Transcriptional and Posttranscriptional Regulation of *CSF-1* Gene Expression in Human Monocytes

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Regulation of *CSF-1* gene expression was investigated in human monocytes. *CSF-1* transcripts were at low or undetectable levels in resting monocytes. However, in monocytes treated with 12-*O*-tetradecanoylphorbol-13-acetate (TPA), *CSF-1* mRNA was increased by 3 h and reached maximal levels by 12 h of drug exposure. When nuclear run-on assays were used, *CSF-1* gene transcription was also at low or undetectable levels in resting monocytes but was activated after TPA exposure. TPA-treated monocytes exposed to actinomycin D further demonstrated that the half-life of the *CSF-1* mRNA is 0.9 h. The results also demonstrated that the protein synthesis inhibitor, cycloheximide (CHX), increases *CSF-1* mRNA levels in both resting and TPA-treated monocytes. These effects of CHX occurred in the absence of detectable increases in *CSF-1* gene transcription. Moreover, treatment of monocytes with CHX and actinomycin D demonstrated that inhibition of protein synthesis is associated with stabilization of the *CSF-1* transcript. Taken together, these findings indicated that *CSF-1* gene expression is controlled at both transcriptional and posttranscriptional levels in human monocytes.

The macrophage-specific colony-stimulating factor (CSF-1, M-CSF) is required for the survival and proliferation of mononuclear phagocytes (19). Moreover, CSF-1 stimulation is sufficient for induction of events leading to the differentiation of human monocytes into macrophages (1, 19). CSF-1 also stimulates the production of interferon, tumor necrosis factor, and colony-stimulating activity in human monocytes (22). In addition, recent studies have demonstrated that CSF-1 plays a role in placental development (11). These effects of CSF-1 are mediated by binding to a single class of high-affinity CSF-1 receptors (19). The CSF-1 receptor and the product of the *c-fms* proto-oncogene have been shown to be closely related or identical molecules (18). In this regard, expression of the *c-fms* gene has been detected in cells derived from the monocyte-macrophage lineage and placenta (8, 9, 16).

Mouse cells that have been shown to produce CSF-1 include uterine epithelium (11), fibroblasts (20), and stromal elements from bone marrow (7). Studies of human cells that produce this factor have been limited, although recent work has demonstrated that CSF-1 is produced by cells of the monocyte lineage (4, 5, 10, 14). Certain human pancreatic carcinomas (6, 12) and a variety of other human carcinoma cell lines (J. Horiguchi et al., unpublished data) also express *CSF-1* transcripts. Multiple species of *CSF-1* mRNA have been detected in the MIA PaCa pancreatic carcinoma cells (6, 12), while expression of this gene in human monocytes (4, 5, 10, 14) and myeloid leukemic cells (13) is predominantly as a 4.0-kilobase (kb) transcript. *CSF-1* gene expression is often detectable in cells differentiated along the monocytic lineage which also express *c-fms* RNA (21), thus suggesting that CSF-1 functions in an autocrine fashion. The mechanisms responsible for the regulation of *CSF-1* expression, however, have not been addressed in previous studies.

As we have already demonstrated, *CSF-1* mRNA is at low or undetectable levels in resting monocytes (4, 5). However,

treatment of resting monocytes with TPA (12-*O*-tetradecanoylphorbol-13-acetate) results in the induction of *CSF-1* gene expression at the RNA and protein levels (5). In the present studies, *CSF-1* transcripts were similarly increased by 3 h of TPA treatment and reached maximum levels by 12 h of drug exposure (Fig. 1A). TPA had no effect on levels of actin transcripts (data not shown). Run-on transcription assays were performed to determine the relative rates of *CSF-1* gene transcription in isolated nuclei from resting and TPA-treated monocytes. *CSF-1* gene transcription was undetectable in these resting monocytes (Fig. 1B). In contrast, treatment with TPA for 12 h was clearly associated with increased transcription of this gene (Fig. 1B). These findings were associated with detectable actin, but not β -globin, gene transcription in both resting and TPA-treated monocytes (Fig. 1B). Moreover, the increase in *CSF-1* gene transcription was associated with secretion of CSF-1 protein into the culture supernatants (5; data not shown). The transcriptional induction of *CSF-1* gene expression in monocytes prompted further studies of the stability of *CSF-1* mRNA. Monocytes were treated with TPA for 12 h and then exposed to actinomycin D for various intervals. Under these experimental conditions, levels of *CSF-1* mRNA decreased progressively with a half-life of 0.9 h (determined by densitometric measurements), while there was little, if any, effect on actin mRNA levels (Fig. 1C).

TPA-treated monocytes were next exposed to cycloheximide (CHX) to determine whether *CSF-1* mRNA levels are regulated by the synthesis of a labile protein. *CSF-1* mRNA levels were increased 17-fold in monocytes treated with both TPA and CHX, compared with cells exposed to TPA alone (Fig. 2A, lanes 4 and 6). Moreover, levels of *CSF-1* transcripts were increased 20-fold by CHX in TPA-induced monocytes which had also been treated with actinomycin D to inhibit transcription (Fig. 2A, lanes 3 and 5). These findings suggested that CHX stabilized the *CSF-1* transcript and had little, if any, effect on the rate of *CSF-1* gene transcription in TPA-treated monocytes. Indeed, run-on

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FIG. 1. Effects of TPA on *CSF-1* expression in resting human monocytes. Peripheral blood monocytes were obtained from "plateletpheresis" by-products from normal blood donors by using a previously described method (15). (A) The resting or deactivated monocytes were treated with 33 nM TPA for the indicated times.

assays demonstrated similar rates of *CSF-1* gene transcription in TPA- and TPA-CHX-treated monocytes (*CSF-1*/actin run-on signal ratio by using the assay described in the legend to Fig. 1B; TPA, 0.22; TPA-CHX, 0.30). Resting, rather than TPA-induced, monocytes were also exposed to CHX to further examine the effects of this agent on *CSF-1* expression. A 3-h exposure to CHX resulted in the detection of *CSF-1* mRNA (Fig. 2B). Moreover, treatment of resting monocytes with CHX and actinomycin D demonstrated that the half-life of *CSF-1* mRNA was prolonged to approximately 2.5 h (Fig. 2B). This finding indicated that CHX stabilized *CSF-1* transcripts by a posttranscriptional mechanism.

Run-on assays were also performed to determine whether CHX treatment was associated with increased rates of *CSF-1* gene transcription. Resting monocytes were treated with CHX for 1 and 3 h to compare *CSF-1* mRNA levels (Fig. 3A) and transcriptional rates of this gene (Fig. 3B). *CSF-1* mRNA was at a low but detectable level in these monocytes (Fig. 3A). Furthermore, the level of these transcripts was increased by 5.3- and 23-fold after CHX exposures of 1 h and 3 h, respectively. This increase at 3 h was comparable to that obtained after similar exposures of TPA-treated monocytes to CHX (Fig. 2A). *CSF-1* gene transcription was also at low levels in these monocytes. However, in contrast to treatment with TPA, treatment with CHX was not associated with a significant increase in transcription of this gene (Fig. 3B). In this regard, the *CSF-1*/actin run-on signal ratios were similar for control and CHX-treated monocytes (Fig. 3B). These findings confirmed that *CSF-1* mRNA levels are controlled by posttranscriptional mechanisms in CHX-treated monocytes.

These results thus demonstrate that transcription of the *CSF-1* gene is at low to undetectable levels in resting monocytes and that treatment with TPA is associated with transcriptional activation of this gene. This increase in *CSF-1* gene transcription was accompanied by the appearance of *CSF-1* transcripts and release of CSF-1 protein into the culture supernatant. The results also indicate that posttranscriptional mechanisms control *CSF-1* gene expression. Thus, inhibition of protein synthesis with CHX increased the level of *CSF-1* transcripts by 17-fold in TPA-activated monocytes. This effect was related to *CSF-1* mRNA stabilization, since CHX also increased the level of *CSF-1* transcripts by 20-fold in transcriptionally inactive cells inhibited by actinomycin D. Similar increases in *CSF-1* mRNA (23-fold) were observed for resting monocytes treated with CHX. More-

Total cellular RNA (12 μ g) was isolated by the guanidine thiocyanate-cesium chloride method and analyzed as described previously (15). The transcripts were visualized by autoradiography following hybridization to the 32 P-labeled 0.6-kb *AccI-EcoRI* fragment of a human *CSF-1* cDNA (6). (B) Nuclei from resting and 12-h TPA-treated monocytes were isolated for preparation of 32 P-labeled nuclear RNA (15). A linearized JW102 plasmid carrying a human β -globin gene (23), the chicken β -actin pA1 plasmid DNA (3), and the 4.6-kb human *CSF-1* plasmid DNA (6) were run on a 1% agarose gel, transferred to nitrocellulose paper by Southern blotting, and hybridized to similar amounts of 32 P-labeled nuclear RNA (15). (C) Total cellular RNA was isolated from monocytes treated with 33 nM TPA and 5 μ g/ml actinomycin D (ACT) per ml for the indicated times. RNA (12 μ g) was analyzed as in panel A by hybridization to the 32 P-labeled 0.6-kb fragment of a human *CSF-1* cDNA (6) or to a 32 P-labeled 1.8-kb *PstI* fragment of the chicken β -actin pA1 plasmid (3). Densitometric tracings of the *CSF-1* mRNA signals indicated a half-life of 0.9 h.

FIG. 2. Effects of CHX on *CSF-1* expression in resting and TPA-treated monocytes. (A) Resting monocytes were treated with 33 nM TPA, 10 µg of CHX per ml, and 5 µg of actinomycin D (ACT) per ml for the indicated times before isolation of total cellular RNA. The RNA (12 µg) was then hybridized to ³²P-labeled fragments of the human *CSF-1* cDNA or the β-actin plasmid as described in the legend to Fig. 1. (B) Resting monocytes were treated with 10 µg of CHX per ml and 5 µg of ACT per ml for the indicated times. Total cellular RNA (12 µg) was hybridized to the ³²P-labeled *CSF-1* and actin probes. The half-life of the *CSF-1* transcript was 2.5 h on the basis of densitometric tracings of the *CSF-1* mRNA signals. Similar results were obtained in three separate experiments.

FIG. 3. Effects of CHX on *CSF-1* mRNA levels and rates of *CSF-1* gene transcription. (A) Resting monocytes were treated with 10 µg of CHX per ml for 1 and 3 h. Total cellular RNA was then hybridized to the ³²P-labeled *CSF-1* probe. (B) Nuclei from the same monocytes used for the studies shown in Fig. 3A were isolated for preparation of ³²P-labeled nuclear RNA (15). Linearized plasmids were run on 1% agarose gels, transferred to nitrocellulose filters, and hybridized to similar amounts of ³²P-labeled nuclear RNA as described in the legend to Fig. 1. The *CSF-1*/actin signal ratio was 0.25 for control monocytes and 0.27 for monocytes treated with CHX for 3 h. Similar findings were obtained in five separate run-on experiments.

over, CHX had no detectable effect on rates of *CSF-1* gene transcription for resting or TPA-treated monocytes. These findings indicated that *CSF-1* gene expression is also regulated at a posttranscriptional level by a labile protein.

Certain cytokines and proto-oncogenes contain AU-rich sequences in the 3' untranslated regions of their mRNAs (2). These genes are expressed transiently, while AU-rich sequences have not been found in genes which have stable patterns of expression (2). These sequences may represent recognition sites for mRNA-processing pathways which degrade specific transcripts (2, 17). Furthermore, the sensitivity of these pathways to CHX has suggested that RNases may be responsible for the instability of these transcripts. For example, transient expression of the *c-fos* gene (15) and the tumor necrosis factor gene (15a) has been shown to be regulated at the posttranscriptional level in human monocytes by labile proteins. Although the *CSF-1* transcript lacks the long AU-rich sequences, several short AU-rich stretches are present (6, 24). These short stretches may, therefore, contribute to the instability of the *CSF-1* mRNA. Taken together, the present findings thus suggest that *CSF-1* gene expression is controlled by both transcriptional and posttranscriptional mechanisms in human monocytes. This control of *CSF-1* gene expression may contribute to the autocrine regulation of monocyte growth and differentiation.

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LITERATURE CITED

- Becker, S., M. K. Warren, and S. Haskill. 1987. Colony-stimulating factor-induced monocyte survival and differentiation into macrophages in serum-free cultures. *J. Immunol.* **139**: 3703–3709.
- Caput, D., B. Beutler, K. Hartog, R. Thayer, S. Brown-Shimmer, and A. Cerami. 1986. Identification of a common nucleotide sequence in the 3' untranslated region of mRNA molecules specifying inflammatory mediators. *Proc. Natl. Acad. Sci. USA* **83**:1670–1674.
- Cleveland, D., M. Lopata, R. MacDonald, N. Cowan, J. Rutter, and M. Kirschner. 1980. Number and evolutionary conservation of alpha- and beta-tubulin and cytoplasmic beta and gamma-actin genes using specific cloned cDNA probes. *Cell* **20**:95–105.
- Horiguchi, J., M. K. Warren, and D. Kufe. 1987. Expression of the macrophage-specific colony-stimulating factor in human monocytes treated with granulocyte-macrophage colony-stimulating factor. *Blood* **69**:1259–1261.
- Horiguchi, J., M. K. Warren, P. Ralph, and D. Kufe. 1986. Expression of the macrophage specific colony-stimulating factor (CSF-1) during human monocytic differentiation. *Biochem. Biophys. Res. Commun.* **141**:924–930.
- Kawasaki, E. S., M. B. Ladner, A. M. Wang, J. Van Arsdell, M. K. Warren, M. Y. Coyne, V. L. Schweickart, M.-T. Lee, K. J. Wilson, A. Boosman, E. R. Stanley, P. Ralph, and D. F. Mark. 1985. Molecular cloning of a cDNA encoding human macrophage-specific colony-stimulating factor (CSF-1). *Science* **230**:291–296.
- Lanotte, M., D. Metcalf, and T. M. Dexter. 1982. Production of monocyte/macrophage colony stimulating factor by preadipocyte cell lines derived from murine marrow stroma. *J. Cell. Physiol.* **112**:123–127.
- Muller, R., J. Tremblay, E. Adamson, and I. Verma. 1983. Tissue and cell type specific expression of two human c-onc genes. *Nature (London)* **304**:454–456.
- Nienhuis, A. W., H. F. Bunn, P. H. Turner, T. Gopal Venkat, W. G. Nash, S. J. O'Brien, and C. J. Sherr. 1985. Expression of the human c-fms proto-oncogene in hematopoietic cells and its deletion in the 5q- syndrome. *Cell* **42**:421–428.
- Oster, W., A. Lindemann, S. Horn, R. Mertelsmann, and F. Herrmann. 1987. Tumor necrosis factor (TNF)-alpha but not TNF-beta induces secretion of colony stimulating factor for macrophages (CSF-1) by human monocytes. *Blood* **70**:1700–1703.
- Pollard, J. W., A. Bartocci, R. Arcenci, A. Orlofsky, M. B. Ladner, and E. R. Stanley. 1987. Apparent role of the macrophage growth factor, CSF-1, in placental development. *Nature (London)* **330**:484–486.
- Ralph, P., M. K. Warren, M. Lee, J. Csejtey, J. Weaver, H. Broxmeyer, D. Williams, E. Stanley, and E. Kawasaki. 1986. Inducible production of human macrophage growth factor, CSF-1. *Blood* **68**:633–639.
- Rambaldi, A., N. Wakamiya, E. Vellenga, J. Horiguchi, M. K. Warren, D. Kufe, and J. Griffin. 1988. Expression of the macrophage colony-stimulating factor and c-fms genes in human acute myeloblastic leukemia cells. *J. Clin. Invest.* **81**:1030–1035.
- Rambaldi, A., D. C. Young, and J. D. Griffin. 1987. Expression of the M-CSF (CSF-1) gene by human monocytes. *Blood* **69**: 1409–1413.
- Sariban, E., R. Luebbbers, and D. Kufe. 1988. Transcriptional and posttranscriptional control of *c-fos* gene expression in human monocytes. *Mol. Cell. Biol.* **8**:340–346.
- Sariban, E., R. Luebbbers, and D. Kufe. 1988. Transcriptional and posttranscriptional regulation of tumor necrosis factor gene expression in human monocytes. *J. Clin. Invest.* **81**:1506–1510.
- Sariban, E., T. Mitchell, and D. Kufe. 1985. Expression of the c-fms proto-oncogene during human monocytic differentiation. *Nature (London)* **316**:64–66.
- Shaw, G., and R. Kamen. 1986. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* **46**:659–667.
- Sherr, C. J., C. W. Rettenmir, R. Sacca, M. F. Roussel, A. T. Look, and E. R. Stanley. 1985. The c-fms proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. *Cell* **41**:665–676.
- Stanley, E., L. Guilbert, R. Tushinski, and S. Bartelmez. 1983. CSF-1: a mononuclear phagocyte lineage-specific hematopoietic growth factor. *J. Cell. Biochem.* **21**:151–159.
- Stanley, E. R., and P. M. Heard. 1977. Factors regulating macrophage production and growth: purification and some properties of the colony stimulating factor from medium conditioned by mouse L cells. *J. Biol. Chem.* **252**:4305–4312.
- Wakamiya, N., J. Horiguchi, and D. Kufe. 1987. Detection of c-fms and CSF-1 RNA by in situ hybridization. *Leukemia* **1**: 518–520.
- Warren, M. K., and P. Ralph. 1986. Macrophage growth factor CSF-1 stimulates human monocyte production of interferon, tumor necrosis factor and colony stimulating activity. *J. Immunol.* **137**:2281–2285.
- Wilson, J., L. Wilson, J. de Riel, L. Villa-Komaroff, A. Efstratiadis, B. Forget, and S. Weismann. 1978. Insertion of synthetic copies of human globin genes into bacterial plasmids. *Nucleic Acids Res.* **5**:563–581.
- Wong, G., P. A. Temple, A. C. Leary, J. S. Wittek-Giannotti, Y. Yang, A. B. Ciarletta, M. Chung, P. Murtha, R. Kriz, R. J. Kaufman, C. R. Ferenz, B. S. Sibley, K. J. Turner, R. M. Hewick, S. C. Clark, N. Yanai, H. Yokota, M. Yamada, M. Saito, K. Motoyoshi, and F. Takaku. 1987. Human CSF-1: molecular cloning and expression of 4-kb cDNA encoding the human urinary protein. *Science* **235**:1504–1508.