[CANCER RESEARCH 51, 1202–1209, February 15, 1991]

Relationship of Acivicin-induced Monocytoid Differentiation of Human Myeloid Leukemia Cells to Acivicin-induced Modulation of Growth Factor, Cytokine, and Protooncogene mRNA Expression[1]

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ABSTRACT

We have previously noted that the glutamine antagonist acivicin (αS,5S-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) induces monocytoid differentiation of freshly isolated human myeloid leukemia cells and HL-60 cells. This study was designed to determine the effects of acivicin on the levels of HL-60 cell mRNA transcripts of several cytokines, growth factors, and protooncogenes implicated in the control of hematopoietic cell proliferation and differentiation. Control HL-60 cells did not express mRNA for granulocyte-colony-stimulating factor, granulocyte-macrophage-colony-stimulating factor, interleukin 3, or interleukin 6, and acivicin or phorbol myristate acetate did not induce their expression. Phorbol myristate acetate reduced expression of c-*myc*, c-*myb*, and heat shock protein 70 and enhanced those of macrophage-colony-stimulating factor and c-*fms*. Acivicin caused a decreased expression of c-*myc*, and an increased expression of mRNA for interleukin 1β and tumor necrosis factor α (TNF-α). The drug also caused an initial increase in c-*myb*, followed by a subsequent decrease below baseline levels. Supernatants and lysates of acivicin-treated HL-60 cells contained increased levels of interleukin 1β. Both TNF-α and interleukin 1β have been shown previously to influence hematopoietic cell differentiation. In our experiments, exogenous interleukin 1 added to HL-60 cells did not induce differentiation, but the combination of interleukin 1 and TNF synergistically enhanced the process. Pretreatment of the cells with TNF enhanced their responsiveness to subsequent treatment with interleukin 1. Our results demonstrate that the glutamine antagonist acivicin modulates HL-60 cell expression of TNF-α, interleukin 1β, c-*myc*, and c-*myb* and suggest that interleukin 1β and TNF-α might (in an autocrine manner) cause the differentiation.

INTRODUCTION

ANLL[3] is a malignant disease characterized by an increase in the number of bone marrow immature myeloid cells and a decrease in the number of mature, functional leukocytes. This maldistribution of cells is apparently a consequence of a block in the maturation (differentiation) of the precursor cells (1, 2). A number of chemicals, drugs, and biological agents have been identified which induce differentiation of human myeloid leukemia cell line cells and freshly isolated ANLL cells (1, 3). We have discovered recently that freshly isolated ANLL cells and HL-60 leukemia cells differentiate into monocytic cells in response to essential amino acid deprivation (4) or to culture with the glutamine antagonist αS,5S-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (acivicin) (5).

The mechanisms by which various treatments induce the irreversible commitment to monocytic differentiation are not clear. Differentiation of ANLL cells is a complex process. Various "natural" factors (including GM-CSF, M-CSF, G-CSF, IL-3, IL-1, γ-interferon, and TNF) are known to influence proliferation and differentiation. The purpose of this study was to determine the mechanism by which acivicin induces monocytic differentiation of HL-60 cells. We have investigated the effects of acivicin on HL-60 cell expression of mRNA for several genes potentially important in controlling these processes. Results demonstrate that acivicin decreases expression of mRNA for c-*myc* and c-*myb*, and increases that for the cytokines TNF-α and IL-1β. TNF and IL-1 function additively or synergistically to induce the monocytic differentiation. Although the changes noted in TNF and IL-1 might simply represent results of the differentiation processes, the acivicin-induced HL-60 cell maturation may occur through an autocrine process caused by induction of TNF-α and IL-1β mRNA expression and protein production, with subsequent modulation of the differentiation process.

MATERIALS AND METHODS

Culture and Cell Line. HL-60 cells were from the American Type Culture Collection (Rockville, MD). The cells were used at passage numbers of less than 60 (usually less than 55) and were routinely passed at a concentration of 1 to 2 × 10^5/ml in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Sterile Systems, Logan, UT), 100 units/ml penicillin, and 100 mg/ml streptomycin. Tissue culture medium and sera were tested periodically and found to contain less than 0.1 EU/ml lipopolysaccharide as determined by *Limulus* amebocyte lysate testing (QCL-1000 from Whittaker Bioproducts, Walkersville, MD).

RNA Analyses. RNA was isolated using the CsCl/guanidinium isothiocyanate method of Chirgwin *et al.* (6), with phenol/chloroform extraction of residual proteins (7), RNA (20 μg/lane) was electrophoresed in a formaldehyde gel (8, 9) and then transferred to Optibind membranes by the method of Southern (10). Double stranded cDNA probes were labeled with [α-^{32}P]dCTP by nick translation, and oligonucleotide probes were end-labeled with [α-^{32}P]dATP. The blotted RNA was prehybridized for 24 h at 42°C in a solution of 50% deionized formamide, 5× solution of SSC (pH 7.0), 50 mM NaH_2PO_4 (pH 6.8), 5× Denhardt's (1× being 0.02% Ficoll, polyvinylpyrrolidone, and bovine serum albumin fraction V), 0.5% SDS, and 150 to 175 μg/ml of denatured, sheared, sonicated salmon testis DNA. Hybridization was done at 42°C for 24 h in fresh buffer containing 1 to 2 million cpm of probe/ml. The filters were washed in 2× SSC-0.1% SDS at room temperature for 10 min, then in 1.5× SSC-0.1% SDS at room temperature for 40 min, and again in the latter buffer at 42°C for 35 min. Additional washing in 0.1× SSC-0.1% SDS at room temperature or 50°C was occasionally required to reduce background. The filters were then visualized by autoradiography using Kodak X-OMAT AR film.

Received 7/11/90; accepted 12/3/90.

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[1] This work was supported in part by the VA Research Service, the James Swiger Hematology Research Fund, and Grants P01 AI23308, P01 32682, and P50 AR39162 from the NIH.

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[3] The abbreviations used are: ANLL, human acute nonlymphocytic leukemia; GM-CSF, granulocyte-macrophage colony stimulating factor; M-CSF, macrophage colony stimulating factor; IL, interleukin; TNF, tumor necrosis factor; cDNA, complementary DNA; SSC, saline sodium citrate; SDS, sodium dodecyl sulfate; ATCC, American Type Culture Collection; HSP, heat shock protein; ELISA, enzyme-linked immunosorbent assay; PMA, phorbol myristate acetate; NSE, nonspecific esterase; 1 × SSC, 0.15 M NaCl, 15 mM sodium citrate, pH 7.0.

Equality of loading of RNA in different lanes in each experiment was assured by visualization of gels stained with acridine orange or by hybridization to 28S rRNA.

To assess RNA stability (half-life), actinomycin D (10 μg/ml) was added to control or acivicin-treated cultures 48 h after initiation of the culture. Total cellular RNA was then isolated at designated time points, and mRNA expression was examined by the Northern analysis technique. In nuclear run-on experiments, control HL-60 cells and those treated with 10 μg/ml acivicin were harvested after 48 h in culture and washed twice with phosphate-buffered saline (4°C, pH 7.2) with 0.04% EDTA. Nuclei were isolated according to the method of Bitter and Roeder (11) with vortexing to disrupt plasma membranes in a 0.5% Nonidet P-40 hypotonic solution. Isolated nuclei were suspended in 40% glycerol-2 mM $MgCl_2$-2 mM dithiothreitol-20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.6) and stored at −70°C until use (12). Nascent RNA transcripts were labeled with [α-^{32}P]UTP and treated with RNase-free DNase I (BRL) and proteinase K according to the method of Ausubel *et al.* (7). To confirm RNA polymerase II activity, a sample of nuclei from acivicin-treated cells was treated with 2 μg/ml α-amanitin. After phenol-chloroform extractions and precipitation with *Escherichia coli* tRNA and ethanol, the RNA was resuspended in 100 mM NACl-1 mM EDTA-10 mM Tris (pH 8.0) and centrifuged through "Quick Spin" Sephadex G-50 columns (Boeheringer Mannheim). The eluate was treated with 0.2 N NaOH neutralized with 0.24 M4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and precipitated with ethanol. An equal number of counts per filter were loaded from their respective samples for hybridization.

Probes. The probe for c-*myc* was the 1.4-kilobase *Cla*I-*Eco*RI fragment of the human c-*myc* third exon genomic clone JN-1 in *Acc*I-*Eco*RI cut SP6/T7-19 plasmid (12); c-*myb* was the 2.6-kilobase *Eco*RI fragment of the human c-*myb* gene pHM2.6 clone in the pKH47 plasmid (ATCC) (13); HSP-70 was the 2.3-kilobase *Bam*HI-*Hind*III fragment of the human HSP-70 gene pH2.3 clone purified from the pAT153 plasmid (ATCC) (14); TNF-α was the 0.6-kilobase *Eco*RI-*Hind*III fragment (with the trp promoter) of the human TNF-α gene in the SP6/T7-19 plasmid from Dr. S. McCachren (Duke University) (15); M-CSF was a 48-nucleotide probe corresponding to bases 344–391 (16); G-CSF was a 48-nucleotide probe corresponding to bases 122–169 (17); GM-CSF was a 0.8-kilobase *Eco*RI-*Aha*III fragment of the human GM-CSF gene in the SP64CSF plasmid (18); IL-3 was a 48-nucleotide oligonucleotide corresponding to bases 223–270; IL-1β was a 1.0-kilobase *Hind*III-*Eco*RI fragment of the human IL-1β gene in the SP6/T7 plasmid; IL-6 was a 1.2-kilobase *Eco*RI fragment of the human IL-6 gene in the PXM plasmid at the *Xho*I site; and v-*fms* was a 1.4-kilobase pst-I v-*fms* cDNA fragment purified from the pSM3 plasmid (ATCC) (19). The approximate sizes of the predominant mRNA transcript of the various genes are as follows: c-*myc*, 2.4 kilobases; c-*myb*, 3.8 kilobases; HSP-70, 2.6 kilobases; TNF-α, 1.6 kilobases; M-CSF, 4.2 kilobases; G-CSF, 1.6 kilobases; GM-CSF, 1.0 kilobases; IL-3, 1.2 kilobases; IL-1β, 1.7 kilobases; IL-6, 1.3 kilobases; c-*fms*, 4.0 kilobases; 28S rRNA, 4.9 kilobases; and 18S rRNA, 1.8 kilobases.

IL-1 and TNF Assays. Functional IL-1 was measured using the mouse thymocyte mitogenesis assay (20), and functional TNF was measured using the L929 lysis assay in the presence of actinomycin D (21). Antigen levels of TNF-α and IL-1β were done using commercially available ELISA kits: R&D Systems (Minneapolis, MN) for TNF; and Cistron (Boston, MA) for IL-1β.

Materials. Acivicin was from Upjohn (Kalamazoo, MI). The nick translation system, restriction enzymes, DNase I, proteinase K, and the 3′-terminal deoxynucleotidyl transferase kit for end labeling were from BRL (Gaithersburg, MD). Transcription nucleotides were from Boeheringer Mannheim (Indianapolis, IN). [α-^{32}P]UTP was from ICN (Costa Mesa, CA). [α-^{32}P]dCTP was from New England Nuclear (Boston, MA). α-^{32}P-dATP was from Amersham (Arlington Heights, IL). Agarose was from Bio-Rad (Richmond, CA). Optibind (reinforced nitrocellulose) membranes were from Schleicher and Schuell (Keene, NH). Recombinant human TNF-α was from Genentech (South San Francisco, CA) and Cetus (Emeryville, CA), and recombinant human IL-1α was from Hoffman La Roche (Nutley, NJ). Goat monospecific anti-human IL-1β and T-α IgG antibodies were from AMGEN (Thousand Oaks, CA). All other materials were from Sigma Chemical Company (St. Louis, MO).

RESULTS

Using acivicin at 10 μg/ml (56 μM), a dose that we earlier demonstrated would induce maximal monocytic differentiation of HL-60 cells as manifested by an inhibition of cell proliferation, increased expression of monocyte antigens and chemotactic peptide receptors, increased expression of the monocyte enzyme-nonspecific esterase, increased capacity for hydrogen peroxide secretion, and morphological similarity to monocytes/macrophages (5)], we examined the steady state expressions of the cells of several different genes that we thought would be potentially important in initiating and/or sustaining differentiation into the monocyte-like cells. Control HL-60 cells did not express mRNA for G-CSF, GM-CSF, IL-3, or IL-6, and acivicin or PMA treatment did not induce their expression. PMA reduced expression of c-*myc*, c-*myb*, and HSP-70 and enhanced that of M-CSF and v-*fms*. Acivicin caused a decrease in the expression of c-*myc* mRNA (with decreases notable by 48 or 72 h in different experiments) and induced expression of mRNA for IL-1β and TNF-α. Acivicin initially enhanced expression of c-*myb* mRNA (24 and 48 h), followed by a decline at 72 h to levels less than control cells. Fig. 1 demonstrates a Northern analysis of RNA from control and acivicin-treated cells studying expression of c-*myb* and IL-1β, and Fig. 2 shows a composite of results from studies with all of the probes.

Studies using actinomycin D treatment of control and acivicin-treated cells were done to determine if acivicin modified the half-lives of the mRNAs. The IL-1β or TNF-α mRNA half-lives in acivicin-treated cells were 105 and 15 min, respectively. Half-lives could not be compared to control cells since there was no basal expression of mRNA for IL-1β or TNF-α. With c-*myc*, the mRNA half-life in treated cells (15 min) was unchanged, while the c-*myb* mRNA half-life was slightly prolonged as compared to that of control cells (90 *versus* 70 min). Run-on transcription assays using cDNA probes for IL-1β and TNF-α demonstrated that the acivicin treatment induced the new transcription of IL-1β and TNF-α mRNAs in the nuclei isolated at that time point (48 h) (Fig. 3). At this time point, there was no difference in c-*myc* hybridization by control and acivicin-treated cells (data not shown). This demonstrates that the increased hybridization by RNA from acivicin-treated cells for IL-1β or RNA-α cDNA did not represent nonspecific cross-reactivity with the SP6/T7 plasmid and that they represented specific new transcription of these genes. The increase in synthesis of IL-1β and TNF-α mRNAs was inhibited by α-amanitin (data not shown) indicating that it was mediated by RNA polymerase II.

To determine if the acivicin treatments induced the production of IL-1β or TNF-α protein, we measured the levels of these monokines in supernatant fluids and cell lysates by functional assays and ELISAs. Using the mouse thymocyte mitogenesis assay for IL-1 and the L929 lysis assay for TNF, no activity was detected in supernatants or cell lysates of control HL-60 cells or HL-60 cells treated for 3 days with 10 μg/ml acivicin. ELISA studies showed no detectable TNF-α in either of the cells, but IL-1β was detected in control samples, and this level was greatly enhanced in supernatants and lysates of acivicin-treated cells (Fig. 4). In cultures done using neutralizing

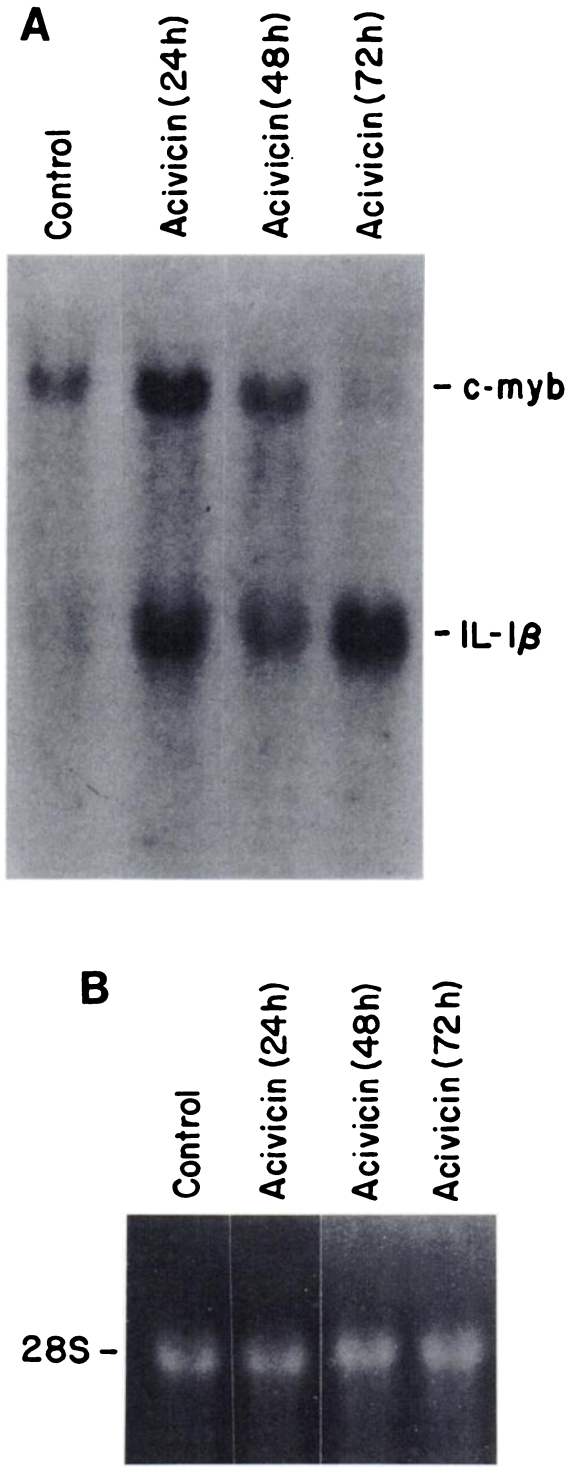

Fig. 1. Expression of c-*myb* and IL-1β mRNA by control and acivicin-treated HL-60 cells. *A*, autoradiograph resulting from the analysis using cDNAs for c-*myb* and IL-1β; *B*, acridine orange-stained gel of the RNA from the cells demonstrating the approximate equal loading of lanes based on 28S RNA staining.

amounts of anti-IL-1β and TNF-α antibodies (alone or in combination), there was no inhibition of the differentiating effects of acivicin (data not shown).

In order to assess the abilities of TNF and IL-1 to interact in inducing differentiation of HL-60 cells, the cells were incubated with TNF-α ± IL-1α for 3 days, and differentiation was then measured. As noted in Fig. 5, cell proliferation was not altered. However, IL-1 or TNF alone caused a very small increase in the number of NSE-positive cells and an increase in the abilities of the cells to produce hydrogen peroxide in the presence of 200 nM PMA. When the cells were cultured with both TNF and IL-1 simultaneously, there was a synergistic enhancement of NSE expression and peroxide formation ability (Fig. 5). None of the treatments with TNF and/or IL-1 caused vacuolization of the cytoplasms, condensation of the cell chromatin, appearance of morphology similar to monocytes on Wright's stain, or adherence of the cells to the plastic substrate. This, along with the lack of inhibition of proliferation, indicates that TNF and/or IL-1 do not cause "complete" or "terminal" differentiation of the cells. We have noted before that acivicin does cause growth cessation, cytoplasmic vacuolization, and a monocytoid appearance of the HL-60 cells (5). To measure the relative importances of the sequence of treatment with TNF or IL-1, HL-60 cells were incubated with the desired cytokine for 3 days, washed, recultured for 3 more days with the appropriate factor, and then assessed for differentiation. Results show that the treatments did not alter cell number, but they did induce changes in NSE expression and peroxide formation ability (Fig. 6). In general, the maximum enhancement of these parameters was seen in those cells cultured initially with TNF and then subsequently with IL-1. These results suggested that TNF might have modified the responsiveness of the HL-60 cells to IL-1.

DISCUSSION

Genes for the colony-stimulating factors GM-CSF, G-CSF, and IL-3 (multi-CSF) were examined because of their obvious relevance to myeloid growth and differentiation (22). M-CSF and c-*fms* [which encodes for the M-CSF receptor (23)] were especially pertinent since acivicin selectively causes differentiation to monocyte-like cells (5). IL-6 has been noted to induce differentiation of myeloid leukemia cells (24–26); therefore it was also a candidate. HSP-70 is one of several proteins which is preferentially induced in many different cells after stress of various types (*e.g.*, heat or starvation) (27), and it has been noted to be modulated in differentiating leukemia cells (28, 29). Since treatment of HL-60 cells with acivicin represents a significant "stress" for these cells, it was possible that HSP-70 might play a role in the differentiation changes noted. However, acivicin did not modify the steady state expression of GM-CSF, G-CSF, IL-3, M-CSF, c-*fms*, IL-6, or HSP-70.

The acivicin-induced decrease in c-*myc* expression was expected, since virtually all agents [with the possible exception of TNF (12)] which cause decreased proliferation and increased differentiation of HL-60 cells interrupt transcription of this gene (30–32). c-*myc* encodes a nuclear protein which can bind to DNA; this DNA binding may be important in modulating gene expression (33). c-*myb* is a gene found in many different types of cells (including hematopoietic cells); it encodes for a DNA-binding protein which may modulate the expression of other genes (34). Others have noted that the expression of c-*myb* mRNA is decreased during the differentiation of mouse erythroleukemia cells treated with hexamethylbisacetamide (35) or human myeloid leukemia cell line cells treated with other differentiating agents (32, 36, 37). Our experiments showed that HL-60 cells induced to differentiate to monocytes by treatment with acivicin had a slightly increased expression of c-*myb* at 24 and 48 h after initiation of culture and then a decrease in c-*myb* expression below baseline level at 72 h.

Both TNF and IL-1 have been shown to influence the regulation of normal hematopoietic cell growth and differentiation

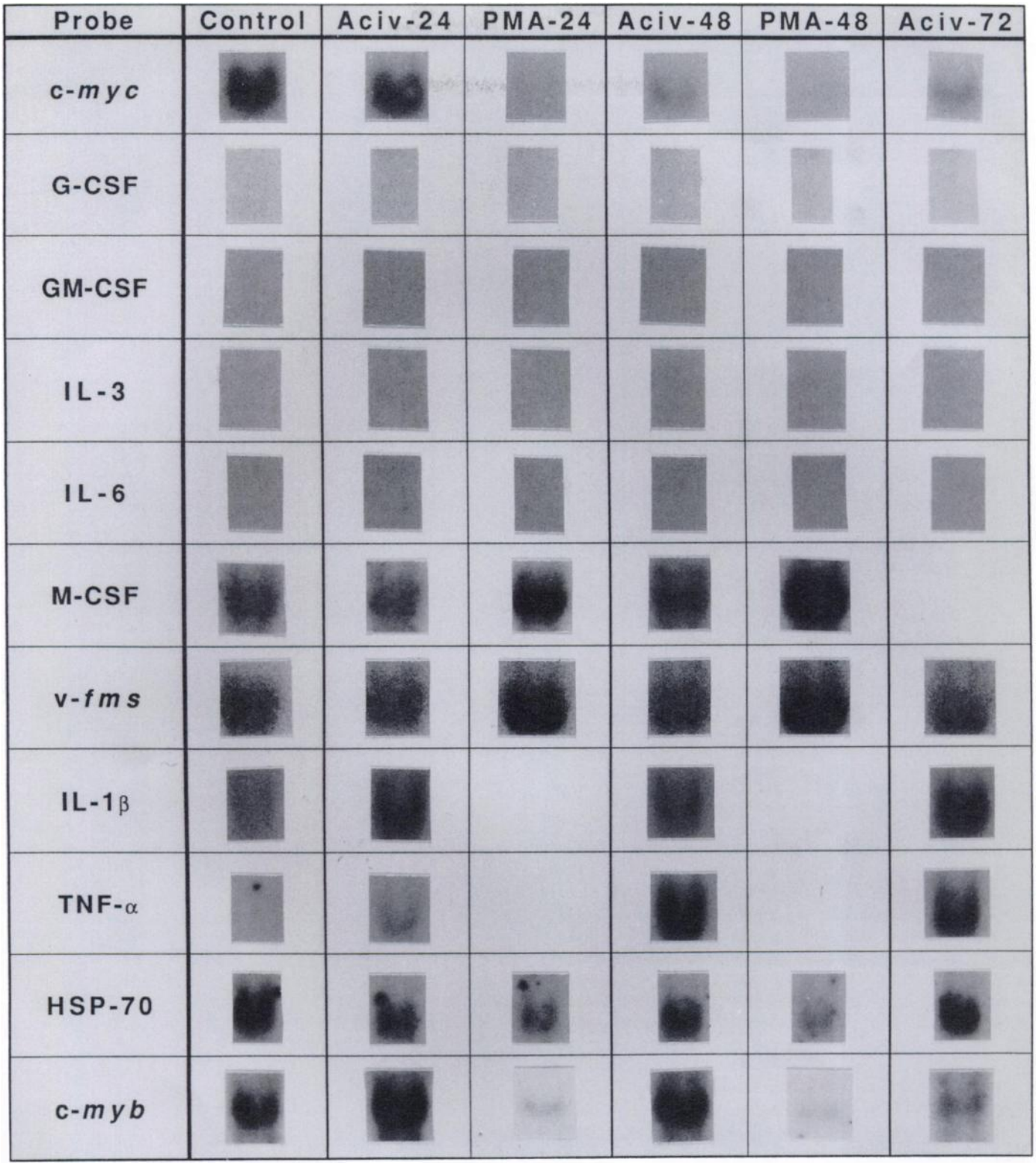

Fig. 2. Expression of mRNA by control, acivicin-treated, and PMA-treated HL-60 cells. Cells were incubated with no additive (control), 10 μg/ml acivicin, or 16 nM PMA for the designated number of days, and then expression of the different mRNAs was examined by Northern analysis. Each *block* is from the appropriate area (relative to 28S and 18S rRNA) of an individual Northern analysis of a single experiment arranged in the overall composite, with each experimental sample being relative to the appropriate untreated control.

(38–40); therefore it was important to examine these. Our work demonstrated that acivicin treatment increased mRNA levels for these two monokines and (based on mRNA half-life and nuclear run-on studies) that the increased levels were the results of new transcription of mRNA. We found increased levels of IL-1β in supernatant fluids and cell lysates of the acivicin-treated cells, but we could not detect TNF; it is possible that the assays were not sensitive enough or that the mRNA was being transcribed but the protein product was not being translated.

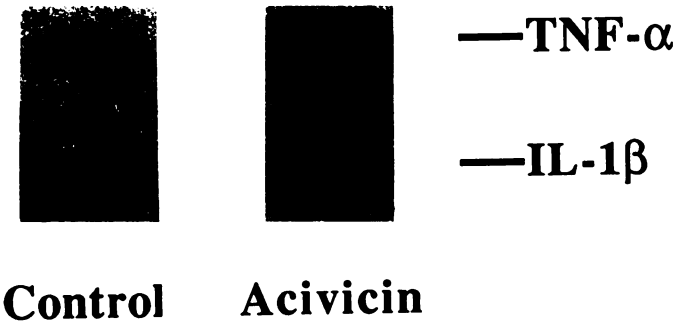

Fig. 3. Continuation of mRNA synthesis in nuclei from control or acivicin-treated HL-60 cells. Cells were incubated for 48 h with nothing (control) or 10 μg/ml acivicin, nuclei were then harvested, and continued transcription of mRNA for TNF-α or IL-1β was analyzed by the "run-on" transcription technique.

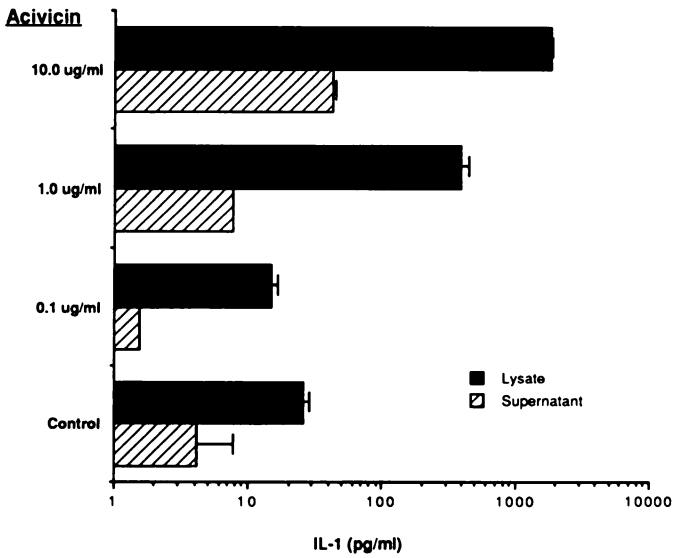

Fig. 4. IL-1β levels in supernatants or lysates of HL-60 cells (control or acivicin-treated). The cells were incubated for 3 days with the designated amount of acivicin, and then IL-1β content was determined by ELISA.

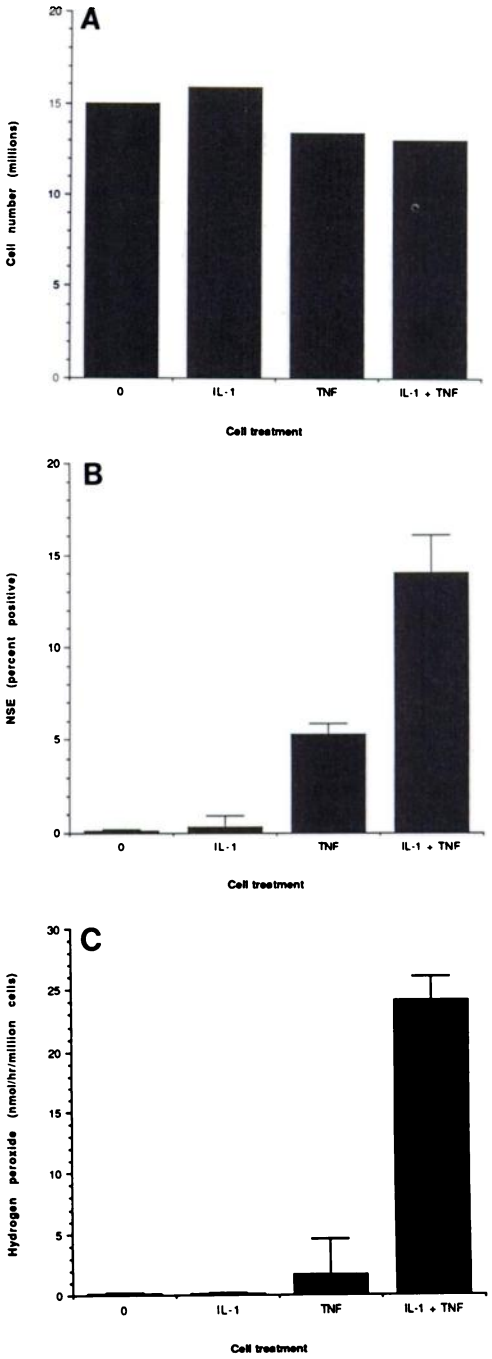

Fig. 5. Differentiation of HL-60 cells induced by exogenous TNF-α and/or IL-1α. Cells were incubated for 3 days with or without 100 units/ml TNF-α ± 100 units/ml (SD)IL-1α and then analyzed for cell count (*A*), nonspecific esterase positivity (*NSE*) (*B*), and PMA-induced hydrogen peroxide production (*C*). *Bars*, SD.

Although it is possible that the acivicin-induced IL-1 and TNF appear simply as a result of the monocytoid differentiation, we postulate that IL-1 and TNF produced by the acivicin-treated HL-60 cells may cause an autocrine type of leukemia cell differentiation and contribute to the observed effects of acivicin. It is important to note that the declines in the expression of c-*myc* and c-*myb* mRNAs occurred relatively late after acivicin treatment (at 48 and 72 hours). Reports from other investigators have noted that decreases in the expression of c-*myc* and c-*myb* mRNA resulting from treatment with TNF-α occurred rapidly (within 24 h) (12, 31, 32). We think that this delayed decrease after acivicin treatment is due to the time needed for acivicin to induce the endogenous generation of IL-1β and TNF-α which then cause the changes in mRNA expression. Experiments done to inhibit induced IL-1β and/or TNF-α by inclusion in the cultures of antibodies against either cytokine, however, did not alter the acivicin differentiation effects. As noted previously for IL-3 (41) and platelet-derived growth factor (42), endogenously produced factors might act on the cells without being secreted; thus, antibodies might not have had access to the cytokines. Alternately (in the more typical autocrine scenario), they could be secreted and then bind to their membrane receptors, initiate the differentiation (43), and escape the effects of the antibodies. While some investigators have demonstrated that IL-1 causes differentiation of the mouse ANLL M1 cells (44) and that TNF and IL-1 can act synergistically in causing this monocytic differentiation of M1 cells (45), others have shown that IL-1 causes proliferation without differentiation of human ANLL cells (46). We note here that IL-1 alone does not induce differentiation of HL-60 cells but that it does act additively or synergistically with TNF to enhance the differentiation process. This positive interplay between TNF and IL-1 could be important in the acivicin-induced HL-60 cell differentiation. It is important to note, however, that IL-1 and/or TNF cannot reproduce the full differentiating effects of acivicin (they do not inhibit proliferation, whereas acivicin does).

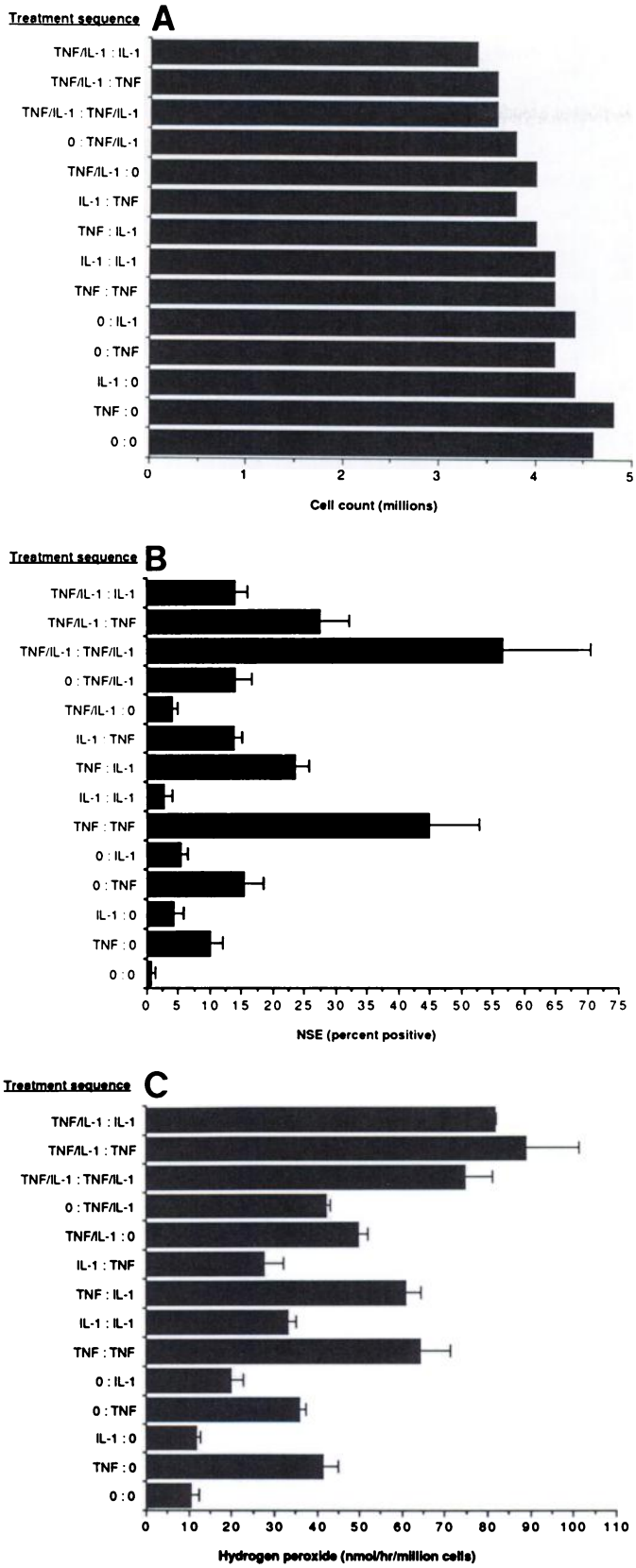

Fig. 6. Importance of the sequence of treatment with exogenous TNF-α and/or IL-1α on the induction of HL-60 cell differentiation. Cells were treated for 3 days with or without 100 units/ml TNF-α ± IL-1α, washed, cultured for another 3 days with or without 100 units/ml TNF-α ± IL-1α, and then analyzed for cell count (*A*), nonspecific esterase positivity (*NSE*) (*B*), and PMA-induced hydrogen peroxide production (*C*). The treatment sequence is designated as "additives first 3 days:additives second 3 days" (for example "TNF/IL-1:TNF/IL-1" means that TNF and IL-1 were present in the first 3 days and in the second 3 days).

The precise mechanisms by which glutamine antagonists cause differentiation of HL-60 cells is unknown. Acivicin inhibits the actions of several glutamine-dependent enzymes resulting in decreased levels of GTP and CTP in the treated cells (47, 48). We have shown before that exogenously added cytosine or guanosine partially blocks the acivicin effects (5). Other investigators have shown that tiazofurin (an inhibitor of inosine monophosphate dehydrogenase which also causes lowered levels of GTP in treated cells) causes HL-60 cell and freshly isolated ANLL cell differentiation *in vitro* (49, 50). There is also evidence that tiazofurin may induce differentiation of the ANLL cells in vivo and be an effective treatment (51, 52). Kharbanda *et al.* (53) noted that tiazofurin induced a new species of c-*myb* mRNA in HL-60 cells. Although we noted quantitative changes in c-*myb* mRNA in acivicin-treated HL-60 cells, we did not see the alternate sized mRNA. Olah *et al.* (54) have postulated that tiazofurin-induced alterations in GTP levels in erythroleukemia cells might change cellular differentiation through pathways involving *ras* genes. Our studies suggest that IL-1β and TNF-α expressed by the acivicin-treated cells could possibly be effectors in causing the differentiation.

Acivicin (and the glutamine antagonists, in general) represents a drug capable of inducing the favorable differentiation of ANLL cell line cells as well as freshly isolated ANLL cells (5). A thorough knowledge of the mechanism(s) by which the drug induces this differentiation may help us understand basic mechanisms of cellular growth and differentiation and should provide valuable information regarding the design of rationale treatment for ANLL in humans. Our work demonstrates that the monocytic differentiation of HL-60 cells induced by treatment with acivicin is accompanied by changes in mRNA expression of several genes. Others have noted that freshly isolated human leukemia cells variably express mRNA for different protooncogenes and cytokines including IL-6, IL-1, and TNF (55, 56). Several agents that cause differentiation of HL-60 cells to monocytoid cells or that modify their function induce the expression of TNF-α [PMA (57), TNF-α, TNF-β, γ-interferon (58), 1,25-dihydroxyvitamin D_3 (59), bryostatin (60), endotoxin or mellitin (61), or leukotriene B_4 (62)] or IL-1 [PMA or endotoxin (63), or 1,25 dihydroxyvitamin D_3 (64)]. Our work suggests that the acivicin-induced changes in IL-1β and TNF-α expression could play causal roles in the induction of the differentiation processes. Although not examined in our experiments, leukemia cell-elaborated TNF and/or IL-1 could also influence leukemia cell growth and differentiation indirectly by acting on stromal cells (*e.g.*, endothelial cells or fibroblasts) to cause them to secrete other bioactive factors (65, 66).

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Relationship of Acivicin-induced Monocytoid Differentiation of Human Myeloid Leukemia Cells to Acivicin-induced Modulation of Growth Factor, Cytokine, and Protooncogene mRNA Expression

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Cancer Res 1991;51:1202-1209.