

Induction of platelet-derived growth factor gene expression during megakaryoblastic and monocytic differentiation of human leukemia cell lines

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Platelet-driven growth factor (PDGF) is one of the most important polypeptide growth factors in human serum. It is composed of two polypeptide chains linked by disulfide bonds. The B-chain is encoded by the *c-sis* proto-oncogene, which is expressed in several malignant and non-malignant cells including K562 cells differentiating towards megakaryoblasts. Expression of the A-chain has been reported to occur in human solid tumor cell lines independently of *c-sis* expression. We report here the non-coordinate expression of the A- and B-chains in human leukemia cell lines. The PDGF-A and B-chain (*c-sis*) RNA expression as well as secretion of PDGF polypeptides are induced in the K562 cell line upon induction of megakaryoblastic differentiation with 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) whereas erythroid differentiation induced with sodium butyrate is accompanied by *c-sis* expression only. Simultaneously with megakaryoblastic differentiation the RNA level for another platelet protein, the transforming growth factor-beta was also increased, but in a complex manner. The promyelocytic leukemia cell line HL-60 does not express PDGF-A RNA, whereas the promonocytic cell line U937 does. Preferential induction of the A-chain RNA is obtained in both cell lines after treatment with TPA which causes monocytic differentiation. PDGF-A expression in HL-60 cells is also observed after treatment with the tumor necrosis factor-alpha but granulocytic differentiation of HL-60 cells induced with dimethyl sulfoxide or the granulocyte colony-stimulating factor is not associated with PDGF gene expression.

Key words: erythroleukemia cells/megakaryoblastic differentiation/monocytic differentiation/platelet-derived growth factor/tumor promoter

Introduction

Abundant evidence indicates that cellular proto-oncogenes play important roles in the control of cell growth and differentiation (see Kahn and Graf, 1986 for references). Many of the oncogene-encoded protein products have been implicated in hormonal signalling systems that regulate cell growth and differentiation (Bishop, 1985; Müller, 1986). One such protein is the product of the *c-sis* proto-oncogene, which encodes the B-chain of PDGF (Doolittle *et al.*, 1983; Waterfield *et al.*, 1983). Expression of the *c-sis* gene has been reported in glioblastoma and osteosarcoma cell lines (see Heldin and Westermark, 1984), and in normal endothelial (Barrett *et al.*, 1984; Collins *et al.*, 1985) and

placental cells (Goustin *et al.*, 1985). The major sources of PDGF *in vivo* are, however, the platelets and macrophages (see Ross *et al.*, 1986). The site of synthesis of PDGF stored in platelet alpha-granules is not known, but activation of the *c-sis* gene has been seen during megakaryocytic differentiation of the hematopoietic cell line K562 (Tabilio *et al.*, 1983; Colamonici *et al.*, 1986). This cell line, which shows predominantly erythroid characteristics (Andersson and Gahmberg, 1980), is derived from a chronic myeloid leukemia (CML) patient in blast crisis, contains the Philadelphia chromosomal translocation (Lozzio and Lozzio, 1975) and an activated and amplified *c-abl* oncogene and tyrosine kinase (Collins and Groudine, 1983; Konopka *et al.*, 1984). Expression of the *c-sis* gene and secretion of PDGF also occurs in activated macrophages (Martinet *et al.*, 1985; Shimokado *et al.*, 1985).

Mononuclear phagocytes are effectors of inflammatory responses and important modulators of immune functions of both T- and B-lymphocytes. In recent years, a number of human tumor cell lines that possess monocytic and myeloid characteristics but vary in their degree of differentiation have been established and studied in culture. Two well-known leukemia cell lines of human origin which can be differentiated into monocyte-macrophages are the promyelocytic leukemia cell line HL-60 (Collins *et al.*, 1978) and the histiocytic lymphoma cell line U937 (Sundström and Nilsson, 1976). The HL-60 cells can be differentiated into macrophages by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or into granulocytic cells by dimethyl sulfoxide (DMSO) (Collins *et al.*, 1978; Rovera *et al.*, 1979). The U937 cells represent promonocytes, and can be induced to differentiate further by treatment with TPA (Nilsson *et al.*, 1980). During the differentiation of HL-60 and U937 cells dramatic changes in the expression of various phenotypic properties and genes occur. Thus, for example, the expression of cellular oncogenes *c-myc* and *c-myb* is decreased (Westin *et al.*, 1982; Reitsma *et al.*, 1983; Craig and Bloch, 1984; Bentley and Groudine, 1986), while increases occur in the expression of *c-fos* (Müller *et al.*, 1984; Mitchell *et al.*, 1986), interferon-beta and HLA-genes (Resnitzky *et al.*, 1986), and of the cellular oncogene *c-fms* (Sariban *et al.*, 1985), which encodes the receptor for the granulocyte-macrophage colony-stimulating factor (CSF-1) (Sherr *et al.*, 1985). The HL-60 cells also activate the pp60^{c-src} tyrosine kinase (Barnekow and Gessler, 1986; Gee *et al.*, 1986) and express *c-sis* when they have been induced to differentiate into monocytes (Pantazis *et al.*, 1986).

We report here the induction of expression of PDGF RNA and protein during TPA-induced megakaryoblastic differentiation of K562 cells and after induction of monocytic differentiation of HL-60 and U937 cells in culture.

Results

Regulation of PDGF gene expression in differentiating K562 cells

We first asked whether megakaryoblastic differentiation of K562 cells is associated with induction of PDGF-A mRNA expression.

For induction of megakaryoblastic differentiation, K562 cells were grown to a density of 300 000 cells/ml and 3×10^{-9} M TPA dissolved in DMSO was added to the culture medium. Parallel control cell cultures were treated with corresponding concentrations of DMSO. Cells were monitored for their differentiation-associated properties and harvested for RNA isolation at varying times after the addition of TPA. Polyadenylated RNA from differentiating and control cells was electrophoresed, blotted and hybridized with the PDGF-A cDNA probe. Figure 1 shows the results of RNA analysis from differentiating and control cultures during three subsequent days of treatment. It can be seen from the figure that there is very little PDGF-A expression in the DMSO-treated K562 cells, but a faint 2.3-kb PDGF-A RNA is visible in cells treated for 1 day with TPA (Figure 1). After longer exposures of the autoradiogram additional faint signals could be seen in this sample at 1.9 and 2.8 kb. In contrast, in the DMSO-treated cells the PDGF-A RNA expression remains barely detectable. Similar, but much more intense RNA bands hybridize with the PDGF-A probe in samples from cultures differentiated for 2 and 3 days (Figure 1). The relative increase in steady-state PDGF-A RNA levels in TPA-treated cells compared with DMSO-treated cells, estimated by quantitative dot-blot hybridization and scanning densitometry of the autoradiograms, is 5-, 40- and 50-fold on days 1, 2 and 3 of treatment respectively.

It has been reported by Colamonici *et al.* (1986) that K562 cells induced to differentiate towards megakaryoblasts with TPA express *c-sis* RNA. Indeed, upon induction of differentiation with TPA we observed a characteristic 3.8-kb *c-sis* signal in RNA extracted from K562 cells (Figure 1, middle panel). Hybridization analyses further indicated that the time-course of the expression of PDGF-A and *c-sis* (PDGF-B) RNAs was closely correlated during the differentiation of the K562 cells: the *c-sis* signal was also weak in cells differentiated for 1 day but increased in intensity during the subsequent days of induction, as has been reported by Colamonici *et al.* (1986). For comparison, the Northern blot was hybridized with a probe for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Figure 1, lower panel), which was uniformly expressed in the same samples, thus confirming the specificity of altered PDGF gene expression during differentiation.

During TPA-induced differentiation the cells became positive for several markers of megakaryocytic differentiation. Four days after induction ~4.4% of the DMSO-treated cells scored above the arbitrary limit for positive fluorescence when stained with C-17, an antibody against the platelet gp IIIa antigen (Tetteroo *et al.*, 1983), whereas 94.6% of the TPA-treated cells distributed above this limit (Figure 2). Similarly, 6.8% of the DMSO-treated and 84% of the TPA-treated cells scored positive with the anti-platelet antibody FMC 27 (Zola *et al.*, 1984), and a positive conversion was also seen when the cells were stained for the factor VIII-related antigen with the immunoperoxidase method: after 3 days induction, 50% of TPA-treated cells were positive whereas DMSO-treated and untreated cells remained negative. On the other hand, as has been shown earlier (Siebert and Fukuda, 1986), the TPA-treated cells became negative for glycophorin RNA, a marker for erythroid differentiation which remained constant in DMSO-treated cells (data not shown).

The PDGF-A and B genes are non-coordinately expressed in response to the various inducers

We next studied the specificity of the PDGF-A RNA accumulation using different inducers of differentiation. While TPA causes

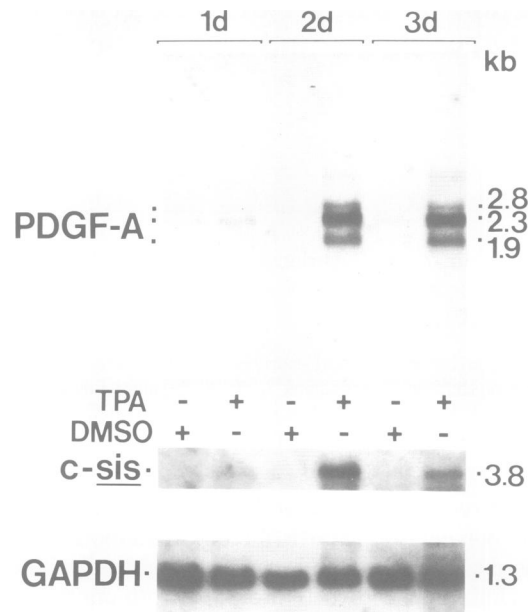


Fig. 1. Analysis of PDGF-A and B (*c-sis*) RNAs from K562 cells during TPA-induced differentiation. The cells were collected 1, 2 and 3 days after addition of TPA or DMSO to the culture medium. Polyadenylated RNA extracted from the cells was analyzed by Northern blotting and hybridization with the PDGF-A, *c-sis* and, as a control, with the GAPDH probe, as shown. The approximate sizes of the RNA bands are given in kilobases (kb) on the right.

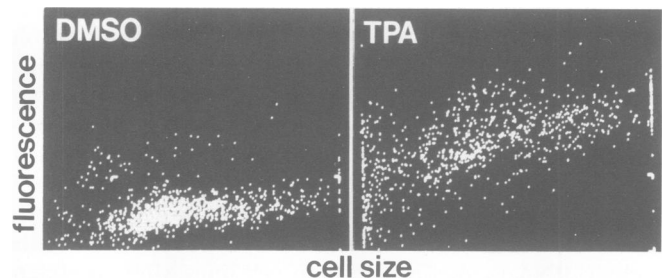


Fig. 2. Detection and quantitation of platelet glycoprotein IIIa on TPA-differentiated K562 cells. The cells were grown in the presence of TPA or DMSO for 3 days, stained with the C-17 monoclonal antibodies for gpIIIa and analyzed for cell size and fluorescence intensity with a Beckton and Dickinson FACS IV flow cytometer. The small arrowhead in the right-hand margin denotes the (arbitrary) limit for positive fluorescence.

megakaryoblastic differentiation of the K562 cells with loss of erythroid properties (Tabilio *et al.*, 1983; Gahmberg *et al.*, 1984; Tetteroo *et al.*, 1984; Colamonici *et al.*, 1986) retinoic acid, sodium butyrate and hemin cause erythroblastic differentiation (Rutherford *et al.*, 1979; Andersson and Gahmberg, 1980) seen, for example, as red color of the cell pellets due to their hemoglobin content. Hybridization analysis of RNA from cells treated for 2 days with the various inducers is shown in Figure 3. It is evident from the results that, among the inducers used, only TPA causes accumulation of PDGF-A RNA (Figure 3, upper panel).

c-sis RNA signals were also obtained from K562 cells treated with retinoic acid or sodium butyrate which induce erythroid differentiation of these cells (Figure 3, middle panel, lanes R and B). On the contrary, K562 cells induced to further erythroid differentiation with hemin did not express *c-sis* transcripts (lane H).



Fig. 3. Analysis of the PDGF RNAs in K562 leukemia cells. Cells treated with 100 μ M hemin (H), 3 nM TPA (T), 0.1 μ M retinoic acid (R) or 1 mM sodium butyrate (B) for 2 days were analyzed by Northern blotting and hybridization with the PDGF-A, c-sis and GAPDH probes. Note that the PDGF-A RNAs are expressed only in TPA-treated cells.

Again, expression of GAPDH RNA was not altered by the various treatments (lower panel), but glycophorin RNA increased with sodium butyrate treatment (data not shown).

Expression of the 8.5 kb *bcr-c-abl* oncogene mRNA of the K562 cells (Shtivelman *et al.*, 1985; Grosveld *et al.*, 1986) remained constant throughout the TPA experiment (Figure 4), despite cellular differentiation and the associated marked reduction of cell growth rate. On the other hand, complex changes occurred in mRNA for another platelet protein, the transforming growth factor-beta. TPA increased the 2.5-kb TGF-beta RNA ~4- to 6-fold during 4 days of differentiation as determined by densitometric scanning and comparison with GAPDH RNA. In addition, the expression of a 2.3-kb TGF-beta RNA, an inconspicuous minor component in untreated cells, increased 6- to 12-fold during the same period (arrowhead in Figure 4).

TPA-differentiated K562 cells secrete PDGF

In order to confirm that the PDGF messenger RNAs were expressed as PDGF protein we performed immunoprecipitations with a polyclonal anti-PDGF antiserum from metabolically labeled K562 cell cultures treated for 3 days with DMSO or TPA. Cells were labeled with radioactive cysteine and culture media were precipitated sequentially with normal rabbit serum and with the anti-PDGF antiserum. Control precipitations were performed using labeled culture media of RD rhabdomyosarcoma cells which produce large amounts of PDGF (Betsholtz *et al.*, 1986). Two polypeptides of ~17 000 mol wt were precipitated with PDGF antiserum from culture media of RD cells and TPA-treated K562 cells (Figure 5, double arrowhead). These polypeptides

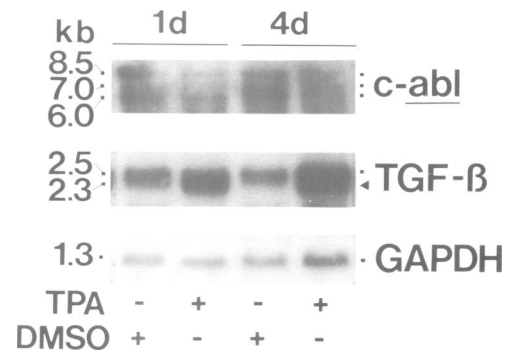


Fig. 4. Analysis of the *bcr-c-abl* and TGF-beta RNAs in differentiating K562 cells. The cells were grown in the presence of TPA or a corresponding concentration of DMSO. RNA extracted at 1 and 4 days of induction was hybridized with the *v-abl* and TGF-beta probes and with the GAPDH probe. The chimeric *bcr-c-abl* mRNA is of 8.5 kb mol. wt and the *c-abl* mRNAs of 7.0 and 6.0 kb. In shorter exposures and other autoradiograms the TGF-beta RNA signal resolved into two bands at 2.5 and 2.3 kb. Note that predominantly the 2.3 kb mRNA is increased during the experiment (arrowhead).

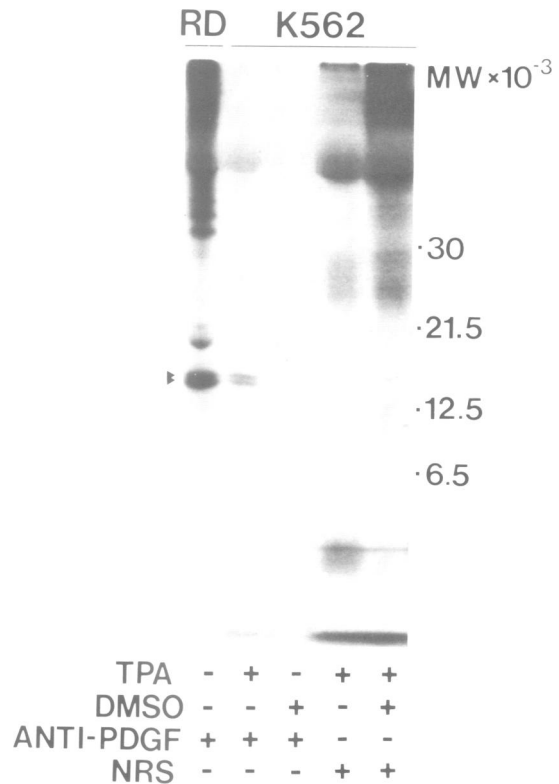


Fig. 5. Immunoprecipitation of PDGF from labeled culture media of RD and K562 cells. The K562 cultures were treated for 3 days with DMSO or TPA, and labeled with [³⁵S]cysteine. Immunoprecipitation of culture media was performed sequentially with normal rabbit serum (NRS) and with anti-PDGF antiserum, as shown. Immunoprecipitated PDGF from similarly labeled culture media of RD rhabdomyosarcoma cells (Betsholtz *et al.*, 1986) served as a mobility marker for PDGF polypeptides (double arrowhead on the left). The mobilities of other mol. wt markers are shown on the right.

migrated with a relative mobility of 31 kd in non-reducing conditions (data not shown). DMSO-treated K562 cultures contained no such polypeptides (Figure 5). Thus the expression of PDGF RNAs is also associated with the secretion of two PDGF polypeptides by TPA-differentiated K562 cells.

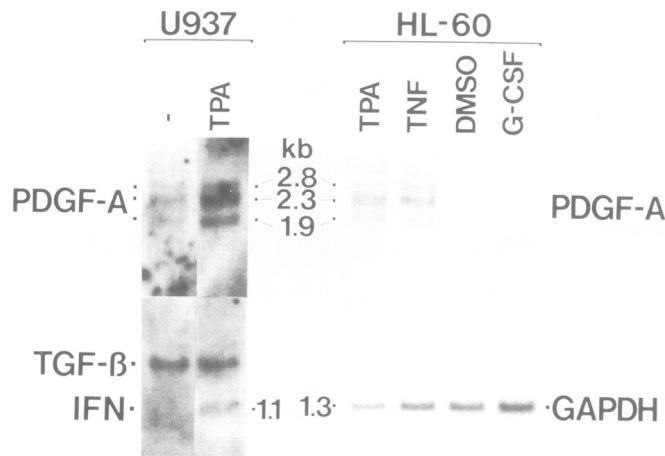


Fig. 6. Expression of PDGF-A in U937 and HL-60 cells. RNA was isolated from untreated U937 cells (–) or from cells treated for 2 days with 50 ng/ml TPA, blotted and analyzed with the PDGF-A, and, as controls, the TGF-beta and beta2-interferon probes. Note that an interferon RNA (IFN) is induced in U937 cells differentiated for 2 days, as has been described (Resnitzky *et al.*, 1986). Shown on the right is a similar analysis of PDGF-A and GAPDH RNA in HL-60 cells treated with 20 ng/ml TPA, 50 ng/ml of TNF, 1.25% DMSO or 2000 U/ml of recombinant human G-CSF.

Expression of PDGF-A is associated with monocytic differentiation of leukemia cells

PDGF and *c-sis* RNA are produced by activated macrophages (Martinet *et al.*, 1985; Shimokado *et al.*, 1985) and the HL-60 and U937 cells have previously been shown to express *c-sis* RNA upon TPA-induced monocytic differentiation (Colamonici *et al.*, 1986; Pantazis *et al.*, 1986). We were interested in the contribution of the PDGF-A gene in PDGF secretion during the process of differentiation.

The left panel of Figure 6 shows that in U937 cells the expression of PDGF-A RNA was enhanced during TPA-induced differentiation. The simultaneous appearance of the beta-2 interferon RNA is in line with previous findings (Resnitzky *et al.*, 1986) and confirms the stage of differentiation of the cells. In contrast, expression of the 2.5-kb TGF-beta mRNA was not changed. Elevated PDGF-A RNA levels persisted in U937 cells throughout their differentiation for at least 4 days. HL-60 cells stimulated with TPA or the tumor necrosis factor alpha (TNF-alpha), which has been reported to induce monocytic differentiation of these cells (Takeda *et al.*, 1986) also expressed PDGF-A RNA when tested 2 and 18 h after addition of the agents (Figure 6).

We could also confirm that *c-sis* RNA appeared in the differentiating HL-60 cells at ~18 h after induction, as has been reported by others (Pantazis *et al.*, 1986). However, the *c-sis* signal in monocytic HL-60 cell RNA was at least 10-fold weaker than in megakaryoblastic K562 cells and at least 8-fold weaker than the PDGF-A signal when tested with equivalent amounts of both probes labeled in a single nick-translation reaction (data not shown). In contrast, no PDGF-A RNA was seen in DMSO- or the granulocyte-colony-stimulating-factor (G-CSF)-stimulated HL-60 cells (Figure 6) (Fujisawa *et al.*, 1986), which acquired morphological features of granulocytic cells or in leukemic cells from single cases of myelomonocytic and monocytic (FAB classes

M4 and M5) leukemia and fresh alveolar macrophages from bronchoalveolar lavage.

Discussion

Platelets contain PDGF, but its site of synthesis has not been proven as megakaryocytes are difficult to obtain for studies of this matter. Cell lines that can be induced to differentiate towards megakaryoblasts offer a model to study this question. The results of studies with the cell line K562 reported in this paper suggest that the gene encoding the two PDGF chains are both expressed during megakaryoblastic differentiation whereas PDGF-A is not expressed in K562 cells differentiating along the erythroid lineage. K562 cells also secrete immunoprecipitable PDGF protein after TPA induction. These observations provide provisional evidence of PDGF synthesis in differentiating megakaryocytes.

We also report here that TPA- or TNF-alpha-induced differentiation of the human promyelocytic HL-60 leukemia cell line along the monocyte-macrophage pathway is associated with a transient activation of the PDGF-A gene. In contrast, no PDGF-A expression was detected during DMSO- or G-CSF-induced granulocytic differentiation of HL-60 cells.

Previous studies have shown that the K562 cell line, which was established from a patient in blast crisis of CML (Lozzio and Lozzio, 1975) has predominantly an erythroid differentiation potential (Andersson *et al.*, 1979; Gahmberg *et al.*, 1979). However, the K562 cells have retained some of the multipotentiality of CML cells (Tabilio *et al.*, 1983). Although specific markers of three different cell lineages can be expressed in K562 cells reflecting a degree of lineage infidelity, TPA markedly induces megakaryocytic differentiation while concomitantly diminishing the expression of myeloid markers and glycophorin as well as globin synthesis (Gahmberg *et al.*, 1984; Tetteroo *et al.*, 1984; Siebert and Fukuda, 1986). This was also reflected in our analysis of the appearance of platelet antigens in TPA-treated K562 cells. The association of K562 cell differentiation with *c-sis* expression and the megakaryoblastic specificity of PDGF-A induction is in striking agreement with the view that human megakaryocytes produce heterodimeric PDGF (Heldin *et al.*, 1985). However, regarding the nature of PDGF produced by K562 cells, our immunoprecipitation analysis is uninformative as we do not have A- and B-chain-specific antibodies.

Interestingly, expression of the TGF-beta RNA was also increased by megakaryocytic differentiation although in a complex manner that will require further study. We do not know the identity of the 2.3-kb TGF-beta RNA species, but Derynck *et al.* (1986) have also detected novel TGF-beta RNAs in PMA-treated HL-60 cells. However, changes reported here may relate to megakaryoblastic differentiation, since platelets have been shown to contain in their alpha-granules high concentrations of this polypeptide growth modulator (Assoian *et al.*, 1983). The expression of the *bcr-c-abl* oncogene mRNA (Shtivelman *et al.*, 1985; Grosveld *et al.*, 1986) remained unaltered suggesting that an active *c-abl* oncogene is compatible with cell differentiation in the K562 *in vitro* model. This result is in agreement with deductions from the cytopathology of CML: despite the activation of the *c-abl* oncogene, CML cells undergo nearly normal maturation (Greaves, 1986).

Although expression of PDGF-A was seen during TPA-induced differentiation of K562, U937 and HL-60 cells, we cannot conclude that induction of PDGF-A is necessary for differentiation

of these cells. For example, although transient *c-fos* proto-oncogene expression accompanies the TPA-induced monocytic differentiation of U937 and HL-60 cells, the *c-fos* induction by TPA can be dissociated from other parameters of cell differentiation (Müller *et al.*, 1985; Mitchell *et al.*, 1986). It is unlikely that PDGF expression has a role in the differentiation process, as monocytic cells apparently are devoid of PDGF receptors (Heldin *et al.*, 1981b). The results suggest to us that the turn-on of the expression of PDGF genes reflects the state of gene expression in megakaryocytic and monocytic cells as these genes were also turned on transiently by TNF-alpha in HL-60 cells, but not, for example, by TPA in the A549 lung carcinoma cells (unpublished data of Tomi Mäkelä and the authors). However, we could not detect PDGF-A RNA in fresh myelomonocytic (M4) or monocytic (M5) leukemia cells or pulmonary macrophages from bronchoalveolar lavage. We therefore consider it likely that TPA treatment also induces some kind of activation of the differentiating monocytic cells. We have not yet tested this hypothesis with freshly isolated peripheral blood cells.

It should be emphasized that the present results were obtained with established, highly aberrant leukemia cell lines that show, for example, abnormalities of karyotype and partial infidelity in cell lineage-specific antigen expression (Greaves, 1986). Due to limitations of differentiation and growth potential of the corresponding normal cells in cell culture, the validation of similar changes occurring during the differentiation of normal bone marrow megakaryoblasts will require, for example, sensitive *in situ* hybridization analyses.

The mechanisms of PDGF-A expression in TPA-treated leukemia cells is at present unknown. Major effects of TPA are thought to be mediated through protein kinase C (Nishizuka, 1984), but activation of the C kinase may not be sufficient for induction of HL-60 cell differentiation (Kraft *et al.*, 1986). Further studies are in progress to elucidate the requirements for induction of PDGF in U937 and HL-60 cells and the specificity of this effect using mutant cell clones and phorbol analogs as well as inhibitors of differentiation.

The mononuclear phagocytes play important roles in host defense mechanisms, acting mainly as scavenger and secretory cells. Cells of the mononuclear phagocyte system circulate in the blood as monocytes and differentiate into macrophages in tissues such as liver, lung, peritoneum or spleen (Van Furth, 1980). It has been estimated that PDGF could constitute as much as 50–70% of the total macrophage-derived mitogenic activity (Shimokado *et al.*, 1985). Platelets and mononuclear phagocytes are among the first cells to invade sites of endothelial and epithelial injury. As PDGF is a key mitogen for mesenchymal cells, it has been implicated in various functions of platelets and macrophages such as in wound repair, tissue remodeling, and pathological conditions such as granulation tissue, atherosclerosis, and maybe even myeloproliferative diseases (Ross *et al.*, 1986).

Materials and methods

Culture and treatment of the cells

The K562 (Lozzio and Lozzio, 1975), U937 (Sundström and Nilsson, 1976), HL-60 (Collins *et al.*, 1978), RD (ATCC CCL 136) and A549 (ATCC CCL 185) cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. For large-scale experiments, 175-cm³ Nunclon flasks or 850-cm³ Falcon roller bottles were used. For differentiation induction experiments cell density was adjusted to ~300 000 cells/ml. TPA (Sigma) was dissolved in DMSO or ethanol at 1 mg/ml, and stored in -20°C protected from light. The human recombinant TNF-alpha and G-CSF (r Met Hu G-CSF, 9.8 × 10⁷ U/A) were kind gifts from Drs Marja Jäättelä and Fuminaro Takaku

(Fujisawa *et al.*, 1986) respectively. Fresh leukemia cells were enriched from the peripheral blood of patients with myelomonocytic and monocytic leukemia by centrifugation in Ficoll-Paque (Pharmacia, Uppsala, Sweden).

Isolation and spot hybridization analysis of RNA

Polyadenylated RNA was isolated from 10⁷–10⁸ cells by direct binding from cell lysates to oligo(dT)-cellulose as detailed elsewhere (Saksela *et al.*, 1985). Polyadenylated RNA (2 µg) was precipitated, dried, dissolved in 12 × SSC:40% formaldehyde, heated for 5 min at 60°C, diluted serially, and spotted on nitrocellulose. The filter was baked, prehybridized and hybridized as described below.

Electrophoresis and blotting

RNA was dissolved in sample buffer containing 20 mM morpholinepropanesulfonic acid (pH 7.0), 1 mM EDTA, 5 mM sodium acetate, 50% (v/v) formamide and 2.2 M formaldehyde. Samples containing 5 µg of polyadenylated RNA were heated for 10 min at 60°C and electrophoresed in formaldehyde-agarose gels. Electrophoresis buffer was the same as the sample buffer. The position of rRNA was visualized by ethidium bromide staining, and the RNA was blotted to Biotransfer membranes (Pall Corp., Glen Cove, NY) in 20 × SSC.

Molecular probes

The PDGF-A probe used in the present studies was a radioactively labeled 1.3-kb *EcoRI* insert of human PDGF-A-chain cDNA cloned into the vector pUC-13 (Betsholtz *et al.*, 1986). The *c-sis* probe was either a 1.3-kb *BglII*–*BamHI* fragment of plasmid pSM-1 (Robbins *et al.*, 1981) or in some experiments a radioactively labeled 1.9-kb *v-sis*-specific fragment of the simian sarcoma virus (kindly prepared by Päivi Koskinen). For control hybridization we used the human glyceroldehyde 3-phosphate-dehydrogenase gene (Dani *et al.*, 1984; a kind gift from Dr Phillip Jeanteur, Laboratoire de Biologie Moléculaire, Université du Languedoc, Montpellier, France). The essentially full-length TGF-beta1 cDNA clone (Derynck *et al.*, 1985) was a kind gift from Dr Rik Derynck (Genetech, Inc., South San Francisco, CA). The *v-abl*-specific probe was a 1.9-kb *SstI*–*HindIII* fragment of the Abelson murine leukemia virus (Goff *et al.*, 1980). The human beta2-interferon and glycophorin A cDNAs were kind gifts from Drs Pravinkumar Sehgal and Lester May (The Rockefeller University, New York) and from Dr Siebert (La Jolla Cancer Research Foundation, CA) respectively.

Hybridization analysis

Nick-translation reactions were carried out according to manufacturer's instructions (Amersham, UK), and hybridizations were performed as in our previous studies (Saksela *et al.*, 1985). Stringent washes of hybridized filters were performed in 0.1 × SSC, 0.1% SDS, at 68°C for 1 h. Non-stringent washes (*v-sis* and *v-abl* probes) were done in 1 × SSC, 0.1% SDS, at 60°C for 1 h. For denaturation of DNA-RNA hybrids, the filters were boiled for 5 min, plotted dry on filter paper, preincubated in hybridization mixture, and rehybridized. Filters were reused for up to six times without detectable loss of signal. Some autoradiographic exposures were scanned with an Ultrascan XL laser densitometer (LKB Products, Bromma, Sweden). The relative intensities of the bands were determined in comparison with the invariant GAPDH signal from the same filter.

Immunological reagents

The C-17 monoclonal antibodies against platelet glycoprotein IIIa obtained as ascitic fluid (Tetteroo *et al.*, 1984) were a kind gift from Dr Lansdorp (Amsterdam, The Netherlands) and the FMC 27 antibody (Zola *et al.*, 1984) from Dr Zola (Adelaide, Australia). The rabbit IgG against factor VIII-related antigen was from Dakopatts a/s, Copenhagen, Denmark. The anti-PDGF antiserum was prepared as described by Heldin *et al.* (1981a). The immunoperoxidase and immunofluorescence stainings as well as flow cytometric analysis followed standard protocols.

Metabolic labeling and immunoprecipitation analysis

For immunoprecipitation, ~2 × 10⁷ cells were incubated overnight in 5 ml of minimal essential medium lacking cysteine, but containing instead 200 µCi/ml [³⁵S]cysteine (600 Ci/mmol, Amersham). The medium and the cells were separated by centrifugation, and immunoprecipitation was performed as described by Betsholtz *et al.* (1986). The immunoprecipitated proteins were analyzed by SDS-gel electrophoresis followed by fluorography.

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Note added in proof

After submission of this manuscript we found that the *bcr-c-abl* tyrosine kinase is extinguished in the TPA-treated K562 cells.