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α_2-Macroglobulin Synthesis by the Human Monocytic Cell Line THP-1 Is Differentiation State-Dependent

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Abstract Human α_2-macroglobulin (α_2M) is a broad spectrum proteinase inhibitor and cytokine carrier synthesized by a number of cell types including monocytes and macrophages. In this study, we report on the expression of α_2M by THP-1 cells. This monocytic cell line can be differentiated into a macrophage-like phenotype by treatment with interferon-γ (IFN-γ) or phorbol 12-myristate 13-acetate (PMA). α_2M was synthesized by THP-1 cells at a rate of 75 ng/10^6 cells/24 h, as determined by Western blot analysis. After treating the cells with 500 U/ml of IFN-γ or with 100 ng/ml PMA, the synthesis rate increased to 219 ng/10^6 cells/24 h and to 179 ng/10^6 cells/24 h, respectively. The same agents also increased α_2M expression, as determined by Northern blot analysis. When the α_2M receptor antagonist, receptor associated protein (RAP), was included in the THP-1 medium, the amount of α_2M recovered in the conditioned medium increased. This result suggests that THP-1-secreted proteinases react with secreted α_2M and that the resulting complexes are catabolized by the α_2M receptor, which is also called low density lipoprotein receptor-related protein (LRP). We conclude that α_2M synthesis by THP-1 cells depends on the state of cellular differentiation. Reaction of α_2M with secreted proteinases may have minimized previous estimates of the rate of synthesis of α_2M by certain cells in culture. J. Cell. Biochem. 67:492–497, 1997. © 1997 Wiley-Liss, Inc.

Key words: interferon-γ; PMA; proteinase inhibitor; cytokine; low density lipoprotein receptor-related protein; receptor-associated protein

Human α_2-macroglobulin (α_2M) is a 718 kDa homotetrameric glycoprotein which is synthesized by hepatocytes and found in the plasma at high concentrations (2–5 μM) [Sottrup-Jensen, 1987; Borth, 1992]. In cell culture, α_2M binds to and alters the activity of specific cytokines [Gonias, 1992]. α_2M also functions as a broad-spectrum proteinase inhibitor, reacting with proteinases from all four mechanistic classes [Barrett and Starkey, 1973]. After reaction with proteinases, α_2M is converted into a receptor-recognized conformation and rapidly catabolized by the α_2M receptor, low density lipoprotein receptor-related protein (LRP) [Gonias, 1992].

By neutralizing transforming growth factor-β (TGF-β), α_2M upregulates expression of inducible nitric oxide synthase in macrophages [Lysiak et al., 1995] and platelet-derived growth factor (PDGF) α-receptor in vascular smooth muscle cells [Weaver et al., 1995] in vitro. These activities suggest that α_2M may play a role in the progression of atherosclerosis. Whether α_2M expresses comparable gene regulatory activities in vivo probably depends on the concentration of α_2M which accumulates in the pericellular spaces. In the blood vessel wall and in other tissues, α_2M accumulates due to permeation across endothelial cell barriers or local synthesis. Cell types that may synthesize and secrete α_2M within tissues include adrenocortical cells [Li Shi et al., 1990], astrocytic cells [Gebicke-Haerter et al., 1987; Keohane et al., 1990; Fabrizi et al., 1994], fibroblasts [Mosher and Wing, 1976], and macrophages [Hovi et al., 1977; White et al., 1980].

Synthesis of α_2M by monocytes and macrophages was originally reported by Hovi et al. [1977] and White et al. [1980]. More recently, Bonner et al. [1989, 1989a] demonstrated that α_2M is secreted by alveolar macrophages in culture and binds PDGF which is synthesized by the same cell type. Thus, α_2M which is syn-

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thesized by cells in culture may regulate the ability of those cells to respond to autocrine-synthesized cytokines. Some α-macroglobulins, such as rat α_2M, are acute-phase reactants which are synthesized at greatly increased levels in response to cytokines such as interleukin-6 and interferon-γ (IFN-γ) [Hattori et al., 1987; Wegenka et al., 1993; Yuan et al., 1994]. By contrast, synthesis of human α_2M is generally assumed to be constitutive, although increased α_2M expression has been demonstrated in astrocytoma cells [Fabrizi et al., 1994] and HepG2 [Kordula et al., 1992] cells treated with IFN-γ.

In this study, we examined the synthesis of α_2M by the human monocytoid cell line, THP-1. We found that these cells express substantial levels of α_2M and that the level of expression is regulated by IFN-γ and by phorbol 12-myristate 13-acetate (PMA). Furthermore, our studies demonstrated that receptor-associated protein (RAP), an LRP antagonist, increases the accumulation of α_2M in THP-1 conditioned medium. We propose a model in which α_2M is secreted, activated, and catabolized by a single cell-type in a cell culture system.

METHODS

Reagents

Recombinant human IFN-γ was purchased from Genzyme Diagnostics (Cambridge, MA). PMA was obtained from Sigma (St. Louis, MO). Polyclonal anti-α_2M antibody was from DAKO Corporation (Carpinteria, CA). Probes for Northern blot analysis included an 850 base pair *SacI* fragment of the full length α_2M cDNA obtained from the ATCC and an *EcoRI/XhoI* fragment of the human LRP cDNA obtained from Dr. J. Herz (University of Texas Southwestern Medical Center). RAP is a 39 kDa LRP ligand which blocks the binding of all known ligands, including activated α_2M [Strickland et al., 1991]. RAP was prepared as a glutathione *S*-transferase fusion protein, as described previously [Herz et al., 1991]. Low endotoxin (≤0.1 ng/ml) bovine serum albumin (BSA) was obtained from Sigma.

Cell Culture

The human monocytic cell line THP-1 was obtained from ATCC and cultured in RPMI 1640 (Sigma) with 10% fetal calf serum and 1% penicillin-streptomycin (PS) at 37°C, in 5% CO_2, and 95% humidity. Before performing experiments, THP-1 cells were washed three times with serum free RPMI (SFM) and then incubated in the same medium for 24 h. The cells were transferred to six-well or 24-well plates at a density of 1×10^6 cells/ml in SFM with 0.1% BSA and treated with IFN-γ (500 U/ml), PMA (100 ng/ml), RAP (180 nM), or vehicle. Incubations were allowed to progress for 6, 12, 18, or 24 h before measuring α_2M synthesis.

Western Blot Analysis of α_2M

THP-1 cells were recovered by gentle scraping and separated from conditioned medium (CM) by centrifugation. The cell pellets were retained for RNA isolation. CM was concentrated 10× using Centricon concentrators with 30 kDa exclusion filters (Amicon, Beverly, MA) and subjected to SDS-PAGE. In order to quantitate α_2M in CM, different amounts of purified human α_2M (1–100 ng) were subjected to SDS-PAGE at the same time. After electrotransfer to nitrocellulose (Millipore Corp., Bedford, MA), the membranes were blocked with 5% nonfat dry milk, 0.1% Tween-20 in 20 mM sodium phosphate, 150 mM NaCl, pH 7.4 for 1 h, incubated with polyclonal α_2M antibody (1:1,000 dilution), washed, and incubated with peroxidase-conjugated goat anti-rabbit IgG (Sigma). Secondary antibody was detected by enhanced chemiluminescence (Amersham Corp).

Northern Blot Analysis of α_2M mRNA

Total cellular RNA was isolated from cells by Trizol extraction (Life Technologies Inc., Grand Island, NY). Equal amounts of RNA (20 μg) were then subjected to 1% agarose gel electrophoresis and electrotransferred to Zeta probe membranes (Bio-Rad, Richmond, CA). cDNA probes were labeled with [α-^{32}P]dCTP (Dupont-NEN, Boston, MA) by random oligonucleotide-primed synthesis (Life Technologies, Inc.) and hybridized with membranes at 42°C in 5 × SSPE, 5 × Denhardt's solution, 50% formamide, 0.1% SDS, and 100 μg/ml salmon sperm DNA for 24 h. The membranes were washed twice with 5 × SSPE, 0.5% SDS at room temperature, followed by two washes in 0.1 × SSPE, 1.0% SDS for 20 min each at 65°C. All blots were hybridized with a [α-^{32}P]dCTP-labeled cDNA probe for phosphoglyceraldehyde dehydrogenase (PGAD), as a control for RNA load. Specific hybridization was quantitated by PhosphorImager analysis.

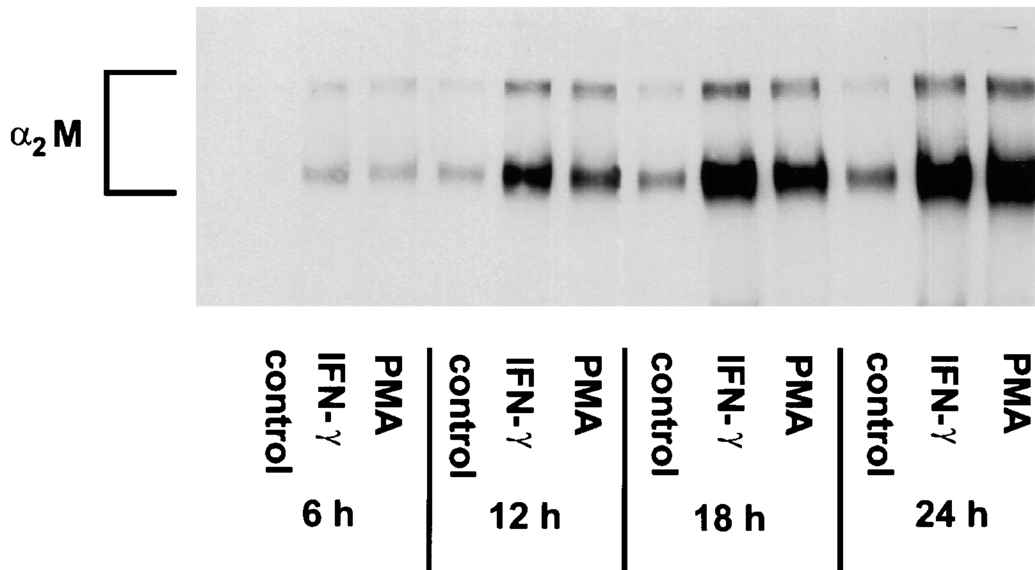

Fig. 1. Representative Western blot of conditioned media from THP-1 cells treated with IFN-γ or PMA. Control cells were maintained in SFM. The time of incubation prior to measuring α_2M is shown.

RESULTS

IFN-γ Up-Regulates Secretion of α_2M

Western blot analysis of THP-1 CM demonstrated that α_2M was synthesized by the cells and accumulated during a 24 h incubation (Fig. 1). The α_2M was recovered in two low mobility bands corresponding to disulfide-linked subunit-dimers and subunit-tetramers [Sottrup-Jensen, 1987]. The latter form of α_2M is preferentially recovered after reaction with proteinase [Wang et al., 1983]. Within 24 h, the concentration of α_2M in the CM was 75 $\pm$ 15 ng/ml (n = 8). This corresponded to a synthesis rate of 75 ng/10^6 cells/24 h.

Cells treated with IFN-γ (500 U/ml) for 12–24 h showed a 2.8–3.0-fold increase in the level of secreted α_2M, compared with control cultures (Fig. 2). At 24 h, the concentration of α_2M in the CM of IFN-γ-treated cells was 219 $\pm$ 16 ng/ml. The IFN-γ-induced increase in α_2M synthesis was highly significant ($P < 0.01$). Treatment of THP-1 cells with PMA, a known stimulator of protein kinase C activity and promoter of THP-1 cell differentiation, also up-regulated α_2M secretion. The level of α_2M which accumulated in PMA-treated cultures was similar to that observed in IFN-γ-treated cultures.

IFN-γ Up-Regulates α_2M mRNA Expression

THP-1 cells that were treated with IFN-γ for 4–24 h expressed increased steady-state levels of α_2M mRNA, as determined by Northern blot analysis (Fig. 3). PMA also increased the level of α_2M mRNA. At 24 h, the increases were 3.1 $\pm$ 0.5-fold and 3.5 $\pm$ 0.7-fold for IFN-γ-treated and PMA-treated cells, respectively (n = 4).

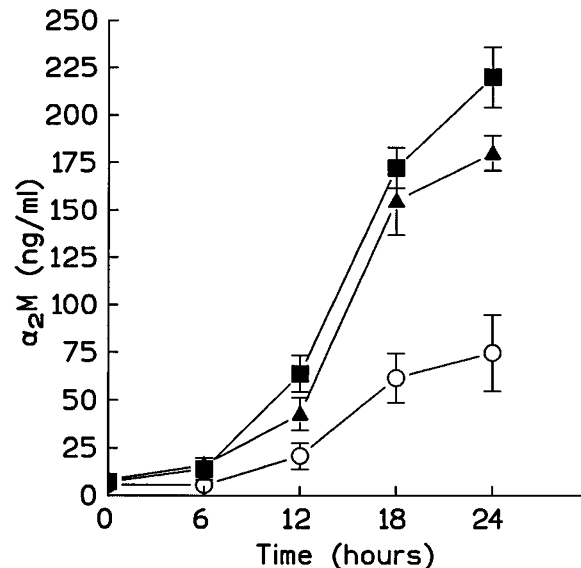

Fig. 2. α_2M levels in the conditioned media of THP-1 cells as detected by Western blot analysis. THP-1 cells were treated with IFN-γ (■), PMA (▲), or maintained in SFM without differentiating agents (○) for up to 24 h. The concentration of α_2M in the conditioned media was then determined (mean $\pm$ SEM, n = 4).

Effects of IFN-γ on LRP mRNA

We previously demonstrated that IFN-γ substantially down-regulates expression of LRP by

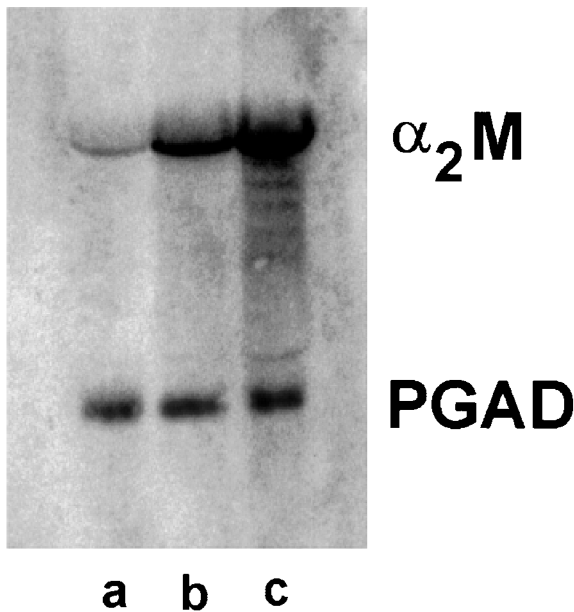

Fig. 3. Representative Northern blot of THP-1 RNA from cells that were treated for 24 h with: (a) SFM; (b) 500 units/ml of IFN-γ; or (c) 100 ng/ml PMA.

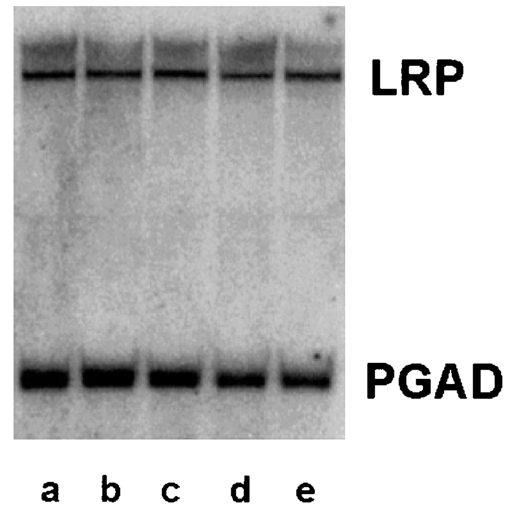

Fig. 4. Northern blot analysis of RNA from THP-1 cells treated with increasing concentrations of IFN-γ for 24 h. The blot was probed for LRP mRNA and PGAD mRNA. The concentrations of IFN-γ were: (a), 0 units/ml; (b), 10 units/ml; (c), 100 units/ml; (d), 500 units/ml; and (e), 1000 units/ml.

the murine macrophage-like cell line, RAW 264.7, and by murine bone marrow macrophages in primary culture [LaMarre et al., 1993]. This result was recently confirmed in studies with human monocyte-derived macrophages [Garner et al., 1997]. Decreased expression of LRP by IFN-γ-treated THP-1 cells could increase the survival of activated α_2M and thus contribute to the increase in the level of α_2M detected by Western blot analysis. To test this hypothesis, we studied the effects of IFN-γ on LRP expression in THP-1 cells. As shown in Figure 4, THP-1 cells express LRP mRNA; however, surprisingly, expression was not regulated by IFN-γ in this cell type. Thus, LRP down-regulation did not contribute to the IFN-γ-induced increase in α_2M accumulation in the CM.

Effects of RAP on the Accumulation of α_2M in THP-1 CM

RAP is a 39 kDa protein which associates with LRP and blocks binding of all other known LRP ligands, including activated α_2M. When THP-1 cells were incubated in RAP-supplemented SFM, accumulation of α_2M in the CM increased 1.7-fold (Fig. 5). In control experiments, RAP had no effect on the level of α_2M mRNA. Furthermore, RAP which was boiled did not affect α_2M accumulation, indicating that

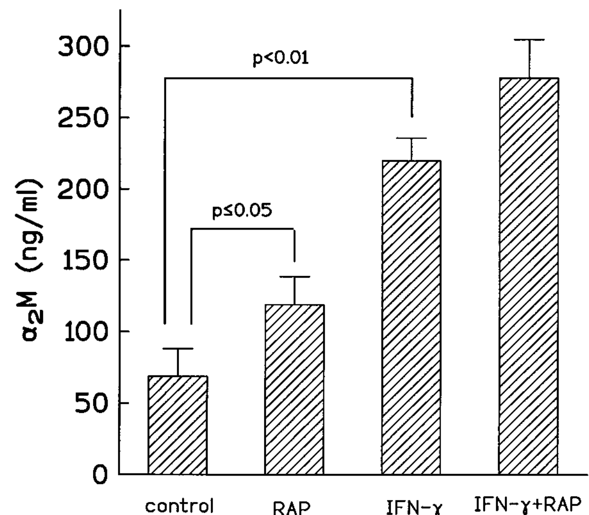

Fig. 5. Effects of RAP on the recovery of α_2M in THP-1 CM. Cells were treated with IFN-γ for 24 h in the presence and absence of RAP or maintained in SFM (control) in the presence and absence of RAP. Recovery of α_2M in the CM was detected by Western blot analysis (mean ± SEM, n = 4).

the activity of RAP was not due to endotoxin contamination.

DISCUSSION

THP-1 cells are human promonocytic leukemia cells which grow in suspension but differentiate into adherant cells with macrophage-like phenotypes when treated with PMA [Tsuchiya et al., 1982] or IFN-γ [Kimball et al., 1995]. The

studies presented here demonstrate that THP-1 cells synthesize α_2M and that the level of expression is increased in response to differentiating agents. Although this is the first report of regulated expression of human α_2M in a monocytoid/macrophage-like cell line, other studies have suggested that differentiated macrophages may express increased levels of α_2M. Hovi et al. [1977] found that α_2M synthesis by adherant peripheral blood mononuclear leukocytes increases with time in culture and is accompanied by apparent morphological transformation of the cells into macrophages. Furthermore, in the studies by Bonner and colleagues [1989, 1989a], rat macrophages, which secreted α-macroglobulin, had been activated by treatment with carbonyl iron spheres.

Most macrophages and monocytoid cells secrete high levels of proteinases [Bjornberg et al., 1995; Malik et al., 1996]. In unpublished studies, we have demonstrated that THP-1 cells express matrix-degrading metalloproteinase-9 (MMP-9) and MMP-2, confirming the work of others [Bjornberg et al., 1995]. Since α_2M is a broad-spectrum proteinase inhibitor, recognizing proteinases from all four major mechanistic classes [Barrett and Starkey, 1973], we hypothesized that α_2M, which is secreted by THP-1 cells might react with THP-1-secreted proteinases. The resulting complexes would then be recognized by LRP and catabolized, thereby decreasing recovery of α_2M in the CM. To test this hypothesis, we supplemented the culture medium with 180 nM RAP. This protein binds to LRP with high affinity (reported K_D values of 1–14 nM) and blocks the binding and catabolism of all other known LRP ligands [Herz et al., 1991; Strickland et al., 1991]. In the presence of RAP, recovery of α_2M in the CM was significantly increased. These data suggest that THP-1 cell-synthesized α_2M reacts with proteinases secreted by the same cells and is then catabolized by LRP.

α_2M accumulation in the CM of IFN-γ-treated THP-1 cells was increased by RAP; however, the increase was not statistically significant ($P > 0.05$). This may have been due to the very high baseline level of α_2M secretion by the IFN-γ-treated cells, so that the fraction of the α_2M reacting with proteinases was decreased compared with the fraction reacting in control cultures. We do not know at this time how IFN-γ affects the expression of various proteinases that react with α_2M.

By Northern blot analysis, we demonstrated that IFN-γ does not regulate LRP expression in THP-1 cells. This distinguishes the THP-1 cell line from other monocyte/macrophage-like cells that have been studied to date, including RAW 264.7 cells, murine bone marrow macrophages, and human peripheral blood monocyte-derived macrophages [LaMarre et al., 1993; Garner et al., 1997]. In the RAW 264.7 cell line, IFN-γ regulation of LRP expression occurs at the level of transcription [Hussaini et al., 1996]. Thus, it is unlikely that THP-1 cell LRP is regulated at a level which is not detected by Northern blot analysis.

In conclusion, we have demonstrated regulated expression of α_2M by a human monocytoid cell line. The α_2M is apparently functional from the standpoint of proteinase inhibition and ability to interact with LRP. Local synthesis of α_2M by macrophages in atherosclerotic lesions and at sites of inflammation may contribute to the regulation of cytokine activity.

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