c-fos Oncogene Expression in Human Hematopoietic Malignancies Is Restricted to Acute Leukemias With Monocytic Phenotype and to Subsets of B Cell Leukemias

By Antonio Pinto, Giulia Colletta, Luigi Del Vecchio, Rita Rosati, Vincenza Attadia, Renato Cimino, and Alfonso Colombatti

To evaluate relationships between c-fos proto-oncogene expression and specific lineages of hematopoietic differentiation we analyzed the constitutive and TPA-induced expression of the c-fos gene in a wide variety of fresh human leukemic cells.

High constitutive c-fos expression was detected in acute leukemias with monocytic phenotype (FAB M4/M5) and in subsets of B lymphoid leukemias, some of which coexpressed B lymphocytic and monocytic markers. Conversely, low basal levels of c-fos transcripts were found in pure acute granulocytic leukemias (FAB M1/M2/M3), in erythroleukemias (FAB M6), in the great majority of B, and in all T lymphoid leukemias. TPA-induced c-fos expression seems to correlate with monocytoid differentiation only when sustained levels of transcripts (ie, detectable for at least 24 hours) were detected. Sustained c-fos expression was in fact observed only in those myeloid or lymphoid cells that acquired a stable monocyte-like phenotype in response to the phorbol ester. These results indicate that high constitutive c-fos expression may identify myelomonocytic-oriented forms of leukemia, specific subsets of B lymphoid malignancies, and at least some cells terminally differentiated in vitro to a monocyte-like phenotype. c-fos oncogene expression can therefore be regarded as an additional marker for the subclassification of human leukemias.

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Changes in oncogene expression have been described in hematopoietic cells as linked to cell growth or differentiation. In vitro induction of differentiation of murine and human leukemic cells by agents such as dimethylsulfoxide (DMSO), retinoic acid (RA), tetradecanoil phorbol acetate (TPA), 1,25-(OH)2 D3, hexamethylenebisacetamide (HMBA), or physiologic regulators like colony-stimulating factor (CSF), is in fact associated with a decreased expression of the previously overexpressed oncogenes c-myec and c-myb. These changes have been related to the observed inhibition of cell proliferation rather than to the induction of differentiation toward a specific lineage. Only recently a number of reports have appeared that link the expression of cellular oncogenes to a specific pathway of hematopoietic differentiation. Increasing amounts of two cellular oncogene transcripts, c-fos and c-fms, accumulate in hematopoietic cells as the monocytic maturation proceeds. Induction of differentiation of murine myelomonocytic cells WEHI-3 into macrophages by CSF and actinomycin D is accompanied by a remarkable increase in c-fos transcripts, maximal three days after induction. Furthermore a rapid induction of the c-fos oncogene has been reported during TPA-induced monocytic differentiation in human cell lines HL-60 and U-937. In this case the kinetics of induction was quite different, since c-fos transcripts reach their maximal levels 30' to 40' following TPA exposure and decrease rapidly thereafter to levels significantly superior to those of uninduced cells. Accordingly, high levels of c-fos RNA transcripts have been detected in primary cultures of mouse peritoneal macrophages. On the other hand, no increase in c-fos transcripts was observed when HL-60 cells were induced to differentiate into granulocytes by DMSO. These observations suggest that c-fos expression is associated with differentiation along the monocyte/macrophage pathway and that the fos oncogene may play a role in different events occurring during monocytic maturation.

Most of these studies have been conducted on established leukemic cell lines, adapted to grow in vitro for a long time, whose behavior may not necessarily reflect all events occurring in leukemic cells in vivo. Conversely, the analysis of fresh cell samples from a wide variety of leukemias, a disease regarded as a clonal expansion of cells reflecting the phenotypes of normal hematopoietic cells at various stages of maturation, may represent a more useful approach by which to investigate the lineage-specific expression of the fos oncogene and may provide some hints on the pathophysiology of hematologic malignancies. Our results show that constitutive c-fos expression in human hematopoietic tumors is restricted to myeloid cells displaying markers of monocytic differentiation and to subsets of B cell malignancies, some of which coexpress lymphocytic and monocytic markers.

MATERIALS AND METHODS

Isolation of leukemic cells. Fresh leukemic cells were obtained from the peripheral blood or bone marrow of patients diagnosed as having acute nonlymphoid leukemia (ANNL), acute lymphoid...
leukemia (ALL), chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), and hairy cell leukemia (HCL). All samples were collected before chemotherapy and after informed consent. Diagnosis was based on cell morphology plus standard cytochemical stainings, and acute leukemias were classified according to the French-American-British (FAB) criteria in acute myeloid/granulocytic leukemia (AML; FAB M1, M2, M3), acute mononoblastic leukemia (AMML; FAB M4), acute monocytic leukemia (AMoL; FAB M5), and acute erythroleukemia (AEL; FAB M6). Only patients who had at least 80% of leukemic cells in peripheral blood or bone marrow were chosen as donors.

Mononuclear cells were purified on a Ficoll-Hypaque (FH) gradient and subjected to two cycles of adherence on a plastic culture dish for one hour at 37°C. Such procedure yielded a population of leukemic cells more than 90% pure and virtually free of contaminating normal monocytes as revealed by May-Grünwald-Giemsa (MGG) and cytochemical stainings of cytoxin preparations. Leukemic cells were processed for RNA extraction soon after Ficoll purification to evaluate constitutive c-fos expression or were cultured in the presence of TPA as described below.

Culture conditions and in vitro differentiation. Fresh leukemic cells were seeded at a concentration of 1 x 10^6/mL in tissue culture dishes ( Falcon) in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS, Flow Laboratories Inc, Irvine Ayrshire, UK) and 4 mmol/L glutamine. TPA (Sigma Chemical Co, St. Louis) dissolved in DMSO was added to a final concentration of 20 ng/mL. Control cultures were grown in 0.002% DMSO. After 30 or 24 hours of incubation at 37°C in a humidified atmosphere of 5% CO2 in air, control and TPA-treated cells were collected, washed twice in cold RPMI, and processed for RNA extraction as described below. Control and TPA-treated myeloid cells were stained for sodium fluoride (NaF) inhibitable naphthylacetate-esterase (a-NAE) and napthol-ASD-chloroacetate esterase (N-ASD-CAE) as previously described. The percentage of adherent cells in myeloid leukemic cells following TPA exposure was evaluated as previously reported. U-937 cells were maintained in RPMI 1640 complete medium and subcultured twice weekly.

Immunophenotypic characterization of leukemic cells. Leukemic cells were characterized by a panel of monoclonal antibodies (MoAbs) either immediately after Ficoll purification or after culture in the presence or absence of TPA. MoAbs recognizing antigenic specificities of different blood cell lineages and grouped into “clusters of differentiation” (CD) according to criteria of the III International Conference and Workshop on Leukocyte Antigens (Oxford, 1986) were either a gift of Dr G. Rovera from Wistar Institute, Philadelphia (SS-25, SS-7) or were obtained through commercial sources: OKM1, OKB2, OKT1, OKT6, OKT8, OKT3, OKT4 (Ortho Pharmaceuticals Corp, Raritan, NJ); anti-HLA-DR, Leu-1 (HLA-DR), Leu-1, anti-IL-2R (interleukin 2 receptor), anti-TrR (transferrin receptor) (Becton Dickinson Co, Sunnyvale, CA); B4, MO2, MY7, MY9 (Coulter Immunology, Hialeah, FL); BA-2 (p24 Ag), 33.1 (HLA-DQ), BA-3 (CALLA) (Hybritech Inc, San Diego); fluorescein-conjugated (FITC)-conjugated goat (FAB'α) antihuman Ig, IgA, IgM, IgD, IgG, k (Kallestad, Austin, TX). In all samples 0.5 x 10^6 leukemic cells in a volume of 100 μL were incubated at 4°C for 30 with different MoAbs at the appropriate dilution. Cells were then washed three times with phosphate-buffered saline (PBS) and incubated at 4°C for an additional 30 min with FITC-conjugated goat antimouse Ig (Ortho). After three more washes in PBS, cells were analyzed by means of a flow cytometer (Ortho, Spectrum, IL). For cytoplasmic Ig (CyIg) analysis, cytospin preparations were fixed in methanol/acetone (3:1) for 5' at -20°C prior to FITC-conjugated goat anti-human IgM application. Cells were then observed under an epifluorescence UV microscope (Zeiss).

RNA extraction and hybridization. Total cellular RNA was extracted from leukemic cells either soon after Ficoll purification or after 30' and 24 hours of culture in the presence of 20 ng/mL TPA by the guanidinium hydrochloride method. Total RNA (5 μg/lane) was fractionated on 1% agarose gel containing 2.2 mol/L formaldehyde in MOPS buffer and transferred onto nitrocellulose filter.

The RNA bound to nitrocellulose was prehybridized for four hours at 42°C (in 1% glycine, 40% formamide, 5X SSC, 50 mmol/L NaH2PO4, pH 6.5, 250 μg/mL sonicated salmon sperm DNA, 1X Denhardt's solution and hybridized for 12 to 16 hours at 42°C (in 50% formamide, 2X SSC, 40 mmol/L NaH2PO4, pH 6.5, 200 μg/mL salmon sperm DNA, 10% dextran sulphate, 10 mmol/L EDTA, 0.1% SDS) with 3 x 10^6 cpm of 32P nick-translated purified DNA fragments (2 x 10^6 cpm/μg of DNA). The oncogene-specific probes used were v-fos (pBR322 1.0 Kb Pstl insert) and v-ras (clone HiHi 3 pBR322 1.0 Kb Hinc II fragment). The size of the c-fos transcripts was determined relative to 18S and 28S RNA markers, which were assumed to correspond to 1.8 and 4.5 Kb, respectively. For dot-blot hybridization studies different amounts of total cellular RNA were denatured and diluted in 10X SSC and spotted onto nitrocellulose by means of a dot-manifold apparatus (Schleicher & Schuell). Filters were then baked and hybridized to 6 x 10^6 cpm of oncogene-specific 32P nick-translated probes as described for Northern blots. Filters were washed twice in 2X SSC at room temperature for 20 minutes, two times in 0.5X SSC, 0.1% sodium dedecyl sulfate (SDS) at 50°C for 30 minutes, and finally four times in 2X SSC at room temperature for ten minutes. Levels of c-fos RNA were expressed as arbitrary units (au) relative to the levels of c-fos RNA present in uninduced U-937 cell line set arbitrarily to 1.0. The specificity of the hybridization experiments with the c-fos probe and the possibility of RNA miscalibration or of a TPA-induced aspecific increased or decreased transcription were assessed by hybridizing the same blots with a different probe specific for the c-ras oncogene that represented the internal calibration of the system.

RESULTS

Basal expression of the c-fos oncogene in myeloid cells. Constitutive expression of c-fos oncogene in human myeloid cells was investigated by evaluating the presence of c-fos-specific transcripts in leukemic samples from 16 patients with various forms of ANLL. An example of dot-blot hybridization assay of RNA extracted from myeloid leukemias is shown in Fig 1. Cells obtained from leukemia subtypes FAB M4 and M5 expressed levels of c-fos transcripts in patient No. 2) are reported, along with the two erythroleukemic samples (FAB M1, M2, M3) and the two erythroleukemic samples (FAB M6) studied expressed lower levels of c-fos transcripts (0.2 to 1.9 au). Conversely c-ras expression did not show any significant variation among the different leukemia samples (Fig 1).

In Table 1 relative levels of c-fos expression in all 17 leukemic samples (both peripheral blood and bone marrow were examined in patient No. 2) are reported, along with α-NAE activity and OKM1/CD11b and MO2/CD14 MoAbs binding. The range of c-fos expression within the AMML/AMo group (FAB M4, M5) varied from 3.8 to 9.9 au. On the other hand, pure granulocytic leukemias (FAB M1, M2, M3) and the two erythroleukemic samples (FAB M6) studied expressed lower levels of c-fos transcripts (0.2 to 1.9 au).

By comparing the levels of c-fos expression with the differentiation markers on leukemic cells, it appears that there was a strong correlation between the presence of “true” monocytic markers (α-NAE and MO2/CD14) and high...
Table 1. c-fos Expression in Human Myeloid Leukemic Cells

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Cell Type</th>
<th>OKM1</th>
<th>MO2</th>
<th>α-NAE</th>
<th>c-fos (au)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>AML M2 (PB)</td>
<td>28*</td>
<td>2</td>
<td>7†</td>
<td>1.9‡</td>
</tr>
<tr>
<td>2</td>
<td>AML M2 (PB)</td>
<td>48</td>
<td>10</td>
<td>10</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>AML M1 (PB)</td>
<td>3</td>
<td>1</td>
<td>11</td>
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</tr>
<tr>
<td>4</td>
<td>AML M2 (PB)</td>
<td>30</td>
<td>35</td>
<td>12</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>AML M3 (BM)</td>
<td>18</td>
<td>1</td>
<td>NT</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>AML M2 (PB)</td>
<td>22</td>
<td>12</td>
<td>NT</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>AMML M4 (PB)</td>
<td>36</td>
<td>48</td>
<td>51</td>
<td>6.1</td>
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<td>8</td>
<td>AMML M4 (PB)</td>
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<td>9</td>
<td>AMML M4 (BM)</td>
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<td>NT</td>
<td>50</td>
<td>3.8</td>
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<tr>
<td>10</td>
<td>AMML M4 (PB)</td>
<td>18</td>
<td>50</td>
<td>48</td>
<td>4.6</td>
</tr>
<tr>
<td>11</td>
<td>AMol M5 (PB)</td>
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<td>78</td>
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<tr>
<td>12</td>
<td>AMol M5 (BM)</td>
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<td>69</td>
<td>80</td>
<td>6.2</td>
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<tr>
<td>13</td>
<td>AMol M5 (PB)</td>
<td>60</td>
<td>58</td>
<td>70</td>
<td>8.9</td>
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<td>14</td>
<td>AMol M5 (PB)</td>
<td>43</td>
<td>61</td>
<td>63</td>
<td>9.9</td>
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<td>15</td>
<td>AEL M6 (BM)</td>
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<td>NT</td>
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<td>1.8</td>
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<tr>
<td>16</td>
<td>AEL M6 (BM)</td>
<td>28</td>
<td>1</td>
<td>NT</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Abbreviations: α-NAE, α-naphthyl acetate esterase; AML, acute myeloid leukemia (FAB M1, M2, M3); AMML, acute myelomonocytic leukemia (FAB M4); AMoL, acute monocytic leukemia (FAB M5); AEL, acute erythroleukemia (FAB M6); PB, peripheral blood; BM, bone marrow; NT, not tested.

*Percent of positive cells determined by indirect immunofluorescence using an Ortho Spectrum III flow cytometer. Fresh leukemic cells were analyzed soon after Ficoll purification.

†Percent of positive cells. Fresh leukemic cells were analyzed soon after Ficoll purification.

‡Levels of RNA quantitated by dot-blot hybridization followed by densitometric scanning of autoradiographic spots and expressed as arbitrary units (au) relative to the levels of c-fos RNA present in uninduced U-937 cell line set arbitrarily to 1.0. Values > 2 au are encased.

The table provides data on the expression of c-fos in human myeloid leukemic cells, with columns for patient number, cell type, and levels of mRNA expression (c-fos). The data are organized in a tabular format, allowing for comparison across different samples.

Basal levels of c-fos and c-rasK1 oncogene expression in human myeloid leukemia cells as detected by dot-blot hybridization. RNA samples were obtained from the following samples (see Table 1): AML FAB M2 (patient no. 1, patient no. 2/PB), AMML FAB M4 (patient no. 8, patient no. 10), AMol FAB M5 (patient no. 11, patient no. 14), AEL FAB M6 (patient no. 15), AML FAB M1 (patient no. 3). For details on RNA isolation and hybridization, see Materials and Methods.

Among lymphatic leukemias without concomitant expression of monocytic markers, high levels of c-fos transcripts.
Fig 2. Basal levels of \(c\)-\textit{fos} and \(c\)-\textit{ras}\textsuperscript{K\textdagger} oncogene expression in human lymphoid leukemia cells as detected by dot-blot hybridization. RNAs were obtained from the following samples (see Table 2): cALL (patient no. 18, patient no. 19), B CLL (patient no. 24, patient no. 26), B-CLL (patient no. 26, patient no. 23), HCL (patient no. 29), CALL t(4;11) (patient no. 20). For details on RNA isolation and hybridization, see Materials and Methods.

were detected only in the most immature of the cases examined, i.e., a B-committed ALL (patient No. 17) and in cells from a PLL (patient No. 28). Both acute and chronic T lymphoid leukemias showed a \(c\)-\textit{fos} expression slightly higher than uninduced U-937 cells (<2.0 au).

c-\textit{fos} expression during TPA-induced monocytic and lymphoid differentiation. Cells from 22 patients with different forms of leukemias (Table 3) were exposed in vitro to 20 ng/mL TPA, and total cellular RNA was extracted at 30' and 24 hours and hybridized to \textit{fos} and \textit{ras}\textsuperscript{K\textdagger} probes. Specific
RNA levels were analyzed by Northern blotting hybridization and quantitated by densitometric scanning of dot-blot autoradiographic films and expressed as times of increase relative to levels of uninduced cells. Addition of TPA to six of 8 myeloid leukemia cell cultures resulted in an overall rapid increase (up to 24.3-fold) in c-fos transcripts, which was maximal after 30' and which decreased thereafter, reaching at 24 hours levels superior (twofold to 13-fold) to those of the uninduced cells (Table 3). Unexpectedly, a drastic reduction of c-fos transcripts following TPA treatment was observed in cells from patients No. 3 (FAB M1) and No. 11 (FAB M5). A representative Northern blot showing the major specific 2.2 kb c-fos band in FAB M2 cells after TPA exposure is given in Fig 3. RNA extracted from myeloid leukemic cells at later times (up to five days) following TPA induction showed levels of c-fos threefold to fivefold those of uninduced cells (data not shown). c-ras levels did not change significantly after TPA induction (data not shown). At three days after TPA addition, up to 70% to 90% of the cells of all myeloid leukemia patients, except patient No. 3, displayed strong adherence to plastic surface. In addition, in all AML (FAB M1, M2) and AMML (FAB M4) samples, the percentage of cells showing NaF inhibitable α-NAE activity increased sharply three days after TPA exposure, whereas N-ASD-CAE activity was reduced in all AML and AMML cells except in the case of patient No. 3. The expression of myelomonocytic differentiation antigens was also modulated by TPA. The reactivity with the OKM1/CD11b and S5.25 antibodies, both reactive with late myeloid cells and mature monocytes, was in fact greatly increased in myeloid leukemia samples (FAB M2, M4, M5). The reactivity with the monocyte-restricted antibody MO2/CD14 increased in AMML/AMOL cells or was induced in AML cells following TPA treatment, whereas the expression of the immature myeloid antigen recognized by the S5.7 antibody was generally decreased (data not shown). Interestingly, as seen for the other parameters evaluated, TPA did not affect the antigenic pattern of cells from patients No. 3 and 11, whose immature phenotype remained unmodified.

**Table 3. c-fos Expression in Human Leukemic Cells During TPA-Induced Differentiation**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>AML</th>
<th>AMML</th>
<th>AML1</th>
<th>PL1</th>
<th>ALL</th>
<th>ALL1</th>
<th>CBL</th>
<th>CBL1</th>
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<tbody>
<tr>
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<td>13.6*</td>
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<tr>
<td>2</td>
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</tr>
</tbody>
</table>

**Abbreviations:** AML, acute myeloid leukemia (FAB M2); AMML, acute myelomonocytic leukemia (FAB M4); AMOL, acute lymphoid leukemia (FAB L1, L2); CLL, chronic lymphocytic leukemia; PLL, prolymphocytic leukemia; HCL, hairy cell leukemia.

*Relative levels of c-fos RNA in leukemic cells exposed for 30 minutes and 24 hours to 20 ng/mL TPA. RNA levels have been quantitated by densitometric scanning of autoradiographic films and are expressed as folds of increase relatively to the levels of uninduced cells (ie, cells cultured for 30 minutes and 24 hours in TPA-free medium).

†(4,11) translocation.

![Fig 3. Expression of c-fos oncogenes in human leukemia cells before and after TPA-induced differentiation as detected by Northern blot hybridization.](image-url)
Treatment with 20 ng/mL TPA of cell from various forms of lymphoid leukemias resulted in a general induction of c-fos expression only in B cell malignancies. As the representative Northern blot in Figure 3 shows, the specific 2.2 Kb c-fos band, already present at high basal levels in ALL1, PLL1, and to a lesser extent in B CLL3 (patients No. 17, 28, and 25 respectively), increased after 30' of TPA treatment and disappeared at 24 hours. Relative levels of c-fos expression in lymphoid leukemias quantitated from dot blots and indicated as fold of increase compared to the untreated controls are shown in Table 3. On the whole, TPA induced c-fos expression in nine of 11 samples of B lymphoid leukemias, whereas a drastic reduction in levels of c-fos was triggered by TPA in all three T cell malignancies. In the HCL cells, c-fos levels, already constitutively high, were unaffected by exposure to phorbol ester. The degree of induction in B cell malignancies ranged between 2.1-fold and 21-fold. After 24 hours of TPA treatment c-fos transcription returned to levels equal to or lower than those detected prior to induction, with the exception of cells from patients No. 20, 26, and 27.

TPA treatment also resulted in the modification of the surface phenotypes in B and T cell leukemias. Antigens of B lineage differentiation such as HLA-DR, HLA-DQ, and B4 were either increased in intensity or induced. B cell activation antigens, such as IL 2 receptor, 33.1 (DQ-like) and BA2 (p24 Ag), were all induced by TPA, whereas antigens characteristic of immature stages of B cell differentiation, such as OKB2, disappeared following treatment. These changes are suggestive of a TPA-induced differentiation of leukemic cells toward mature elements of the B lineage. The myelomonocytic markers (OKM1/CD11b, MY7/CDw13, MY4/CD14, Leu-M5/CD11d) detected in the subset of leukemias with high constitutive c-fos expression were similarly increased after TPA treatment along with the induction of the monococyte-restricted antigen MO2/CD14 and of markers of B lineage activation/differentiation (IL 2R, HLA-DR, HLA-DQ, BA2, and B4). The two acute T cell leukemias responded to TPA with the disappearance of the immature T lymphocyte antigen (OKT6) and with an increased intensity of the pan-T molecules T11 and T1. In the T CLL, a remarkable increase in both percentage of positivety and intensity of T11 and T1 and decreased T4 expression were induced. In all the T cell leukemias the receptor for IL 2 was strongly induced by TPA. These changes are compatible with differentiation into mature T lymphocytes.

**DISCUSSION**

Recent studies have suggested that the product of the c-fos gene may be involved in the differentiation of cells in the monocytic lineage.12-15 High levels of fos transcripts have been found in primary cultures of normal murine macrophages,12 and c-fos transcription and c-fos protein were both induced when human (U-937, HL-60) and murine (WEHI-3) leukemic cell lines were triggered to differentiate into macrophages by agents such as TPA12,14,15 and CSF.16 Nevertheless transient activation of c-fos transcription was shown to be in itself insufficient to commit U-937 cells to differentiate when stimulated by serum or diacylglycerol (DAG), and differentiation of HL-60 to macrophages by 1,25-(OH)2D3 was not accompanied by c-fos induction.26 Therefore the role of c-fos expression in the differentiation of hematopoietic cells remains unclear. On the other hand, the definition of the lineage-specific expression of the fos proto-oncogene may be important in understanding hematopoietic differentiation and may provide new insights into the pathophysiology of leukemias. Consequently c-fos expression was studied at basal levels and after treatment with TPA in a wide variety of fresh cell from patients with different hematopoietic malignancies representing different stages and lineages of blood cell differentiation.17,18 Our results show that a clear connection can be traced between c-fos expression and monocytic differentiation. In fact, high basal levels of c-fos transcripts were detected in myeloid leukemic cells showing markers of monocytic differentiation (FAB M4, M5), whereas very low amounts of transcripts, if any, were found in granulocytic oriented forms of myeloid leukemias (FAB M1, M2, M3) and in erythroleukemia cells (FAB M6). The expression of the monocytic-restricted antigen MO2/CD14 but not that of the panmyeloid OKM1/CD11b strongly correlated with higher c-fos RNA levels. This correlation was also supported by the results obtained after TPA treatment of ANLL cells in which differentiation along the monocytic pathway was accompanied by a remarkable increase in c-fos transcripts, resembling the kinetics of induction already described for TPA-treated U-93713 and HL-6014 cells. In fact, also in our hands a sustained expression of the c-fos gene, detectable for at least 24 hours, was found in those samples inducible to monocytic differentiation by TPA. That monocytic differentiation and c-fos expression are correlated was also suggested by the finding that in the two myeloid leukemic samples that did not show any differentiation in response to TPA, no increase in c-fos levels was induced by the phorbol ester.

The lineage-specificity of c-fos expression was strengthened by the low levels of constitutive expression found in lymphoid malignancies displaying pure B and T lymphocyte markers. Surprisingly, higher basal levels, comparable to those of monocytic-oriented forms of ANLL, were found in a group of lymphatic leukemia cells coexpressing B lymphocytic and myelomonocytic antigenic markers and also in the most undifferentiated form of ALL, a B-committed ALL, and the most immature form of chronic lymphoid leukemia, a PLL.

A rapid and transient c-fos expression was induced by TPA in almost all (nine of 11) B lymphoid samples, independently of the basal level of expression, and was accompanied by the induction of markers of B cell activation/differentiation. Yet sustained levels of c-fos expression were detected only in those cases within B cell leukemias in which induction of myelomonocytic markers was detected after TPA treatment.

The correlation between sustained c-fos expression and monocytic differentiation also seems to be confirmed by different data showing that treatment of both human leukemic cell lines and primary cultures with cytokine arabinoside and 5-aza-2'-deoxycytidine, which exert a molecular mechanism of action different from TPA, results, along with the appearance of a monocytic phenotype, in a progressive
increase of steady-state levels of c-fos RNA, maximal three to six days after the beginning of treatment. It is of interest to notice that in T cell leukemias treatment with TPA, although inducing T cell maturation, rather decreased c-fos expression. Since normal T cells were reported to respond to TPA with increased c-fos transcription, our data suggest that in leukemic T lymphocytes c-fos expression may be regulated differently.

The finding that in fresh human leukemic cells c-fos expression is restricted to a monocytoid phenotype in ANLL and to a subset of B cell leukemias that express the oncogene either constitutively or following activation by TPA could suggest different interpretations. A possibility is that it represents a phenomenon of "lineage infidelity", in which a subset of leukemic B cells coexpresses B lymphocytic and monocytic markers. Alternatively, the restricted c-fos expression in human leukemias may mirror a stage of hematopoietic differentiation in which features of monocytic and B lymphocytic lineages are physiologically and transiently coexpressed and frozen in leukemic cells. It is therefore tempting to speculate that the B lymphoid disorders expressing constitutively high levels of c-fos, ie, ALs with the t(4,11) translocation, HCLS, and some B-CLLs, are those that have been recognized for a long time as showing a number of monocytic features (phagocytosis, enzyme activity, differentiation antigens) along with clear B cell markers (rearranged Ig genes, pan-B antigens, IL 2 receptor). In conclusion, our results suggest that high constitutive levels of c-fos RNA may identify myelomonocytic oriented forms of leukemia in addition to particular subsets of B lymphoid malignancies and some cells terminally differentiated in vitro to a monocye-like phenotype. In this view c-fos expression may be regarded as an additional marker for subclassification of human leukemias.

REFERENCES

c-fos oncogene expression in human hematopoietic malignancies is restricted to acute leukemias with monocytic phenotype and to subsets of B cell leukemias

A Pinto, G Colletta, L Del Vecchio, R Rosati, V Attadia, R Cimino and A Colombatti