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Interferon α Selectively Affects Expression of the Human Myeloid Cell Nuclear Differentiation Antigen in Late Stage Cells in the Monocytic but not the Granulocytic Lineage

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Abstract The human myeloid cell nuclear differentiation antigen (MNDA) is expressed constitutively in cells of the myeloid lineage, appearing in myeloblast cells in some cases of acute myeloid leukemia and consistently being detected in promyelocyte stage cells as well as in all later stage cells including peripheral blood monocytes and granulocytes. The human myeloid leukemia cell lines, HL-60, U937, and THP-1, express similar levels of immunochemically detectable MNDA. Although, the level of MNDA mRNA in primary monocytes is very low it was up-regulated at 6 h following the addition of interferon α. The effect of interferon α on the MNDA mRNA is also observed in the cell lines HL-60, U937, and THP-1. The MNDA mRNA level in primary granulocytes was unaffected by addition of interferon α and other agents including interferon γ, endotoxin, poly (I) · poly (C), and FMLP. The MNDA mRNA level in the myeloid cell lines was also unaffected by the latter four agents. Induction of differentiation in the myeloid cell lines with phorbol ester induces monocyte differentiation which was accompanied by a decrease in MNDA mRNA level. This reduced level of mRNA could then be elevated with subsequent interferon α treatment. The effects of phorbol ester on MNDA mRNA appeared to be associated with induced differentiation since inhibiting cell proliferation did not alter the level of MNDA mRNA and cell cycle variation in MNDA mRNA levels were not observed. The ability of interferon α to up-regulate MNDA mRNA in phorbol ester treated myeloid cell lines is consistent with the observations made in primary monocytes. Granulocyte differentiation induced by retinoic acid treatment of HL-60 cells did not alter the MNDA mRNA level which was also unchanged following subsequent treatment with interferon α. The lack of interferon α effects on retinoic acid treated HL-60 cells is consistent with its inability to influence MNDA mRNA level in primary granulocytes.

Key words: granulocytes, monocytes, human myeloid cell lines, retinoic acid, phorbol ester, mRNA

The human myeloid cell nuclear differentiation antigen (MNDA) was discovered in our laboratory using immunochemical screening [Goldberger et al., 1984]. Those experiments were designed to identify nuclear proteins expressed specifically in the human myeloid lineage being induced at a specific stage of myeloid cell differentiation. The MNDA was the only antigen detected which exhibited a strict stage and myeloid lineage specific pattern of expression [Goldberger et al., 1984, 1986]. Our extensive microsequence analysis of the MNDA [Burrus et al., 1992] and the subsequent characterization of an *MNDA* cDNA [Briggs et al., 1992] revealed nucleic acid and amino acid sequence similarity to a family (*Ifi-200*) of interferon-inducible mouse genes [Choubey et al., 1989]. Consistent with these observations, we also noted the presence of an interferon stimulated response element (ISRE) in the 5′ untranslated region of the *MNDA* cDNA [Briggs et al., 1992]. We have also demonstrated that the level of the 1.8 kb MNDA mRNA can be up-regulated specifically by interferon α in myeloid cell lines and primary myeloid cells that express the MNDA [Briggs et al., 1992, 1993]. In other of our reports we demonstrated that the constitutive level of MNDA expression was limited to cells in the human myeloid lineage as assessed at both the antigen and mRNA

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levels [Briggs et al., 1993; Burrus et al., 1992; Goldberger et al., 1986].

A second human gene, *IFI 16* [Trapani et al., 1992], is related to the *MNDA* but, is expressed constitutively in lymphocytes, and can be induced in myeloid cells by interferon γ. The MNDA and IFI 16P contain a 61% amino acid sequence identity over the NH_2-terminal 400 residues [Briggs et al., 1993]. The MNDA and the IFI 16P also contain domains of 200 amino acid residues shared by the interferon regulated *Ifi-200* gene family in the mouse [Briggs et al., 1992; Burrus et al., 1992; Trapani et al., 1992]. Three of the related human and mouse gene products have been localized to the nucleus [Choubey and Lengyel, 1992, 1993; Goldberger et al., 1984] and two products exhibit DNA binding activity [Choubey and Lengyel, 1993; Gaczynski et al., 1990]. The constitutive expression of the human genes, *MNDA* and *IFI 16*, is limited to blood cells which suggests an association with cell specific responses to interferons. The effectiveness of interferons in inducing or up-regulating MNDA mRNA appears to be cell type restricted, being limited to myeloid cells that constitutively express MNDA [Briggs et al., 1992, 1993].

The present experiments address the ability of interferon α to up-regulate MNDA mRNA level in primary myeloid cells (monocytes and granulocytes) and in myeloid leukemia cell lines before and after induced differentiation. Our results demonstrate that the effects of interferon α on MNDA mRNA levels in myeloid cells differ depending upon whether the cells are in the monocyte or granulocyte lineages. Primary granulocytes and the myeloid leukemia cell line HL-60 induced for granulocyte differentiation do not alter their MNDA mRNA level in response to interferon α. On the other hand, uninduced myeloid cell lines or lines induced to differentiate into monocytes, as well as, primary monocytes respond to interferon α by up-regulating the MNDA mRNA.

MATERIALS AND METHODS

Human Cell Lines

The HL-60 human promyelocytic leukemia cell line was obtained from the American Type Culture Collection (ATCC) (Rockville, MD) and grown in suspension culture in RPMI 1640 medium with 20% heat-inactivated fetal bovine serum. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air and were subcultured to 2×10^5 cells/ml when the cell density exceeded 1×10^6 cells/ml. The U937 histiocytic lymphoma cell line was also obtained from the ATCC and grown in a suspension in RPMI 1640 in 15% fetal bovine serum under the same conditions as described for the HL-60 cells. The U937 cells were subcultured to 1×10^5 cells/ml when the density exceeded 1×10^6 cells/ml. Both the HL-60 and U937 cells were subcultured every 3–4 days and the HL-60 cells used in these experiments were maintained for less than 50 passages. The human monocytic leukemia cell line, THP-1, was obtained from the ATCC and maintained under similar conditions as described above for HL-60 and U937 cells except that the fetal bovine serum level was reduced to 10% and the THP-1 cells were subcultured to 3.5×10^5 cells per ml before reaching 1×10^6 cells per ml. All culture supplies were purchased from GIBCO (Gaithersburg, MD) unless stated otherwise.

Granulocytes and Monocytes

One unit of blood was obtained from normal volunteers using procedures approved by the Vanderbilt University Medical Center Institutional Review Board. The heparin anticoagulated normal blood was separated over Ficoll-Paque® (Pharmacia LKB Biotechnology, Piscataway, NJ, density 1.077 g/ml) and the light density mononuclear cell fraction and the red blood cell/granulocyte pellet fraction were harvested. The red blood cell pellet was diluted 1:1 with Hank's balanced salt solution (HBSS) and then 6% dextran (volume equal to ¼ of the diluted red cell pellet) was added and mixed. The red cells were allowed to sediment for 60 min and the overlying solution which contained the granulocytes was harvested by aspiration. The granulocytes were collected by centrifugation (600*g*, 7 min) and contaminating red cells lysed with one volume of dH_2O (30 sec). The granulocytes were diluted with HBSS, collected by centrifugation (600*g*, 7 min), and resuspended in 200 ml of HBSS. The granulocytes were then added to four 150 mm tissue culture dishes (Sarstedt, Newton, NC). After 20 min, the nonadherent cells were removed by aspirating the overlying HBSS and 50 ml of fresh HBSS was added to each dish. Examination of these cultures revealed that they were consistently greater than 99% pure granulocytes based on cell morphology. At this point in the experi-

ments, various agents were introduced into the cultures.

The light density mononuclear cell fraction was suspended in Iscove's modified Dulbecco's medium (IMDM, Sigma Chemical Co., St. Louis, MO) with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT) and incubated 1 h at 37°C in a 5% CO_2 atmosphere in 225 cm^2 tissue culture flasks (Costar, Cambridge, MA, two flasks of suspended mononuclear cells obtained from *one unit* of blood). After incubation, the nonadherent cells were removed by aspirating the overlying solution and the flasks were rinsed with IMDM. The monocytes remained attached to the flasks and 50 ml of IMDM containing 10% fetal bovine serum was added to each flask. Examination of the cultures indicated that they are consistently greater than 95% monocytes based on cell morphology and esterase staining. These cultures were maintained at 37°C in 5% CO_2 atmosphere for the times indicated before adding test agents.

Inducers of Differentiation and Activators of Myeloid Cell Function

Monocyte/macrophage differentiation was induced with 1.6×10^{-8} M 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in HL-60, U937 and THP-1 cells 1 day after subculturing. TPA-treated cells were completely growth arrested within 24 h and cell morphology changes observed were consistent with macrophage differentiation. Cells of all three lines become adherent to the flasks within 1 h of adding TPA.

Granulocyte differentiation was induced in HL-60 cells with trans-retinoic acid (1×10^{-6} M, Sigma Chemical Co., St. Louis, MO) 1 day after subculturing. Cultures were monitored for effects on cell growth by performing daily cell counts. Cell growth ceased after 3 days of retinoic acid treatment. After 4 days of retinoic acid treatment, the cultures contained greater than 60% NBT-positive cells measured as previously described [Goldberger et al., 1984].

The leukemia cell lines (HL-60, U937, and THP-1) and the primary monocytes and granulocytes were treated under the conditions and for the times indicated with recombinant human interferon γ (Hoffmann-LaRoche, Inc., Nutley, NJ) and/or recombinant human interferon α A (Hoffmann-LaRoche, Inc.). The recombinant interferon α and γ were used at 2,000 units/ml unless indicated otherwise. The U937 cell line was treated with the chemotactic peptide, N-formyl-Met-Leu-Phe (FMLP) for the indicated times. Primary granulocytes and the U937 cells were exposed to endotoxin (5 μg/ml, Sigma Chemical Co., St. Louis, MO) and to double stranded RNA, poly (I) · poly (C) (30 μg/ml, Pharmacia Biotech Inc., Piscataway, NJ) for the indicated times.

Cell Cycle Synchronization

HL-60 human promyelocytic leukemia cells were synchronized by two rounds of 0.1 mM thymidine block as reported [Holthuis et al., 1990]. Cells in rapid growth phase were treated with thymidine for 16 h, harvested by centrifugation, and resuspended in fresh medium for 9 h. The cells were then treated with thymidine for an additional 9 h. S phase cells were harvested 4 h after release of the second thymidine block and G_1 cells were harvested 12 h following release. Proliferation inhibited cells were harvested prior to release of the second thymidine block.

Northern Blotting

Total RNA was isolated from the primary cells and the cell lines using a modification [Zie and Rothblum, 1991] of the acidic guanidinium thiocyanate method of Chomczynski and Sacchi [1987]. Adherent primary cells and the adherent TPA treated cell lines were lysed in the culture flasks after removing the medium. The lysed cells were poured from the flasks and processing continued as in the case with non-adherent suspension cultured cells [Zie and Rothblum, 1991]. The total RNA samples were separated on a 1% agarose, formaldehyde gel [Sambrook et al., 1989], and electrotransferred to activated nylon membrane as described previously [Briggs et al., 1992]. A 954-base asymmetric sense PCR product of the *MNDA* cDNA [Briggs et al., 1992] and a human β-*actin* cDNA probe (Clontech Laboratories, Inc., Palo Alto, CA) were labeled by random priming [Feinberg and Vogelstein, 1984] with incorporation of [α^{32}P] dATP (3,000 Ci/mmole, ICN Biomedicals, Inc., Irvine, CA). The probes were hybridized to the RNA blots at 43°C in 50% formamide, 0.25 M NaCl, 0.12 M Na_2HPO_4 (pH 7.4), and 7% SDS. The hybridized membranes were rinsed in 2× SSPE [Sambrook et al., 1989], 0.1% SDS for 15 min twice at room temperature, and then stringently washed at 65°C in 0.1× SSPE, 0.1% SDS for 20 min. The blots were exposed to Kodak XAR film at −70°C. The signal for the constitutively expressed β-ac-

tin mRNA or the ethidium bromide stained 28S and 18S ribosomal RNAs are provided as an indication of variations in sample loading.

A 30 mer antisense oligodeoxynucleotide, AAGCATGGGTAATTCAGCCAGGCCT-CAGCC complementary to the human 2′5′ Oligoadenylate synthetase mRNA [Shiojiri et al., 1986] was synthesized and purified by cartridge (Clontech). The 3′ OH end was labeled using [α^{32}P] dATP at a 2.5 molar ratio to oligo in the presence of terminal deoxynucleotidyl transferase. The probe was hybridized to the blot in 0.25 M NaCl, 0.12 M Na_2HPO_4 (pH 7.4) and 7% SDS at 43°C. The stringent wash of the bound oligo probe was at 47°C for 20 min in 2× SSPE, 0.1% SDS.

RESULTS

Our procedure for isolating monocytes involves their separation from total peripheral blood mononuclear cells by adherence. The adherence of primary monocytes is accompanied by changes in cell physiology that are consistent with macrophage differentiation [Hammerstrøm, 1979]. Under specific conditions the differentiation of monocytes to macrophages in vitro is complete after 10 days [D'Onofrio and Paradisi, 1983]. After either 6 h or 1 day of adherence the isolated monocytes significantly up-regulate the MNDA mRNA steady state level in response to interferon α (Fig. 1). The induction of MNDA mRNA to a higher level in monocytes maintained in culture for 1 day versus that observed in cells maintained for only 6 h is an indication that interferon α effects on MNDA mRNA steady state level is more pronounced once monocytes begin macrophage differentiation (Fig. 1). Our procedure for isolating primary granulocytes also involved the use of a final cell adherence step. Unfortunately, it was not possible to maintain the adherent granulocytes for 24 h due to low cell recovery which results from their short life expectancy. Therefore, our analysis of MNDA mRNA levels in granulocytes was limited to cells maintained for a maximum of 6 h in culture. The level of MNDA mRNA in granulocytes was not up-regulated with interferon α treatment or in response to endotoxin, poly (I) · poly (C) (Fig. 2), interferon β, or interferon γ (data not shown).

The human myeloid leukemia cell lines, HL-60, U937, and THP-1, express a constitutive level of MNDA mRNA and this level is up-regulated after 6 h of interferon α treatment [Briggs et al., 1992, 1993] (Fig. 3 and data not shown). The up-regulation of the MNDA mRNA level by interferon α in the myeloid cell lines is transient and after 18 h the steady state mRNA level has returned to normal (Fig. 4). On the

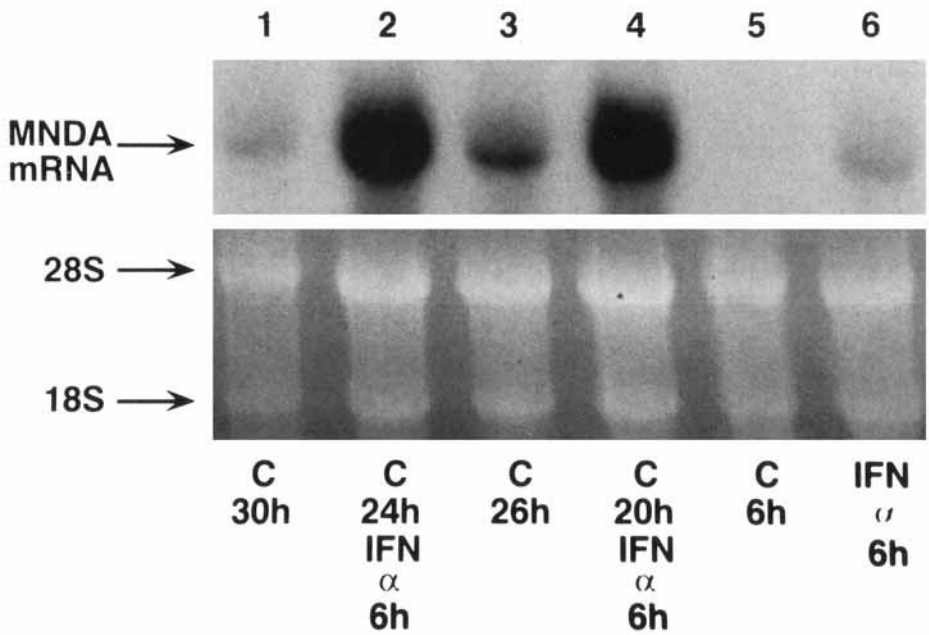

Fig. 1. Northern blot analysis of the 1.8 kb MNDA mRNA in total RNA isolated from adherent primary monocytes maintained in culture for 30 h (lane 1), 26 h (lane 3), and 6 h (lane 5). Recombinant interferon α was added for the final 6 h in cultures maintained for 30 h (lane 2), 26 h (lane 4), and 6 h (lane 6). Ethidium bromide staining of the samples showing the 28S and 18S ribosomal RNAs (lower panel).

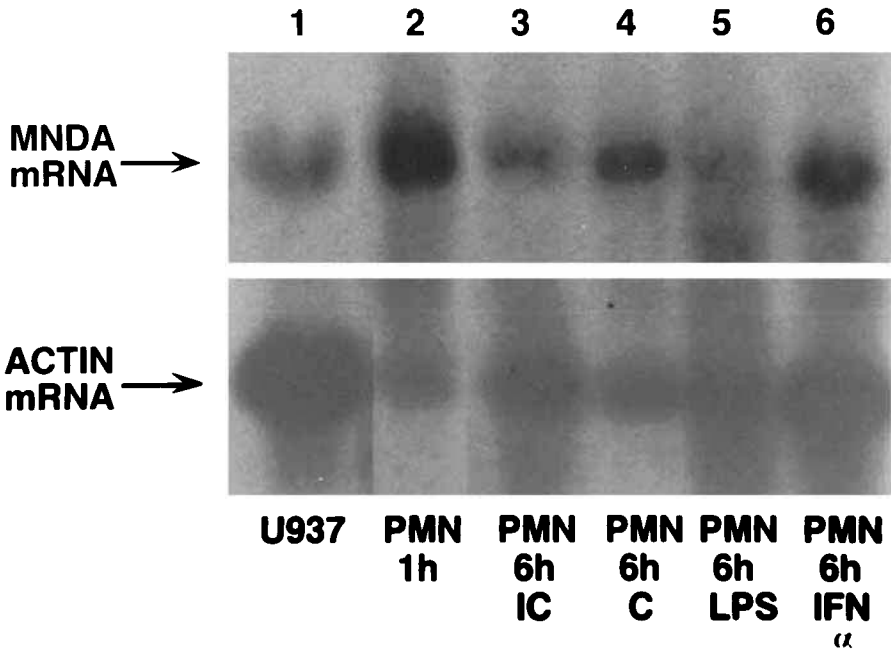

Fig. 2. Northern blot analysis of the MNDA mRNA in primary granulocytes. Control U937 cells (lane 1). Total cellular RNA was analyzed from untreated granulocytes maintained for 1 h (lane 2) or 6 h (lane 4) in culture. Granulocytes were also exposed for 6 h in culture to poly (I) · poly (C) (lane 3), endotoxin (LPS, lane 5), and recombinant interferon α (lane 6). β-actin mRNA signals (lower panel).

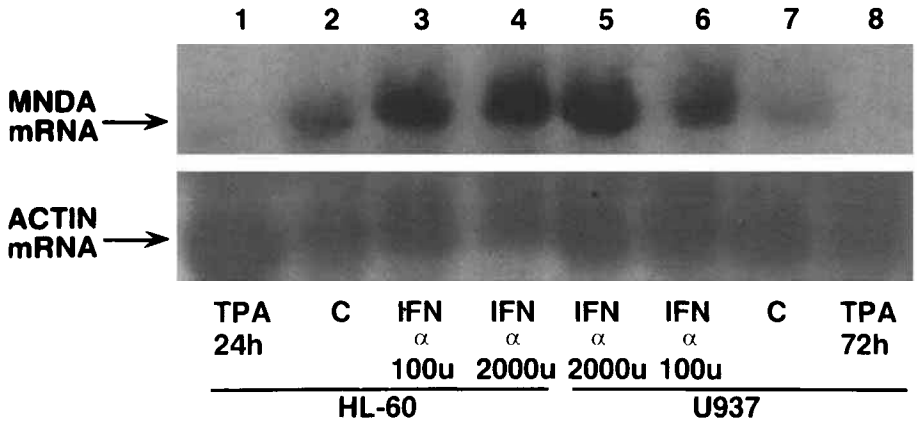

Fig. 3. Northern blot analysis of the MNDA mRNA in total cellular RNA isolated from HL-60 and U937 cells. RNA from untreated HL-60 cells (lane 2), HL-60 cells treated with TPA for 24 h (lane 1), and HL-60 cells treated with recombinant interferon α for 6 h at 100 units/ml (lane 3) or 2,000 units/ml (lane 4). Total RNA from untreated U937 cells (lane 7), U937 cells treated with TPA for 72 h (lane 8), and U937 cells treated with recombinant interferon α for 6 h at 100 units/ml (lane 6) or 2,000 units/ml (lane 5). β-actin mRNA signals (lower panel).

other hand, the phorbol ester, TPA, down-regulates the constitutive levels of MNDA mRNA in the human myeloid leukemia cell lines which persists for at least 3 days (Fig. 3). Subsequent addition of interferon α to TPA-treated HL-60 cells (Fig. 5) and U937 and THP-1 cells (data not shown) up-regulates the depressed level of MNDA mRNA. The ability of interferon α to increase the steady-state level of MNDA mRNA in the TPA-treated cells was, to a modest extent, augmented by pretreating the cells with interferon γ (Fig. 5). Pretreatment of U937 cells with interferon γ also augmented the subsequent up-regulation of MNDA mRNA level even when the cells were not induced to differentiate with TPA (data not shown). However, interferon γ treatment alone had no effect on MNDA mRNA levels in cells induced (Fig. 5) or not induced (data

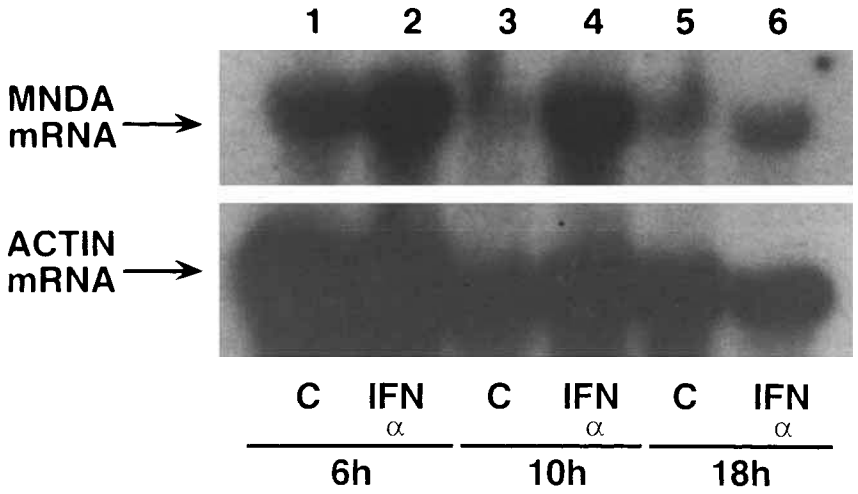

Fig. 4. Northern blot analysis of the MNDA mRNA in total cellular RNA from untreated U937 cells maintained for 6 h (lane 1), 10 h (lane 3), or 18 h (lane 5) and from U937 cells treated with recombinant interferon α for 6 h (lane 2), 10 h (lane 4), or 18 h (lane 6). β-actin mRNA signals (lower panel).

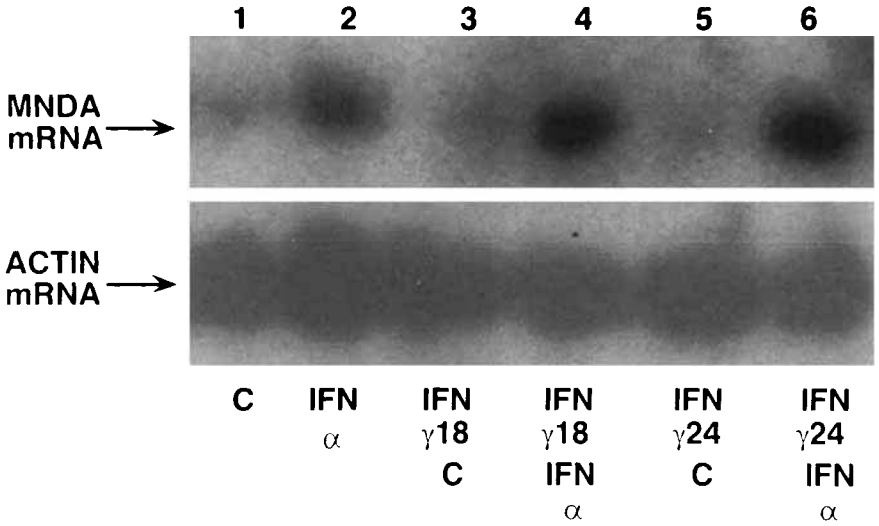

Fig. 5. Northern blot analysis of the MNDA mRNA level in HL-60 cells exposed to TPA for 48 h. Total cellular RNA isolated from cells with no additional treatment (lane 1), HL-60 cells treated with TPA (48 h) and recombinant interferon α over the last 6 h of culture (lane 2), HL-60 cells treated with TPA (48 h) and recombinant interferon γ over the last 24 h of culture (lane 3), HL-60 cells treated with TPA (48 h), recombinant interferon γ over the last 24 h of culture and exposed to recombinant interferon α over the last 6 h of culture (lane 4), HL-60 cells treated with TPA (48 h), and recombinant interferon γ over the last 30 h of culture (lane 5), HL-60 cells treated with TPA (48 h), recombinant interferon γ over the last 30 h of culture and exposed to recombinant interferon α over the last 6 h of culture (lane 6). β-actin mRNA signals (lower panel).

not shown) with TPA. The ability of interferon α and TPA to alter the steady-state level of MNDA mRNA is specific since other agents that affect myeloid cell function, FMLP, endotoxin, poly (I) · poly (C), and interferon γ, did not affect the MNDA mRNA level in U937 cells (data not shown).

Cell differentiation to maturity is generally associated with the cessation of cell proliferation [Stein et al., 1990]. The TPA induction of monocyte/macrophage differentiation of the myeloid leukemia cell lines is also associated with a rapid decline in cell proliferation. The possibility that the down-regulation of MNDA mRNA in TPA-treated cells was due to the interruption of cell proliferation was examined in HL-60 cells blocked at the G_1/S phase transition point in the cell cycle by treatment with thymidine to inhibit DNA synthesis. This approach induced cell cycle synchrony in the HL-60 cells permitting us to

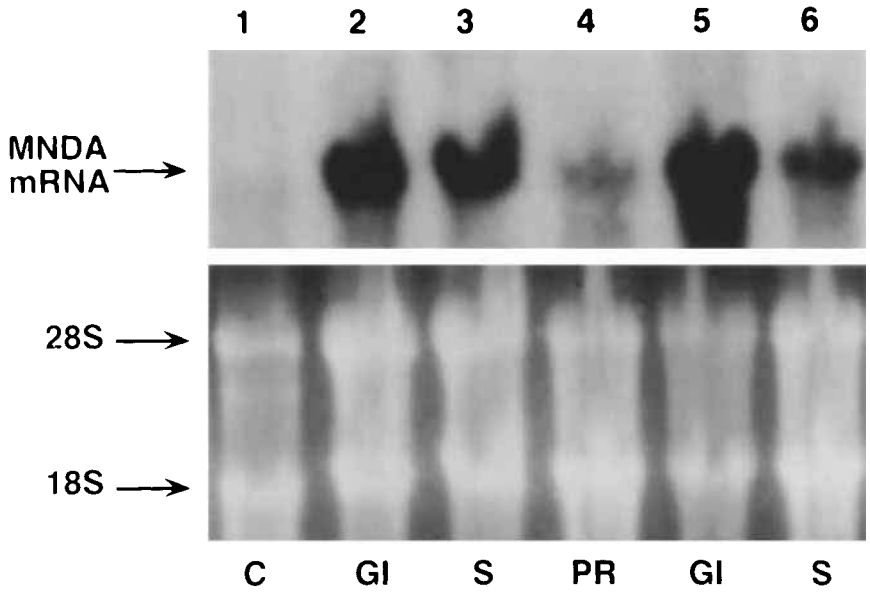

Fig. 6. Northern blot analysis of the MNDA mRNA in total cellular RNA from HL-60 cells growth arrested (lane 4) and in the G_1 (lanes 2 and 5) and S (lanes 3 and 6) phases of the cell cycle. The MNDA mRNA level in the untreated HL-60 cells is provided in lane 1. Ethidium bromide staining of total RNA showing the 28S and 18S ribosomal RNAs (lower panel).

determine cellular levels of MNDA mRNA following release from thymidine block as the cells passed through S phase, G_2, mitosis, and G_1 (Fig. 6). The MNDA mRNA level in the growth arrested cells was not depressed relative to the cells in the untreated culture (Fig. 6, compare lane 4 with lane 1). The level of MNDA mRNA is elevated in cells after release from the block; however, MNDA mRNA levels are similar during the cell cycle (compare G_1 and S phase signals in Fig. 6).

The effects of a block in cell proliferation or a change in differentiation on MNDA mRNA level was also addressed in experiments where retinoic acid was used to induce granulocyte differentiation in HL-60 cells. Retinoic acid treated and untreated HL-60 cells were harvested at 1, 2, and 3 days. No changes were observed in the MNDA mRNA level over the 3 day period of retinoic acid-induced granulocyte differentiation (data not shown). In addition, the retinoic acid treated HL-60 cells were exposed for 10 h to interferon α (2,000 units/ml) on days 1, 2, and 3 (Fig. 7). In these cases, the interferon α treatments had no effect on the MNDA mRNA levels (Fig. 7). Since retinoic acid treatment also halts HL-60 cell proliferation and does not affect MNDA mRNA level, the drop in MNDA mRNA level observed following TPA treatment of HL-60 cells (Fig. 3) is most likely not the result of the loss in proliferative activity. The loss in the ability to up-regulate MNDA mRNA in the retinoic acid treated HL-60 cells is a change that specifically effects the MNDA since the 2'5' oligoadenylate synthetase mRNA was induced by interferon α in HL-60 cells induced with retinoic acid for 1, 2, or 3 days (Fig. 7).

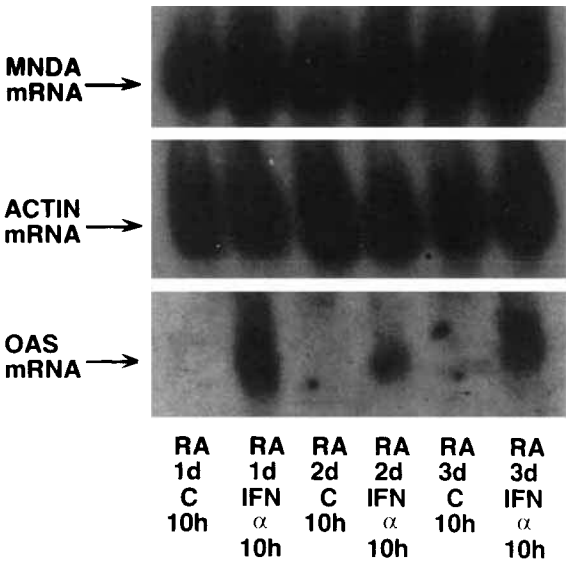

Fig. 7. Northern blot analysis of HL-60 cells treated with retinoic acid for 1 day (lanes 1 and 2), 2 days (lanes 3 and 4), and 3 days (lanes 5 and 6). During the last 10 h of retinoic acid treatment, no additions were made to the cultures (lanes 1, 3, and 5) or recombinant interferon α was added (lanes 2, 4, and 6). The MNDA mRNA signal (upper panel), the β-actin constitutive mRNA (middle panel) and the human 2'-5' oligoadenylate synthetase (OAS) mRNA signal (lower panel).

DISCUSSION

The regulation of the MNDA expression is apparently under the control of a hierarchy of mechanisms. One level of regulation establishes a lineage specific pattern which has been documented previously through immunochemical detection of MNDA by immunoblotting and immunocytochemical detection and through Northern blot analysis of the 1.8 kb MNDA mRNA [Goldberger et al., 1984, 1986; Briggs et al., 1992, 1993]. The demonstration of specificity of expression using any method depends on the sensitivity of the test thus, it is only possible, at this time, to state that MNDA is preferentially expressed constitutively in cells of the human myeloid lineage. Our previous research also indicated that MNDA positive myeloid cells significantly up-regulated the steady-state MNDA mRNA level in response to interferon α, whereas cells which did not exhibit detectable MNDA mRNA or antigen activity did not respond to interferon α by inducing or up-regulating the steady-state level of MNDA mRNA [Briggs et al., 1992]. Our results now demonstrate that the up-regulation of MNDA mRNA is transient and augmented by interferon γ pretreatment as is the case for other interferon-inducible genes [Sen, 1991; Sen and Lengyel, 1992]. The present results also indicate that detecting a constitutive level of MNDA mRNA or antigen does not ensure that the cell will up-regulate the MNDA mRNA level in response to interferon α since primary granulocytes or HL-60 cells induced for granulocyte differentiation showed no significant alteration in the MNDA mRNA level following interferon α treatment. The change in the HL-60 cell response to interferon that accompanies retinoic acid-induced granulocyte differentiation affects the MNDA gene specifically as the mechanism of inducing the 2′5′ oligoadenylate synthetase gene by interferon α remained functional.

Phorbol ester treatment of the myeloid leukemia cell lines HL-60, U937, and THP-1 led to rapid cessation of cell proliferation, adherence and monocyte/macrophage differentiation. The steady-state level of MNDA mRNA decreased in these cells following TPA treatment which is consistent with the much lower level of constitutively expressed MNDA mRNA in primary monocytes than in the untreated myeloid leukemia cell lines. As is the case in primary monocytes, the TPA-induced myeloid leukemia cell lines responded to interferon α treatment and significantly up-regulated the MNDA mRNA level. Although TPA-treated cell lines exhibited decreased levels of MNDA mRNA, this effect was apparently related to the monocyte/macrophage-induced differentiation and not the inhibition of cell proliferation. In proliferating HL-60 cells, the levels of the MNDA mRNA were similar during the cell cycle and both double thymidine block and retinoic acid treatment inhibited cell proliferation, but neither treatment decreased the constitutive level of MNDA mRNA. The results show that both primary monocytes and TPA-induced HL-60 cells contain low or undetectable levels of the MNDA mRNA which is up-regulated in both cells by interferon α. Since the uninduced HL-60, U937, and THP-1 cells also respond to interferon α treatment and up-regulate the MNDA mRNA, our results indicate that interferon α up-regulates the MNDA mRNA specifically in the human monocytes and also in earlier stage cells capable of differentiating into either monocytes or granulocytes.

Interferon α has shown efficacy in the treatment of patients with certain types of leukemia. The therapeutic effectiveness of interferon α in chronic myelogenous leukemia may be especially relevant to the present results and the elucidation of the myeloid cell specific effects of interferon α could contribute to more effective use of interferon α in cancer therapy. In this regard, the identification and characterization of the regulatory elements responsible for the unusually specific regulation of MNDA constitutive expression and the specific effects of interferon α on MNDA expression is an important goal.

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