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Rapid turnover of low density lipoprotein receptor in human monocytic THP-1 cells

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We examined whether human monocyte-derived macrophages had low density lipoprotein (LDL) receptors with a short life span. The human monocytic leukemia cell line, THP-1, was highly differentiated when treated with phorbol ester. LDL receptors degraded rapidly with half-lives of 3–4 h in THP-1 cells before phorbol ester treatment. During the transition into monocytic cells, expression of the LDL receptor gene was not affected. However, relative degradation rates of LDL receptors normalized by those of cellular total proteins were about twice as fast in phorbol ester-treated THP-1 cells compared to untreated cells.

Low density lipoprotein receptor; Human monocytic leukemia THP-1 cell

1. INTRODUCTION

The accumulation of foam cells is an important cholesterol-loaded step for atherosclerosis [1,2]. It has been proposed that human monocyte-derived macrophages store cholesterol ester and form foam cells when exposed to native or modified low density lipoprotein (LDL), but modified LDL forms foam cells more efficiently than native LDL [2–4]. Goldstein et al. [3] have originally demonstrated a relevant hypothesis that macrophages express scavenger receptors which can recognize acetylated LDL but only few receptors by native LDL.

The human monocytic leukemia cell line, THP-1, is a useful model cell for studying foam cell formation. THP-1 cells are differentiated into macrophage-like cells when stimulated with phorbol ester [5]. THP-1 cells degrade native LDL through the LDL receptor, but the differentiated cells degrade chemically modified LDL such as acetylated LDL and lose their ability to degrade the native LDL [6]. The scavenger receptor which binds acetyl LDL is functional in delivering cholesterol to phorbol ester-stimulated THP-1 cells [7]. Since similar levels of native LDL receptor mRNA are observed in THP-1 cells before and after treatment with phorbol ester [8], one could argue that expression of native LDL receptor is decreased in the differentiated human macrophages. The decreased expression of native LDL receptors might be due to posttranscriptional control such as stability of mRNA or metabolic stability of native LDL receptor, rather than transcriptional control.

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We have recently reported that mouse macrophage-like J774.1 cells have metabolically unstable LDL receptors with 2–3 h half-lives [9,10], and the rapid turnover of LDL receptors in macrophages appears to occur after exiting the Golgi apparatus, possibly during transport of the LDL receptor to the plasma membrane [10,11]. In this study, we examined whether this rapid turnover of native LDL receptors is involved in decreased expression in human macrophages derived from THP-1.

2. MATERIALS AND METHODS

2.1. *Cell lines and culture*

Human monocytic THP-1 cells were cultured in RPMI-1640 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 5% fetal calf serum in the presence of 100 μg/ml kanamycin [5].

2.2. *Materials*

[^{35}S]methionine (618 Ci/mmol) and [α-^{32}P]dCTP (6000 Ci/mmol) were from New England Nuclear (Boston, MA); proteinA-Sepharose was from Pharmacia Fine Chemicals (Uppsala, Sweden); phorbol 12-myristate,13-acetate (PMA) was from Sigma Chemical Co. (St. Louis, MO). Lipoprotein-depleted serum (LPDS) and human LDL were prepared by ultracentrifugation using a standard technique [11,12].

2.3. *DNA probes*

Human LDL receptor cDNA was obtained form Drs. D. Russell and J. Goldstein [13,14]. Transforming growth factor-beta (TGF-β) cDNA was kindly donated by Dr. R. Derynck (Genentech, Inc., CA) [15] and human platelet-derived growth factor-A chain (PDGF-A) cDNA was obtained from Dr. C. Betscholtz (Uppsala University, Uppsala, Sweden) [16].

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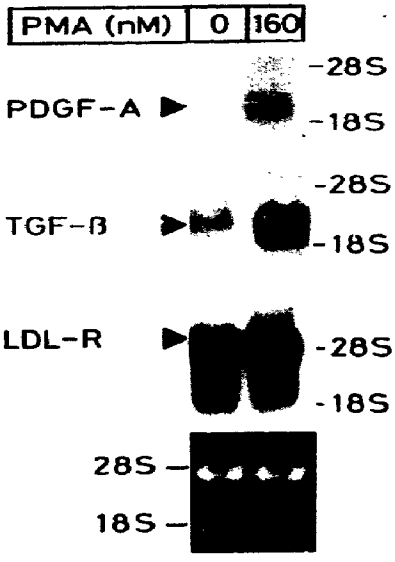

Fig. 1. Effect of phorbol ester on PDGF-A, TGF-β and LDL receptor mRNA synthesis in THP-1 cells. THP-1 cells were treated with 160 nM PMA for 24 h. Total RNA was extracted, fractionated on a 1% agarose gel, and transferred to a Nytron filter. Northern blotting was performed with ^{32}P-labeled PDGF-A, TGF-β, and LDL receptor (LDL-R) cDNA. 28 S and 18 S rRNAs were presented after staining with ethidium bromide.

2.4. *Northern blot analysis*

Northern blot analysis was carried out as previously described [17,18]. Total RNAs were isolated from THP-1 cells treated with or without PMA, dissolved in sterile distilled water, fractionated on a 1% agarose gel containing 2.2 M formaldehyde, then transferred to a Nytran filter (Schleicher and Schuell). The filter was hybridized to various ^{32}P-labeled DNA probes in Hybrisol (Oncor) for 24 h at 42°C. The filter was washed at room temperature in 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0) and 0.1% SDS (sodium dodecyl sulfate), then washed at 65°C in 0.2 ×SSC (0.04 M NaCl, 0.003 M sodium citrate, pH 7.0) and 0.1% SDS. Autoradiography was performed using Kodak XAR film.

2.5. *Metabolic labeling, immunoprecipitation, and polyacrylamide gel electrophoresis of LDL receptor and total cellular proteins*

The cells were incubated in methionine-free MEM containing 5% LPDS and 300 μCi/ml [^{35}S]methionine for 2 h. Pulse-chase experiments were performed as follows. After 2 h of labeling, cells were washed with PBS and incubated in 2 ml RPMI-1640 medium containing 10% LPDS supplemented with 1 mM unlabeled methionine. After

PMA (nM)	0				160			
Chase (hr)	0	1	3	6	0	1	3	6

Fig. 2. Turnover of [^{35}S]methionine-labeled LDL receptor in THP-1 cells treated with or without phorbol ester. After incubation with or without PMA 160 nM for 24 h in THP-1 cells, the cells were pulse-labeled for 2 h with 300 μCi/ml of [^{35}S]methionine then chased for the indicated times. Immunoprecipitated receptors were subjected to SDS-PAGE and fluorography. Arrow indicates the LDL receptor (LDL-R).

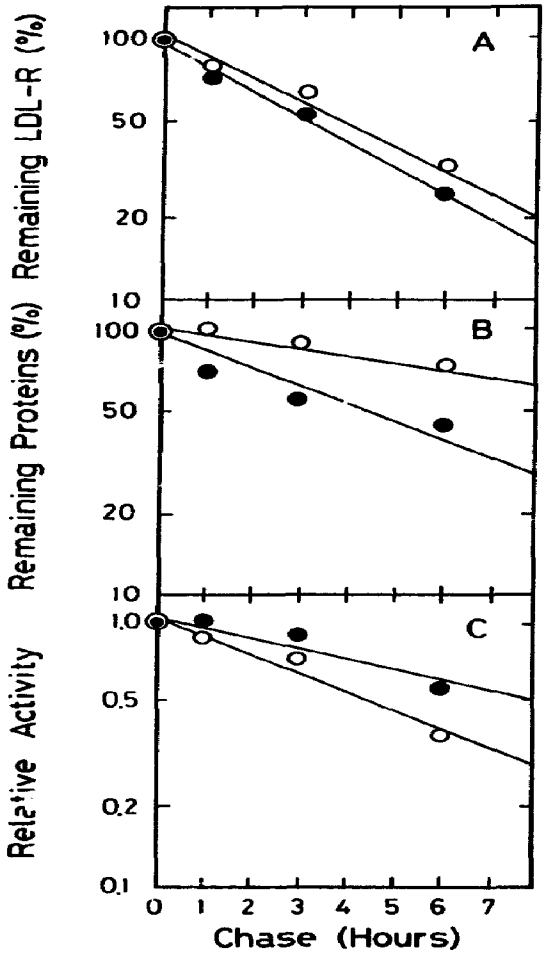

Fig. 3. Comparison of turnover rates of LDL receptor and total cellular proteins before and after adding phorbol ester to THP-1 cells. (A) Quantification of mature LDL receptor remaining at each time as presented in Fig. 2 was plotted by densitometric scanning. Remaining LDL receptor activity was expressed as a percentage of the control value without chase. (B) Cellular proteins labeled for 2 h with [^{35}S]methionine were further chased for various periods, and radioactivity of acid-insoluble fractions was determined. The cellular proteins remaining were expressed as the percentage of control value without chase. (C) Specific activity of the remaining LDL receptor is expressed by dividing each value of the remaining receptor in (A) by that of the remaining cellular protein (B). Untreated (●) and PMA-treated (○) THP-1 cells.

the chase, the cells were solubilized with detergents and LDL receptors were immunoprecipitated with anti-C antibody as described previously [11,12,14]. Immunoprecipitates were electrophoresed on 6% polyacrylamide gels and fluorographed as described previously [12,14]. LDL receptor levels were quantified by densitometric scanning. To follow degradation of total cellular proteins, cells were labeled with [^{35}S]methionine for 2 h, followed by a chase with unlabeled 1 mM methionine as described above. At the indicated periods, the cells were solubilized and the radioactivity in the 10% trichloroacetic acid-insoluble fractions on glass-fiber filters was determined.

3. RESULTS AND DISCUSSION

Human monocytic leukemia THP-1 cells could differentiate into macrophage-like cells in the presence of a phorbol ester such as PMA [5]. Expression of LDL receptors changes during THP-1 cell differentiation [6,7]. A large number of genes are specifically induced as monocytes differentiate into macrophages. Expression of both PDGF-A and TGF-β genes is induced in PMA-treated THP-1 cells [19]. We examined whether cellular mRNA levels of PDGF-A and TGF-β genes were altered during the transition from monocytes to macrophages under our culture conditions. Steady-state levels of PDGF-A and TGF-β mRNA were significantly increased after exposure to PMA for 24 h (Fig. 1). As seen in Fig. 1, steady-state levels of LDL receptor mRNA were not increased when treated with PMA for 24 h. Our results were consistent with a previous report [8].

We then examined metabolic turnover of LDL receptors in THP-1 cells before and after treatment with PMA. Figure 2 demonstrates the apparent expression of native LDL receptors in THP-1 cells before and after treatment with PMA. Figure 2 also demonstrates similar degradation rates of LDL receptors at 3–4 h in THP-1 cells before and after exposure to PMA (see also Fig. 3A). LDL receptors in THP-1 cells are very unstable in comparison with human fibroblasts and other fibroblasts [9,10,20]. It is likely that degradation of cellular proteins in differentiated macrophages proceeds more slowly than that in proliferating monocytes. We compared the degradation rates of the cellular proteins in THP-1 cells before and after PMA exposure. The half-life of cellular proteins in THP-1 cells treated with PMA was about 10 h, whereas that of untreated THP-1 cells was about 5 h (Fig. 3B). The relative degradation rate of the remaining LDL receptor is presented in Fig. 3C. The turnover rates of LDL receptors in THP-1 cells before and after PMA exposure were divided by those of the total cellular proteins. The relative activity of the LDL receptor showed that LDL receptors of PMA-treated THP-1 cells degraded two times faster than those of untreated cells. LDL receptors may be more rapidly transported in the Golgi apparatus of phorbol ester-treated human macrophages, resulting in rapid degradation of the receptors as we previously reported [9,10].

Hara et al. [6] have indicated that the degradation activity of LDL is dramatically abolished in PMA-treated THP-1 cells. However, the precise mechanism for the decreased degradation of LDL is not yet known. In contrast, Auwerx et al. [8] have reported that cellular levels of LDL receptor mRNA are comparable in untreated and PMA-treated THP-1 cells, consistent with our data in Fig. 1. The decreased binding activity of the native LDL in PMA-treated THP-1 cells might be at least partly due to a rapid degradation of the native LDL receptor. Further study is required to understand whether the rapid turnover of the native LDL receptor affects expression of modified LDL receptors in human macrophages.

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