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Evidence for Distinct Regulation Processes in the Aclacinomycin- and Doxorubicin-Mediated Differentiation of Human Erythroleukemic Cells

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ABSTRACT. Human erythroleukemic K 562 cells were induced to differentiate along the erythroid lineage by anthracycline antitumor drugs, such as aclacinomycin (ACLA) and doxorubicin (DOX). Subsequent stimulation of heme and globin synthesis led to a differential quantitative expression of hemoglobins. Gower 1 (ϵ_2, ζ_2) was the major type for ACLA and X (ϵ_2, γ_2) for DOX. Although ACLA and DOX increased both the expression of γ-globin and porphobilinogen deaminase mRNAs, striking differences were observed in the expression of erythropoietin receptor mRNAs and in erythroid transcription factors GATA-1 and NF-E2, known to play a key role in erythroid gene regulation. Indeed, ACLA induces an increase either in the binding capacity of GATA-1 and NF-E2 or in the accumulation of erythropoietin receptor, GATA-1 and NF-E2 transcripts. In contrast, their expression with DOX was not significantly modified compared to uninduced cells, except for a slight decrease in NF-E2 expression on day 3. In conclusion, these data show that: 1. increased expression of erythroid transcription factors and erythroid genes are associated only with ACLA treatment, and 2. although cytotoxicity of both ACLA and DOX is certainly dependent on DNA intercalation, regulation of differentiation processes by these two drugs involves distinct mechanisms. BIOCHEM PHARMACOL 51;6:839–845, 1996.

KEY WORDS. aclacinomycin; doxorubicin; GATA-1; NF-E2; erythroid genes; K 562

Several anticancer agents commonly used in conventional cytotoxic chemotherapy are also able to induce tumor cell differentiation. In this respect, anthracyclines, in particular ACLA‡ and DOX, appear as good differentiating inducers in myeloid and erythroid leukemias, although their effect depends on the cell line involved (for review, see [1]). These drugs have already been shown to affect cell proliferation by different processes. Several targets have been proposed to explain their cytotoxic effects: intercalation into DNA [2], interference with DNA-topoisomerases [3], and interaction with the cell membrane [4, 5]. Nevertheless, if their cytotoxic mechanisms seem to be established, their differentiating processes remain unclear.

In previous studies, we and others demonstrated that ACLA and DOX led to erythroid differentiation of the human erythroleukemia cell line K 562 [6, 7]. At subtoxic concentrations, both drugs induced the appearance of hemoglobinized cells, but acted differently on the balance between growth and differentiation. Although cell growth and differentiation were tightly coupled after DOX treatment, the two processes were not related when ACLA was used as a differentiating agent, and in this case cell differentiation was activated without requiring the total arrest of cell growth [8]. In addition, the expression of membrane EPO-R, another marker of the erythroid lineage, increased after ACLA treatment (400 sites/cell vs fewer than 50 in the control) in contrast to DOX, which did not significantly change this receptor expression [9]. All these data led us to hypothesize that ACLA and DOX induced erythroid differentiation by distinct mechanism(s). In the present study, we compare effects of each drug on hemoglobin synthesis and at the transcriptional level by following early and late erythroid gene expression, such as EPO-R, globin, and PBGD, an enzyme of the heme synthesis. We concurrently examine the expression of erythroid transcription factors GATA-1 and NF-E2, known to play a central role in the regulation control of the erythroid gene promoters [10, 11]. Data indicate that these drugs exhibit marked differences in how they stimulate erythroid differentiation. ACLA, in contrast to DOX, is shown to act mainly at the transcriptional level *via* overexpression of erythroid transcription factors.

MATERIALS AND METHODS

Chemicals

DOX (Farmitalia Carlo Erba, Milan, Italy) and ACLA (Laboratoires Roger Bellon, Paris, France) were reconstituted in

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‡ *Abbreviations:* ACLA, aclacinomycin; DOX, doxorubicin; EPO-R, erythropoietin receptor; PBGD, porphobilinogen deaminase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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sterile water according to the manufacturer's instructions and were diluted in culture medium immediately before use. All other chemicals were of reagent grade.

Cell Culture

K 562 cells were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum and 2 mM L-glutamine in a 5% CO_2 humidified atmosphere.

Heme Labeling

Heme synthesis was followed by ^{55}Fe metabolic labeling using a ^{55}Fe-transferrin complex, as previously described [12]. On day 3, cells (1.5×10^6 cells) were washed three times in ice cold PBS and heme was extracted with acid butanone, as described by Krystal *et al.* [13]. Radioactivity was measured in β-scintillation and results were expressed in dpm by μg of proteins.

Hemoglobin Analysis

Cells 5×10^6 labeled as described above, were washed 3 times in ice cold PBS and lysed in 50 μL of 10 mM KCN using 3 cycles of freezing and thawing. Cytosolic supernatants (100,000 × g) were analysed by nondenaturating electrophoresis on 6% polyacrylamide gels in Tris-glycine buffer (25 mM Tris, 200 mM glycine at pH 8.6). Autoradiograms were analyzed by densitometry.

Protein Synthesis

Protein synthesis was evaluated by leucine incorporation. L-[3,4,5-^{3}H] (4 μCi) leucine (150 Ci/mmol, Amersham) were added over 1 hr to 10^6 K 562 cells previously washed in PBS and diluted in 200 μL leucine-free modified Eagle medium. As previously described [14], the cell lysis supernatant was analyzed on 12.5% polyacrylamide gel electrophoresis. Autoradiograms were quantified by densitometry.

Oligonucleotides

Sequences of DNA fragments used in these experiments were as follows (top strand): human γ-globin promoter from −201 to −156 [15]; 5′-CCTTCCCCACACTATCTCAATGCAAA TATCTGTCTGAAACGGTCCC-3′, human PBGD promoter from −170 to −142 [16]; 5′-CCTCCAGTGACTCAGCACA GGTTCCCCAG-3′. All fragmentswere ^{32}P-labelled at the 5′ ends with T4 polynucleotide kinase and [γ-^{32}P]ATP according to standard procedures.

Nuclear Protein Extraction and Electrophoretic Mobility Shift Assay

Nuclear proteins of 4×10^6 K 562 cells, differentiated or not, were prepared according to Schreiber *et al.* [17] in the presence of protease inhibitors (leupeptin, aprotinin, o-phenanthroline, and phenylmethylsulfonyl fluoride). *In vitro* binding capacity to their consensus sequences and electrophoretic runs were performed as previously described by Mantovani *et al.* [18].

mRNA Identification by Northern Blot Analysis

Total RNAs were prepared by phenol-chloroform extraction of acid-guanidinium thiocyanate lysates according to Chomczynsky and Sacchi [19]. RNA (10 or 20 μg) were run in 1.5% agarose gel, transferred onto nylon membrane (Hybond N, Amersham), as previously described [20]. The randomly labeled DNA probes used for hybridization were as follows: human Aγ-globin corresponding to the 2.7 kb EcoRI genomic fragment [21], human cDNA PBGD [22], EPO-R [23], GATA-1 [24], GAPDH (Clontech, Ozyme, Montigny le Bretonneux, France) probes, and the murine cDNA NF-E2 probe [10].

RESULTS

Effects of ACLA and DOX on Hemoglobin Synthesis

K 562 cells were treated for 3 days with ACLA 20 nM or DOX 40 nM, concentrations that induced optimum differentiation as assessed by benzidine staining (67% and 51% of hemoglobin producing cells for ACLA and DOX respectively vs 1% for control cells). At the translational level, quantification of the electrophoretic analysis of proteins labeled with ^{3}H-leucine during the differentiation time-course (Table I) revealed that ACLA led to a transient decrease in total protein synthesis on day 1. On days 2 and 3, it was stimulated 2- to 3-fold compared to the values observed for uninduced cells on the same day. Over the same period, we observed a strong stimulation of globin chains 6- to 10-fold higher than the control cells on days 2 and 3. DOX treatment induced an inhibition (50%) of the total protein pool as early as day 1 that was slightly amplified on days 2 and 3 (60%). Nevertheless, the rate of globin chains increased 2-fold during the same time. Although these drugs affected the total translational activity in a different way, we noted a preferential globin chain synthesis which, however, was not as significant after DOX treatment.

To follow heme synthesis and to identify the hemoglobin types produced after induction, the porphyrinic bulk was la-

TABLE 1. Effect of ACLA and DOX on total and globin protein synthesis

Inducers		Days of culture 1	2	3
Control	Globins*	74	47	40
	Total proteins*	1428	1289	1020
ACLA (20 nM)	Globins	17	287	388
	Total proteins	752	2857	2966
DOX (40 nM)	Globins	50	90	83
	Total proteins	726	491	412

* [^{3}H]Leucine-labeled proteins were analyzed on SDS-polyacrylamide gel (12.5%) electrophoresis (gel patterns not shown). After autoradiography, the relative proportion of total and globin proteins were calculated from the areas of the densitometry peaks. Results are from one experiment (two other experiments gave similar results).

belled by incorporation of ^{55}Fe. Heme synthesis was also stimulated by both drugs as estimated by measuring the ^{55}Fe incorporated after heme extraction (105 ± 10 dpm/μg of protein in control cells and 525 ± 60 dpm/μg of protein in ACLA-treated vs 303 ± 12 dpm/μg of protein in DOX-treated cells).

The analysis of the labelled hemoglobins was performed by polyacrylamide gel electrophoresis in nondenaturating conditions. The autoradiograph analysis (Fig. 1) shows that the embryonic Gower 1 (ϵ_2, ζ_2) was mainly detected in uninduced cells. When the cells were induced with ACLA, the Gower 1 was still the major hemoglobin to be synthesized; new types Gower 2 (ϵ_2, α_2), Portland (γ_2, ζ_2), X (ϵ_2, γ_2), and Fetal F (α_2, γ_2) were also detected. In DOX-induced cells, similar types were produced, but the major one was the X type. In all cases, the hemoglobins synthesized by K 562-differentiated cells were exclusively embryonic and fetal in type, despite the origin of the line from an adult (the β-globin gene was repressed [25]).

Expression of γ-globin, PBGD, and EPO-R mRNAs During ACLA- or DOX-Induced Differentiation

Having observed the changes in hemoglobin and in EPO-R synthesis in K 562-treated cells, we investigated modifications to individual species of mRNAs involved in differentiated phenotype expression. The γ-globin and PBGD genes, two representative genes that participate in the hemoglobin synthesis, were selected to follow the gene expression implied in this metabolism. As shown in Fig. 2, the γ-globin transcripts were significantly increased by ACLA or DOX treatment in a similar way. This increase was time-dependent, appeared by the first day and was maintained on the third day when the maximum of hemoglobin producing cells was reached. PBGD mRNA expression was also stimulated by both drugs. The response was close to the maximum by the first day, but the stimulation induced by DOX was less efficient than that of ACLA (2.5- vs 4-fold on day 3). Such results indicate that these two anthracyclines promote mRNA synthesis necessary to hemoglobin production. In contrast, only ACLA significantly enhanced the EPO-R mRNA steady state level and DOX did not modify it (Fig. 2). The rate of EPO-R transcripts reached its maximum on day 1 (5 fold increase in comparison to uninduced cells). These data confirmed our previous results obtained by measuring the number of erythropoietin binding sites during the differentiation time course (400 sites per cell after ACLA treatment vs fewer than 50 in uninduced cells) [9].

Expression of Erythroid Transcription Factors GATA-1 and NF-E2 During ACLA- or DOX-Induced Differentiation

Because the transcription of the erythroid genes studied is known to be positively controlled by the specific erythroid transcription factors GATA-1 and NF-E2, we examined their expression by mobility shift assays and Northern blotting. Compared to uninduced cells, ACLA-mediated differentiation led to an enhancement of the GATA-1 and NF-E2 binding

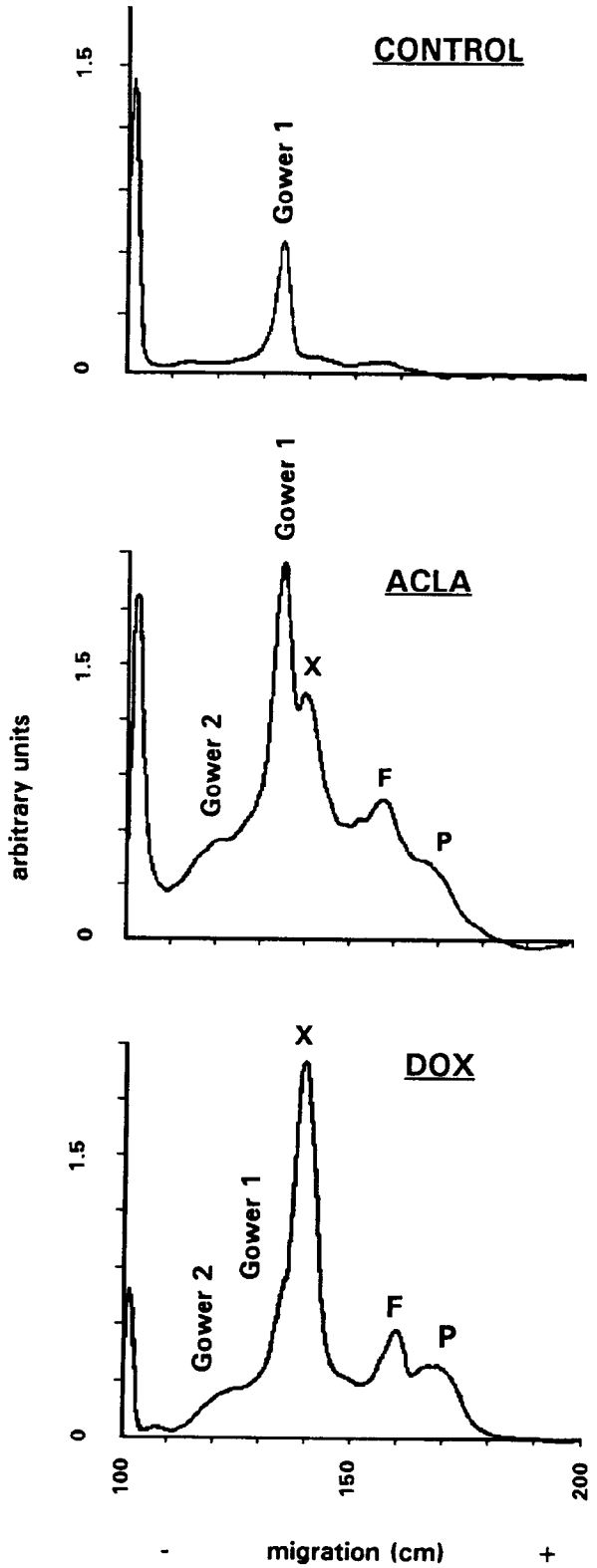

FIG. 1. Electrophoretic analysis of hemoglobins of K 562 cells induced to differentiate by ACLA or DOX. Cells were treated for 72 hr with DOX 40 nM or ACLA 20 nM and labeled during the same period with [^{55}Fe] transferrin [12].

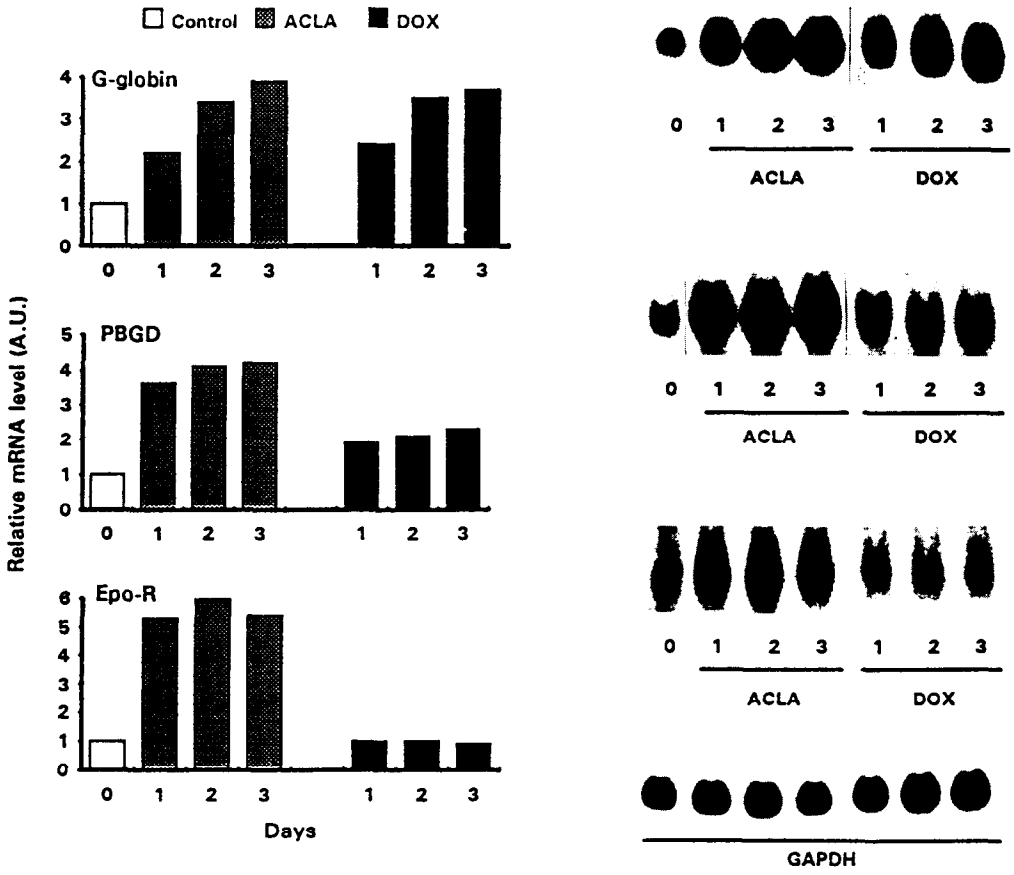

FIG. 2. Expression of γ-globin, PBGD, and EPO-R mRNAs in K 562 cells induced to differentiate by ACLA or DOX. Northern blots were performed as described in Materials and Methods and quantified by densitometry. Controls for the level of RNA loaded in each lane are represented by the level of GAPDH gene expression.

capacity (1.5-fold and 3.5-fold, respectively on day 3) (Figs. 3A and 4A). In contrast, DOX did not significantly modify it and even a decrease in the NF-E2 binding capacity was observed on day 3 (Fig. 4A). Such results are clearly confirmed by Northern blot analysis (Figs. 3B and 4B). Concerning the ubiquitous factors OTF-1 and AP-1, neither drug has a marked effect on their binding capacity except for a decrease in AP-1 observed on day 3 in DOX-treated cells.

Taken together, these results indicate that ACLA activates erythroid transcription factor genes whereas DOX does not, suggesting that distinct regulation mechanisms were involved in the activation of erythroid gene expression.

DISCUSSION

Anthracyclines, currently used in conventional chemotherapy as cytotoxic agents, have been shown to induce *in vitro* differentiation of various malignant tissues including leukemic cells [1], solid tumors such as neuroblastoma cells [26], and melanoma cells [27]. Induction of differentiation has also been reported in animal tumors [1] and in humans [28] but, in such cases, it is still difficult to evaluate the contribution of the antitumor or the differentiating effect of the drug. In addition, most of these approaches describe the appearance either of morphological changes or differentiation markers, but remain imprecise on the molecular basis of this effect.

In this study, we used erythroleukemic K 562 cells as a model cell culture system to study molecular aspects of erythroid differentiation induced by ACLA and DOX. Data clearly indicate that these antitumor drugs induced hemoglobin by affecting erythroid gene expression in different ways. Such discrepancies in their differentiating effects have already been reported in human leukemic HL 60 cells, where ACLA and DOX induced differentiation along different pathways: granulocytic for ACLA [29, 30] and monocytic for DOX [30]. We have previously shown that, in K 562 cells, the drugs affect the balance between growth and differentiation differently [8]. ACLA did not require a total arrest of cell growth to achieve its optimal differentiating effect but DOX did.

At the transcriptional level, both anthracyclines specifically enhanced globin chain synthesis. However, their effects on nonglobin synthesis were quite different: after electrophoretic analysis of proteins labelled with ^{3}H-leucine, we observed that DOX strongly inhibited nonglobin protein synthesis, whereas ACLA stimulated it on days 2 and 3. Heme synthesis was also stimulated as shown by the incorporation of ^{55}Fe followed by

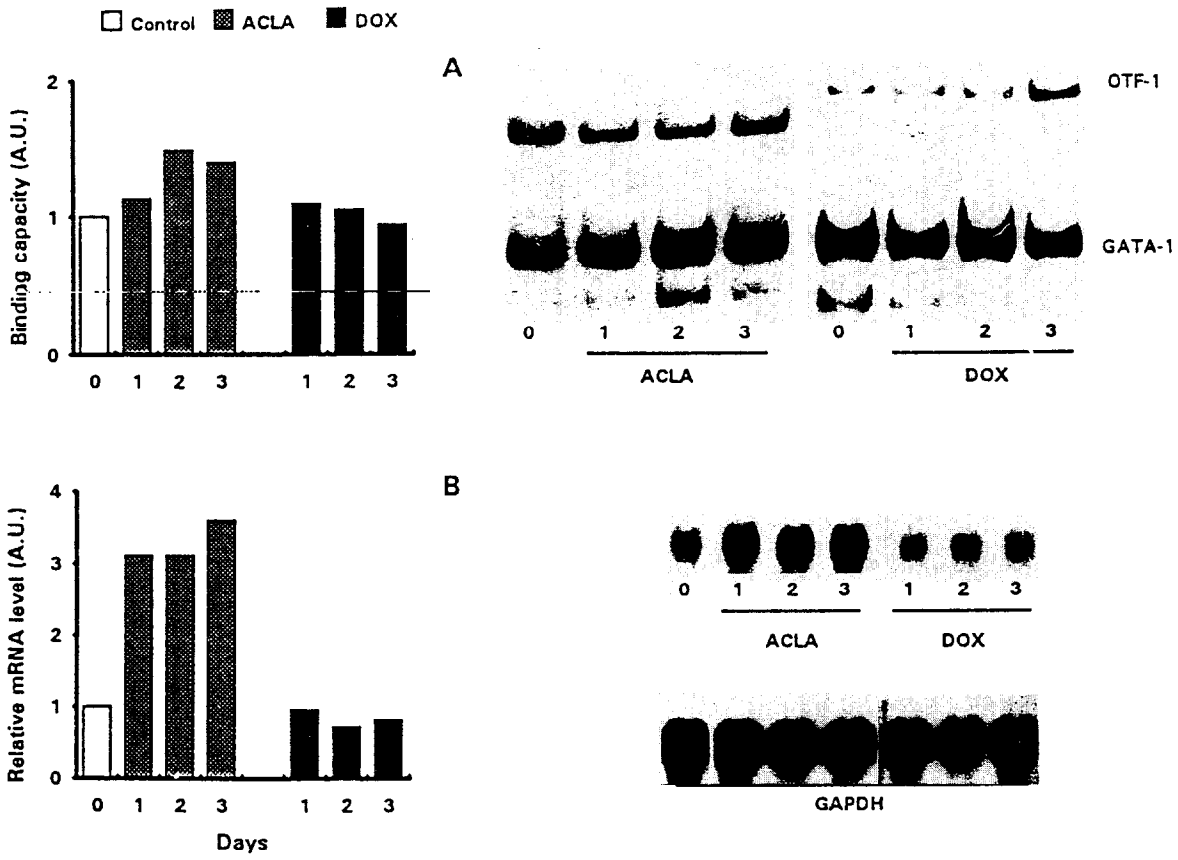

FIG. 3. Expression of the GATA-1 transcription factor in K 562 cells induced to differentiate by ACLA or DOX. Bandshift data (A) and Northern blots (B) were performed as described in Materials and Methods and were quantified by densitometry.

heme extraction. Compared to normal erythropoiesis and to murine erythroleukemic cell [31], heme also plays a key role in hemoglobin production in our model. Indeed, the use of a specific inhibitor of this biosynthetic pathway, such as succinylacetone (a δ-amino-levulinate dehydratase inhibitor), completely abolished the recruitment of hemoglobinized cells induced by ACLA and DOX treatment (data not shown).

As previously reported with hemin [32], ACLA and DOX caused partial switching of hemoglobin. New types of embryonic hemoglobin (Gower 2, X, Portland) and fetal hemoglobin F were observed vs Gower 1 in control cells. With ACLA, the major hemoglobin was still Gower 1 whereas hemoglobin X was predominant with DOX, indicating quantitative rather than qualitative changes.

Specific activation of globin and heme synthesis was confirmed at the transcriptional level because ACLA and DOX strongly stimulated γ-globin and PBGD mRNAs (approximately 4-fold on day 3 for ACLA and 2.5-fold for DOX). In contrast, only ACLA, and not DOX, is able to increase EPO-R transcripts. These data are in agreement with those showing an increase in membrane EPO-R only in ACLA induced cells [9]. They also suggest that the differentiating effect of ACLA could be mediated by such receptors because they have been reported to play a key role in the differentiation of murine erythroleukemic cells [33].

Because erythroid gene transcription has been shown to be regulated by the two major erythroid transcription factors GATA-1 and NF-E2, we next examined their expression during ACLA and DOX treatment. Binding sites for GATA-1 have been detected on virtually all erythroid genes [11], whereas those for NF-E2 appear to be more restricted to the locus control region (LCR) of α- and β-globins and to the promoters of enzymes of the heminic pathway (PBGD or ferrochelatase) [10]. Gel shifts and Northern blot analysis indicate that only ACLA induces an overexpression of GATA-1 and NF-E2. Expression of the ubiquitous factors OTF-1 and AP-1, detected only by gel shift, were not significantly modified. Because GATA-1 and NF-E2 are generally described as positive regulators of erythroid differentiation, it is tempting to speculate that, in the case of ACLA, their enforced expression could activate the transcription of targeted genes in tumor cells. In contrast, DOX could regulate globin or PBGD gene expression independently of GATA-1 or NF-E2 activation. To explain such a distinct activation of the erythroid program by these drugs, investigations are now in progress at the transcriptional and posttranscriptional level.

Although ACLA is used less than DOX in conventional chemotherapeutic protocols, it could prove interesting for clinical trials of differentiation therapy at low-dose administration, as supported by *in vitro* and *in vivo* results in acute

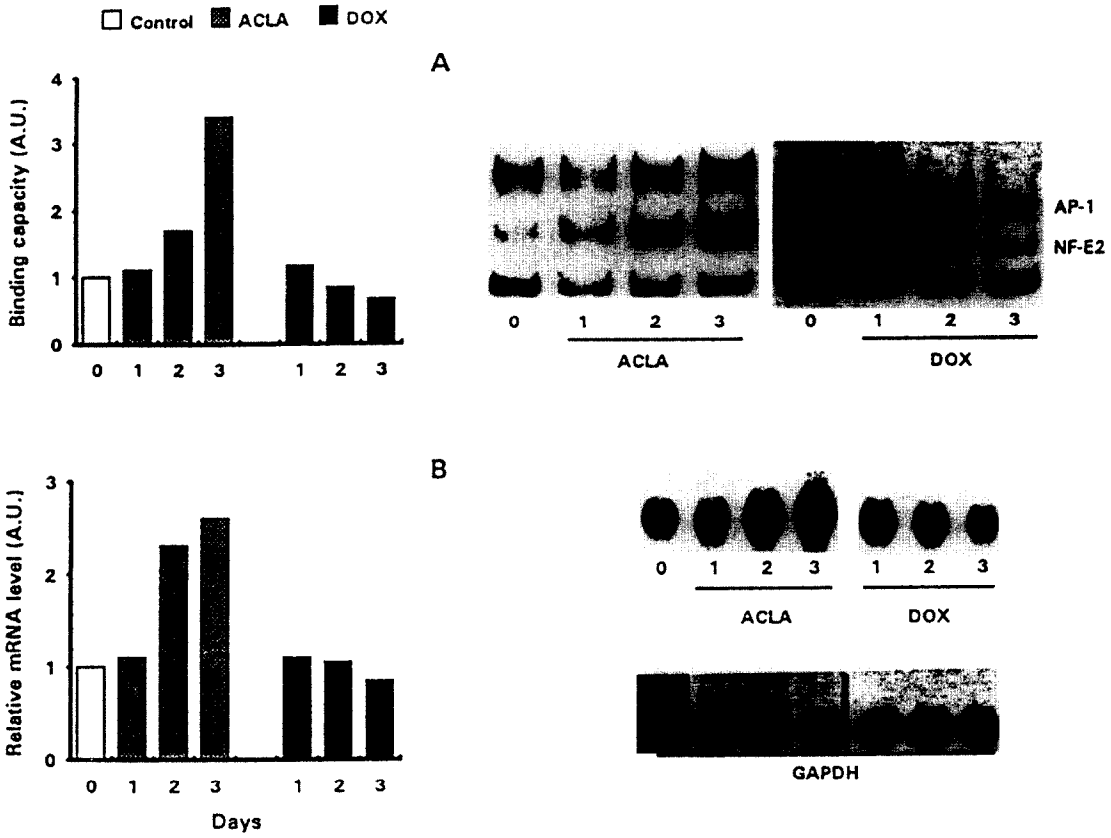

FIG. 4. Expression of the NF-E2 transcription factor in K 562 cells induced to differentiate by ACLA or DOX. Bandshift data (A) and Northern blots (B) were performed as described in Materials and Methods and were quantified by densitometry.

myeloid leukemia and myelodysplastic syndrome [28, 34]. Moreover, the specific effects of ACLA on transcription factors could contribute to the development of a new drug design that would be useful in the targeted regulation of specific cellular genes. As underlined by recent papers [35, 36, 37], this approach opens the way to a new pharmacological strategy for the control of proliferation and differentiation of tumor cells.

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References

1. Casazza AM, Anthracyclines as inducers of tumor cell differentiation. In: *Bioactive Molecules vol.* **6** (Ed. Lown JW), pp. 715–734. Elsevier Science, Amsterdam, 1988.
2. Gabbay EJ, Grier D, Fingerle RE, Reimer R, Levy R, Pearce SW and Wilson WD, Interaction specificity of the anthracyclines with deoxyribonucleic acid. *Biochemistry* **15:** 2062–2069, 1976.
3. Potmesil M, DNA topoisomerase II as intracellular target in anthracycline treatment of cancer. In: *Anthracycline and Anthracenedione-based Anticancer Agents* (Ed. Lown JW), pp. 447–466. Elsevier Science, Amsterdam, 1988.
4. Jeannesson P, Trentesaux C, Gérard B, Jardillier JC, Ross KL and Tökès ZA, Induction of erythroid differentiation in human leukemic K 562 cells by membrane-directed action of adriamycin covalently bound to microspheres. *Cancer Res* **50:** 1231–1236, 1990.
5. Morin MJ and Sartorelli AC, Inhibition of glycoprotein biosynthesis by inducers of HL-60 cells differentiation, aclacinomycin A and marcellomycin. *Cancer Res* **44:** 2807–2812, 1984.
6. Jeannesson P, Ginot L, Manfait M and Jardillier JC, Induction of hemoglobin synthesis in human leukemic K562 cells by adriamycin. *Anticancer Res* **4:** 47–52, 1984.
7. Mazouzi Z, Francastel C, Tapiero H and Robert-Lézénès J, Growth inhibition of doxorubicin-resistant K562 leukemia cells by anthracyclines: Absence of correlation with erythroid differentiation. *J Cell Pharmacol* **2:** 157–164, 1991.
8. Ngo Nyoung M, Trentesaux C, Aries A, Carpentier Y, Jardillier JC, Gorisse MC and Jeannesson P, Effect of aclacinomycin-doxorubicin association on differentiation and growth of human erythroleukemic K562 cells. *Anticancer Res* **14:** 1203–1208, 1994.
9. Jeannesson P, Trentesaux C, Ngo Nyoung M, Mayeux P, Jacquot R and Jardillier JC, Induction of erythropoietin receptors during aclacinomycin-mediated erythroid differentiation of K562 leukemia cells. *Leukemia* **5:** 14–18, 1991.
10. Andrews NC, Erdjument-Bromage H, Davidson MB, Tempst P and Orkin SH, Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein. *Nature* **362:** 722–728, 1993.
11. Weiss JW and Orkin SH, GATA transcription factors: Key regulators of hematopoiesis. *Exp Hematol* **23:** 99–107, 1995.
12. Trentesaux C, Gérard B, Mayeux P, Jeannesson P, Jacquot R and

Jardillier JC, Differentiating activity of adriamycin in human erythroleukemic cells: Effect on globin and heme synthesis. *Biochim Biophys Acta* **1012:** 161–165, 1989.
13. Krystal G, Eaves AC and Eaves CJ, A quantitative bioassay for erythropoietin, using mouse bone marrow. *J Lab Clin Med* **97:** 144–157, 1981.
14. Mayeux P, Félix JM, Billat C and Jacquot R, Induction by hemin of proliferation and of differentiation of progenitor erythroid cells responsible for erythropoietin. *Exp Hematol* **14:** 801–808, 1986.
15. Broggini M, Ponti M, Ottolenghi S, D'Incalci M, Mongelli N and Mantovani R, Distamycins inhibit the binding of OTF-1 and NFE-1 transfactors to their conserved DNA elements. *Nucleic Acids Res* **17:** 1051–1059, 1989.
16. Mignotte V, Elouet JF, Raich N and Romeo PH, Cis and trans-acting elements involved in the regulation of the erythroid promoter of the human porphobilinogen deaminase gene. *Proc Natl Acad Sci USA* **86:** 6548–6552, 1989.
17. Schreiber E, Matthias P, Muller MM and Schaffner W, Rapid detection of octamer binding proteins with 'min-extracts' prepared from small number of cells. *Nucleic Acids Res* **17:** 6419, 1989.
18. Mantovani R, Malgaretti N, Nicolis S, Ronchi A, Giglioni B and Ottolenghi S, The effects of HPFH mutations in the human γ-globin promoter on binding of ubiquitous and erythroid specific nuclear factors. *Nucleic Acids Res* **16:** 7783–7797, 1988.
19. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium-thiocyanate-phenol chloroform extraction. *Anal Biochem* **162:** 156–159, 1987.
20. Trentesaux C, Ngo Nyoung M, Aries A, Morceau F, Ronchi A, Ottolenghi S, Jardillier JC and Jeannesson P, Increased expression of GATA-1 and NFE-2 erythroid-specific transcription factors during aclacinomycin-mediated differentiation of human erythroleukemic cells. *Leukemia* **7:** 452–457, 1993.
21. Slightom JL, Blechl AE and Smithies O, Human fetal G gamma- and A gamma-globin genes: complete nucleotide sequences suggest that DNA can be exchanged between these duplicated genes. *Cell* **21:** 627–638, 1980.
22. Raich N, Mignotte V, Dubart A, Beaupain D, Leboulch P, Romana M, Chabret C, Charnay P, Papayannopoulou T, Goossens M and Romeo PH, Regulated expression of the overlapping ubiquitous and erythroid transcription units of the human porphobilinogen deaminase (PBG-D) gene introduced into non-erythroid and erythroid cells. *J Biol Chem* **264:** 10186–10192, 1989.
23. Migliaccio AR, Jiang Y, Miggliaccio G, Nicolis S, Crotta S, Ronchi A, Ottolenghi S and Adamson JW, Transcriptional and post-transcriptional regulation of the expression of the erythropoietin receptor (Epo-R) gene in human Epo-responsive cell lines. *Blood* **82:** 3760–3769, 1993.
24. Nicolis S, Bertini C, Ronchi A, Crotta S, Lanfranco L, Moroni E, Giglioni B and Ottolenghi S, An erythroid specific enhancer upstream to the gene encoding the cell-type specific transcription factor GATA-1. *Nucleic Acids Res* **19:** 5285–5291, 1991.
25. Fordis CM, Anagnou NP, Dean A, Nienhuis AW and Schechter AN, A β-globin gene, inactive in the K562 leukemic cell, functions normally in a heterologous expression system. *Proc Natl Acad Sci USA* **81:** 4485–4489, 1984.
26. Rocchi P, Ferreri AM, Simone G and Prodi G, Epirubicin-induced differentiation of human neuroblastoma cells *in vitro*. *Anticancer Res* **7:** 247–250, 1987.
27. Supino R, Mariani M, Colombo A, Prosperi E, Croce AC and Bottiroli G, Comparative studies on the effects of doxorubicin and differentiation inducing agents on B16 melanoma cells. *Eur J Cancer* **28A:** 778–783, 1992.
28. Sato S, Sakashita A, Ishiyama T, Nakamaki T, Hino KI, Tomoyasu S, Tsuruoka N, Homma Y and Hozumi M, Possible differentiation treatment with aclacinomycin A in acute myelomonocytic leukemia refractory to conventional chemotherapy. *Anticancer Res* **12:** 371–376, 1992.
29. Wilson RG, Feit S, Vossugh S and Randolph V, Aclacinomycin A and adriamycin induction of differentiation of HL-60 and ML-1 cells. *Proc Am Assoc Cancer Res* **27:** 749, 1986.
30. Stöcker U, Schaefer A and Maquardt H, DMSO-like rapid decrease in *c-myc* and *c-myb* mRNA levels and induction of differentiation in HL-60 cells by the anthracycline antitumor antibiotic aclarubicin. *Leukemia* **9:** 146–154, 1995.
31. Sassa S and Kappas A, Induction of δ-aminolevulinate synthetase and porphyrins in culture liver cells maintained in chemically defined medium. *J Biol Chem* **252:** 2428–2436, 1977.
32. Yamato K and Koeffler HP, Induction of terminal differentiation of human leukemic cells by chemotherapeutic and cytotoxic drugs. In: *Mechanisms of Differentiation* vol. **II** (Ed. Fisher PB), pp. 81–91. CRC Press, Boston, 1990.
33. Chiba T, Ikawa Y and Todokoro K, GATA-1 transactivates erythropoietin receptor gene, and erythropoietin receptor-mediated signals enhance GATA-1 gene expression. *Nucleic Acids Res* **19:** 3843–3848, 1991.
34. Shibuya T, Teshima T, Harada M, Taniguchi S, Okamura T and Niho Y, Treatment of myelodysplastic syndrome and atypical leukemia with low dose aclarubicin. *Leukemia Res* **14:** 161–167, 1990.
35. Peterson MG and Tupy JL, Transcription factors: a new frontier in pharmaceutical development? *Biochem Pharmacol* **47:** 127–128, 1994.
36. Arcamone F, Design and synthesis of anthracycline and distamycin derivates as new, sequence-specific, DNA-binding pharmacological agents. *Gene* **149:** 57–61, 1994.
37. Pomerantz JL, Sharp PA and Pabo CO, Structure-based design of transcription factors. *Science* **267:** 93–96, 1995.