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Regulation and Specificity of MNDA Expression in Monocytes, Macrophages, and Leukemia/B Lymphoma Cell Lines

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Abstract The expression of the human myeloid cell nuclear differentiation antigen (MNDA) was observed specifically in cells of the granulocyte–macrophage lineage in our earlier reports. The specificity of MNDA expression for cells in the granulocyte–macrophage lineage was reexamined in cell lines established from patients with Philadelphia chromosome-positive chronic myeloid leukemia. Cell lines that expressed MNDA exhibited myeloid cell features and granulocyte or monocyte differentiation could be induced in vitro, while cell lines exhibiting properties of very early stage cells or multipotential cells did not express MNDA. Cells originating from cases of Burkitt's lymphoma were negative. By contrast, three lymphoblastoid cell lines (immortalized in vitro with Epstein-Barr virus) were weakly positive and MNDA was up-regulated by interferon-α (IFN-α) treatment. As we reported previously, MNDA mRNA level in adherent monocytes is elevated by IFN-α; in this study, we further assessed MNDA expression in in vitro monocyte-derived macrophages. Three additional agents (endotoxin, phytohemagglutinin, and phorbol ester) and other conditions that affect function, cytokine production, differentiation, and/or growth of monocytes were examined for their ability to alter MNDA expression. The results varied with the agent, cell type, and stage of differentiation. Changes in MNDA expression occurred slowly (hours to days), suggesting that MNDA could mediate changes realized over a long period. The results also reveal a discordance in certain MNDA positive cells between steady-state levels or changes in levels of protein and mRNA indicating that the regulation of MNDA expression occurs at more than one point. Changes in MNDA expression are consistent with a role in opposing macrophage differentiation and activation of monocytes/macrophages.

Key words: human, myeloid, nuclear, differentiation, chronic myeloid leukemia, Burkitt's lymphoma, Epstein-Barr virus, interferon-α, PHA, phorbol ester

The human myeloid cell nuclear differentiation antigen (MNDA) was discovered in this laboratory and is a human granulocyte–monocyte/macrophage (GM) specific nuclear protein detected initially in the late myeloblast and monoblast stages of differentiation. We subsequently showed that the expression of MNDA in acute leukemia followed a lineage-specific pattern of expression [Cousar and Briggs, 1990]. Cases of acute myeloid leukemia were consistently MNDA positive when myeloid cell differentiation was evident, while cases of lymphocytic leukemia were negative [Cousar and Briggs, 1990]. The T-cell lymphoma, Molt 4, and Burkitt's lymphoma (BL), Raji, lines were also negative [Goldberger et al., 1986]. Human leukemia cell lines exhibiting characteristics of immature GM cells (KG-1a) or exhibiting properties of cells in lineages other than granulocytes and macrophages (K562) were also negative [Goldberger et al., 1986]. MNDA expression continues through production of peripheral blood granulocytes and monocytes [Briggs et al., 1994a].

We recently mapped the MNDA gene to chromosome 1q21–22 within a large linkage group conserved between mouse and human [Briggs et al., 1994b]. A cluster of interferon-inducible genes is located in this region and each gene

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contains one or two copies of a conserved 200 amino acid (aa) region [Briggs et al., 1994b]. A single copy of this 200-aa region appears in the MNDA. One of the related mouse genes contains a single copy of the conserved 200-aa region and exhibits macrophage-restricted interferon-induced expression [Tannenbaum et al., 1993]. A second human gene that contains two copies of the 200-aa conserved region shows constitutive expression in lymphoid cells and can be induced in myeloid cells by interferons [Trapani et al., 1992]. The results indicate that some members of a cluster of genes containing a conserved 200-aa region(s) are expressed and regulated specifically in blood cells.

Although the related human and mouse 200 cluster genes share the large 200-aa conserved region, and in all the genes, expression is affected by interferons, each gene has unique properties with regard to pattern of expression and regulation. The present results confirm the specificity of MNDA expression in cells exhibiting characteristics of differentiating granulocytes and monocytes. However, Epstein-Barr virus (EBV) immortalization of human B lymphocytes, lymphoblastoid cell lines (LCL), exhibit constitutive MNDA expression as well as the ability to up-regulate MNDA expression in response to interferon-α (IFN-α). While MNDA expression continues in adherent monocytes maintained in culture, the level of expression progressively decreases but can be up-regulated by IFN-α and dramatically down-regulated by phorbol ester 12-*O*-tetradecanolyphorbol-13-acetate (TPA) and phytohemagglutinin (PHA). Endotoxin (LPS) treatment had no effect on MNDA expression. The time course over which changes are observed in MNDA expression suggests that MNDA does not mediate signal transduction into the nucleus and is most likely not directly affected through activation of a number of signal transduction pathways. The results are consistent with the possibility that MNDA opposes the activation of monocyte function and differentiation into macrophages.

MATERIALS AND METHODS

Cells and Reagents

The Philadelphia chromosome (Ph^1)-positive leukemia cell lines (K562 [Lozzio et al., 1981], BV173 [Pegoraro et al., 1983], RWLeu 4 [Lasky et al., 1990], and EM2 [Keating et al., 1983]), myeloid cell lines (HL-60 and U937 [Briggs et al., 1994a]), LCLs (Pala and ML [Miller et al., 1993]), and BL cell line (Ramos) were grown in RPMI-1640 medium with 10% or 15% (v/v) heat-inactivated bovine calf serum in 5% CO_2 atmosphere at 37°C. The LCLs Pala and ML were obtained from Dr. Geraldine Miller (Vanderbilt University, Nashville, TN), and the Ramos cells were obtained from the American Type Culture Collection (Rockville, MD).

The isolation and maintenance of adherent monocytes from normal donor blood have been described previously [Briggs et al., 1994a]. In experiments involving the detection of MNDA by immunoblotting, the adherent monocytes were maintained in 100 × 20-mm tissue culture dishes. Cells harvested from one dish provided sufficient material for immunoblot detection. Experiments involving the detection of MNDA mRNA required that the adherent monocytes be maintained in 225-cm^2 tissue culture flasks (Costar, Cambridge, MA). Total RNA harvested from cells maintained in two flasks provided sufficient RNA for Northern blot analysis.

Phytohemagglutinin (PHA) (M form) (Gibco, Grand Island, NY) was used at a concentration of 1 ml/dl for the times specified. Lipopolysaccharide (LPS) (Sigma Chemical Co., St. Louis, MO) was used at 1 μg/ml. The phorbol 12-myristate 13-acetate, TPA (Sigma), was used in cell lines at the concentrations specified. Double-stranded RNA, poly (I) · poly (C) (Pharmacia Biotech Inc., Piscataway, NJ) was used at 30 μg/ml. The recombinant IFN-αA (Hoffmann-LaRoche, Nutley, NJ) and immunoaffinity-purified natural human IFN-α (Interferon Sciences, New Brunswick, NJ) were used at 2,000 U/ml. 1,25-Dihydroxyvitamin D3 (Hoffmann-LaRoche) was used at 5×10^{-8} M.

Conditioned medium was prepared from cultures of adherent monocytes treated with PHA for 24 h. The culture medium was collected and particulate material removed by centrifugation (1,000*g*, 3 min). The supernatant was stored at −20°C and used at 5% or 10% (v/v) in U937 cell cultures.

Immunoblotting

The whole cell immunoblot assay [Goldberger et al., 1986] involves the injection of suspended, washed cells into lysis buffer maintained in a dry bath (100°C) and incubation for an additional 3 min at 100°C. The DNA in the samples was sheared by ultrasound [Goldberger et al., 1986] before electrophoretic separation and transfer to nitrocellulose membrane [Goldberger et al.,

1986]. Cells in suspension culture were counted before lysis and volumes of sample containing 1×10^5 cells were analyzed. The samples of Raji and subclones, 11.5.8, 13.6.7, and LCL, X50-7 [Allday and Farrell, 1994; Allday et al., 1993], were provided by Martin Allday, Ludwig Institute for Cancer Research (London, UK). The experiments using adherent monocytes involved introducing equal numbers of normal donor mononuclear cells in 100 × 20-mm tissue culture dishes [Briggs et al., 1994b]. The adherent monocytes were harvested by lysis of the cells on the dishes, after washing in phosphate-buffered saline (PBS), using lysis buffer [Goldberger et al., 1986] heated in the dry bath (100°C). The lysed cells were scraped from the dish and returned to the dry bath (100°C) for 3 min. The samples were then processed as described above (suspended cells). Within each experiment, equal numbers of cells were harvested at each time point or condition as indicated. Since the adherent monocytes could not be counted, paired samples were also electrophoretically analyzed and stained with Coomassie Brilliant Blue, in order to assess variability in sample loading.

Northern Blotting

Total cellular RNA was isolated using RNAzol (Biotex, Houston, TX) as recommended by the supplier. The electrophoresis, blotting, probes, and hybridization conditions were defined previously [Briggs et al., 1994a]. Each experiment was repeated at least once.

RESULTS

A whole cell immunoblot analysis was used to assess MNDA expression in Ph[1]-positive leukemia cell lines (Fig. 1). MNDA was detected in the two lines that exhibit properties of cells in the G-M lineage (RWLeu4 and EM2) [Keating et al., 1983; Lasky et al., 1990] at a level similar to that in the positive control HL-60 cells (Fig. 1). The lines that exhibit properties of multipotential progenitor cells (K562) [Lozzio et al., 1981] or represent undifferentiated blasts (BV173) [Pegoraro et al., 1983] were MNDA negative (Fig. 1). Both of the MNDA expressing Ph[1]-positive cell lines respond to the induction of granulocyte or monocyte/macrophage differentiation (Keating et al., 1983; Lasky et al., 1990) and the induction of differentiation in these MNDA expressing cells did not alter the level of immunochemically detectable MNDA (Fig. 1, cf. lanes 2 and 3 with lanes 1 and 11 with lane 10), as was reported previously for other lines [Briggs et al., 1994a].

Although MNDA has only been detected in normal and leukemic cells of the GM lineage, human B lymphocytes immortalized in vitro with EBV, LCLs, express MNDA (Fig. 2). Both Pala (Fig. 2, lanes 2 and 3) and X50.7 are LCLs (Fig. 2, lane 8) and exhibited constitutive levels of MNDA somewhat less than that observed in the positive control U937 cells (Fig. 2, lane 1). The constitutive level of MNDA expression was enhanced in the Pala cells following exposure of the cells to IFN-α (Fig. 2, lane 3). A third LCL, ML, showed the same level of constitutive MNDA expression and elevation of antigen following IFN-α treatment (data not shown). By contrast, cell lines established from cases of BL did not express a constitutive level of MNDA either in cells positive for EBV (Fig. 2, lane 7) or in those which are EBV negative (Fig. 2, lanes 9 and 10). In addition, MNDA expression could not be induced in BL lines with IFN-α (Fig. 2, lane 10). In the case of the EBV-positive Raji BL cells, a large segment of the EBNA 6 coding sequence is deleted and no EBNA 6 protein is detected in these cells. The 11.5.8 clone of Raji cells expresses EBNA 6 after co-transfection of an EBNA 6 gene under the control of the SV40 early promoter with a selectable marker (Allday et al., 1993) and MNDA expression was not observed (Fig. 2, lane 5).

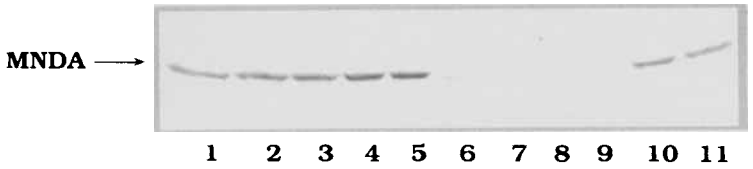

Fig. 1. Whole cell immunoblot analysis of the M_r 55,000 MNDA in human leukemia cell lines. Untreated RWLeu4 cells (*lane 1*), RWLeu4 cells exposed to 5×10^{-8} M 1,25-dihydroxyvitamin D_3 for 72 h (*lane 2*), or RWLeu4 cells exposed to 1×10^{-8} M TPA for 24 h (*lane 3*). HL-60 cells untreated (*lane 4*) or exposed to 1×10^{-8} M TPA for 24 h (*lane 5*). K562 cells untreated (*lane 6*) or treated with 1×10^{-8} M TPA for 24 h (*lane 7*). BV173 cells untreated (*lane 8*) or treated with 1×10^{-7} M TPA for 24 h (*lane 9*). EM2 cells untreated (*lane 10*) or treated with 3×10^{-8} M TPA for 24 h (*lane 11*).

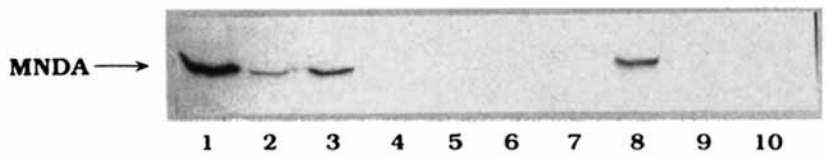

Fig. 2. *Whole cell immunoblotting analysis of the M_r 55,000 MNDA in human leukemia cell lines. Untreated U937 positive control cells (lane 1), LCL Pala cells untreated (lane 2) or treated with recombinant interferon-α (IFN-α) for 8 h (lane 3), untreated negative control K562 cells (lane 4), an EBNA 3C/6-positive clone, 11.5.8, of BL Raji (lane 5) and an EBNA 3C/6-negative clone, 13.6.7, of Raji (lane 6), Raji cells (lane 7), LCL X50.7 cells (lane 8), and the Epstein-Barr virus (EBV)-negative BL Ramos cells untreated (lane 9) or treated with IFN-α for 8 h (lane 10).*

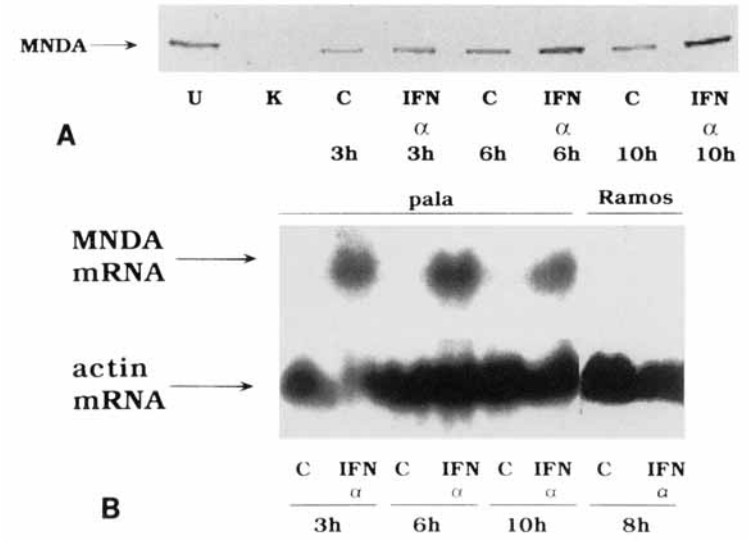

Fig. 3. *Whole cell immunoblot analysis of MNDA and Northern blot analysis of MNDA mRNA levels in human lymphoid cell lines.* **A:** *Immunoblot detection of the M_r 55,000 MNDA in positive control U937 cells (U), negative control K562 cells (K), untreated LCL Pala cells (C) (lanes 3,5,7), and Pala cells treated with interferon-α (IFN-α) for 3 h (lane 4), 6 h (lane 6), or 10 h (lane 8). Paired cultures of untreated Pala cells (lanes 3,5,7) were harvested at each time point simultaneously with the treated cultures (lanes 4,6,8).* **B:** *MNDA mRNA levels (top) in LCL Pala cells treated with recombinant IFN-α for 3 h (lane 2), 6 h (lane 4), or 10 h (lane 6). The level of MNDA mRNA in paired untreated cultures of Pala cells (lanes 1,3,5). MNDA mRNA level in the untreated Epstein-Barr virus (EBV)-negative BL Ramos cells (lane 7) or Ramos cells treated for 8 h with recombinant IFN-α (lane 8). Twenty μg of total RNA was loaded in each lane. The β-actin mRNA signals (bottom).*

The effect of interferon α on the level of immunochemically active MNDA in the LCL Pala cells was examined over an extended time course. No change was observed at 0.5, 1, or 2 h (data not shown). After 3 h of exposure to IFN-α, the level of MNDA was increased (Fig. 3A) and remained elevated for 48 h (data not shown). The effectiveness of IFN-α in elevating MNDA immunochemical activity in the LCL was specific in that LPS, TPA, and poly (I) · poly (C) did not alter MNDA (data not shown). IFN-α treatment of the Pala cell line up-regulated the steady-state level of MNDA mRNA (Fig. 3B). Neither LPS nor TPA treatment affected MNDA mRNA level in Pala cells (data not shown). The BL cell line Ramos is MNDA-negative and MNDA mRNA was not induced by IFN-α (Fig. 3B). The association between constitutive MNDA expression and ability to up-regulate MNDA in response to IFN-α, as observed in the LCLs, is consistent with previous results [Briggs et al., 1994b]. The results obtained with the LCLs also suggest that the level of the constitutive steady-state level of MNDA mRNA is not important in determining the cellular responsiveness to interferon α. As observed in Figure 4A, the normal untreated human monocyte exhibited a very low level of MNDA mRNA (lanes 1–3) compared to the signal in the control U937 cell RNA (lane 9). However, monocytes maintained in culture for a day up-regulate the steady-state level of MNDA mRNA dramatically after 6 hours of interferon α treatment (Fig. 4A, lanes 7 and 8). No concomitant change in the MNDA antigen level was observed (data not shown).

The ability of IFN-α to elevate the steady-state level of MNDA mRNA was a unique effect and the use of LPS, an activator of monocyte/

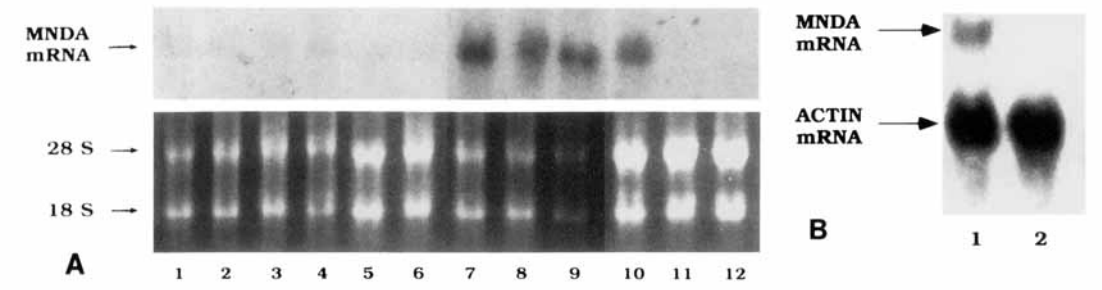

Fig. 4. Northern blot analysis of MNDA mRNA levels in normal human donor adherent monocytes. **A:** MNDA mRNA levels (upper panel) in adherent monocytes maintained for 24 h without treatment (*lanes 1,2,3*), exposed to lipopolysaccharide (LPS) for the last 6 h of the 24-h culture period (*lanes 4,5,6*) or treated with recombinant (*lane 7*) or natural (*lane 8*) interferon-α (IFN-α) for the final 8 h of the 24-h culture period. Total RNA from the positive control U937 cells (*lane 9*). Adherent monocytes maintained for 72 h untreated (*lane 10*) or exposed to phytohemagglutinin (PHA) over the 72-h period (*lanes 11,12*). Twenty μg of total RNA was analyzed in each lane, except in the case of the positive control U937 RNA, where only 10 μg was loaded due to the relatively high MNDA mRNA signal in the U937 cells. The ethidium bromide staining of samples (*bottom*) is provided to indicate the variability in sample loading and quality of the RNA samples. **B:** MNDA mRNA levels (*top*) in adherent monocytes maintained for 96 h untreated (lane 1) or treated with 1.6×10^{-5} M TPA (*lane 2*) over the last 24 h of the 96-h culture period. TPA was equally as effective in reducing MNDA mRNA level in adherent monocytes treated with 2×10^{-9} M (data not shown). Twenty μg of total RNA was analyzed in each lane. The β-actin signals (*bottom*).

macrophage function, was ineffective in altering MNDA mRNA level in adherent monocytes (Fig. 4A, lanes 4–6). Both TPA and PHA (Liebler et al., 1994), other effectors of monocyte/macrophage function, repressed MNDA mRNA levels in adherent monocytes (Fig. 4A, cf. lanes 11 and 12 with 10, and Fig. 4B). Experiments designed to reveal the effectiveness of TPA and PHA in repressing the level of MNDA mRNA were facilitated by treating adherent monocytes maintained in culture for several days. Adherent monocytes maintained for three or more days in culture exhibit a higher constitutive level of MNDA mRNA over that detected in freshly isolated monocytes or after 1 day in culture (Fig. 4A, cf. lanes 1, 2, and 3 with lane 10). The drop from a relatively high constitutive level of MNDA mRNA in the adherent monocytes to an undetectable level in response to TPA or PHA is obvious in cells that have been maintained in culture for over 3 days (Fig. 4A,B). The elevation in MNDA mRNA level detected in adherent monocytes after days in culture was not accompanied by a change in immunochemically detected MNDA (Fig. 5). The level of immunochemically detected MNDA was remarkably consistent in cultured monocytes but appeared to drop after 6 days in culture (Fig. 5). In addition, adherent monocytes treated with TPA or PHA showed no immediate decrease in immunochemically detectable MNDA (Fig. 6).

While previous research showed that the level of MNDA mRNA decreased in U937, HL-60 and THP-1 cells treated with TPA [Briggs et al., 1994a], both LPS and PHA had no consistent

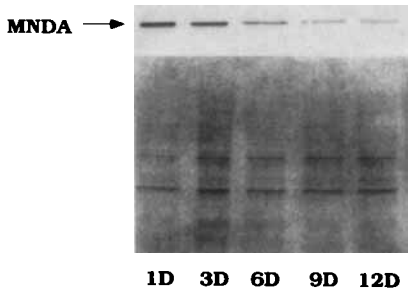

Fig. 5. Whole cell immunoblot analysis of the M_r 55,000 MNDA (*top*) in adherent monocytes from normal human donor blood. Cells harvested from dishes after 1–12 days (D) in culture. A duplicate set of samples were electrophoretically separated and stained with Coomassie Brilliant Blue (*bottom*), in order to monitor variability in sample loading.

effect on U937 cell MNDA mRNA level and immunochemical activity (data not shown). In addition, the use of conditioned medium from PHA-treated adherent monocytes had no effect on MNDA mRNA level in U937 cells (Fig. 7).

DISCUSSION

The present study assesses MNDA expression in a variety of blood cells not evaluated previously [Briggs et al., 1994a,b; Goldberger et al., 1986]. In addition, the characterization of the effects of agents and conditions that alter monocyte/macrophage function, cytokine production, growth and/or differentiation was completed in order to identify the function of the MNDA. The human cell lines EM-2 and RWLeu 4 are Ph^1-positive cell lines established from patients with CML [Keating et al., 1983; Lasky et al., 1990].

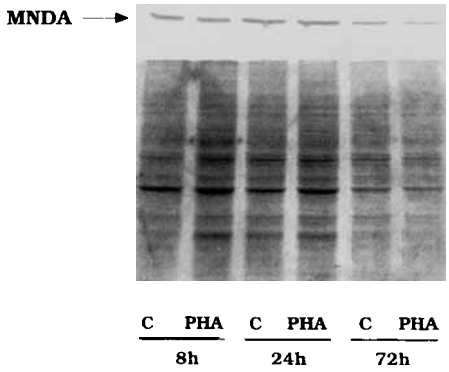

Fig. 6. Whole cell immunoblot analysis of the M_r 55,000 MNDA (*top*) in adherent monocytes from normal human donor blood treated with phytohemagglutinin (PHA). The level of the MNDA in adherent monocytes treated for 8 h, 24 h, and 72 h with PHA was compared to the level of MNDA in cells in untreated paired cultures (C) harvested at the same time points. Paired samples were electrophoretically separated and stained with Coomassie Brilliant Blue (*bottom*) in order to monitor variability in sample loading.

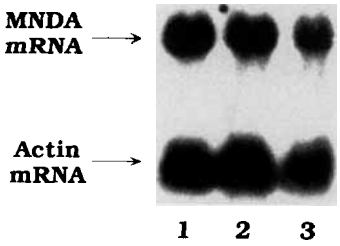

Fig. 7. Northern blot analysis of MNDA mRNA level (*top*) in U937 cells treated with conditioned medium from phytohemagglutinin (PHA)-treated adherent monocytes. Untreated U937 cells (*lane 1*), U937 cells treated for 24 h with 10% PHA conditioned medium (*lane 2*) or U937 cells treated with 5% PHA conditioned medium for 24 h (*lane 3*). Twenty μg of total RNA was analyzed in all lanes. The β-actin mRNA signals (*bottom*).

Both lines are capable of granulocyte or monocyte differentiation in response to the appropriate inducing agents [Keating et al., 1983; Lasky et al., 1990]. The expression of MNDA in these lines is consistent with MNDA positivity in HL-60, THP-1, and U937 cells that also differentiate along the GM lineage. By contrast, the MNDA-negative cell lines BV173 and K562 do not complete G-M differentiation in response to inducing agents. Although both are Ph^1-positive, the BV173 has features of an undifferentiated blast cell [Pegoraro et al., 1983], whereas the K562 cells are multipotential with features of progenitor cells of a number of lineages [Lozzio et al., 1981]. The pattern of expression of MNDA in the human Ph^1-positive lines is consistent with the specificity for cells in the GM lineage that differentiate in response to appropriate agents.

Burkitt's lymphoma cells that are EBV-positive or -negative were nonreactive for MNDA, which is consistent with the specificity of MNDA expression for cells capable of GM differentiation. However, B lymphocytes immortalized with EBV in vitro, LCL, were consistently positive for MNDA. The significance of the unexpected MNDA expression in LCLs is unclear. However, a number of differences in cellular physiology between the LCL and the BL have been documented. The behavior of LCL and BL cells is different following injection of each cell into athymic mice where the BL cells grow and the LCL do not [Tosato et al., 1994]. Differences in the expression of EBV genes and host cell genes between BL cells and LCL have also been documented [Allday et al., 1993; Joske and Knecht, 1993; Renzo et al., 1993]. In general, a greater number of genes that have been examined and originate from either genome are detected in LCLs versus the BL lines, and the expression of MNDA may be a consequence of the diversity of gene expression in the LCLs. Significant differences have been reported in the induction of the lytic cycle in EBV-infected BL cells and LCLs [Bogedain et al., 1994]. On the other hand, "illegitimate transcription" was used to account for a low basal level of collagen gene expression in LCL [Chan and Cole, 1991; Dharmavaram et al., 1993] and to describe the phenomenon whereby the expression of a highly tissue-specific gene can be detected in a cell not known to express the gene of interest [Chelly et al., 1989; Sarkar and Sommer, 1989]. The procedure for detecting these low abundance transcripts involved PCR amplification and was thought to detect the presence of a single transcript in 500–1,000 cells. Clearly the detection of MNDA expression in LCL using whole cell immunoblot analysis or Northern blot analysis of total RNA is not consistent with the MNDA originating from a rare or low-abundance transcript. The possibility of a relationship between MNDA expression in LCLs and the detection of the phagocyte NADPH oxidase [Volpp and Lin, 1993] should be considered. In addition, the LCL has a more blast-like phenotype than the BL lines and MNDA expression could be related to this stage of B cell differentiation. Although the mechanism for altering MNDA expression in the LCLs is unknown, the change also allows the MNDA to be up-regulated specifically by

IFN-α in the LCL, confirming the association between constitutive MNDA expression and sensitivity to IFN-α observed simultaneously at the mRNA and protein levels.

The effectiveness of IFN-α in up-regulating MNDA mRNA in human monocytes has been reported previously [Briggs et al., 1994a,b]. The specificity of the effects of IFN-α on MNDA expression in monocytes was more thoroughly examined through an analysis of the effects of three agents that stimulate phagocytic cell activity, cytokine production, growth, and/or differentiation. LPS treatment had no effect on the steady-state level of MNDA mRNA. The mouse D3 gene shares structural features with MNDA in addition to the common 200-aa region [Briggs et al., 1994b; Tannenbaum et al., 1993], but D3 is inducible in mouse macrophages by LPS as well as IFN-α or IFN-γ [Tannenbaum et al., 1993]. At the level of mRNA, both D3 and MNDA genes are up-regulated in a monocyte/macrophage lineage-specific manner, but the difference in response to activating agents indicates that the two gene products have different functions.

Treatment of adherent monocytes with PHA, an agent that induces cytokine gene expression in these cells [Liebler et al., 1994], led to a dramatic decrease in MNDA mRNA level (Fig. 4). Unfortunately, the effects of PHA on the expression of other genes that contain the conserved 200-aa region has not been reported. Monocytes treated with PHA and/or LPS differentially alter the expression of the chemotactic genes IL-8 and MCP-1, which provided the basis for concluding that each agent acts through different signaling pathways [Liebler et al., 1994]. The differential effects of these two agents on MNDA mRNA level is consistent with those findings. The ability of PHA to decrease MNDA mRNA level in adherent monocytes was not reproduced in U937 cells. Since monocytes isolated from blood by adherence will contain a few contaminating cells it was important to consider the possibility that cytokines produced by the contaminating cells in the monocyte cultures were directly responsible for the drop in MNDA mRNA. Conditioned medium was harvested from PHA treated adherent monocytes and added to U937 cell cultures and no effect was observed on MNDA mRNA level (Fig. 7). Conditioned medium from PHA treated lymphocytes contains a factor(s) that inhibited U937 cell proliferation and induced differentiation [Clement et al., 1988]. If these factors were also present in the conditioned medium due to lymphocyte contamination, they did not alter MNDA expression. The ability of PHA to decrease MNDA mRNA in adherent monocytes, but not in U937 cells, is apparently related to inherent differences in the promonocytic U937 cell and the in vitro differentiating monocyte derived macrophage.

The dramatic effect of TPA on MNDA mRNA level in adherent monocytes is similar to the effect of TPA on MNDA expression in human myeloid cell lines [Briggs et al., 1994a]. TPA is used at the micromolar level to initiate an immediate respiratory burst in phagocytic cells that have acquired this capability and, when used at nanomolar concentrations, TPA initiates differentiation in myeloid cell lines. In addition, TPA at the nanomolar concentration promotes the viability and recovery of long-term cultures of human monocyte derived macrophages [Markovich et al., 1994]. This treatment of adherent monocytes also facilitates the ability of the cells to secrete TNFα (Markovich et al., 1994). The effectiveness of TPA to eliminate the MNDA mRNA signal in monocytes was observed following treatment at the micromolar or nanomolar levels (Fig. 4B). While the relationship between the loss of detectable MNDA mRNA and the effects of TPA on the monocytic cells is unclear, it appears that the effectiveness of nanomolar concentrations of TPA to affect viability/differentiation may be more relevant since the changes in MNDA mRNA observed in the cell lines occurs when these cells are not capable of producing a respiratory burst.

While the LCLs express MNDA and up-regulate MNDA expression in response to interferon α, these cells do not decrease MNDA mRNA level in response to TPA treatment as is observed in the adherent monocytes and MNDA expressing myeloid cell lines. The results confirm that a constitutive level of MNDA expression is associated with an ability to up-regulate MNDA expression in response to IFN-α. It has not been possible to induce MNDA mRNA expression using IFN-α in cells that do not constitutively express a detectable level of MNDA mRNA or antigen. The results indicate that in the cells examined MNDA is not an interferon inducible gene, but that a constitutively expressed level can be up-regulated by IFN-α. By contrast, constitutive expression of MNDA does not correlate with the ability of agents to down-regulate MNDA mRNA level. Although TPA

treatment down-regulates MNDA mRNA in adherent monocytes and in human myeloid cell lines, the MNDA mRNA level in LCLs is unaffected by TPA treatment. In addition, PHA dramatically reduces MNDA mRNA in adherent monocytes, but has no effect on expression in U937 cells. Agents such as PHA and TPA, which activate signal transduction pathways different from IFN-α, do not elicit the same changes in different MNDA expressing cells. It appears that the stage of cell differentiation and cell type determines the effectiveness of agents other than IFN-α to alter MNDA expression.

The observations that TPA and PHA are capable of dramatically reducing the level of MNDA mRNA in adherent monocytes but have no apparent immediate effect on level of antigen indicate that MNDA is a relatively stable protein. The possibility that immunochemical detection does not correlate with actual level of protein is unlikely, as the immunoblot analysis employed in these experiments detects recombinant MNDA produced in bacteria [D. Xie, R.C. Briggs, and J.A. Briggs, unpublished observations]. The apparent lack of effect of elevating MNDA mRNA in adherent monocytes on level of antigen is also consistent with stability of the MNDA in peripheral blood monocytes. The decline in antigen level in adherent monocytes maintained for 12 days in culture without a concomitant drop in mRNA level indicates that the rate of translating MNDA mRNA and/or stability of the protein changes with macrophage differentiation.

Overall, the effects of agents that dramatically alter the level of MNDA mRNA in the adherent monocyte have no immediate effect on antigen level. The results indicate that MNDA is not involved in rapid cellular responses to activators of a number of signal transduction pathways. Previous characterization of a strictly nuclear intracellular localization of MNDA is also an indication that MNDA is not a mediator transducing signals into the nucleus. The drop in immunochemically detectable MNDA after 6 days of maintaining adherent cells in vitro indicates that MNDA expression changes during the differentiation of monocytes into macrophages. The process of in vitro differentiation of monocytes is accompanied by changes in morphology, functional activity, and surface phenotype [Andreesen et al., 1990; Bauer et al., 1989; D'Onofrio and Paradisi, 1983]. The in vitro differentiation process of adherent macrophages is inhibited by IFN-α [Becker, 1984; Andreesen et al., 1990]. IFN-α has also been observed to have an inhibitory effect on respiratory burst and glucose metabolism in macrophages [Conde et al., 1994]. Since inducers of monocyte/macrophage cytokine production and differentiation (TPA and PHA) down-regulate MNDA expression, it is possible that MNDA is a repressor of monocyte/macrophage activation and differentiation, but that its effects are not immediately realized.

With regard to MNDA expression in the LCLs, interferon α up-regulates the MNDA mRNA and protein steady-state levels within three hours of treatment. IFNα up-regulation of MNDA mRNA in monocytes required at least six hours and a concomitant change in antigen was not detected. While the role of MNDA in either cell is unknown, the monocyte results indicate that the effects of altering MNDA protein expression is a delayed response or that a second signal is required to alter translation, such as is the case with ferritin [Coulson and Cleveland, 1993]. The relatively rapid effect of IFN-α on both MNDA protein and mRNA in the LCLs may facilitate the subsequent evaluation of MNDA function.

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