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Differentiation of Human Promonocytic Leukemia U-937 Cells with DNA Topoisomerase II Inhibitors: Induction of Vimentin Gene Expression

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SUMMARY

The administration of the DNA topoisomerase II inhibitors 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) (10^{-7} M), VP-16 (2×10^{-7} M), or novobiocin (1.5×10^{-4} M) reduces the growth activity of human promonocytic leukemia U-937 cells, by arresting them preferentially at the G_2 (*m*-AMSA and VP-16) or at the G_1 and G_2 (novobiocin) phases of the cell cycle. Under these conditions, *m*-AMSA and VP-16 induce the differentiation of the cells efficiently, as proved both by an increase in the production of reactive oxygen species and by the activation of the surface expression of CD11b and CD11c, two differentiation-specific antigens. Novobiocin also induces the expression of those differentiation markers, but to a lesser extent. Analyses by Northern blot indicate that the topoisomerase II inhibitors reduce the levels of c-*myc* and β-actin mRNA and increase the levels of vimentin mRNA. The expression of vimentin is also stimulated at the protein level, as indicated by immunofluorescence assays. This represents one of the few known instances in which topoisomerase inhibitors stimulate gene expression in eukaryotic cells.

The potential use of differentiation inducers in the therapy of leukemia is a subject of increasing interest. This refers to certain cytostatic/cytotoxic drugs that are capable of causing cell maturation when applied at concentrations that are lower, and hence less toxic, than those usually employed to kill cells (1). DNA topoisomerase inhibitors have proven to be clinically important antitumor drugs, based on their ability to preferentially kill rapidly growing neoplastic cells (2, 3). In addition, the possible value of these inhibitors as maturation inducers is now being considered (4, 5). For instance, Constantinou *et al.* (4) reported that novobiocin caused the differentiation of human myelomonocytic HL-60 cells, with this effect being apparently related to the anti-topo II activity of the drug. However, they also found that other topo II inhibitors had little, if any, capacity to cause maturation.

In this report, we analyze the ability of *m*-AMSA, etoposide (VP-16), and novobiocin to induce *in vitro* the differentiation of human myeloid cells, using as a model the human histiocytic leukemia U-937 cell line (6). Both *m*-AMSA and VP-16 are known to be specific inhibitors of eukaryotic topo II (7, 8). On the other hand, novobiocin inhibits eukaryotic topo II activity (9), but it also affects other enzyme activities (10, 11). We examine here possible changes in cell growth rates, the production of oxygen species, and the expression of differentiation-associated antigens induced by the above mentioned topo II inhibitors. Also, we analyze the expression of genes such as c-*myc* and vimentin, which behave as molecular markers for the differentiation of myeloid cells.

Materials and Methods

Cell Culture and Drug Treatment

U-937 cells were grown in RPMI 1640 medium, supplemented with 10% heat-inactivated fetal calf serum, 5 mM HEPES buffer, and 0.2% (w/v) sodium bicarbonate, in a humidified 5% CO_2 atmosphere at 37°. Cells were seeded in 100-mm plastic dishes at concentrations of 2–3 × 10^5 cells/ml and were maintained in continuous logarithmic growth by passage every 2–3 days. TPA and novobiocin were obtained from Sigma Chemical Co. (St. Louis, MO). *m*-AMSA, as a lactate derivative (Lamasine), and VP-16 (Vepesid) were generous gifts from Bristol Myers, S.A.E. DFMO was a generous gift from the Merrell Dow Research Institute (Strasbourg, France). TPA was dissolved in dimethyl sulfoxide

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ABBREVIATIONS: topo II, type II topoisomerase; *m*-AMSA, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; DFMO, DL-α-difluoromethylornitine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; PIPES, 1,4-piperazinediethanesulfonic acid; kb, kilobases.

at 1.5×10^{-3} M. VP-16 was dissolved at 3.4×10^{-2} M in an organic solvent containing (per ml): citric acod (2 mg), benzylalcohol (30 mg), polysorbate 80 (80 mg), polyethylene glycol 300 (650 mg), in 30% ethanol. At the final concentrations used here, these solvents were without significant effects on cell growth and viability. *m*-AMSA, novobiocin, and DFMO were dissolved in RPMI medium at 10^{-3} M, 10^{-2} M, and 10^{-1} M, respectively. Cell number and viability were checked using an hemocytometer and trypan blue exclusion, respectively.

Flow Cytometry Determinations

Cell cycle distribution. To measure the DNA content, cells were incubated for 15 min at 4° in RPMI medium containing 0.05% (w/v) NP40 and 50 μg/ml propidium iodide. DNA histograms were then generated with an EPICS-CS flow cytometer (Coulter Científica, Mostoles, Spain), with an argon laser operated at 200 mV and excitation wavelength of 488 nm.

Measurement of H_2O_2 production. The production of H_2O_2 was measured essentially as described by Bass *et al.* (12). Namely, cells (10^6/ml) were first incubated for 15 min at 37° with 5×10^{-6} M 2′,7′-dichlorofluorescein diacetate, in PBS containing 0.1% (w/v) gelatin and 5×10^{-3} M glucose, and then stimulated for 30 min at 37° with 3×10^{-7} M TPA. The fluorescence was estimated by flow cytometry, as described above.

Detection of surface antigens. To detect the expression of cell surface antigens, indirect immunofluorescence determinations were carried out, using the MAbs Bear1 (anti-CD11b) (13) and HCl/1 (anti-CD11c) (14). Cells were labeled with the MAb for 30 min at 4°. After two washes with RPMI medium, FITC-labeled sheep anti-mouse IgG (Amersham, UK) was added and the incubation was continued for an additional period of 30 min at 4°. After the cells were washed twice with RPMI medium, their fluorescence was estimated by flow cytometry, as described above.

Measurement of vimentin content. To measure the cytoskeleton-associated vimentin, cells were fixed for 5 min at room temperature with 3.7% (v/v) formaldehyde, in a buffer containing 2 mM $MgCl_2$, EGTA, and 100 mM PIPES, pH 6.8, and then permeabilized for 5 min at room temperature with 0.2% (v/v) Triton X-100, 3.7% (v/v) formaldehyde, in the same buffer. After two washes with RPMI medium, cells were incubated with a mouse anti-vimentin MAb (Amersham), washed twice with RPMI medium, and incubated with FITC-labeled sheep anti-mouse IgG for 45 min at 37°. After two washes with RPMI medium, the cell fluorescence was estimated by flow cytometry, as described above.

DNA Decatenation Assay

U-937 cells (about 2×10^7) were centrifuged and washed once with cold PBS. Then, the nuclei were isolated and extracted as described by Heartlein *et al.* (15). The protein content of the nuclear extracts was determined by the method of Bradford (16). Topo II activity in the nuclear extracts was determined immediately by measurement of the capacity to decatenate catenated kinetoplast DNA from *Leishmania donovani*. Briefly, reaction mixtures of 20 μl contained 13.5 μl of reaction buffer (50 mM Tris, pH 7.5, 85 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA), 30 μg/ml bovine serum albumin, 1 mM ATP, 0.3 μg of catenated kinetoplast DNA, and serial dilutions of nuclear extracts. The mixture was incubated for 30 min at 37°, and then the samples were electrophoresed in 1% (w/v) agarose gels. The gels were stained with ethidium bromide, destained, and photographed over a UV light source.

DNA Sedimentation Assay

U-937 cells were labeled for 3 days with [*methyl*-^{3}H]thymidine (2 Ci/mmol; Amersham), in the absence or in the presence of drugs. Then, the cells were washed with PBS and lysed, and the DNA (approximately 5×10^4 cpm) was separated in alkaline sucrose gradients, following the procedure described by Kalwinsky *et al.* (17).

RNA Blot Assays

Total cytoplasmic RNA was prepared as described in a previous work (18). RNA samples (15 μg/lane) were denatured, electrophoresed in 1.1% (w/v) agarose-formaldehyde gels (19), and blotted onto nylon membranes (Hybond-N; Amersham). RNA blots were prehybridized, hybridized with excess ^{32}P-labeled probes, washed under highly stringent conditions (20), and finally autoradiographed. The probes used were the 1.1-kb human vimentin-specific *Xho*I fragment of p4F1 plasmid (21); the 1.5-kb *Cla*I-*Eco*RI fragment of pMC413rc plasmid, which contains the third exon of human c-*myc* (22); and the 0.66-kb mouse β-actin-specific *Kpn*I-*Bgl*II fragment of pAL41 plasmid (23). The fragments were labeled to approximately 10^9 cpm/μg of DNA with [α-^{32}P] dCTP (3000 Ci/mmol; New England Nuclear), by random hexanucleotide priming (24).

Results

Cell growth inhibition. Fig. 1a shows the effect of different concentrations of *m*-AMSA, VP-16, and novobiocin on the growth of U-937 cells. We found that the doses of 10^{-7} M *m*-AMSA, 2×10^{-7} M VP-16, and 1.5×10^{-4} M novobiocin greatly reduced cell growth activity, as determined by cell number increase. These concentrations were usually adopted for further experiments. Higher drug concentrations blocked the growth more efficiently but in addition caused excessive cell death.

Examination of cell cycle distribution showed that *m*-AMSA- and VP-16-treated cells accumulated preferentially at the postreplicative stage, as revealed by the increased number of cells with G_2 DNA content. On the other hand, novobiocin-treated cells accumulated in G_1 and, to a lesser extent, also in G_2, whereas the number of cells with intermediate DNA content (S phase) was greatly reduced (Fig. 1b).

Observations by light microscopy and flow cytometry showed that treatment with *m*-AMSA or VP-16 resulted in an increase in cell size, as compared with cells with G_2 DNA content in untreated populations. This alteration was not observed in novobiocin-treated cells (results not shown).

Topo II inhibition. To examine the efficiency of novobiocin as a topoisomerase inhibitor in U-937 cells, nuclear extracts were obtained from either untreated cells or cells treated for 3 days with different concentrations of the drug, and the topo II activity in the extracts was determined as the capacity to decatenate kinetoplast DNA. As shown in Fig. 2, the decatenation reaction was greatly reduced by the drug, even at the lowest concentration tested (10^{-4} M). This suggests that novobiocin, under the conditions used here, inhibits topo II activity efficiently.

The capacity of *m*-AMSA and VP-16 to inhibit topo II activity in U-937 cells was studied by determination of DNA strand scission, which is known to be a result of the drug-induced stabilization of the cleavable DNA-enzyme complex (7, 8). With this aim, cells were labeled for 3 days with [^{3}H] thymidine, either in the absence (control) or in the presence of 10^{-7} M *m*-AMSA or 2×10^{-7} M VP-16. Next, cells were lysed, and the DNA was fractionated by sedimentation in alkaline sucrose gradients. The resulting radioactivity profiles are shown in Fig. 3. Most of the DNA from control cells sedimented at the middle of the gradient (peak at fraction 17), although an amount of lower molecular weight species (peak at fraction 4) was also obtained. This latter peak probably represents radiolysed DNA, produced by the extended period of labeling, because it was not observed when untreated cells were labeled for short time periods (data not shown). Treatments with *m*-AMSA

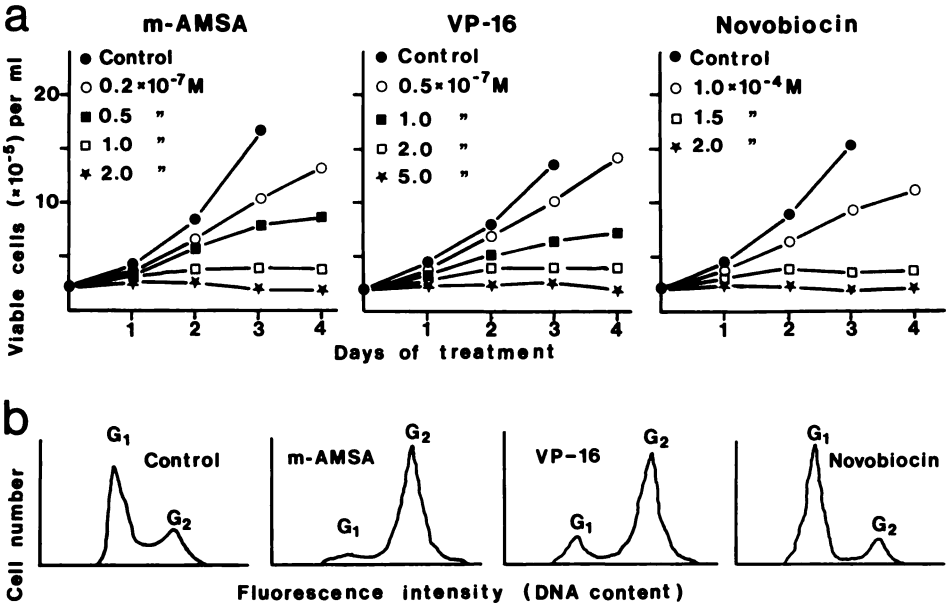

Fig. 1. Effect of *m*-AMSA, VP-16, and novobiocin on U-937 cell proliferation. a, Increase in the number of viable cells in cultures treated with different concentrations of the drugs. Results are the mean of two different experiments. b, Cell cycle distribution of untreated cells (control) and cells treated for 3 days with 10^{-7} M *m*-AMSA, 2×10^{-7} M VP-16, or 1.5×10^{-4} M novobiocin. The histograms were generated by flow cytometry analyses of propidium iodide-stained cells.

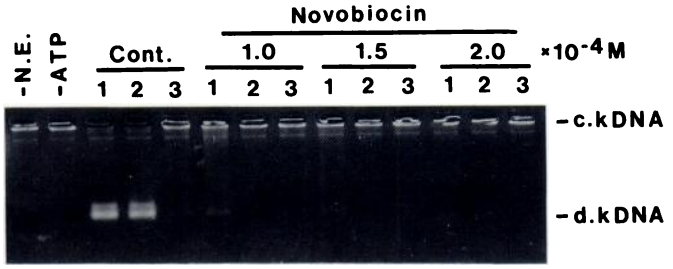

Fig. 2. Topo II activity in nuclear extracts from U-937 cells. Nuclear extracts were obtained from either untreated cells (*Cont.*) or cells treated for 3 days with different concentrations of novobiocin. Serial dilutions of the extracts, containing 1.6 μg (*lanes 1*), 0.8 μg (*lanes 2*), or 0.4 μg (*lanes 3*) of protein, were used as a source of topo II in decatenation assays of kinetoplast DNA networks. After electrophoresis in agarose gels, the DNA was visualized using UV light. *c.kDNA*, catenated kinetoplast DNA; *d.kDNA*, decatenated kinetoplast DNA; *–N.E.*, incubation without nuclear extract; *–ATP*, incubation with nuclear extract from untreated cells (1.6 μg of protein), without exogenous ATP.

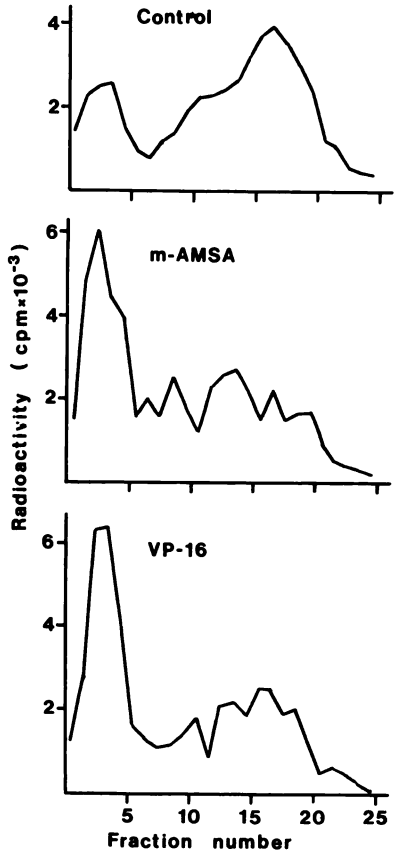

Fig. 3. Induction of DNA strand scission. Cells labeled for 3 days with [^{3}H]thymidine, either in the absence (control) or in the presence of 10^{-7} M *m*-AMSA or 2×10^{-7} M VP-16, were lysed, and the DNA size distributions were determined by sedimentation in alkaline sucrose gradients.

or with VP-16 caused a shift of the DNA to lower molecular weight species. This indicates that, under the conditions used here, the drugs effectively produce strand scission.

Cell differentiation induction. The question of whether the topo II inhibitors induce the functional differentiation of U-937 cells was approached by measurement of the capacity to generate reactive oxygen derivatives (12). For this purpose, cells were treated for 3 days with either 10^{-7} M *m*-AMSA, 2×10^{-7} M VP-16, or 1.5×10^{-4} M novobiocin. As a negative control, cells were treated with 5×10^{-3} M DFMO, a specific inhibitor of the enzyme ornithine decarboxylase. DFMO inhibits cell growth by arresting the cells at G_1 but does not induce differentiation (25).[1] The results in Fig. 4 show that both *m*-AMSA and VP-16 increased greatly the capacity of the cells to produce hydrogen peroxide. This capacity was also increased by novobiocin, but to a lesser extent. As expected, DFMO did not modify the production of H_2O_2 significantly.

The differentiation of U-937 cells by topo II inhibitors was also phenotypically characterized, by measurement of the surface expression of CD11b and CD11c, two maturation-associated antigens of human myeloid cells (13, 14). Fig. 5a shows the changes in the expression of these antigens upon treatment for 3 days with 10^{-7} M *m*-AMSA, 2×10^{-7} M VP-16, or 1.5×10^{-4} M novobiocin. As a negative control we also included DFMO (5×10^{-3} M), whereas as a positive control cells were treated for 2 days with 3×10^{-8} M TPA, a potent maturation-

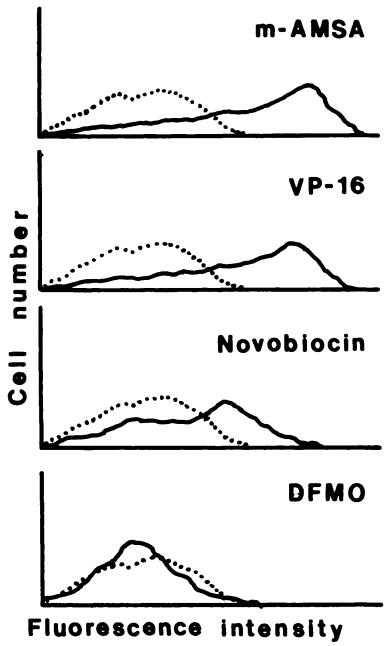

Fig. 4. Generation of hydrogen peroxide by U-937 cells. Untreated cells (control) and cells treated for 3 days with 10^{-7} M *m*-AMSA, 2×10^{-7} M VP-16, 1.5×10^{-4} M novobiocin, or 5×10^{-3} M DFMO were first loaded with 2′,7′-dichlorofluorescein diacetate and then stimulated with TPA. The fluorescence generated was estimated by flow cytometry. · · · ·, Control cells; ——, drug-treated cells.

inducing agent. We found that the expression of CD11b and CD11c was clearly stimulated by *m*-AMSA and VP-16, although to lower levels than with TPA. However, their expression was not significantly stimulated by novobiocin. As expected, DFMO had no effect on the expression of these antigens.

Fig. 5b shows the changes in the number of cells expressing CD11b and CD11c antigens upon treatment for 3 days with different concentrations of the topo II inhibitors. The fraction of positive cells was increased in a dose-dependent manner by *m*-AMSA and VP-16. Novobiocin produced only a slight increase at the maximum concentration tested.

Taken together, these results indicate that *m*-AMSA, VP-16, and, to a lesser extent, novobiocin induce the differentiation of U-937 cells.

Changes in gene expression. The differentiation of myeloid cells has been related to changes in the expression of specific genes (26). In addition, there is evidence suggesting that topo II activity might be required for gene transcription (27). Thus, we found it of interest to analyze the action of topo II inhibitors on gene expression in U-937 cells. With this aim, Northern blot assays were carried out to measure possible changes in the levels of c-*myc*, β-actin, and vimentin RNA levels upon treatment for 3 days with different concentrations of *m*-AMSA, VP-16, and novobiocin. The results in Fig. 6 show that these agents decrease the levels of c-*myc* and β-actin mRNA, whereas they greatly increase the levels of vimentin mRNA.

Next, we analyzed the time course changes in the levels of c-*myc* and vimentin RNA upon treatment with 10^{-7} M *m*-AMSA. We also measured the time course changes in the expression of the differentiation-specific CD11b and CD11c antigens. The results in Fig. 7 show that the level of c-*myc* RNA was already greatly decreased at 12 hr of treatment, preceding the stimulation of the antigens. On the other hand, a significant increase in the level of vimentin RNA was first detected at 48 hr of treatment, when CD11b and CD11c were already being expressed at high levels. Thus, c-*myc* inhibition appears as an early event, whereas vimentin induction seems to be a late marker of the *m*-AMSA-induced differentiation process.

Finally, we analyzed the changes in vimentin protein content upon treatment of U-937 cells for 3 and 4 days with either 10^{-7} M *m*-AMSA or 1.5×10^{-4} M novobiocin, by means of immunofluorescence combined with flow cytometry. As a control, we also measured the protein content in cells treated with 5×10^{-3} M DFMO. The vimentin was made accesible to specific antibodies by formaldehyde fixation followed by Triton X-100 permeabilization of the cells. Thus, the fluorescence values reflect the fraction of nonextractable, cytoskeleton-associated vimentin. The results in Fig. 8 show that treatment with *m*-

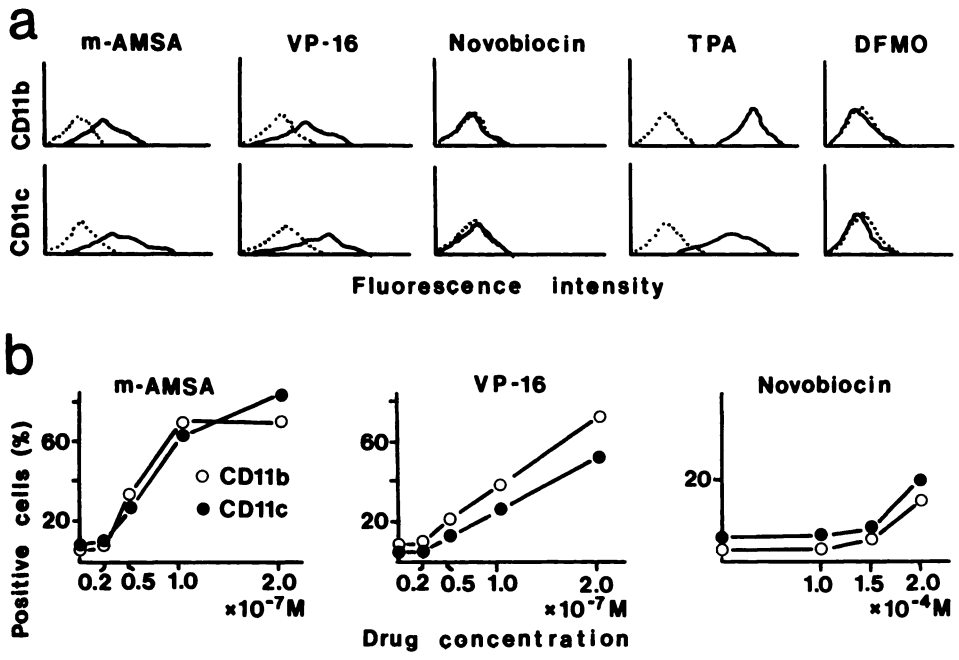

Fig. 5. Expression of cell surface antigens in U-937 cells as determined by flow cytometry. a, Reactivity with Bear1 (anti-CD11b) and HCI/1 (anti-CD11c) MAbs of either untreated cells or cells treated for 3 days with 10^{-7} M *m*-AMSA, 2×10^{-7} M VP-16, 1.5×10^{-4} M novobiocin, 3×10^{-8} M TPA, or 5×10^{-3} M DFMO. · · · ·, Untreated cells; ——, drug-treated cells. b, Percentage of cells expressing CD11b and CD11c antigens upon 3 days of treatment with different concentrations of *m*-AMSA, VP-16, or novobiocin. Both panels show the result of one of two similar experiments.

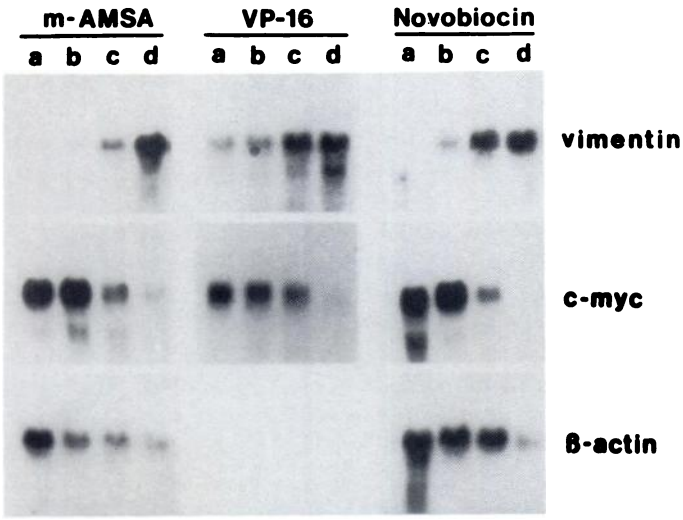

Fig. 6. Changes in the levels of specific mRNAs. Total cytoplasmic RNA was extracted from either untreated cells (*lanes a*) or cells treated with different concentrations of *m*-AMSA, VP-16, or novobiocin. RNA blots were prepared and hybridized sequentially with the indicated clones. *m*-AMSA was used at 2×10^{-8} M (*lane b*), 5×10^{-8} M (*lane c*), and 10^{-7} M (*lane d*). VP-16 was used at 5×10^{-8} M (*lane b*), 10^{-7} M (*lane c*), and 2×10^{-7} M (*lane d*). Novobiocin was used at 10^{-4} M (*lane b*), 1.5×10^{-4} M (*lane c*), and 2×10^{-4} M (*lane d*). Results of one of two similar experiments are shown.

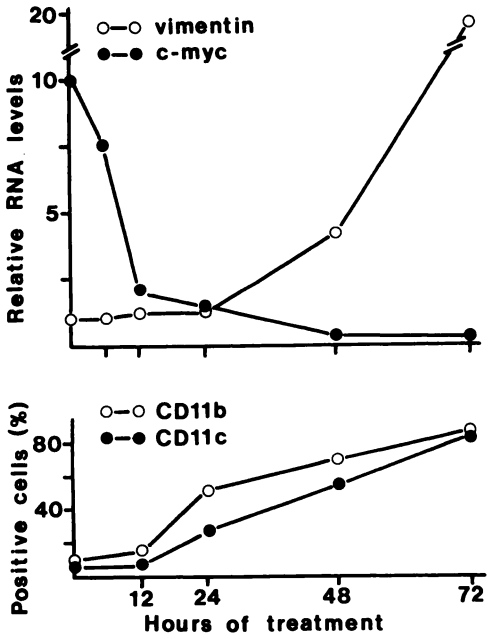

Fig. 7. Time course of changes of specific gene transcript levels and of cell surface antigens. The relative levels of c-*myc* and vimentin RNA (*upper*) and the percentage of cells expressing the CD11b and CD11c antigens (*lower*) were determined at increasing times of treatment with 10^{-7} M *m*-AMSA. The mRNA values were obtained by densitometric readings of Northern blots. c-*myc* and vimentin RNA levels in untreated cells were given arbitrary values of 10 and 1, respectively.

AMSA or novobiocin produced an increase in the fluorescence intensity, compared with untreated cells, demonstrating that the drugs effectively augment the vimentin in the cytoskeleton. In contrast, DFMO, a growth inhibitor that does not induce differentiation (Figs. 4 and 5), failed to increase the vimentin content. These conclusions were confirmed by fluorescence microscopy on cytological preparations (data not shown).

Discussion

DNA topoisomerases are enzymes involved in essential nuclear functions, such as DNA replication, transcription, and also possibly DNA recombination (for reviews see Refs. 28–30). Much of the knowledge of the function of these enzymes is due to the use of specific topoisomerase inhibitors. In this report, we have studied the effects of three agents with anti-topo II activity, namely *m*-AMSA, VP-16, and novobiocin, when applied at low concentrations to human hystiocytic U-937 cells. Both *m*-AMSA and VP-16 are known to interfere with the breakage-reunion reaction of topo II, by stabilizing the cleavable DNA-enzyme complex (7, 8). Novobiocin acts at a prior step, by competing with ATP for binding to the enzyme and, consequently, preventing its activation and reaction with DNA. In addition, the action of novobiocin is not specific, because the drug *per se* affects other enzyme activities (10, 11). The differences in the mechanisms of action of these agents may explain their distinct effects on the cell cycle. Thus, whereas *m*-AMSA and VP-16 arrest U-937 cells preferentially at G_2, novobiocin blocks them at G_1 and G_2. The G_2 blockade caused by the three agents is consistent with the reported requirement for topo II activity for progression of mammalian cells into mitosis (31).

Leukemic cells are blocked at some step of the maturation process and display a high proliferative capacity. The potential value of differentiation inducers as therapeutic agents resides in their ability to overcome the maturation blockade. Our results indicate that *m*-AMSA and VP-16 induce the differentiation of human leukemia U-937 cells efficiently. This was functionally demonstrated by the capacity to produce reactive oxygen species and was phenotypically confirmed by the induction of the expression of differentiation-associated antigens. Moreover, the finding that c-*myc* RNA levels decay soon after drug administration may represent additional evidence at the molecular level, because it has been shown that the expression of this gene is rapidly down-regulated in differentiating myeloid cells (32, 33). Although *m*-AMSA and VP-16 have in common the inhibition of topo II, they differ in other aspects. For instance, the acridine *m*-AMSA intercalates with DNA, whereas the epipodophyllotoxin VP-16 does not. This might suggest that differentiation induction is associated with topo II inhibition. A similar conclusion has been recently proposed by Constantinou *et al.* (4), to explain the novobiocin-induced differentiation of myelomonocytic HL-60 cells. Nevertheless, this proposal must be considered with caution, because differentiation is also induced by other cytostatic agents with different mechanisms of action. As an example, treatment with low doses of S phase inhibitors, such as Cytarabine and hydroxyurea, causes the differentiation of human myeloid cells (34, 35). Also, DNA damage by X-irradiation, which causes G_2 arrest (36), induces differentiation in some animal and plant cell systems (reviewed in Ref. 37). In contrast, novobiocin and DFMO, which arrest cells preferentially at G_1, have a poor or null capacity to induce differentiation (results in this work). Therefore, we should consider the possibility that differentiation is a cell cycle-dependent event, i.e., that cells arresting at specific stages of the growth cycle might suffice to trigger differentiation, independently of the agent used. In this regard, Yen (38) suggested that myeloid cell maturation may be initiated when the normal sequence of DNA replication is interrupted. Also, Wangenheim and Howard (37) proposed that the

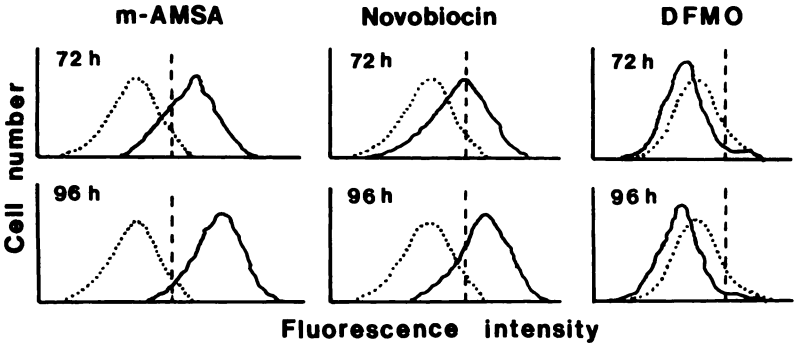

Fig. 8. Changes in cytoskeleton-associated vimentin content. Following formaldehyde fixation, Triton X-100 permeabilization, and incubation with anti-vimentin antibody, the vimentin content in the cells was determined by indirect immunofluorescence combined with flow cytometry. · · · ·, Untreated cells; ——, drug-treated cells. *Dashed vertical lines*, regions corresponding to nonspecific fluorescence, which were given by the distribution of cells incubated only with the second antibody (FITC-labeled anti-IgG).

"unbalanced growth" (excess of cell mass in relation to the degree of ploidy) that results from X-irradiation may be a cause of differentiation. Interestingly, unbalanced growth is often produced by agents that arrest cells at the S phase and at G_2 (Refs. 39 and 40 and observations in this work).

Although novobiocin inhibits cell growth and topo II activity efficiently, it seems to be a poor inducer of U-937 cell differentiation, in comparison with *m*-AMSA or VP-16. As stated above, a possible explanation for such a discrepancy might reside in the manner in which the cell cycle is perturbed, i.e., G_1 versus G_2 arrest. In addition novobiocin directly inhibits RNA polymerase activity (11, 41), independently of the anti-topo II action. Thus, Webb *et al.* (11) demonstrated that novobiocin suppressed the *in vitro* transcription of the mouse metallothionein gene, whereas the more specific topo II inhibitor VM-26 did not. Because the expression of CD11b and CD11c genes in differentiating cells is regulated at the RNA level (42, 43), novobiocin may be preventing the transcriptional activation of these genes and also possibly of others that are critical for differentiation.

The induction of vimentin expression by topo II inhibitors may appear to be a surprising result. In fact, there are indications that topo II activity is necessary for transcription (27) and that antitopoisomerase drugs depress RNA synthesis. For instance, novobiocin blocks the activation of most mitogen-inducible genes in serum-stimulated hamster fibroblasts (18). Also, novobiocin and *m*-AMSA reduce the TPA-driven induction of c-*fos* mRNA in myeloid cells.[1] Nonetheless, topo II inhibitors may activate the expression of heat shock genes in *Drosophila* cells (44). Furthermore, treatment of myeloid cells with maturation inducers, such as TPA (21, 33), butyrate (45), or S phase inhibitors (35), leads to the stimulation of vimentin expression. Therefore, the activation of this gene by anti-topo II agents might represent additional evidence of their capacity to induce differentiation.

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