Transcriptional Activation of the Lipoprotein Lipase and Apolipoprotein E Genes Accompanies Differentiation in Some Human Macrophage-like Cell Lines†

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ABSTRACT: Stimulation of the macrophage-like cell line THP-1 with the phorbol ester phorbol 12-myristate 13-acetate (PMA) resulted in differentiation into cells with many features of macrophages. This differentiation was accompanied by transcriptional activation of the lipoprotein lipase (LPL) and apo E genes and accumulation of their protein products in the media. PMA-induced differentiation of the HEL and HL-60 cell lines was not accompanied by induction of the gene for LPL, whereas the apo E gene was induced slightly in HL-60 cells. By contrast, the gene for superoxide dismutase (SOD-1) was either unaffected (THP-1) or down regulated (HL-60 or HEL cells) by PMA treatment. Induction of LPL mRNA in THP-1 cells was dependent upon the concentration of phorbol ester added. A minimal concentration of 1.6×10^{-8} M PMA was necessary for macrophage differentiation, induction of LPL mRNA, and synthesis of the enzyme. LPL mRNA accumulates within 3 h after stimulation with PMA and attains a maximum concentration after 6 h, thereafter slowly decreasing over the next 3 days. In contrast, the steady-state level of apo E mRNA in the same THP-1 cultures increased gradually over a period of 48 h after induction. These studies thus demonstrate that THP-1 cells are of value as a model to study the quantitative and temporal expression of the LPL and apo E genes during macrophage differentiation.

Macrophages are precursors of arterial wall foam cells and have been suggested to play a pivotal role in the development of atherosclerosis (Brown et al., 1979). The precise mechanism by which macrophages become foam cells is unclear, but cellular lipoprotein receptors have been invoked in this transformation. In contrast to normal low-density lipoproteins (LDL)[1] (Brown & Goldstein, 1983), macrophages internalize chemically modified LDL, such as acetylated or oxidatively modified LDL, by specific scavenger receptors (Goldstein et al., 1979; Via et al., 1985), resulting in massive cholesteryl ester accumulation. The uptake of different very low density lipoproteins (VLDL), including β-VLDL (Goldstein et al., 1980; Innerarity et al., 1986), hypertriglyceridemic VLDL (Gianturco et al., 1982), and normal VLDL (Kraemer et al., 1983) as well as chylomicron remnants (Floren & Chait, 1981), occurs by receptor-mediated mechanisms, frequently resulting in the formation of foam cells in vitro.

Macrophages secrete a variety of biologically active products including coagulation factors, monokines, proteases, and growth factors (Nathan, 1987). Macrophages have been reported to secrete apo E (Kayden et al., 1985; Wang-Iverson et al., 1985), an apolipoprotein believed to be important in local regulation of lipid metabolism by promoting reverse cholesterol transport and by facilitating the interaction of VLDL with lipoprotein receptors on cells (Innerarity et al., 1986). Furthermore, macrophages (Mahoney et al., 1982; Chait et al., 1982) and macrophage-like cell lines (Khoo et al., 1981; Melmed et al., 1983; Tajima et al., 1985) have been shown to secrete the enzyme lipoprotein lipase (LPL), which is responsible for the hydrolysis of triglycerides in VLDL and chylomicrons. LPL-mediated hydrolysis of triglycerides plays a role in the uptake of triglyceride-rich lipoproteins in several cell types including macrophages (Floren et al., 1981; Lindqvist et al., 1983).

The studies reported here address the question of whether phorbol ester induced differentiation into macrophages is accompanied by transcriptional activation of the LPL and apo E genes.

Experimental Procedures

Materials. Culture dishes were obtained from Falcon (Oxnard, CA). Complete RPMI-1640 medium was obtained from M. A. Bioproducts (Walkersville, MD), and fetal calf serum was from Flow Laboratories (Cellect gold, McLean, VA). Phorbol 12-myristate 13-acetate (PMA) was obtained from LC Services Corp. (Woburn, MA). Hybond, nylon membranes for blotting of RNA were from Amersham (Amersham, U.K.), and the random primed DNA labeling kit was from Boehringer Mannheim (Indianapolis, IN).

Cell Culture. HL-60 and THP-1 cells were purchased from the American Type Culture Collection, and the HEL cells were a generous gift from Dr. Thalia Papayannopoulou. Cell lines HL-60 (Collins et al., 1977), THP-1 (Tsuchiya et al., 1980), and HEL (Papayannopoulou et al., 1983) have been described previously and can be induced to differentiate to monocyte-macrophages by treatment with phorbol esters. The transformed cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. These cells were maintained by passage of 2×10^6 cells every 3 days. For most

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[1] Abbreviations: FFA, free fatty acids; LDL, low-density lipoprotein; LPL, lipoprotein lipase; PMA, phorbol 12-myristate 13-acetate; TG, triglyceride; VLDL, very low density lipoprotein; SOD, superoxide dismutase; apo E, apolipoprotein E; ELISA, enzyme-linked immunosorbent assay; kb, kilobase(s); bp, base pair(s); SDS, sodium dodecyl sulfate; DMSO, dimethyl sulfoxide.

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experiments 60×10^6 cells were plated in tissue culture dishes with 147-cm^2 growth area.

Induction of Macrophage Differentiation. For induction, the cells were washed and resuspended in fresh medium containing various concentrations of PMA (freshly prepared from a stock solution of 1.6×10^{-4} M stored at −70 °C). An optimum concentration of 1.6×10^{-7} M was used for subsequent experiments. Control incubations were performed in medium containing 0.1% acetone, the solvent used to dissolve PMA.

Lipoprotein Lipase Activity and Mass Assays. The determination of LPL enzyme activity and mass was performed on 3×10^6 cells per well in six-well multidishes (surface area 9.6 cm^2 each well). The media collected for LPL activity and mass assays were centrifuged at 4 °C, 500*g*, for 10 min to remove cells. The samples were then aliquoted (300 μL) and stored at −70 °C.

To estimate the relative amounts of intracellular and extracellular enzyme in THP-1 cells, culture medium was changed 24 h before assay. The plates were placed for 15 min before the experiment on a slurry of crushed ice and water, at which time half of the medium was removed for assay of LPL activity and mass. Heparin was then added to give a final concentration of 10 units/mL. The remaining medium was collected 15 min after addition of heparin. The difference in LPL mass and activity between the two samples represents the heparin-releasable LPL. After collection of this second medium sample, cells were rinsed twice with cold phosphate-buffered saline (PBS) and cell extracts were prepared as described (Chait et al., 1982).

Lipolytic activity in the media or cell extracts was measured with a triacylglycerol–lecithin emulsion (Iverius & Brunzell, 1985). LPL activity was expressed in nanomoles of free fatty acids (FFA) released per minute per milliliter of medium or cell extract. LPL mass was measured by an ELISA (Scheibel et al., 1987). The LPL ELISA was adapted for use with tissue culture media by preparation of the standards (bovine LPL) in RPMI-1640 medium and by the addition of a blocking step using 3% albumin in PBS on the microtiter plates.

Analysis of RNA. Total cellular RNA from the established cell lines (approximately 60×10^6 cells) and monocyte-derived macrophages (approximately 20×10^6 cells) and from human subcutaneous adipose tissues (50 g) was isolated by the guanidine isothiocyanate–cesium chloride method of Chirgwin et al. (1979). Total RNA was electrophoresed through a 1% agarose–formaldehyde gel (Lehrach et al., 1977), followed by capillary transfer to nylon membranes (Maniatis et al., 1982). The specific mRNA content of the various cell lines was then analyzed by dot blot hybridization using six different quantities (10, 5, 2.5, 0.62, 0.31, and 0.15 μg) of the total cellular RNA (Thomas, 1980).

A human lipoprotein lipase cDNA clone (HLPL26) was used to detect LPL-specific mRNA. HLPL26 is 1.36 kb in length and corresponds to base pairs 271–1630 of the sequence reported by Wion et al. (1987). The clone was isolated in our laboratory by screening a human adipose tissue cDNA library with a bovine LPL cDNA clone, which in turn was isolated from a lactating bovine udder cDNA library with oligonucleotide probes based on the partial amino acid sequence of bovine milk LPL protein fragments (P. H. Iverius, unpublished data). The apolipoprotein E probe is a full-length cDNA probe (isolated in our laboratory from a human liver cDNA library). The superoxide dismutase (SOD-1) probe is a partial cDNA fragment (bp 390–550) derived from clone pS61-10 (Sherman et al., 1983; ATCC 57231). The probes were labeled with [^{32}P]dCTP to specific activities of $(1–2) \times 10^8$ cpm/μg by random primed labeling.

Northern and dot blots were prehybridized for 14–16 h at 42 °C in 50% formamide, 5× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 5× Denhardt's solution [1× Denhardt's = 0.02% each of Ficoll, poly(vinylpyrrolidone), and bovine serum albumin], 0.1% SDS, and 100 μg/mL denatured salmon sperm DNA and then hybridized in the above solution containing 10% dextran sulfate and 10^7 cpm of probe at 42 °C for 16–20 h [a modification of the procedure described in Maniatis et al. (1982)]. Washing of filters after hybridization was done at 65 °C in 0.5× SSC. The filters were then exposed to Kodak XAR film with enhancer screens at −70 °C. The relative concentration of the specific mRNA was determined by densitometric scanning of the autoradiograms and expressed in relative absorbance units corresponding to the area under the curve. The measurements were made within the linear response limits of the X-ray film. Labeled probes were removed from filters by treatment (3–4 times) with boiling distilled water for 10 min prior to hybridization to a second probe.

RESULTS

Induction of Macrophage Differentiation. Induction of macrophage differentiation in THP-1 cells by PMA was characterized by increased adherence of cells to tissue culture dishes (95% of all cells after PMA treatment at 1.6×10^{-7} M during 48 h versus 12% in cells treated with acetone alone), changes in cell morphology, and the enhanced expression of the following macrophage cell surface markers: 5F1 (Bernstein et al., 1982), 60.3 (CDW18; β-chain of the cytoadhesive complex; Beatty et al., 1983), and 4.1 (HLA-DR; Torok-Storb et al., 1983). The minimal dose of PMA necessary to induce partial differentiation into macrophages after 48 h was 1.6×10^{-8} M. Higher doses of PMA (1.6×10^{-7} and 1.6×10^{-6} M) were necessary for complete differentiation within 48 h. We have chosen to use 1.6×10^{-7} M of PMA in subsequent experiments since this is the lowest concentration able to induce complete differentiation.

Induction of LPL mRNA and Protein by PMA. The cell lines HL-60, THP-1, and HEL were tested for their ability to synthesize LPL mRNA and secrete the enzyme into the medium upon induction by PMA (1.6×10^{-7} M during 48 h) to differentiate into macrophages. The results of Northern blot analysis of total cellular RNA and assay of LPL activity in the medium are shown in Figure 1A,B. A dramatic increase in the amounts of LPL-specific mRNA and secreted enzyme activity was observed only in the THP-1 cell line. Four discrete molecular species of LPL mRNA were observed in both THP-1 and adipose tissue cells (approximately 3.75, 3.35, 2.0, and 1.6 kb). The two larger species were more abundant in adipose tissue, while the two smaller species were more abundant in THP-1 cells. As a control the same Northern blot was rehybridized (after removal of the LPL probe) with a labeled cDNA probe for superoxide dismutase (SOD-1). The level of SOD-1 mRNA in THP-1 cells (which is relatively low before induction) does not change upon treatment with PMA (Figure 1C). It is interesting to note however that induction of HEL and HL-60 into macrophages results in a substantial decrease in SOD-1 mRNA (Figure 1C).

Induction of LPL in THP-1 cells is dependent on the concentration of the phorbol ester in the medium. A minimal concentration of 1.6×10^{-7} M PMA was necessary to induce complete macrophage differentiation (measured by cell adhesion) and induction of LPL mRNA and enzyme after 48 h (Figure 2).

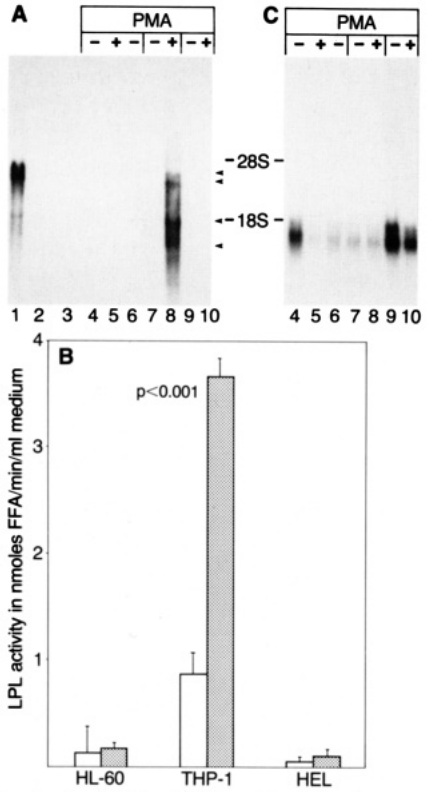

FIGURE 1: Analysis of LPL mRNA and activity in macrophage-like cell lines. (A) Northern blot analysis of LPL mRNA in different cell lines. The macrophage-like cells were grown for 48 h in the presence (+) or absence (−) of 1.6×10^{-7} M PMA. Total RNA (20 μg) was electrophoresed, blotted, and probed according to the procedure described under Experimental Procedures. Lane 1, human adipose tissue; lane 2, $CaCo_2$ cells (a human colon carcinoma cell line; Pinto et al., 1983); lane 3, DNA markers (no RNA); lane 4, untreated HL-60 cells; lane 5, PMA-treated HL-60 cells; lane 6, DMSO (1.2% v/v) treated HL-60 cells; lane 7, untreated THP-1 cells; lane 8, PMA-treated THP-1 cells; lane 9, untreated HEL cells; lane 10, PMA-treated HEL cells. The four discrete molecular species (3.75, 3.35, 2.0, and 1.6 kb) of LPL mRNA are indicated by arrowheads. (B) Accumulation of LPL activity in the culture media of different macrophage-like cells grown in the absence (open bars) or presence (stippled bars) of 1.6×10^{-7} M PMA for 48 h. Lipolytic activity in culture media was estimated as indicated under Experimental Procedures. The bars represent the mean ± SD of three experiments. Only in the THP-1 cells is there a significant difference between uninduced and induced cells. (C) Autoradiograph of the Northern blot described in (A) reprobed with SOD-1 cDNA.

To study the kinetics of accumulation of LPL mRNA and activity, THP-1 cells were exposed to 1.6×10^{-7} M PMA for various lengths of time and assayed for LPL mRNA by dot blot hybridization. LPL mRNA was essentially undetectable before stimulation of the cells with PMA, appeared after 3 h, reached a maximum at 6 h, and then dropped gradually thereafter (Figure 3). A similar pattern of induction was observed when the media in which these cells were grown were assayed for LPL protein (data not shown) or activity (Figure 3A), except that the peak of LPL protein accumulation was at 12 h.

To evaluate intracellular LPL content, all cell surface bound LPL was released by treatment of the cells with heparin. Unstimulated THP-1 cells lack intracellular enzyme, and the enzyme detected in the medium with unstimulated cells was very low (Table I). Only a very limited amount of the LPL enzyme was found in the extracts of induced THP-1 cells (Table I), showing that the majority of the enzyme was secreted. Most of the secreted enzyme was not associated with the cell surface since addition of heparin to the cooled THP-1 cells released only 17% and 19% of the secreted activity and mass, respectively.

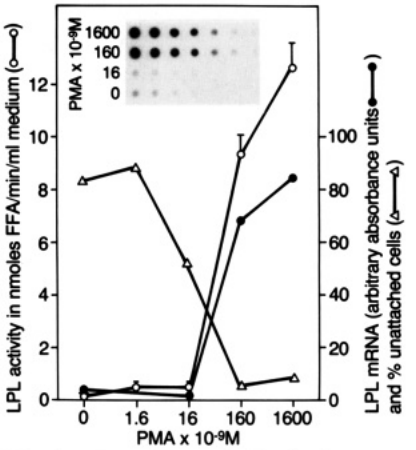

FIGURE 2: PMA dose dependence of cell adhesion, LPL mRNA, and LPL activity accumulation in THP-1 cells. Cells (on quadruplicate dishes each with 20×10^6 cells) were grown for 48 h in the indicated concentration of PMA. Media were then collected for LPL activity assay. Total RNA extracted from three of the four dishes was combined and assayed for LPL mRNA by dot blot hybridization as described under Experimental Procedures. Cell counts of floating and attached cells were performed on the fourth parallel dish. LPL mRNA (●) and LPL activity in the media (○); each point represents the mean ± SD). The extent of attachment of cells to the culture plates is depicted by (△). The inset shows the autoradiograph of the RNA dot blot.

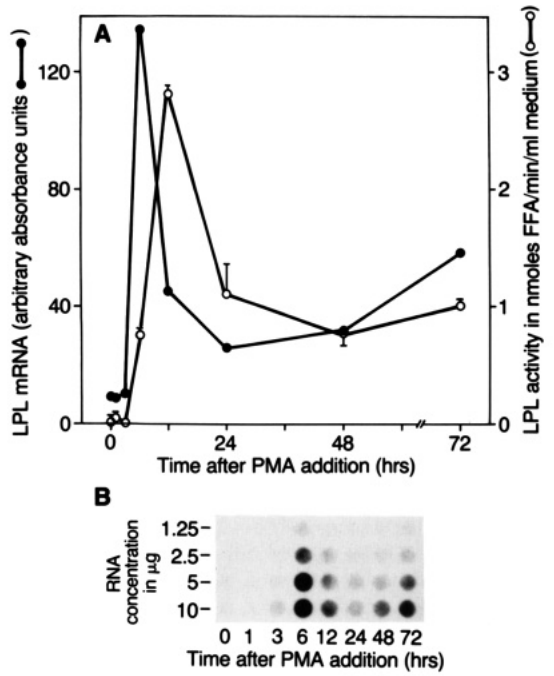

FIGURE 3: Kinetics of accumulation of LPL mRNA and activity upon induction with PMA. (A) Time course of LPL mRNA (●) and LPL activity (○) accumulation in THP-1 cells. Cells (60×10^6) were grown for the indicated time periods in medium containing 1.6×10^{-7} M PMA. Total RNA was isolated as described under Experimental Procedures. Serial dilutions of RNA were dot blotted and hybridized to labeled probe. Media of these cells were collected and assayed for LPL activity. Each time point represents the mean ± SD of three experiments. (B) Autoradiograph of the RNA dot blot. The time after PMA addition and the concentration of RNA spotted are indicated.

Apo E mRNA Accumulation in Macrophage-like Cell Lines. Since apo E has been reported to be secreted by phorbol ester differentiated THP-1 cells (Tajima et al., 1985), we investigated whether this also involved an increase in the

Table I: Intracellular and Extracellular LPL from THP-1 Cells[a]

	LPL activity (nmol of FFA min^{-1} mL^{-1})			
	medium			
	initial	15-min post-Hep	difference	intracellular
no PMA	0.4 ± 0.2	0.3 ± 0.3	−0.1 ± 0.2	0
PMA	13.7 ± 1.0	16.6 ± 1.6	2.4 ± 0.6	2.5 ± 0.2
	LPL mass (ng/mL)			
	medium			
	initial	15-min post-Hep	difference	intracellular
no PMA	8.6 ± 0.9	7.7 ± 0.8	−1.5 ± 0.5	0
PMA	85.2 ± 8.6	104.8 ± 12.2	19.6 ± 3.8	26.6 ± 1.4

[a] Abbreviation: Hep, heparin. The experiment was performed as described under Experimental Procedures on cells cooled on crushed ice and water (4 °C). The results are the mean ± SD of three experiments.

steady-state level of mRNA. No apolipoprotein E specific mRNA was detectable in unstimulated THP-1 cells. However, treatment with PMA resulted in a marked induction of apo E mRNA (Figure 4A). Differentiation of HL-60 cells (but not HEL cells) was accompanied by much less increase in apo E mRNA levels. Unlike the pattern of accumulation of LPL mRNA described above, the induction of apo E mRNA started immediately after PMA addition and continued to increase up to 48 h of incubation (Figure 4B). The dose–response curve was similar to that for LPL (data not shown).

Discussion

In agreement with previous reports, the three cell lines used in this study (THP-1, HL-60, and HEL) assumed macrophage-like characteristics upon induction with phorbol esters (Rovera et al., 1979; Tsuchiya et al., 1980, 1982; Papayannopoulou et al., 1983). Tajima et al. (1985) reported that LPL and apo E accumulate in the media of THP-1 cells upon treatment with PMA. We have shown in this paper that the induction of these proteins by phorbol esters is at the level of mRNA metabolism, involving either transcriptional activation of the genes or increased stability of the mRNA transcripts. The former is believed to be the more likely case, since LPL mRNA levels were extremely low in uninduced cells and reached very high levels within a relatively short period of time (4–6 h) and since phorbol esters were shown to induce transcription of other genes such as the human metallothionein IIA (Imbra & Karin, 1987) and collagenase (Angel et al., 1987). It is interesting that the rapid accumulation of LPL mRNA and the subsequent accumulation of the enzyme in the medium were transient, maximizing at about 6 and 12 h after induction, respectively, and then dropping rapidly to approximately 25% of maximum. The increase in apo E mRNA was linear with time after addition of PMA, reaching a maximum at 48 h.

The difference in abundance of the LPL mRNA species between adipose tissue and THP-1 cells suggests another level of regulation of this protein. The two larger species (approximately 3.75 and 3.35 kb), which are much more prominent in adipose tissue, have been shown to be the result of different polyadenylation sites (Wion et al., 1987). The nature of the other two smaller RNA species (2.0 and 1.6 kb; abundant in THP-1 cells) is unknown at present, although it cannot be totally excluded that these two smaller RNA species are artifacts due to RNA degradation.

The biological significance of this temporal and quantitative regulation of LPL and apo E in macrophage differentiation remains to be determined. Both of these proteins were shown

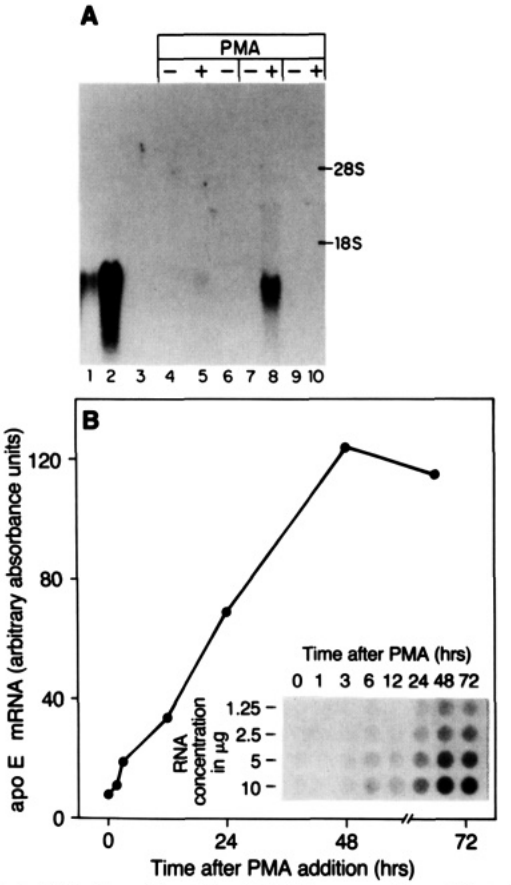

FIGURE 4: Induction of apo E mRNA in macrophage-like cell lines. (A) Northern blot analysis of apo E RNA in different cell lines. The cells were grown in either the presence (+) or absence (−) of 1.6×10^{-7} M PMA. This blot was the same as the one in Figure 1; to strip the LPL probe, the filter was heated 4 times with boiled distilled water. Lane 1, human adipose tissue; lane 2, $CaCo_2$ cells (a human colon carcinoma cell line; Pinto et al., 1983); lane 3, DNA markers (no RNA); lane 4, untreated HL-60 cells; lane 5, PMA-treated HL-60 cells; lane 6, DMSO (1.2% v/v) treated HL-60 cells; lane 7, untreated THP-1 cells; lane 8, PMA-treated THP-1 cells; lane 9, untreated HEL cells; lane 10, PMA-treated HEL cells. (B) Time course of apo E mRNA accumulation as analyzed by dot blotting. Cells were grown for the indicated time periods in 1.6×10^{-7} M PMA. Total RNA was isolated and apo E mRNA was assayed by dot blot hybridization. The quantity of RNA applied per dot is indicated. Inset: Autoradiograph of dot blot.

to be induced as monocytes differentiate into macrophages (Mahoney et al., 1982; Chait et al., 1982; Wang-Iverson, 1985; Werb et al., 1986). The THP-1 cell line, being much faster and easier to grow than monocyte-derived macrophages, therefore offers a unique human model system for investigation of the mechanism underlying regulation of expression of the LPL and apo E genes in relation to differentiation.

Although each of the three cell lines studied can be induced to differentiate into macrophages, they differ widely with respect to induction of both LPL and apo E by PMA. Whereas LPL mRNA is induced in THP-1 cells only, apo E mRNA appears in THP-1 and, to a much lesser extent, in HL-60. SOD mRNA levels, which remain low and unchanged upon treatment of THP-1 cells with PMA, are substantially reduced in HL-60 and HEL cells upon differentiation. These observations are consistent with the view that phorbol esters influence the expression of different genes by activating a common mediator, which in turn modulates the activity of a variety of enhancer-binding transcription proteins (Elsholtz et al., 1986; Comb et al., 1986; Lee et al., 1987; Chiu et al., 1987). Different macrophage-like cell lines may have different subsets of phorbol ester responsive transcription factors. The

three cell lines studied have different clonal origins, THP-1 being derived from a monocyte lineage and the HEL and HL-60 cell lines derived from erythroid and myeloid lineages, respectively.

These studies thus demonstrate that THP-1 cells are of value as a model to study the quantitative and temporal expression of the LPL and apo E genes during differentiation and to determine their potential role in macrophage foam cell formation.

Registry No. PMA, 16561-29-8; LPL, 9004-02-8.

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