones of the HEL cell line responded with different degrees of hemoglobin production to the various inducers of hemoglobin synthesis. We have made a similar observation with respect to TGF_{β}; one HEL clone in our possession did not produce hemoglobin in response to TGF_{β}, while another HEL clone was induced by TGF_{β}. The latter HEL

clone has been designated HEL-T. The HEL line that was non-responsive to TGF_{β} was also non-responsive to hemin whereas the HEL-T cells did produce hemoglobin following exposure to either agent.

The three cell surface markers, glycophorin A, transferrin receptor and Hle-1 have shown to be modulated in normal erythroid cell differentiation (10). We have shown that the expression of both glycophorin A and transferrin receptor in the HEL-T cells was increased by day 5 following exposure to $TGF_{\beta 1}$. However, we cannot rule out that the increase in transferrin receptor was due to cell proliferation rather than to the erythroid program of differentiation. The megakaryoctic related marker GPIIb IIIa co-expressed on the HEL-T cells was unaffected by $TGF_{\beta 1}$ treatment.

Essentially all compounds that have been used to induce hemoglobinization in the erythroleukemia cell lines are chemical in nature and many may have no physiological relevance (i.e. DMSO, hexamethylene bisacetamide), whereas others, such as hemin and σ-aminolevulinic acid, do have a physiological role (9). To our knowledge this is the first report to show that a physiological relevant polypeptide, other than erythropoietin, can independently induce hemoglobin synthesis in human erythroleukemia cell line.

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