Activation of 2',5'-Oligoadenylate Synthetase Activity on Induction of HL-60 Leukemia Cell Differentiation

EDWARD L. SCHWARTZ* AND LAURA A. NILSON†

*Department of Oncology, Albert Einstein College of Medicine and Montefiore Medical Center,
111 East 210th Street, Bronx, New York 10467*

Received 23 March 1989/Accepted 14 June 1989

A 27-fold increase in 2',5'-oligoadenylate synthetase activity, an enzyme associated with the antiproliferative actions of interferon (IFN), was observed after treatment of HL-60 human leukemia cells with dimethyl sulfoxide (DMSO), an inducer of granulocytic differentiation of the cells. Enzyme activity was elevated after 24 h of exposure to DMSO, was maximal at 48 hours, and declined thereafter. A comparable increase was observed after treatment with 1 U of alpha interferon (IFN- α) per ml or 8 U of beta interferon (IFN- β) per ml. Elevated levels of expression of other IFN-inducible genes, including type I histocompatibility antigen (HLA-B) mRNA and 2',5'-oligoadenylate phosphodiesterase activity, were also observed with DMSO treatment. DMSO-treated HL-60 cells had an increased amount of a 1.8-kilobase mRNA for oligoadenylate [oligo(A)] synthetase when compared with that of control cells; both DMSO- and IFN-treated HL-60 cells also expressed 1.6-, 3.4-, and 4.3-kilobase mRNA. The increase in both oligo(A) synthetase activity and mRNA levels was inhibited by polyclonal antiserum to human IFN- α ; however, no IFN- α mRNA could be detected in the cells. Antiserum to IFN- β or gamma interferon (IFN- γ) had no effect on oligo(A) synthetase expression or activity nor was there any detectable IFN- β_1 or IFN- β_2 mRNA in the cells. The anti-IFN- α serum did not block the elevation of HLA-B mRNA in DMSO-treated cells. These observations suggest that the increased expression of oligo(A) synthetase in DMSO-treated cells may be mediated by the release of an IFN- α -like factor; however, the levels of any IFN- α mRNA produced in the cells were extremely low.

Interferons (IFNs) are modifiers of a variety of cellular functions and exert antiproliferative effects on cells in culture (2). Their potential role as naturally occurring negative growth factors is supported by evidence that IFNs are released in an autocrine manner upon mitogenic stimulation of human fibroblasts (20, 21, 40) and other cell types (4, 5). The induction of beta interferon subtype 2 (IFN- β_2) (interleukin-6) under these conditions has been suggested to represent a homeostatic mechanism to control cell proliferation (20, 21, 40). The autocrine production of IFN- β -related interferons was also observed after the induction of differentiation of leukemic and normal hematopoietic cells, where upon they participate in the production of cell cycle arrest associated with differentiation (9, 14, 18, 26, 28, 34, 39). In these and other studies, a close link between either exogenous or autocrine IFN and the reduction in expression of the oncogene *c-myc* has also been observed (7, 16, 28).

The cellular actions of the IFNs are mediated by the activation of a distinct group of genes (17, 30). The increase in synthesis of some of these genes is due to increased rates of transcription, in some instances beginning within 10 min of IFN exposure and in other cases persisting for several days (17). The proteins induced upon IFN exposure include the following: enzymes, such as multiple forms of 2',5'-oligoadenylate (2-5A) synthetase, 2-5A phosphodiesterase, RNase L, and eucaryotic initiation factor P1/eIF-2 kinase; proteins of known functions, such as class I and II histocompatibility antigens; and a number of proteins of unknown functions (for recent reviews, see references 22 and 35). Of particular interest is the 2-5A synthetase activity, which polymerizes ATP into a series of 2',5'-linked oligomers with

the structure pppA(2'p5'A)_n (22, 35). The 2-5A oligomers activate an endogenous ribonuclease (RNase L), leading to the degradation of both viral and cellular RNAs and possibly participating in the antiviral and antiproliferative actions of IFN (22).

Loss of self-renewal capacity is one of the changes in cell growth regulation that accompanies the maturation of HL-60 leukemia cells (8, 37, 38). Induction of granulocytic differentiation of the cells simultaneously activates both negative (e.g., increased serum dependence for proliferation) and positive (e.g., enhanced mitogenic responsiveness to growth factors) proliferation controls (31). A rapid increase in the number of cell surface growth factor receptors likely accounts for the latter observation (10, 31); the mechanisms for the rapidly diminished autonomous growth characteristics and eventual terminal division of the cells upon dimethyl sulfoxide (DMSO) treatment have not been determined. In this communication, we describe the activation of members of the IFN-responsive family of genes after DMSO treatment of HL-60 cells.

MATERIALS AND METHODS

Cell culture. HL-60 human promyelocytic leukemia cells (3) were maintained in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum (FBS) (GIBCO Laboratories, Grand Island, N.Y.). Cells were passaged weekly and screened for mycoplasma contamination; only cells between passage 25 and 50 were used. For growth in serum-free defined medium, cells were washed twice and resuspended in RPMI 1640 medium with insulin and transferrin (at 5 μ g/ml for both) (Sigma Chemical Co., St. Louis, Mo.). Sheep antiserum against human alpha interferon (IFN- α) (G-026-502-568) or human IFN- β (G-028-501-568), rabbit antiserum against human gamma interferon (IFN- γ) (G-034-501-565), and the corresponding control antisera were obtained from

* Corresponding author.

† Present address: Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510.

the Research Resource Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Granulocytic maturation was assessed by the Nitro Blue Tetrazolium assay, as previously described (3). Normal human lymphocytes were incubated (72 h) with 0.1% PHA-P (Difco Laboratories, Detroit, Mich.).

Oligo(A) synthetase activity assay. Approximately 10^7 HL-60 cells were lysed in buffer containing 0.5% Nonidet P-40 and were centrifuged at $2,500 \times g$ for 10 min. Oligoadenylate [oligo(A)] synthetase activity was purified from supernatants of the cell extracts by affinity chromatography on agarose-poly(I)-poly(C) (Pharmacia, Inc., Piscataway, N.J.), as has been previously described (29). Enzyme assays were for 16 h at 30°C in 10 μ l and contained 10 μ Ci of [α - 32 P]ATP (Dupont, NEN Research Products, Boston, Mass.) at a final concentration of 3.5 mM, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 7.5), 120 mM KCl, 5 mM $\text{Mg}(\text{CH}_2\text{COOH})_2$, 7 mM β -mercaptoethanol, and 10% glycerol (29). Samples were treated with 1 U of alkaline phosphatase and analyzed by high-pressure liquid chromatography (HPLC) using an ODS-18 column (Whatman, Inc., Clifton, N.J.) and a 20-min linear gradient of 4 mM KH_2PO_4 (pH 6.8) to 20% methanol at 1.5 ml/min (1). Fractions were collected every 0.5 min, and radioactivity was determined by liquid scintillation counting. Under these conditions, authentic oligo(A) dimer, trimer, and tetramer cores (Pharmacia) had retention times of 10.2, 8.5, and 7.3 min, respectively.

2-5A phosphodiesterase assay. Cell extracts were incubated with 2-5A dimer core (0.1 mM) for 90 min at 37°C in a volume of 25 μ l in the buffer described above (29). Reactions were stopped by boiling, and after a brief centrifugation, the supernatants were analyzed by HPLC, as described above. Enzyme activity was calculated from the conversion rate of 2-5A dimer core to adenosine and AMP, which was linear for at least 2.5 h and which did not occur in the absence of cell extract.

mRNA quantitation. RNA was purified by extraction in the presence of guanidine thiocyanate and fractionated on formaldehyde-containing agarose gels as has been described previously (6). Poly(A) RNA was isolated on oligo(dT) cellulose (6). RNA was transferred to nitrocellulose, and blots were probed with nick-translated DNA: human HLA-B7 antigen cDNA, provided by S. Weissman (32); cDNA for human IFN- α_1 and IFN- β_1 (24), a kind gift from P. Sehgal and L. May; and human actin. Human IFN- β_2 cDNA (40) and human 2-5A synthetase cDNA (25) (both kind gifts from M. Revel) were used to synthesize RNA probes, using T7 RNA polymerase and [α - 32 P]ATP (6). Blots were washed with $0.1 \times \text{SSC}$ ($1 \times \text{SSC}$ is 0.15 M NaCl plus 0.015 M sodium citrate) at 55°C; blots probed with IFN- β_2 and oligo(A) synthetase RNA probes were further incubated with RNase (1 μ g/ml for 5 min at 25°C in $2 \times \text{SSC}$). A 20-mer oligonucleotide (5'-ACAAATGGGAAGAATCTCTC-3'), representing a consensus sequence for the IFN- α gene family (nucleotides 1057 to 1076) (12), was obtained from Synthecell (Rockville, Md.) and was end labeled by the forward kinase reaction; blots probed with this oligomer were washed at 40°C. Blots were exposed to XAR film (Eastman Kodak Co., Rochester, N.Y.) with intensifying screens at -80°C. Autoradiograph films were scanned with a Hoefer densitometer equipped with an HP recording integrator.

RESULTS

Stimulation of 2-5A synthetase activity by DMSO. 2-5(A) synthetase activity was measured in extracts of HL-60 cells

FIG. 1. Oligo(A) synthetase activity in control and DMSO-treated cells. Cell extracts were prepared from 10^7 control (○) or 1.25% DMSO-treated (●) cells at the indicated times. Oligo(A) synthetase activity was purified by affinity chromatography and assayed as described in Materials and Methods. Values are means $\pm$ S.E.M. for three experiments.

treated with DMSO for intervals up to 7 days (Fig. 1). Activity was calculated on the basis of the formation of radiolabeled 2-5A dimers, trimers, and tetramers, as separated by HPLC. Enzyme activity increased beginning after 16 h of DMSO exposure, was maximal at 48 h, and declined thereafter. The activity in DMSO-treated cells was 27-fold higher at 48 h than that in control cells. Granulocytic differentiation of the cells was first observed after 3 to 4 days of DMSO treatment (results not shown). Although oligo(A) synthetase activity progressively increased in control cells as they reached confluency, their values were less than half those of DMSO-treated cells at all times studied (Fig. 1). A comparable increase in activity to that induced by DMSO was observed after the treatment of cells for 48 h with 1 U of recombinant IFN- α per ml or approximately 8 U of recombinant IFN- β per ml (Fig. 2).

Oligo(A) synthetase activity was examined in cells cultured in defined medium, in medium with FBS, or in serum in the presence of the hematopoietic growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF). Proliferation of cells in defined medium over 72 h was approximately half of that of cells in FBS, while the addition of GM-CSF to FBS-containing medium doubled the rate of cell division over 72 h. The relative stimulation of oligo(A) synthetase activity by DMSO was greater in cells maintained in defined medium when compared with that of cells in FBS-containing medium, as a result of the significantly reduced basal level of activity observed in the control cells (Table 1). The addition of human GM-CSF produced a modest reduction in the stimulation of activity by DMSO when compared with that of cells grown with FBS alone. These culture conditions did not affect the extent of granulocytic differentiation observed as judged by staining with Nitro Blue Tetrazolium (Table 1).

RNA was extracted from control, DMSO-, and IFN-treated cells for the analysis of oligo(A) synthetase mRNA expression (Fig. 3). DMSO- and IFN-treated cells had an increased accumulation of a 1.8-kilobase (kb) mRNA when compared with that of control cells. Smaller amounts of 1.6-, 3.4-, and 4.3-kb mRNA were also detected in both DMSO- and IFN-treated cells (Fig. 3).

Effect of DMSO on phosphodiesterase activity and HLA-B mRNA. 2-5A phosphodiesterase activity in cell extracts was

FIG. 2. 2-5A synthetase activity in IFN-treated cells. HL-60 cells were treated for 48 h with the indicated concentrations of recombinant IFN- α_{2a} (●) or IFN- β_1 (○). In some instances, antiserum to IFN- α (▲) or IFN- β (△) (both at 100 neutralizing units per ml) was also added. Activity was measured as described in Materials and Methods.

assayed by measuring the degradation of 2-5A into adenosine and AMP. Activity in control cells increased with time in culture up to 7 days, and there was a 60 to 100% increase in extracts from DMSO-treated cells on days 2 and 4 when compared with control cells (Table 2). Activity in cells treated with DMSO for 7 days was lower than that of the corresponding control cells.

The expression of mRNA for type I histocompatibility antigen was examined in DMSO-treated cells. HL-60 cells expressed a 1.7-kb mRNA for HLA-B antigen (Fig. 4). mRNA levels increased progressively over a 7-day period in both control and DMSO-treated cells but were four- to fivefold higher than DMSO treatment at all time points (Fig. 4 and 5). The majority of the increase in cellular mRNA levels occurred between 48 and 96 h of DMSO treatment.

Stimulation of oligo(A) synthetase activity by DMSO was blocked by anti-IFN serum. The observed stimulation of oligo(A) synthetase activity of cells in defined medium

TABLE 1. Effect of DMSO on 2-5A synthetase activity and cell differentiation^a

Culture condition	2-5A synthetase activity			% Differentiation (mean $\pm$ SEM)	
	Mean $\pm$ SEM		Fold stimulation	Control	DMSO
	Control	DMSO			
Defined medium	24 $\pm$ 9	323 $\pm$ 139	13	7 $\pm$ 3	83 $\pm$ 5
FBS (10%)	91 $\pm$ 21	450 $\pm$ 70	5.0	4 $\pm$ 1	85 $\pm$ 6
FBS + GM-CSF	73 $\pm$ 6	287 $\pm$ 94	3.9	4 $\pm$ 1	70 $\pm$ 9

^a HL-60 cells were suspended in RPMI 1640 medium containing 5 μ g of insulin per ml and 5 μ g of transferrin per ml (defined medium), 10% heat-inactivated FBS, or 10% FBS plus 5 nM human recombinant GM-CSF. Granulocytic differentiation was induced by the addition of 1.25% (vol/vol) DMSO. Cell extracts were prepared on day 3, and 2-5A synthetase activity was measured as described in Materials and Methods and is expressed as picomoles of 2-5A formed per milligram of protein; the values shown are from three to six experiments. The extent of differentiation was determined on day 7 by determining the percentage of positive cells after staining with Nitro Blue Tetrazolium; values shown are from four experiments.

FIG. 3. Oligo(A) synthetase mRNA in HL-60 cells. RNA was extracted from HL-60 cells treated for 48 h as follows: control (C), DMSO (D), or IFN- β at 30 U/ml [I(30)] or 100 U/ml [I(100)]. RNA (8 μ g) was fractionated on an agarose-formaldehyde gel, transferred to nitrocellulose, and probed with a ³²P-labeled RNA probe prepared from a human oligo(A) synthetase cDNA. Autoradiography was for 3 days.

indicates that the action of DMSO does not require an exogenous source of IFN or known stimulants of IFN synthesis. IFN- β_2 (also known as interleukin-6) has been reported to be released in an autocrine manner in response to the treatment of human fibroblasts with growth factors (20, 21, 40). To test for the production of endogenous IFNs, oligo(A) synthetase activity was measured after DMSO treatment in the presence of anti-human IFN sera. Polyclonal antisera to human IFN- α but not to IFN- β or IFN- γ ,

TABLE 2. Effect of DMSO on 2-5A phosphodiesterase activity^a

Day	Activity (pmol/mg per h)	
	Control	DMSO
2	1.3 $\pm$ 0.3	2.6 $\pm$ 0.3 ^b
4	3.5 $\pm$ 0.4	5.6 $\pm$ 1.6
7	6.4 $\pm$ 0.7	1.9 $\pm$ 0.1 ^b

^a Extracts from control and DMSO-treated cells were prepared as described in Materials and Methods. Assays contained cell extract (approximately 75 μ g of protein) and 2-5A dimer core (0.1 mM) and were incubated for intervals up to 120 min at 37°C. After being briefly boiled, samples were analyzed by HPLC for loss of 2-5A and formation of adenosine and AMP; activity is expressed as picomoles of adenosine per milligram of protein per hour.

^b Significantly different from control ($P < 0.05$).

FIG. 4. Effect of DMSO treatment on HLA-B mRNA. RNA was extracted from control (odd-numbered lanes) and DMSO-treated (even-numbered lanes) HL-60 cells on the days indicated at the bottom of the gel. RNA (4 μ g) was analyzed as described in the legend to Fig. 2 and probed with a nick-translated HLA-B cDNA.

significantly reduced oligo(A) synthetase activity (Table 3) in DMSO-treated cells. Enzyme activity was not completely reduced to control levels, however. The concentrations of antisera used were sufficient to completely block the effects of exogenous IFN- α and IFN- β on 2-5A synthetase activity (Fig. 2).

The effect of the anti-IFN antisera on 2-5A synthetase mRNA expression was also examined (Fig. 6). In agreement with their effects on 2-5A synthetase activity, anti-IFN- α serum blocked synthetase mRNA expression, while anti-IFN- γ serum and anti-IFN- β serum (including a fivefold-higher concentration) did not. In contrast, none of the antisera blocked the increase in HLA-B mRNA expression observed in DMSO-treated cells (Fig. 6).

IFN mRNA was not detected in DMSO-treated HL-60 cells. RNAs from control HL-60 and cells treated with DMSO for 0, 16, 24, and 48 h were fractionated and probed with a riboprobe transcribed from a human IFN- β_2 cDNA. No hybridizing RNA was detected in either control or DMSO-

FIG. 5. Quantitation of HLA-B mRNA. The autoradiograph illustrated in Fig. 4 was scanned with a densitometer, and the intensity of the 1.7-kb mRNA band from control (○) and DMSO-treated (●) cells is expressed relative to the control at day 1, which was given a value of 1.

TABLE 3. Effect of anti-IFN antisera on 2-5A synthetase activity of DMSO-treated HL-60 cells^a

Antiserum added	n	2-5A synthetase activity (pmol/mg)
None	10	450 $\pm$ 75
Anti-IFN- α	5	173 $\pm$ 50 ^b
Anti-IFN- β	4	454 $\pm$ 83
Anti-IFN- γ	4	440 $\pm$ 96
Control ^c	5	407 $\pm$ 85

^a HL-60 cells were treated with 1.25% DMSO for 48 h in the presence or absence of sheep (IFN- α and IFN- γ) or rabbit (IFN- β) antisera to human IFNs (120 U/ml) or normal sheep serum. Oligo(A) synthetase activity was measured in cell extracts, as described in the legend to Fig. 1, and is expressed as picomoles of 2-5A per milligram of protein. Activity in control cells (no DMSO treatment) was 50 pmol/mg.

^b Significantly different from no antiserum ($P < 0.05$).

^c Normal sheep serum used at 1:500.

treated cells (Fig. 7). The low levels of constitutive expression of the 1.3-kb IFN- β_2 mRNA in human lymphocytes, as well as the stimulation of expression occurring upon phytohemagglutinin treatment, were detectable (13). Similarly, cells treated for shorter periods of time (i.e., hourly intervals for up to 6 h) had no detectable mRNA for IFN- β_2 (results not shown).

There were also no measurable mRNA hybridizing to either IFN- β_1 or IFN- α in HL-60 cells treated for various times with DMSO (data not shown). In the latter experiments, up to 8 μ g of poly(A)⁺ RNA was used and both a cDNA for human IFN- α_1 and a synthetic 20-mer oligonucleotide were used as probes. The oligomer represents a consensus sequence for the IFN- α gene family, with a minimum of 75 (class II genes) or 85% (class I genes) identity to at least 20 of the individual IFN- α gene family members (12). Probes were labeled to a specific activity of 2×10^8

FIG. 6. Effect of anti-IFN antisera on oligo(A) synthetase and HLA mRNA expression. RNA was extracted from cells treated for 48 h as follows: untreated control (lane 1); DMSO (1.25%) with no antiserum (lane 2), with anti-IFN- α serum (100 U/ml) (lane 3), with anti-IFN- β serum (100 U/ml [lane 4] and 500 U/ml [lane 5]), with anti-IFN- γ serum (100 U/ml) (lane 6), and with normal sheep serum (lane 7). RNA (8 μ g) was fractionated and transferred as described in Materials and Methods. The blot was probed consecutively with nick-translated cDNA for 2-5A synthetase, HLA-B, and actin.

FIG. 7. Lack of IFN- β_2 mRNA in HL-60 cells. RNA was extracted from HL-60 cells treated with 1.25% DMSO for 0 (lane 1), 16 (lane 2), 24 (lane 3), or 48 h (lane 4) and fractionated as described in Materials and Methods. Blots were hybridized with 32 P-labeled human IFN- β_2 cDNA RNA probe and washed and treated with RNase as described in Materials and Methods. As a positive control (36), RNAs from normal (lane 5) and phytohemagglutinin-stimulated (lane 6) human lymphocytes were used.

cpm/ μ g, and the blots were exposed for 7 days; all blots were reprobed with an actin cDNA to confirm the presence of intact mRNA.

DISCUSSION

The HL-60 human leukemia-derived promyelocytic cell line has been used to study the process of terminal cell differentiation (3). The granulocytic maturation these cells undergo in response to chemical agents, such as DMSO, has been well characterized (3). In addition to the induction of a series of lineage-specific phenotypic properties, maturation is associated with the loss of self-renewal capacity in these cells (8, 37, 38). The loss of self-renewal capacity has been reported to be a distinct cellular event that occurs before the commitment of the cells to differentiation (8, 38). In this study, we have observed the stimulation of a family of genes which are associated with IFN treatment, including 2-5A synthetase activity, 2-5A phosphodiesterase activity, and HLA-B antigen expression.

In studies with other cell lines (20, 21, 26, 28, 40), the activation of oligo(A) synthetase has been shown to result from the autogenous production of IFN- β (typically IFN- β_2). There was no detectable mRNA for either IFN- α , IFN- β_1 , or IFN- β_2 in DMSO-treated HL-60 cells. In support of the presence of an autocrine factor, however, was the finding that the increase in 2-5A synthetase activity and synthetase mRNA could be blocked by a polyclonal antiserum to human IFN- α . One explanation to reconcile these findings is the possibility that the antiserum contains activity against other unidentified cytokines, even though it was raised against a highly purified source of natural IFN- α and effectively neutralizes IFN- α (Fig. 2). One of these cytokines could be responsible for the increase in 2-5A synthetase observed. An alternative possibility is that autocrine IFN- α does mediate the effects observed but that an increase in IFN- α mRNA is not necessary for the release of the protein into the medium. Finally, it is possible that the levels of

IFN- α mRNA remain below the level of detection, despite activation by DMSO.

In support of the latter possibility is the observation that the expression of IFN- α mRNA in unstimulated human leukocytes is exceedingly low, substantially less than 0.03 copies per cell (36). Treatment of mouse cells, previously transfected with an inducible human IFN- α gene, with Newcastle disease virus produced an increase in steady-state IFN- α mRNA to approximately 10 to 20 transcripts per cell (27); a similarly large stimulation was observed in human leukocytes infected with the same virus (36). Infection with this virus resulted in the production of up to 4,000 U of IFN- α per ml (23). On the basis of the observed increases in 2-5A synthetase activity, treatment of HL-60 cells with DMSO was estimated to be comparable with incubation with 1 U of IFN- α per ml (Fig. 1 and 3). Hence DMSO-treated cells likely have levels of IFN- α transcripts several orders of magnitude lower than those of the virus-treated cells. A 1-U/ml concentration of IFN- α is equivalent to roughly 150 molecules of IFN produced per cell (assuming a specific activity of 2×10^8 U/mg of a molecular mass of 19 kilodaltons for IFN, and a cell concentration of 10^6 /ml). Since each IFN transcript can produce several hundred IFN protein molecules (36), it is possible that a small, undetectable increase in IFN- α mRNA could produce sufficient IFN to result in the stimulation of 2-5A synthetase activity observed.

On the basis of the differences in the kinetics of expression and on the lack of effect of the anti-IFN- α sera, it is likely that the mechanism for the stimulation of HLA-B antigen mRNA differs from that of oligo(A) synthetase. Similarly, 2-5A phosphodiesterase activity was elevated in DMSO-treated cells on days 2 and 4, but not on day 7.

It has been suggested that 2-5A synthetase activity mediates some of the actions of IFN although it is not sufficient to mediate the antiproliferative actions of IFN (19, 22, 33). Although we have observed the modulation of synthetase activity and the effect of DMSO by factors affecting HL-60 proliferation (e.g., serum or GM-CSF), 2-5A synthetase activity was found not to be strictly correlated with cell proliferation. The degree of stimulation of activity in the HL-60 cells was comparable with that reported to occur during the differentiation of murine erythroleukemia cells (9) and U937 histiocytic lymphoma cells (28, 34). The kinetics of the stimulation of activity in HL-60 cells, however, differed from the erythroleukemia cells in having an earlier onset and differed from the U937 cells in having a shorter duration. The kinetics of activity are also distinct from that of the control cells, in which activity slowly increased as the cells approached confluency. This likely reflects changes in 2-5A synthetase activity occurring at different stages in the cell cycle or the transition from subconfluent active proliferation to confluent stationary phase (15, 18). Such an explanation cannot account for the increased activity in DMSO-treated HL-60 cells at 48 h, since the majority of these cells are still actively dividing; furthermore, the increase at 48 h was more than twice that of the cells at confluency.

Previous studies have shown that IFN- β alone did not affect HL-60 cell proliferation nor induce cell differentiation but did, at high concentrations, confer antiviral activity upon the cells (11). When added in combination with DMSO, however, IFN- β was found to be cytotoxic to HL-60 cells without affecting differentiation; the growth inhibition was observed only when IFN- β was added during the first two days of DMSO treatment (11). These findings, in conjunction with our observations of an overlap in genes induced by

DMSO and IFN, suggest that the induction of the IFN-responsive family of genes is not sufficient to induce differentiation or inhibit HL-60 proliferation but might potentiate other DMSO-induced alterations. Furthermore, if the activation of the IFN-inducible family of genes is necessary for some of the cellular effects of DMSO, it is likely related to the antiproliferative actions of DMSO, rather than to the granulocytic maturation induced.

ACKNOWLEDGMENTS

We thank M. Revel, P. Sehgal, L. May, and S. Weissman for the cDNA probes; G. Wong for the GM-CSF; and Helena Eninger for excellent technical assistance.

This research was supported in part by the Mathers Foundation and grant CH-298 from the American Cancer Society.

LITERATURE CITED

- Brown, R. E., P. J. Cayley, and I. M. Kerr. 1981. Analysis of (2'-5')-oligo(A) and related oligonucleotides by high performance liquid chromatography. *Methods Enzymol.* **79**:208-216.
- Clemens, M. J., and M. A. McNullan. 1985. Regulation of cell proliferation and differentiation by interferons. *Biochem. J.* **226**:345-360.
- Collins, S. J., F. W. Ruscetti, R. E. Gallagher, and R. C. Gallo. 1979. Normal functional characteristics of cultured human promyelocytic leukemia cells (HL-60) after induction of differentiation by dimethylsulfoxide. *J. Exp. Med.* **149**:969-974.
- Content, J., L. DeWit, P. Poupart, G. Opdenakker, J. Van Damme, and A. Billiau. 1985. Induction of a 26-kDa-protein mRNA in human cells treated with an interleukin-1-related, leukocyte-derived factor. *Eur. J. Biochem.* **153**:253-257.
- Creasey, A. A., D. A. Epstein, Y. V. Marsh, Z. Khan, and T. C. Merigan. 1983. Growth regulation of melanoma cells by interferon and (2'-5')oligo-adenylate synthetase. *Mol. Cell. Biol.* **3**:780-786.
- Davis, L. G., M. D. Dibner, and J. F. Battey. 1986. Basic methods in molecular biology. Elsevier, New York.
- Dron, M., N. Modjtahedi, O. Brison, and M. G. Tovey. 1986. Interferon modulation of c-myc expression in cloned Daudi cells: relationship to the phenotype of interferon resistance. *Mol. Cell. Biol.* **6**:1374-1378.
- Fibach, E., T. Peled, and E. A. Rachmilewitz. 1982. Self-renewal and commitment to differentiation of human promyelocytic leukemic cells (HL-60). *J. Cell. Physiol.* **113**:152-158.
- Friedman-Einat, M., M. Revel, and A. Kimchi. 1982. Initial characterization of the interferon secreted during growth and differentiation of Friend erythroleukemia cells. *Mol. Cell. Biol.* **2**:1472-1480.
- Gasson, J. C., S. E. Kaufman, R. H. Weisbart, M. Tomanaga, and D. W. Golde. 1986. High affinity binding of granulocyte-macrophage colony-stimulating factor to normal and leukemic human myeloid cells. *Proc. Natl. Acad. Sci. USA* **83**:669-673.
- Hamburger, A. W., C. P. White, R. E. Sienenlist, J. J. Sedmak, and S. E. Grossberg. 1985. Cytotoxicity of human β -interferon for differentiating leukemic HL-60 cells. *Cancer Res.* **45**:5369-5373.
- Henco, K., J. Brosius, A. Fujisawa, J.-I. Fujisawa, J. R. Haynes, J. Hochstadt, T. Kovacic, M. Pasek, A. Schambeck, J. Schmid, K. Todokoro, M. Walchli, S. Nagata, and C. Weissman. 1985. Structural relationship of human interferon alpha genes and pseudogenes. *J. Mol. Biol.* **185**:227-260.
- Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, S. Tsunawasa, F. Sakiyama, H. Matsui, Y. Takahara, T. Taniguchi, and T. Kishimoto. 1986. Complementary DNA for a novel interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature (London)* **324**:73-76.
- Hovvanesian, A. G., and I. M. Kerr. 1978. Synthesis of oligonucleotide inhibitor of protein synthesis in rabbit reticulocyte lysates analogous to that formed in extracts from interferon-treated cells. *Eur. J. Biochem.* **84**:149-159.
- Jacobsen, H., D. Krause, R. M. Freidman, and R. H. Silverman. 1983. Induction of ppp(A₂'p)_nA-dependent RNase in murine JLS-V9R cells during growth inhibition. *Proc. Natl. Acad. Sci. USA* **80**:4954-4958.
- Jonak, G. J., and E. Knight. 1984. Selective reduction of c-myc mRNA in Daudi cells by human β interferon. *Proc. Natl. Acad. Sci. USA* **81**:1747-1750.
- Kelly, J. M., C. S. Gilbert, G. R. Stark, and I. M. Kerr. 1985. Differential regulation of interferon-induced mRNAs and c-myc mRNA by alpha- and gamma-interferons. *Eur. J. Biochem.* **153**:367-371.
- Kimchi, A. 1981. Increased levels of interferon-induced (2'-5')oligo-isoadenylate synthetase in mature T lymphocytes and in differentiated Friend erythroleukemia cells. *J. Interferon Res.* **1**:559-569.
- Kimchi, A., H. Shure, and M. Revel. 1981. Anti-mitogenic function of interferon-induced (2'-5')oligo(adenylate) and growth-related variations in enzymes that synthesize and degrade this oligonucleotide. *Eur. J. Biochem.* **114**:5-10.
- Kohase, M., D. Henriksen-DeStefano, L. T. May, J. Vilcek, and P. B. Sehgal. 1986. Induction of β 2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. *Cell* **45**:659-666.
- Kohase, M., L. T. May, I. Tamm, J. Vilcek, and P. B. Sehgal. 1987. A cytokine network in human diploid fibroblasts: interactions of β -interferons, tumor necrosis factor, platelet-derived growth factor, and interleukin-1. *Mol. Cell. Biol.* **7**:273-280.
- Lengyel, P. 1981. Enzymology of interferon action—a short survey. *Methods Enzymol.* **79**:135-148.
- Mantei, N., and C. Weissman. 1982. Controlled transcription of a human alpha-interferon gene introduced into mouse L cells. *Nature (London)* **297**:128-132.
- May, L. T., P. B. Sehgal, K. S. LaForge, and M. Inouye. 1983. Expression of native alpha and beta interferon genes in human cells. *Virology* **129**:116-126.
- Merlin, G., J. Chebath, P. Benech, R. Metz, and M. Revel. 1983. Molecular cloning and sequence of partial cDNA for interferon-induced (2'-5')oligo(A) synthetase mRNA from human cells. *Proc. Natl. Acad. Sci. USA* **80**:4904-4908.
- Moore, R. N., H. S. Larsen, D. W. Horohov, and B. T. Rouse. 1984. Endogenous regulation of macrophage proliferative expansion by colony-stimulating factor-induced interferon. *Science* **223**:178-181.
- Ragg, H., and C. Weissman. 1983. Not more than 117 base pairs of 5'-flanking sequence are required for inducible expression of a human IFN-alpha gene. *Nature (London)* **303**:439-442.
- Resnitzky, D., A. Yarden, D. Zipori, and A. Kimchi. 1986. Autocrine β -related interferon controls c-myc suppression and growth arrest during hematopoietic differentiation. *Cell* **46**:31-40.
- Revel, M., D. Wallach, G. Merlin, A. Schattner, A. Schmidt, D. Wolf, L. Shulman, and A. Kimchi. 1981. Interferon-induced enzymes: microassays and their applications; purification and assay of (2'-5')-oligoadenylate synthetase and assay of 2'-5'-phosphodiesterase. *Methods Enzymol.* **79**:149-161.
- Samanta, H., D. A. Engel, H. M. Chao, A. Thakur, M. A. Garcia-Blanco, and P. Lengyel. 1986. Interferons as gene activators. *J. Biol. Chem.* **261**:11849-11858.
- Schwartz, E. L., and A. M. Maher. 1988. Enhanced mitogenic responsiveness to granulocytic-macrophage colony-stimulating factor in HL-60 promyelocytic leukemia cells upon induction of differentiation. *Cancer Res.* **48**:2683-2687.
- Sood, A. W., D. Pereira, and S. M. Weissman. 1981. Isolation and partial nucleotide sequence of a cDNA clone for human histocompatibility antigen HLA-B by use of an oligodeoxynucleotide primer. *Proc. Natl. Acad. Sci. USA* **78**:616-620.
- Tamm, I., B. R. Jasny, and L. M. Pfeffer. 1987. Antiproliferative actions of interferon, p. 25-58. *In* L. M. Pfeffer (ed.), *Mechanisms of interferon actions*, vol. II. CRC Press Inc., Boca Raton, Fla.
- Testa, U., M. Gabbianelli, B. Pasucci, G. Boccoli, F. Louache, and M. N. Thang. 1988. Effect of endogenous and exogenous interferons on the differentiation of human monocyte cell line

- U937. *Cancer Res.* **48**:82–88.
35. **Torrence, P. F. (ed.)**. 1985. How interferon works, p. 77–105. *In* *Biological response modifiers: new approaches to disease intervention*. Academic Press, Inc., New York.
36. **Tovey, M. G., M. Streuli, I. Gresser, J. Gugenheim, B. Blanchard, J. Guymarho, F. Vignaux, and M. Gigou**. 1987. Interferon messenger RNA is produced constitutively in the organs of normal individuals. *Proc. Natl. Acad. Sci. USA* **84**:5038–5047.
37. **Tsiftoglou, A. S., W. Wong, R. Hyman, M. Minden, and S. H. Robinson**. 1985. Analysis of commitment of human leukemia HL-60 cells to terminal granulocytic differentiation. *Cancer Res.* **45**:2334–2339.
38. **von Melchner, H., and K. Hoffken**. 1985. Commitment to differentiation of human promyelocytic leukemia cells (HL-60): an all-or-none event preceded by reversible losses of self-renewal. *J. Cell. Physiol.* **125**:573–581.
39. **Yarden, A., H. Shure-Gottlieb, J. Chebath, M. Revel, and A. Kimchi**. 1984. Autogenous production of interferon- β switches on HLA genes during differentiation of histiocytic lymphoma U937 cells. *EMBO J.* **3**:969–973.
40. **Zilberstein, A., R. Ruggieri, J. H. Korn, and M. Revel**. 1986. Structure and expression of cDNA and genes for human interferon- β 2, a distinct species inducible by growth-stimulatory cytokines. *EMBO J.* **5**:2529–2537.