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Transcriptional Activation of the Lipoprotein Lipase Gene in Macrophages by Dexamethasone†

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ABSTRACT: The effect of dexamethasone on lipoprotein lipase (LPL) gene expression during macrophage differentiation was investigated by using the human monocytic leukemia cell line THP-1 and human monocyte-derived macrophages. Addition of dexamethasone to THP-1 cells increased steady-state levels of LPL mRNA and LPL mass accumulation in the medium during PMA-induced differentiation by 4-fold. Studies with human monocyte-derived macrophages showed a similar effect of dexamethasone on LPL expression. Peak LPL mRNA levels were achieved 24-h post-dexamethasone addition to THP-1 cells. Optimal stimulation of LPL mRNA occurred when dexamethasone was added 24 h after induction with PMA. Thereafter, there was rapid decline in responsiveness to dexamethasone. Induction of LPL mRNA in THP-1 cells was completely blocked by actinomycin D, suggesting that induction was transcription dependent. The stability of LPL mRNA was not influenced by dexamethasone. Treatment of THP-1 cells with PMA led to a 2-fold increase in specific binding of dexamethasone and a 4-fold increase in glucocorticoid receptor mRNA within 12 h. Thus, dexamethasone stimulates LPL gene expression during differentiation of human macrophages, a process that involves induction of glucocorticoid receptor synthesis and activation.

Lipoprotein lipase (LPL)[1] is an extracellular triglyceride hydrolase important in lipoprotein and energy metabolism (Eckel, 1989). It is synthesized by many tissues, but most abundantly in muscle and adipose tissue where its regulation has been extensively studied. In these tissues, LPL is regulated by hormones that control energy storage and utilization, such as insulin, catecholamines, glucocorticoids, thyroxin, and growth hormone (Semenkovich et al., 1989; Ong et al., 1988; Friedman et al., 1978; Ball et al., 1986; Robinson & Speake, 1989; Marikawa et al., 1982; Miller et al., 1989; Ailhaud et al., 1986). LPL also is synthesized and secreted by macrophages (Khoo et al., 1981; Chait et al., 1982; Wang-Inverson et al., 1982; Mahoney et al., 1982). In contrast to the many studies concerning regulation of LPL in adipocytes, little is known about the hormonal regulation of LPL in macrophages. In adipose tissue, LPL activity can be stimulated by insulin (Ong et al., 1988; Semenkovich et al., 1989) and depressed by catecholamines (Friedman et al., 1978; Ball et al., 1986). In contrast, insulin does not appear to regulate LPL in macrophages, while catecholamines result in a slight inhibition of LPL activity (Kawakami et al., 1986; Behr & Kraemer, 1986). The human LPL gene has been sequenced (Deeb & Peng, 1989; Kirchgessner et al., 1989), and two sequence motifs homologous to known glucocorticoid regulatory elements have been found in the 5′ upstream region of the human LPL gene, suggesting that glucocorticoids may regulate LPL gene expression. Both stimulatory (de Gasquet et al., 1975;

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[1] Abbreviation: LPL, lipoprotein lipase.

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Ashby & Robinson, 1986) and inhibitory (Krotkiewski et al., 1976; Bagdade et al., 1976; Krausz et al., 1981) effects of glucocorticoids on adipocyte LPL activity have been reported, while dexamethasone has been shown to decrease LPL expression in murine macrophages (Hamosh et al., 1976; Behr & Kraemer, 1986; Goldman, 1990).

In both adipocytes and macrophages, another major stimulus for LPL expression is cellular differentiation. Upon treatment with phorbol esters, the THP-1 human monocytic leukemia cell line has been shown to differentiate into cells with structural and functional characteristics of macrophages. We previously have shown that LPL gene expression in THP-1 cells was under the control of extracellular signals that activate both protein kinase C and the calcium–calmodulin-dependent protein kinase (Auwerx et al., 1989). In the present study, we have used THP-1 cells and human monocyte-derived macrophages to investigate the mechanism of regulation of LPL gene expression by glucocorticoids. Since the process of differentiation leads to a marked induction of LPL production in adipocytes and macrophages, we have investigated the effect of dexamethasone upon LPL gene expression during phorbol ester induced macrophage differentiation.

Materials and Methods

Cell Culture. THP-1 cells were purchased from the American Type Culture Collection and grown at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium (Whitaker) containing penicillin (100 units/mL) and streptomycin (100 μg/mL) and supplemented with 10% fetal calf serum (low endotoxin from Flow Laboratories). PMA (1.6×10^{-7} M; LC Services Corp.) was used to induce differentiation of THP-1 monocytes into macrophage-like cells (Auwerx et al., 1988). Human monocyte-derived macrophages were isolated by the method of Böyum (1968) and incubated in RPMI 1640 medium supplemented with glutamine (580 μg/mL) and 20% autologous serum for 10–14 days prior to use.

Analysis of LPL Mass. Cells were treated with the noted conditions for the times indicated, after which the medium was removed, flash-frozen, and stored at −70 °C until LPL mass was measured (within 14 days) by a sensitive ELISA technique (Babirak et al., 1989). Prior studies in this laboratory have shown the intracellular mass to be <20% of that in the medium. Therefore, only LPL mass that accumulated in the medium was measured.

Analysis of RNA. For each time point, total RNA was isolated from 3×10^7 THP-1 cells or 1×10^7 human monocyte-derived macrophages by the method of Chomczynski and Sacchi (1987), and Northern blot analysis was performed by electrophoresis through 1.2% agarose–formaldehyde gels (Sambrook et al., 1989), followed by capillary transfer to nylon membranes (Hybond from Schleicher & Schuell). Relative mRNA abundance was quantified by dot-blot hybridization on nylon membranes with subsequent densitometric scanning of autoradiographs. RNA was probed with HLPL26, a 1.36-kb *Eco*RI fragment of the human LPL cDNA clone (Auwerx et al., 1988), a 1.5-kb *Kpn*I–*Cla*I fragment of glucocorticoid receptor α [a kind gift of Dr. Ronald Evans (Salk Institute, San Diego, CA)], and a 0.75-kb *Eco*RI–*Hin*dIII fragment of pA1 (a chicken β-actin cDNA clone). All probes were labeled with ^{32}P to a specific activity of approximately 1×10^{-9} cpm/μg using the Boehringer Mannheim random prime DNA labeling kit. All mRNA values were corrected for variations in actin mRNA.

Dexamethasone Binding. Dexamethasone binding was determined in intact cells by the method of Werb et al. (1978). Briefly, THP-1 cells were incubated with PMA in RPMI 1640 medium with 10% fetal calf serum for the times indicated in the individual experiments. Cells were washed with serum-free medium followed by a 30-min incubation in serum-free medium followed by a second wash. Cells then were incubated in RPMI 1640 with varying concentrations of [^{3}H]dexamethasone (Dupont [6,7-^{3}H]dexamethasone; specific activity 44.7 Ci/mmol) for 70 min, washed extensively with phosphate-buffered saline, and then extracted with ethanol. Scintillation counting of the lipid extract was performed in Ecolume (ICN Biomedicals Inc.) in a Beckman LS 7000 Beta spectrometer. Data were analyzed by saturation and Scatchard analysis (Scatchard, 1949).

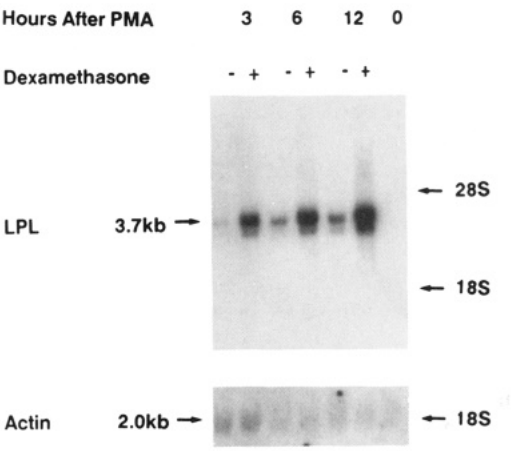

FIGURE 1: Induction of LPL mRNA during macrophage differentiation by dexamethasone. THP-1 cells were induced to differentiate with PMA in the presence or absence of dexamethasone (1×10^{-8} M). At the times indicated after addition of PMA, total RNA was isolated as described under Materials and Methods and subjected to Northern blot analysis. Each lane contained 10 μg of RNA.

Results

Induction of LPL by Dexamethasone. As reported previously (Auwerx et al., 1988), THP-1 cells expressed LPL mRNA during PMA-induced differentiation. Addition of dexamethasone (10^{-8} M) to PMA-treated cells resulted in a superinduction (4-fold) of LPL mRNA steady-state levels (Figure 1), with maximum accumulation occurring at 12 h (Figure 2A). This transient induction followed the same kinetics as those observed with PMA alone. Accumulation of LPL mass in the medium also was markedly stimulated by PMA plus dexamethasone relative to PMA alone (Figure 2B). Increased levels of LPL mass continued for at least 48 h. The difference in timing of peak LPL mRNA and LPL mass indicates both the latency of LPL synthesis and secretion and possibly dissimilar rates of production and degradation of mRNA and mass. No exogenous heparin was added. Superinduction of LPL mRNA and mass by dexamethasone was dose-dependent, showing a maximal increase at 10^{-8} M in THP-1 cells (data not shown). Treatment of human monocyte-derived macrophages with dexamethasone alone increased LPL mRNA by 2–4-fold in various experiments and LPL mass accumulation in the medium by 2-fold (Figure 3).

The time course of the response of differentiating THP-1 cells to dexamethasone was next evaluated. Dexamethasone was added to THP-1 cells at the same time as PMA, or at various times thereafter. The response when dexamethasone was added 24 h after induction with PMA was over 2-fold greater than when dexamethasone was added with PMA (data not shown). Addition of dexamethasone at later times showed less stimulation, indicating that PMA-induced differentiation of THP-1 cells led to the transient expression of a factor that

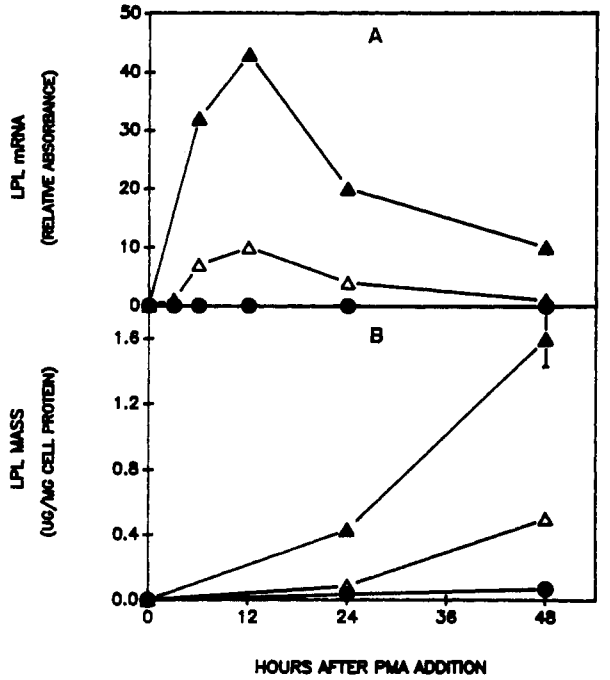

FIGURE 2: Time course of dexamethasone augmentation of LPL expression during THP-1 cell differentiation. THP-1 cells were induced to differentiate with PMA in the presence or absence of dexamethasone (1×10^{-8} M). At the times indicated, total RNA was isolated and subjected to dot-blot analyses, or media were collected for LPL mass measurement as described under Materials and Methods. (A) Time course of LPL mRNA accumulation (normalized to actin mRNA): PMA alone (△); dexamethasone alone (●); or PMA plus dexamethasone (▲). (B) Time course of LPL mass accumulation in media (adjusted for cellular protein). Each point represents the mean ± SD. This is one representative experiment of two.

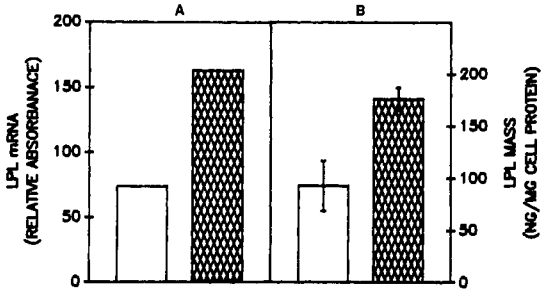

FIGURE 3: Induction of LPL mRNA and mass in human monocyte-derived macrophages by dexamethasone. Fourteen-day-old human monocyte-derived macrophages were incubated in the absence (open bars) or presence (cross-hatched bars) of 1×10^{-8} M dexamethasone for 24 h. Then the media were removed and assayed for LPL mass, and total cellular RNA was extracted for LPL mRNA analysis as described under Materials and Methods. LPL mass is shown as the mean ± SD. This is one representative experiment of three.

mediated maximal glucocorticoid responsiveness. Dexamethasone treatment of both THP-1 and human monocyte-derived macrophages up to 14 days after induction of differentiation continued to stimulate accumulation of LPL mRNA and mass (data not shown).

Induction of LPL by Dexamethasone Is at the Level of Transcription. The increase in the steady-state level of LPL mRNA resulting from treatment with dexamethasone could be due to an increase in either the rate of transcription or the stability of LPL mRNA. No increase in LPL mRNA occurred when actinomycin D was added to differentiated THP-1 cells prior to the addition of dexamethasone (Figure 4A). The effect of actinomycin D on LPL mRNA stability was next determined. When actinomycin D was used to block transcription after maximal PMA induction of LPL mRNA was achieved, the rates of disappearance of LPL mRNA in the presence or absence of dexamethasone were similar (Figure 4B). These results are consistent with transcriptional activation of the LPL gene by dexamethasone.

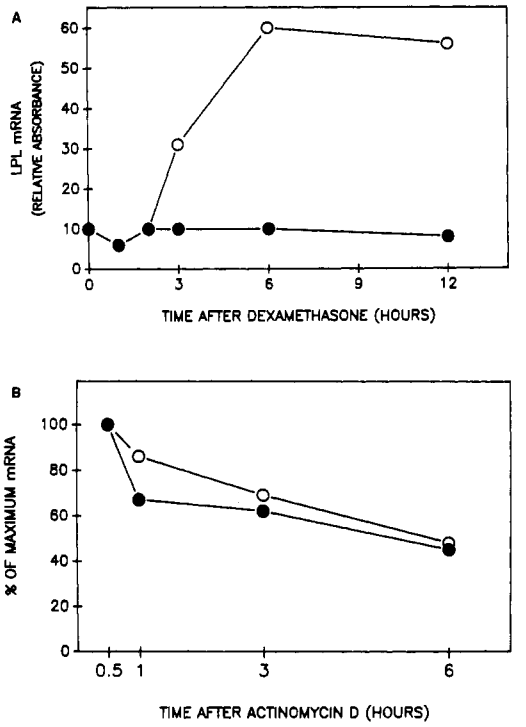

FIGURE 4: Effect of actinomycin D on induction and stability of LPL mRNA. (A) THP-1 cells were incubated with PMA for 24 h. Then 5 μg/mL actinomycin D (●) or carrier (○) was added for 90 min prior to the addition of dexamethasone (1×10^{-8} M). At various time points thereafter, total RNA was isolated, and LPL mRNA was quantitated by dot-blot hybridization. (B) THP-1 cells were treated with PMA (○) or with PMA and 1×10^{-8} M dexamethasone (●) for 12 h prior to the addition of actinomycin D (5 μg/mL). Total RNA was isolated for measurement of LPL mRNA at the time points indicated. This is one representative experiment of two.

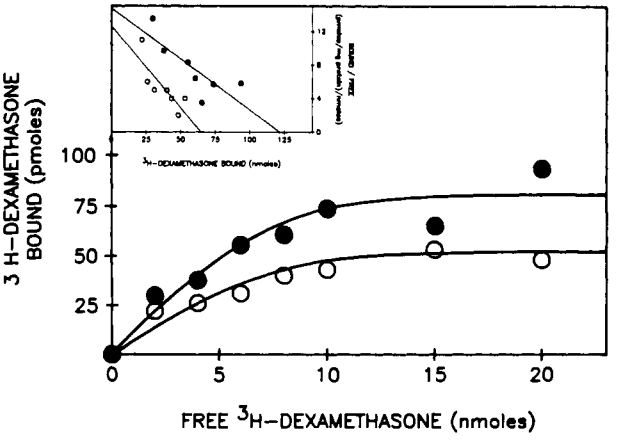

FIGURE 5: Dexamethasone binding during THP-1 differentiation. Cells were treated with PMA and then assayed for [^{3}H]dexamethasone binding at the indicated concentrations at 0 (○) and 24 (●) h as described under Materials and Methods. The inset showed a Scatchard plot derived from these data. This is one representative experiment of four. B_{max} increased by 118 ± 23% ($p < 0.02$ by paired t test).

Induction of the Glucocorticoid Receptor during Cellular Differentiation. To evaluate the possibility that responsiveness of THP-1 cells to dexamethasone resulted from induction of the glucocorticoid receptor by PMA, dexamethasone binding was determined directly in THP-1 cells before and after PMA induction. There was a time-dependent increase in the specific

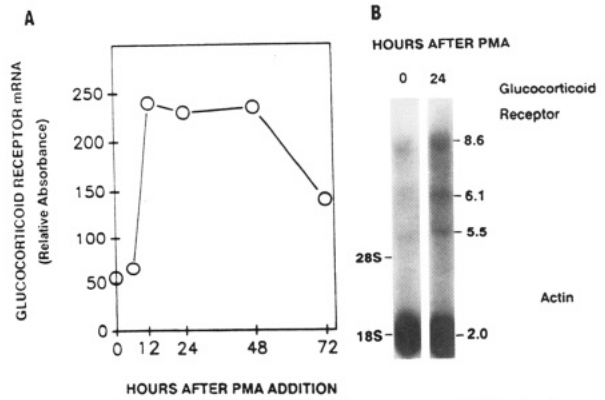

FIGURE 6: Induction of glucocorticoid receptor mRNA during macrophage differentiation. THP-1 cells were treated with PMA, after which total RNA was extracted for evaluation of glucocorticoid receptor mRNA at the times shown. (A) Glucocorticoid receptor mRNA levels (normalized to actin mRNA) as determined by dot-blot hybridization. (B) Northern blot of THP-1 cells that had been exposed to PMA for the indicated time, probed for glucocorticoid receptor and actin mRNA. Each lane contains 30 μg of RNA. This is one representative experiment of three.

binding with an approximate doubling in binding site number (from 13 000 sites at 0 h to 24 000 sites per cell at 24 h) and with a small change in affinity (from a K_d = 5.1 nM at 0 h to K_d = 8.3 nM at 24 h; see Figure 5). This increase in binding sites continued for at least 72 h after exposure of cells to PMA (data not shown). Thus, dexamethasone binding increased in association with increasing dexamethasone responsiveness, but continued beyond the time of maximum responsiveness to dexamethasone. The steady-state level of glucocorticoid receptor mRNA levels also increased more than 4-fold by 12 h after induction of THP-1 cells with PMA (Figure 6). Northern blot analysis showed three species of glucocorticoid receptor mRNA, similar to the results of Hollenberg et al. (1985). These results suggest that increased responsiveness of THP-1 cells to dexamethasone was at least in part due to induction of synthesis of glucocorticoid receptor mRNA and protein.

Discussion

Our results indicate that dexamethasone substantially enhances LPL gene expression during PMA-induced differentiation of the human THP-1 cell into macrophage-like cells. Induction of LPL by dexamethasone was observed in human monocyte-derived macrophages as well, indicating that this effect may occur in vivo. Previously, treatment of murine macrophages with dexamethasone was found to decrease both LPL activity secreted into the incubation medium (Hamosh et al., 1976, 1982; Behr & Kraemer, 1986; Goldman, 1990) and LPL mRNA levels (Goldman, 1990). Studies of the effect of dexamethasone on human macrophages have not been reported previously. These contrasting results may be due to species differences in macrophage lipid metabolism. Rodents do not normally develop atherosclerosis, and arterial lesions that do develop in inbred murine strains do not have the same characteristics of human arteriosclerotic plaques including foam cell accumulation.

We further showed that there was a latent period for the maximal effect of dexamethasone, suggesting the need for production of some factor increasing the cells' ability to respond to glucocorticoids. Therefore, we evaluated the role of the glucocorticoid receptor in mediating the induction of LPL expression by dexamethasone. Glucocorticoid receptor mRNA and the number of glucocorticoid binding sites increased during PMA treatment of THP-1 cells. The finding of an increased number of glucocorticoid receptors in association with differentiation is consistent with the results of Hirai et al. using the HL60 and KG1 myeloid leukemia cell lines (Hirai et al., 1985). However, other investigators using the CM-S human monocyte cell line have found that differentiation of macrophages is associated with decreased glucocorticoid receptor binding (Ranelletti et al., 1986). These differences may be due to the cell lines used, or due to the time of measurement of glucocorticoid binding. Our observation of an increase in glucocorticoid receptor mRNA during differentiation suggests that the increased glucocorticoid binding observed was at least in part due to increased synthesis of glucocorticoid receptors. These findings suggest that induction of the glucocorticoid receptor during macrophage differentiation may be directly involved in the dexamethasone enhancement of LPL transcription. However, glucocorticoid binding was present prior to cellular differentiation (when cells were not responsive to dexamethasone), increased upon PMA treatment, and remained elevated beyond the first 24 h of differentiation, by which time dexamethasone was less effective at stimulating LPL expression. Thus, these results suggest that, in addition to induction of synthesis of glucocorticoid receptors, PMA-induced differentiation involved activation of glucocorticoid receptor molecules perhaps through phosphorylation by protein kinase C which is activated by PMA.

Macrophages appear to play an important role in atherogenesis and are precursors of arterial wall foam cells (Gerrity, 1981). It has been suggested that LPL activity could be involved in lipid accumulation in cells within the atherosclerotic plaque (Zilversmit, 1973; Lindqvist et al., 1983). Since macrophages develop into foam cells early in plaque formation, macrophage LPL may play an important role in the initiation and progression of the atherosclerotic lesion. The role of LPL in macrophage metabolism and that of associated cells within atherosclerotic lesions remain unresolved. LPL is known to be involved in lipid and energy metabolism in muscle and adipose tissues. Extensive in vitro and in vivo studies suggest that the anabolic state is associated with an increase in LPL activity in adipose tissue. Conversely, catecholamines decrease such activity. Glucocorticoid effects on adipocyte LPL remain controversial. Speake et al. have suggested that dexamethasone potentiates the stimulation of LPL activity by insulin (Speake et al., 1986). In muscle, insulin has variable effects on LPL activity possibly depending upon the cellular energy state, while glucocorticoids and catecholamines seem to stimulate LPL activity (Friedman et al., 1978; Miller et al., 1989; Kiens et al., 1989). Thus, in these tissues LPL is regulated by hormones controlling energy metabolism. These hormones may also influence LPL and energy metabolism in macrophages, including those in arteriosclerotic plaques.

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