Induction of acetyl-LDL receptor activity by phorbol ester in human monocyte cell line THP-1

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Abstract Human monocytic cell line THP-1 incubated with as little as 10 ng/ml of phorbol myristate acetate bound and metabolized 1–2 μg of Ac-LDL over a 5-h period. In the absence of phorbol treatment, no specific metabolism of Ac-LDL occurred. Optimal levels of receptor were reached after 72 h of exposure. Induction of receptor was dependent on protein and RNA synthesis and was partially reversed upon removal of the phorbol. Induction of receptor required activation of the protein kinase C pathway. Metabolism of Ac-LDL by THP-1 cells at 37°C was saturated at 25 μg/ml. Binding at 4°C was saturable with an average K_d of 8.0×10^{-9} M. Cell population studies by fluorescent activated cell sorting indicated that approximately 87% of the THP-1 population was expressing scavenger receptor activity 96 h after phorbol treatment as compared to 99% for murine macrophage cell line $P388D_1$. Uptake of Ac-LDL by THP-1 resulted in an 11-fold increase in the rate of cholesterol esterification which was saturable at 50 μg/ml. Incubation of cells for 48 h with 50 μg/ml of Ac-LDL resulted in a 60% increase in free cholesterol and a 10-fold increase in the cholesteryl ester content of the cells. Lipid accumulation in THP-1 cells after Ac-LDL uptake was readily visible by Oil Red-O staining. Solubilization of THP-1 cells, before and after phorbol treatment, followed by ligand blotting with Ac-LDL detected the presence of a 250-kDa protein only in cells treated with phorbol. The protein comigrated with the scavenger receptor derived from mouse macrophage cell line $P388D_1$. **—Via, D. P., L. Pons, D. K. Dennison, A. E. Fanslow, and F. Bernini.** Induction of acetyl-LDL receptor activity by phorbol ester in human monocyte cell line THP-1. *J. Lipid Res.* 1989. **30:** 1515–1524.

Supplementary key words atherosclerosis • cholesterol • protein kinase C • macrophage

Murine macrophages (1,2), human monocytes (3), and endothelial cells (4) have the capacity to metabolize charge-modified low density lipoproteins through a pathway distinct from that for low density lipoproteins. Uptake of lipoproteins through this pathway can result in significant lipid loading of the cells, suggesting a potential role for this pathway in the formation of the foam cells so characteristic of atherosclerotic lesions in vivo. A protein of approximately 250 kDa has been identified as mediating the uptake of Ac-LDL, MDA-LDL (5,6), and oxidized LDL (7,8) in murine macrophages, bovine endothelial cells, and rat liver tissue. The degree of expression of this receptor is not easily manipulated in vitro. Cholesterol loading through uptake of Ac-LDL does not down-regulate receptor levels in primary murine macrophages, macrophage cell lines, or primary human monocytes (1,3). Dexamethasone has been demonstrated to cause a small increase in receptor activity in human monocytes (9), while lipopolysaccharide prevents the induction of receptor expression that accompanies the differentiation of circulating human monocytes to macrophages in vitro (10). Interferon-gamma has been demonstrated to down-regulate scavenger receptor activity in murine macrophages (Via, D., L. Pons, D. Dennison, A. Fanslow, and F. Bernini, unpublished results).

In a previous study, we attempted to regulate the expression of scavenger receptor activity in human monocytic cell lines HL-60 and U-937 by controlling their state of differentiation by the phorbol ester PMA and supernatants of concanavalin A-stimulated lymphocytes, respectively (2). In neither case did induction of macrophage-like characteristics result in expression of scavenger receptor activity. In this report we have examined the effect of the phorbol ester PMA on scavenger receptor activity in the human cell line THP-1. This human monocytic cell line responds to PMA treatment by dramatic changes in cell morphology with acquisition of macrophage-differentiated functions (11). Specific enzymes and proteins are induced, including tissue transglutaminase (12) and lipoprotein lipase (13),

Abbreviations: Ac-LDL, low density lipoprotein modified by acetylation; MDA-LDL, LDL modified by malondialdehyde; PMA, phorbol- 12-myristate-13-acetate; α-PMA, 4α-phorbol-12-myristate-13-acetate; PDD, phorbol-12,13-didecanoate; α-PDD, 4α-phorbol-12,13-didecanoate; DiI, 1,1-dioctyldecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate; FCS, fetal calf serum; apoE, apolipoprotein E; TCA, trichloroacetic acid.

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and apoE secretion is enhanced (13). We now report that PMA treatment of THP-1 cells results in a dramatic induction of scavenger receptor activity mediated by a 250 kDa protein. Induction of scavenger receptor activity leads to increased cholesterol esterification and cholesteryl ester loading of these cells. Induction of receptor is correlated with exposure to compounds that activate the protein kinase C pathway. While these studies were in progress a preliminary report appeared which indicated that PMA induced the uptake of iodinated Ac-LDL and caused a decrease in LDL receptor activity in THP-1 cells (14).

MATERIALS AND METHODS

Cells and cell culture

THP-1 cells were obtained from the American Type Tissue Culture Collection (TIB 202) and grown in HEPES-buffered RPMI-1640 (Gibco) containing 50 μM 2-mercaptoethanol, 10% FCS (Gibco), and 50 μg/ml Garamycin. Phorbol esters, their alpha derivatives, and mezerein were obtained from L. C. Services (Woburn, MA) and stored in small aliquots in DMSO at −80°C. Medium containing phorbols was made fresh for each addition and each frozen stock aliquot was used a maximum of two times. The final DMSO concentration in the medium was limited to 0.1%. Retinoic acid (all-*trans*) was obtained from Sigma and stored in small aliquots at −80°C in ethanol. Media containing the compound was made fresh for each experiment. Murine macrophage cell line P388D$_1$ was cultured in HEPES-buffered RPMI-1640 containing 10% FCS and 50 μg/ml Garamycin.

Lipoproteins

LDL was isolated from normolipemic plasma by centrifugation in KBr gradients (15). LDL was acetylated by the method of Basu et al. (16) and charge modification was monitored by agarose gel electrophoresis. LDL and Ac-LDL were iodinated by using iodine monochloride (17) and ^{125}I (Isotex) to a specific activity of 100–300 cpm/ng for most experiments. For 4°C binding experiments a specific activity of 400–500 cpm/ng was used. Incorporation of radioactivity into lipid was less than 3%. Incorporation of DiI into Ac-LDL was accomplished as previously described (18).

Lipoprotein metabolism

Cells were incubated with the indicated concentrations of radiolabeled lipoproteins for 5 h at 37°C. At the end of the incubation period, the media was processed for determination of TCA-soluble radioactivity as previously described (2). Cell monolayers were washed and dissolved in NaOH to determine bound plus internalized lipoprotein. Cells in suspension, i.e., not incubated with phorbols or incubated for periods of time too brief to allow full attachment, were washed as described by Ho et al. (19) prior to being dissolved in NaOH. Protein content was determined by the method of Lowry et al. (20).

Cholesterol esterification and cholesterol content

Cholesterol esterification rates were measured in cells after 7 days in PMA, followed by an additional 24 h in the presence of 50 μg/ml Ac-LDL. Cells were incubated with 10 mM [^{14}C]oleic acid–BSA complexes for 5 h. The amount of label incorporated into cholesteryl ester was determined after extraction and thin-layer chromatography as previously described (2). Free and total cholesterol quantities were measured fluorimetrically by the method of Gamble et al. (21) using *p*-phenylhydroxy acetic acid as an indicator in hexane–isopropanol 3:2 extracts of cells. Fluorescence was monitored on an SLM Instruments Model 800 fluorimeter in the photon counting mode.

Fluorescence-activated cell sorting and microscopy

Cultures incubated with and without PMA were incubated for 5 h with 20 μg/ml of DiI Ac-LDL (18). At the end of the incubation period, cells were released from the growth surface by incubation with 0.2% EDTA at 37°C for 10 min, followed by gentle scraping with a rubber policeman. Cultures of murine macrophage cell line P388D$_1$ were incubated with DiI-Ac-LDL in parallel. Cells were examined for the distribution of fluorescence on an Ortho Series 50-H cytofluorograph at a flow-rate of 1,000 cells/min. The probe was excited with the 518 nm line of an argon laser. Fluorescence emission was regulated with a 630 nm long pass filter and forward light scatter was used to discriminate cells from debris. For fluorescent microscopy, cells on coverslips were incubated with DiI-Ac-LDL as above and fixed in 3% formaldehyde in PBS. Coverslips were examined on a Leitz fluorescence microscope equipped with a standard rhodamine filter cube.

Oil Red-O staining

Cultures were incubated for 5 days with 50 ng/ml PMA followed by 24 h in medium containing 50 μg/ml Ac-LDL. Cells were fixed and stained with Oil Red O as previously described (2).

Receptor solubilization and purification

THP-1 cells, 1×10^9, were incubated for 5 days with 50 ng/ml PMA. At the end of the incubation period these cells, and an equivalent number of untreated THP-1 cells, and 3×10^8 P388D$_1$ cells were solubilized in 1% TX-100. Scavenger receptor was partially purified by application to a PEI-cellulose column (5) followed by affinity chromatography on Mal-BSA Sepharose. Receptor was eluted with 1.0

M NaCl and electrophoresed on a 6% polyacrylamide slab gel (5). Receptor proteins were electrophoretically transferred to nitrocellulose and active receptor was detected by blotting with Ac-LDL as previously described (5).

RESULTS

To initially examine the effects of phorbol ester on scavenger receptor expression, cells ~1 × 10^6 cells/35-mm plate, were incubated in the presence and absence of 400 ng/ml of PMA for 48 h. These conditions have been demonstrated to halt cell growth and to induce cell to substrate attachment and accompanying morphological and biochemical changes in THP-1 cells (11). Specific degradation of Ac-LDL was not detectable in cells grown in the absence of phorbol, but in the presence of 0.1% DMSO used to solubilize the phorbols. In contrast, PMA-treated cells degraded significant quantities of Ac-LDL (**Fig. 1**). In the three experiments averaged in Fig. 1, the amount of Ac-LDL metabolism was 920 ± 85 ng/mg protein in a 5-h period. Levels of DMSO as high as 1% did not affect the metabolism of Ac-LDL after treatment with phorbols and did not induce the expression of Ac-LDL metabolism in cells not treated with phorbols. Degradation of Ac-LDL was competitively inhibited by a 20-fold excess of Ac-LDL but not LDL. Metabolism of Ac-LDL was independent of the final cell density over the range of 0.3–4.0 × 10^6.

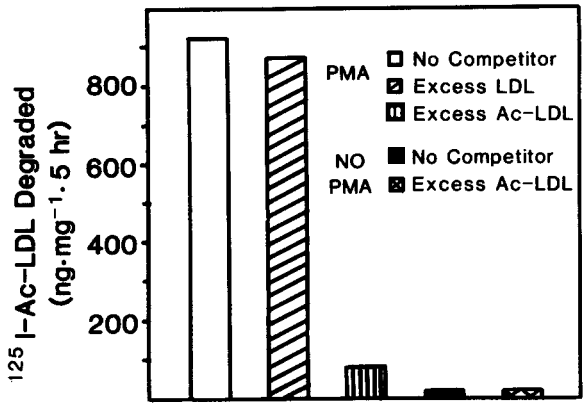

Fig. 1. Induction of Ac-LDL metabolism in THP-1 cells. THP-1 cells, 1 × 10^6, were incubated for 48 h in medium containing 200 ng/ml of PMA. Unattached or dead cells were removed by aspiration of the medium, and fresh medium containing PMA and 20 μg/ml of ^{125}I-labeled Ac-LDL and, where indicated, a 20-fold excess of unlabeled LDL or Ac-LDL was added. Cultures were incubated for 5 h and the media and attached monolayer were processed for determination of degraded and internalized radioactivity as detailed in Materials and Methods. The data represent the average of three experiments with triplicate determinations for each variable in each experiment. The standard deviations for the determinations were from left to right in the figure: ± 75, ± 78, ± 15, ± 10, ± 12. The difference in metabolism between cells treated with phorbol and those untreated was statistically significant at $P < 0.001$.

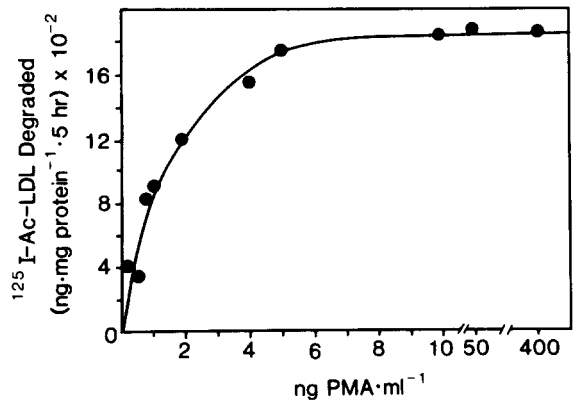

Fig. 2. Dose dependence of PMA induction of Ac-LDL metabolism. THP-1 cells, 1 × 10^6, were plated in the presence of various concentrations of PMA. At the end of 5 days, ^{125}I-labeled Ac-LDL was added to the medium to a concentration of 20 μg/ml in the presence or absence of a 20-fold excess of unlabeled Ac-LDL. After 5 h, the medium was removed and spun to recover any cells that were not attached at the lower doses of PMA. The medium was then processed for the determination of degraded lipoprotein as detailed in Materials and Methods. Attached cells and cells pelleted from the medium were washed as described in Materials and Methods and digested in NaOH for determination of bound plus internalized lipoprotein and for determination of total (suspended plus attached) protein. Metabolism in the presence of excess Ac-LDL has been subtracted. The data represent the average of three experiments with triplicate determinations of each point. The standard deviation was ± 9% or less for each point shown.

The dose dependence of scavenger receptor expression was examined after 5 days over a concentration range of 0.1–400 ng/ml (1.6 × 10^{-10} M–6.4 × 10^{-7} M) PMA. As demonstrated in **Fig. 2**, small amounts of specific degradation were detected with as little as 0.4 ng/ml of PMA. At a level of 10 ng/ml PMA, degradation of ^{125}I-labeled Ac-LDL was saturable and remained virtually unchanged over the range of 10 ng/ml–400 ng/ml. Morphological changes, comprising attachment to the substratum and spreading, were most pronounced above 10 ng/ml PMA. The total amount of degradation of Ac-LDL at saturating levels of PMA varied over the course of 13 months from approximately 900 to 2300 ng/mg protein in a 5-h incubation. However, levels for experiments conducted within 2 to 3 weeks of each other using the same batch of cells varied no more than approximately 10%. The time course of induction of scavenger receptor activity was also examined over a 120-h period of exposure to both 50 ng/ml or 200 ng/ml PMA. At either concentration, specific metabolism of Ac-LDL was maximal at approximately 72 h after PMA addition (**Fig. 3**). There was no significant change in this time course throughout the course of our experiments.

The affinity of the receptor for Ac-LDL was examined by incubation of cells at 4°C with ^{125}I-labeled Ac-LDL. Cells were plated at an initial density of 5 × 10^6 cells per 35-mm plate to achieve a final cell density after 5 days in phorbol of approximately 4 × 10^6 per plate. Cells were incubated for 2 h with the indicated amount of labeled lipoprotein in

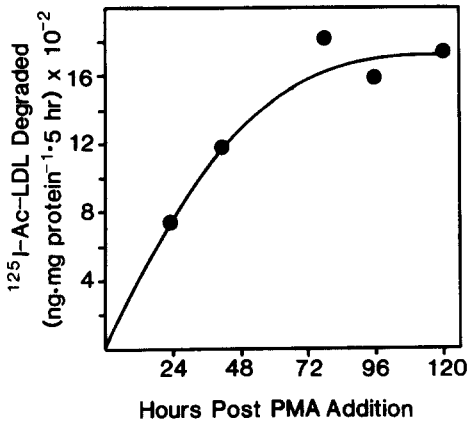

Fig. 3. Time course of induction of Ac-LDL activity. THP-1 cells, 1 × 10^6/plate, were incubated with 50 ng/ml of PMA. At the indicated times, ^{125}I-labeled Ac-LDL was added to a concentration of 20 μg/ml. After 5 h, the media were processed for determination of degraded lipoprotein and cells were washed for determination of internalized lipoprotein and total protein. The data represent the average of three experiments with triplicate determinations of each time point. Nonspecific degradation in the presence of a 20-fold excess of unlabeled lipoprotein has been subtracted. The standard deviation was ± 9% or less at each point measured.

the presence or absence of a 20-fold excess of unlabeled lipoprotein at each point. Under these conditions specific binding was saturable at 6–10 μg/ml (**Fig. 4**) over four experiments. Scatchard analysis indicated a single binding site with an average affinity of 8.02 ± 0.77 × 10^{-9} M. On the basis of these data, each cell has approximately 1500 receptors/cell. When the binding and degradation of Ac-LDL in PMA-treated THP-1 cells was examined at 37°C, saturation occurred between 25 and 50 μg/ml. The time course of degradation was also examined. Specific degradation was detectable within 2 h after addition of lipoprotein and was linear up to 24 h (not shown).

The requirements for the length of continuous phorbol exposure necessary for achieving maximal expression of the scavenger were examined by replacing the PMA medium with normal medium at various intervals. Each sample set was then assayed for scavenger receptor activity 72 h post-reversal. As seen in **Fig. 5**, reversal after 24, 48, or 72 h resulted in approximately a 36% drop in metabolism as compared to the nonreversed control. During the reversal period, a partial reversal of morphology was also noted. Neither the cell number nor the protein content of the plate changed after removal of the phorbol.

The response of the cultures to phorbol treatment appeared to be heterogeneous in that some cells changed morphology completely within 4 h while others required the full 72 h in phorbol to maximize morphological changes. Even after 1 week in culture, not all cells appeared to have totally changed morphology. We therefore examined the population distribution of scavenger receptor activity by incubating phorbol-treated cells with DiI-Ac-LDL followed by fluorescence-activated cell sorter analysis (**Fig. 6**). In a typical experiment, cells treated with 5 ng/ml PMA, a concentration that did not maximize receptor expression, had a mean fluorescent intensity of 277 with 48% of the population being positive for Ac-LDL uptake (Fig. 6, lower left panel). Cells treated with 10–400 ng/ml were 83% positive with a mean fluorescent intensity of 516 (Fig. 6, upper right panel). A positive control population of P388D_1 cells was determined to be 99% positive for Ac-LDL uptake with a mean fluorescence of 525 (Fig. 6, upper left panel). Over the course of three experiments, the percentage of cells positive for Ac-LDL uptake was 44 ± 3.3% at 5 ng/ml PMA, 83 ± 9.2 at 400 ng/ml, and for the P388D_1 cells 98.5 ± 47. In the absence of PMA treatment in all experiments, THP-1 cells had a background level of fluorescence (Fig. 6, lower right panel). Visual inspection of DiAc-LDL-labeled cells by fluorescence microscopy confirmed the presence of nonfluorescent cells in the PMA-treated cultures (**Fig. 7 a,b**). These data suggest that not all cells in this population are capable of responding to PMA by induction of scavenger receptor metabolism. In addition, the cell sorter data suggest that increased metabolism of Ac-LDL, seen as a function of PMA dosage, represents an increase in both the number of positive cells and the degrees of receptor expression.

Metabolism of Ac-LDL by mouse macrophages and primary human monocytes through the scavenger receptor results in increased rates of cholesterol esterification and cholesteryl ester loading of the cells (1–3). In contrast, endo-

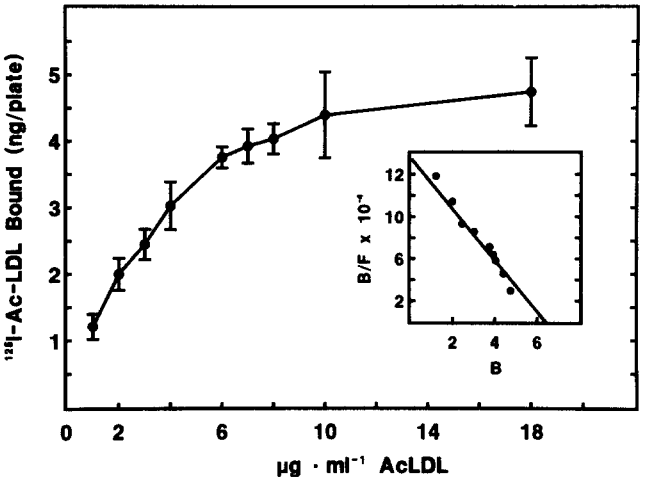

Fig. 4. Saturation binding of Ac-LDL to THP-1 cells. THP-1 cells, 4 × 10^6/plate, were incubated with 50 ng/ml of PMA for 72 h. Cells were cooled to 4°C and then incubated with increasing concentrations of ^{125}I-labeled Ac-LDL in the presence or absence of a 20-fold excess of unlabeled Ac-LDL to determine specific binding. At the end of the incubation period, cultures were washed and cells were dissolved in NaOH for protein determination. The specific binding data represent the average of four experiments with the standard deviations indicated by the error bars. The inset shows the Scatchard plot of the mean of the averaged data.

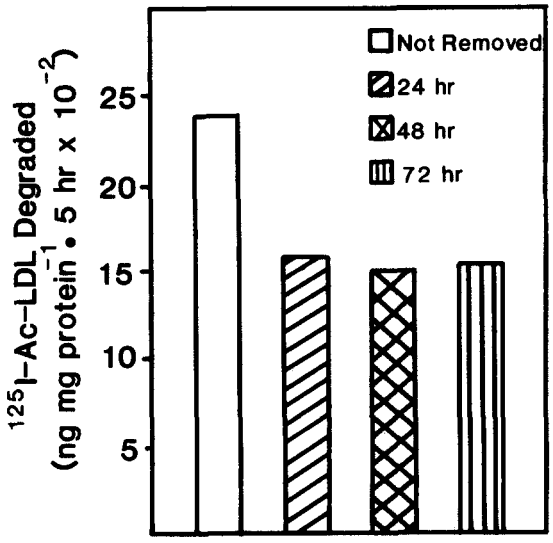

Fig. 5. Effect of phorbol removal on maximal expression of Ac-LDL activity. THP-1 cells were incubated with 50 ng/ml PMA for the indicated periods. The medium was removed, cells were washed, and medium without PMA was added. After an additional 72 h, with one medium change at 48 h, cells were placed in medium containing 20 μg/ml Ac-LDL for 5 h. Degradation of lipoproteins, cell-associated lipoprotein, and total cell protein were determined. Degradation in the presence of a 20-fold excess of lipoprotein has been subtracted. The data represent the average of two experiments with triplicate determinations at each time point. The degree of reversal after 24 h was statistically significant compared to the nonreversed control at the $P < 0.01$ level. There was no statistical difference between the degree of reversal seen at 24–72 h. The standard deviations were, from left to right in the figure, ± 214, ± 126, ± 135, ± 143.

thelial cells exhibit very poor cholesteryl ester accumulation through the scavenger pathway (4,22). To determine cholesteryl ester accumulation of THP-1 cells, cultures were incubated with PMA for 5 days followed by a 24-h incubation with various concentrations of Ac-LDL. When the rate of incorporation of [^{14}C]oleic acid into cholesteryl ester was examined, a saturable increase in esterification rates was observed (**Fig. 8a**). At saturation, an 11-fold increase was observed as compared to PMA-treated cells in the absence of any Ac-LDL. PMA treatment itself was observed to cause a 32% decrease in the rate of endogenous cholesterol esterification in medium containing FCS or lipoprotein-deficient serum (not shown). Free and esterified cholesterol were determined fluorimetrically in PMA-treated cells following a 48-h incubation with various concentrations of Ac-LDL. Free cholesterol rose a total of 1.3-fold while esterified cholesterol rose 10.4-fold (Fig. 8b). The increased cholesteryl ester content was reflected in the increased number of Oil Red-O staining lipid droplets (Fig. 7c) observed as compared to PMA-treated cells incubated in the absence of any Ac-LDL (Fig. 7d).

The induction of scavenger receptor activity in THP-1 cells is dependent on new protein synthesis. When cells are allowed to attach for 4 h in the presence of PMA and cycloheximide is then added to a concentration of 5 μM, a 70% reduction in scavenger receptor activity at 24 h post-addition of PMA was noted. RNA synthesis also seems to be required. Cells exposed to actinomycin D, 0.2 μM, after a 4-h attachment period in PMA, showed an 80% decrease in Ac-LDL metabolism when assayed 24 h post-PMA exposure. Attachment of cells to the substratum was not sufficient to induce receptor synthesis. Cells attached to poly-lysine-coated plates of Cell-Tack-coated plates showed no increase in scavenger receptor activity after 24 h. Induction of scavenger receptor activity in our population of THP-1 cells also appears to be specific for the inducting agent. A 96-h exposure to retinoic acid, an inducer

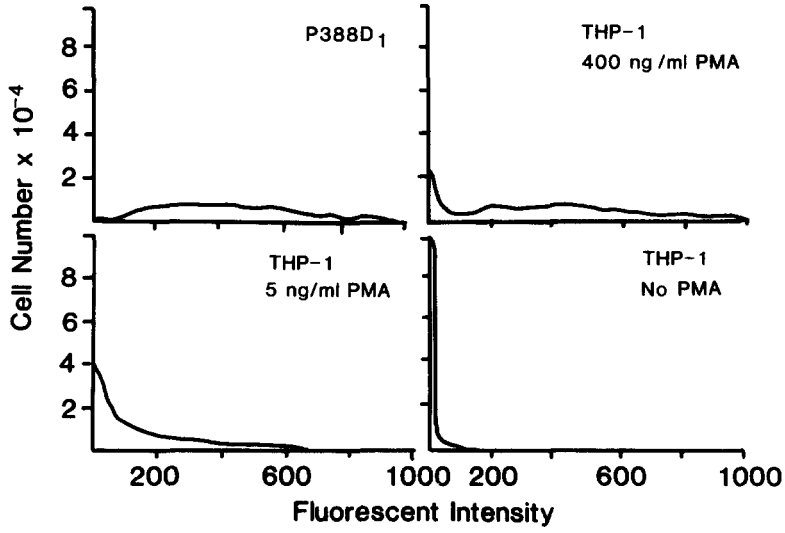

Fig. 6. Fluorescence-activated cell sorter analysis of Di-Ac-LDL uptake in PMA-treated THP-1 cells. Cultures of THP-1 cells, treated for 96 h with 5 ng/ml PMA (lower left panel) or 400 ng/ml PMA (upper right panel) as well as untreated THP-1 cells (lower right panel) and P388D$_1$ cells (upper left panel), were incubated with 20 μg/ml Di-Ac-LDL for 5 h. Cells were removed from the growth surface and analyzed for fluorescence on an Ortho Series 50-H cytofluorograph. Data represent the results of a typical experiment. Similar profiles were observed in two additional experiments.

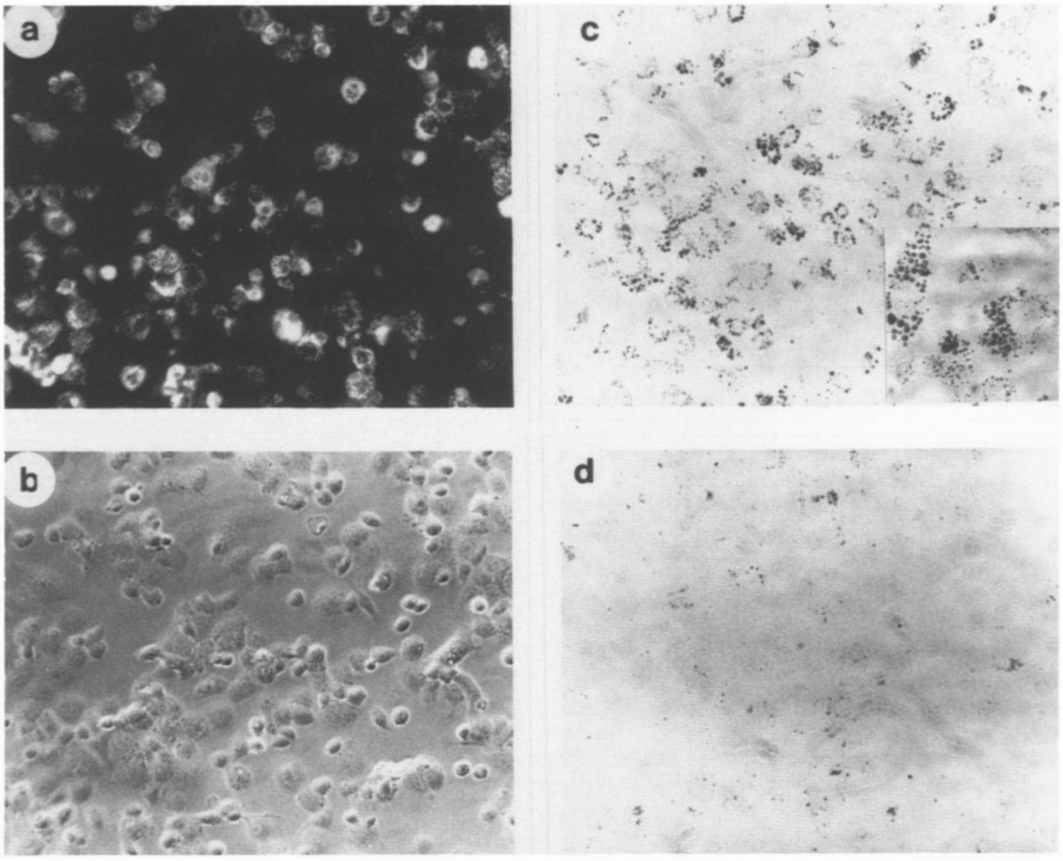

Fig. 7. Visualization of Ac-LDL uptake and lipid droplets in THP-1 cells after phorbol treatment. THP-1 cells grown in the presence of 50 ng/ml PMA for 72 h were incubated with 10 μg/ml DiI-Ac-LDL for 5 h at 37°C. Cells were fixed and examined for fluorescence (7a) and phase contrast morphology (7b). THP-1 cells grown in the presence (7c) or absence (7d) of 50 μg/ml PMA for 72 h were further incubated with 50 μg/ml of Ac-LDL for 48 h. Cells were then examined for lipid inclusions by Oil Red O staining. The results are typical of those observed in four separate experiments.

of differentiated functions in some populations of THP-1 cells (23,24), induced only 2% of the maximal scavenger receptor activity seen in PMA-treated cells.

Phorbol esters bind to and activate protein kinase C in cell membranes and tissues (25–28). In addition, phorbol esters can have direct effects on membrane structure through their hydrophobic acyl side chains (29–31). To determine the role of each of these features of phorbol esters in the activation of scavenger receptor metabolism, a number of phorbol ester and nonphorbol derivatives were examined. As demonstrated in **Table 1**, both PMA and a more hydrophobic phorbol, PDD, induced scavenger receptor activity. In contrast, the 4-alpha derivatives, which virtually match the parent compounds in hydrophobicity but do not activate protein kinase C, were not capable of inducing scavenger receptor activity. A potent nonphorbol activator of protein kinase C, mezerein, was as effective as the phorbol esters in inducing Ac-LDL metabolism in THP-1 cells.

In order to identify the induced protein that binds Ac-LDL in THP-1 cells, cultures were detergent-solubilized and the receptor was partially purified for ligand blotting. Extracts from THP-1 cells with and without PMA treatment were electrophoresed under nonreducing conditions and their corresponding Western transfers were probed for Ac-LDL binding proteins. In the absence of PMA treatment, no Ac-LDL-binding proteins were detected in THP-1 extracts (**Fig. 9**). After 72 h of PMA treatment, a prominent band of Ac-LDL binding at 250,000 daltons was detected in the THP-1 extract from an equivalent number of cells. The band comigrated with the high molecular weight Ac-LDL binding protein extracted from the murine macrophage cell line $P388D_1$.

DISCUSSION

THP-1 cells, originally derived from a human monocytic leukemia (11), respond to PMA treatment by converting from a suspension cell line to one that is adherent and spread. This conversion is accompanied by the acquisition of many monocyte/macrophage characteristics including phagocytosis (11), and enhanced secretion of lipoprotein lipase and apoE (13). The latter two products are also involved

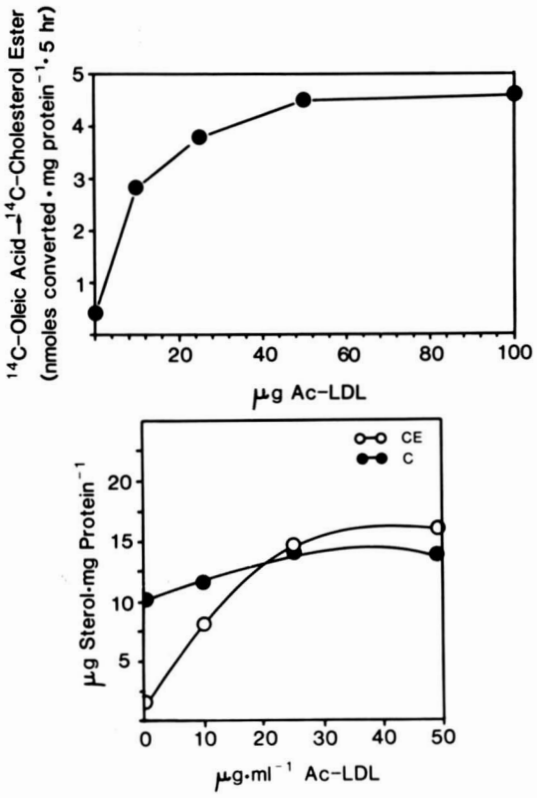

Fig. 8. Increased cholesterol esterification and accumulation in PMA-treated THP-1 cells after Ac-LDL uptake. A: THP-1 cells were incubated with 50 ng/ml PMA for 72 h. Cells were then incubated with fresh PMA containing medium supplemented with various concentrations of Ac-LDL for 24 h. [^{14}C]Oleic acid-BSA complexes were added to a final concentration of 10 mM oleate and cultures were allowed to incubate for 5 h. Lipids were extracted from the cells and incorporation into cholesteryl ester was quantitated by thin-layer chromatography as described in Materials and Methods. The data are the average of three experiments with duplicate plates for each experiment. The standard deviation for all points was 11.5% or less. B: THP-1 cells were incubated for 72 h in medium containing 50 ng/ml PMA and then incubated an additional 48 h in fresh PMA medium containing the indicated amount of Ac-LDL. Lipids were extracted and the free and total cholesterol contents were measured fluorometrically. Cholesteryl ester content was calculated by subtracting the free cholesterol content from the total cholesterol content. The results are the average of three separate experiments with triplicate determinations of each point. The standard deviation was 9.5% or less for each point.

in the metabolism of lipoproteins. In this report we demonstrate the induction in THP-1 cells by PMA of a 250,000 dalton protein that has been previously demonstrated in murine macrophage cells to mediate the uptake and metabolism of Ac-LDL. Metabolism of Ac-LDL was demonstrated to result in increased rates of cholesterol esterification and cholesteryl ester loading of the cells in the form of cytoplasmic lipid droplets. These results confirm and extend the preliminary report of Hara et al. (14), published while the present work was in progress. These workers reported enhanced metabolism of ^{125}I-labeled Ac-LDL in these cells following PMA treatment.

TABLE 1. Induction of scavenger receptor activity by phorbols and nonphorbol protein kinase C activators

Addition	Lipoprotein Degradation
	ng · mg^{-1} · 5 h
None	3 ± 1
PMA	1302 ± 36
α-PMA	21 ± 2
PDD	1688 ± 128
α-PDD	4 ± 2
Mezerein	1701 ± 189

Cells were incubated for 96 h in medium containing 8.1 × 10^{-8} M of each compound. Fresh medium containing 20 μg/ml ^{125}I-labeled Ac-LDL and each compound was added to cultures and allowed to incubate for 5 h. Media were processed for determination of degraded lipoproteins as detailed in Materials and Methods. The data reflect the average of two experiments, with triplicate plates for each point. Nonspecific degradation occurring in the presence of a 20-fold excess of Ac-LDL has been subtracted.

Although PMA can have direct effects on membranes (29–31), it also activates the transcription of a number of genes including those for lipoprotein lipase and apoE (32), as well as those for collagenase (33) and human metallothionen (34). The appearance of scavenger receptor most

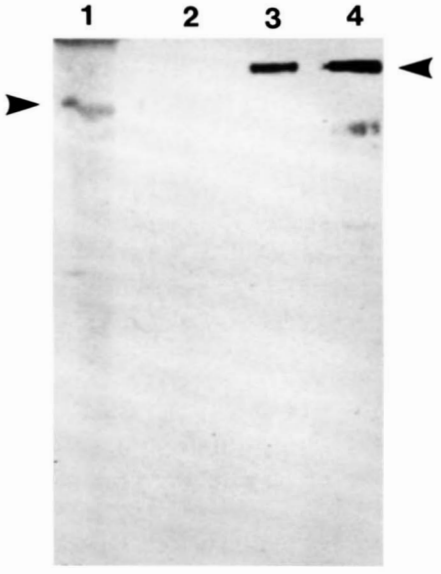

Fig. 9. Ligand blot analysis of detergent extracts from THP-1 cells. THP-1 cells grown in the presence or absence of PMA and a positive control consisting of P388D$_1$ cells were detergent-extracted, and the extracts were passed through a PEI-cellulose ion exchange column and a Mal-BSA Sepharose column to partially purify the scavenger receptor. The NaCl eluates from the column were electrophoresed on 6% PAGE slab gels, transferred to nitrocellulose, and the blots were probed for Ac-LDL binding proteins. Lane 1, prestained myosin molecular weight marker; lane 2, extract equivalent to 5 × 10^8 THP-1 cells grown in normal medium; lane 3, extract equivalent to 5 × 10^8 THP-1 cells grown in the presence of 50 ng/ml PMA for 72 h; lane 4, extract equivalent to 1.5 × 10^8 P388D$_1$ cells. The arrow denotes the position of scavenger receptor. The results are typical of those seen in two separate experiments.

likely reflects induction of expression of the receptor gene. No metabolism of Ac-LDL could be detected in the uninduced cells. In addition, ligand blotting of solubilized uninduced cells did not detect any significant intracellular pools of receptor protein that could be recruited to the cell surface. Preliminary experiments have also demonstrated the dependence of the induction on actinomycin D as well as cycloheximide, so both messenger RNA and protein synthesis seem to be required for expression of activity.

It would also appear that induction of the scavenger receptor in THP-1 cells requires events secondary to activation of the protein kinase C pathway and not just simple interaction of phorbols with the plasma membrane lipid matrix. This is supported by the inability of the 4-alpha derivatives of both PMA and PDD to induce activity, even though both retain the hydrophobic characteristics of the parent compounds required for any direct interaction with membrane lipids.

There is some degree of heterogeneity of expression of receptor within the THP-1 population. Regardless of the length of exposure to PMA, a certain portion of the population was negative for receptor as judged by cell sorting analysis and by fluorescence microscopy. No obvious correlation between the type of morphology displayed, i.e., adherent and round or adherent and spread, and expression of receptor was noted. Heterogeneity in morphology and expression of macrophage functions after PMA treatment have also been noted by others (35). It is also clear that different results after PMA treatment may be obtained depending on the parameter being measured, the inducing agent, and source of the THP-1 cells. With regard to Ac-LDL metabolism, Hara et al. (14) reported that maximal activity was obtained after 24 h in PMA. Mehta and Lopez-Berestein (12) reported complete morphological differentiation of THP-1 cells after as little as a 15-min exposure to PMA. In our studies, maximal scavenger receptor activity and morphological differentiation were consistently obtained only after a 72-h exposure to PMA.

The observation that partial reversibility of both morphology and Ac-LDL metabolism could occur upon removal of phorbol was surprising. Differentiation of other human premonocytic cell lines, such as HL-60, is considered to be a terminal, irreversible differentiation. We considered the possibility that some cell growth resumed after phorbol removal, leading to increased amounts of protein on the plate and thus a reduction in the amount of metabolism reported on a protein basis. However, we found no evidence for reversal of the phorbol-induced growth inhibition on the basis of cell numbers or for any increase in the amount of protein per plate. We can only speculate that a portion of our cells are not fully committed to differentiation after phorbol treatment. This would appear to be a reflection of the heterogeneous nature of the population and the multiple intracellular steps involved in phorbol induction of differentiation.

In contrast to some previous studies that report the induction of specific proteins in THP-1 cells by both PMA and retinoic acid (12), retinoic acid failed to induce scavenger receptor activity in our THP-1 cultures even after prolonged exposure. In agreement with Hemmi and Britman (24), but in contrast to the report of Mehta and Lopez-Berestein (12), no changes in cell morphology and very little change in cell adherence were noted even after 5 days in retinoic acid. Whether or not clones responsive to retinoic acid for scavenger receptor induction can be isolated from the ATTC stocks remains to be determined.

Hara et al. (14) also reported that PMA caused a marked reduction in LDL receptor activity in THP-1 cells after PMA treatment. We and others have noted a similar phenomenon with human monocytic cell lines HL-60 (2) and U937 (2,36). A recent report also suggests that PMA reduces LDL metabolism in human skin fibroblasts (37). Thus, events associated with protein kinase C activation may play a role in regulating the receptor-mediated metabolism of both normal and abnormal LDL in appropriate systems. The precise mechanisms of these PMA effects on lipoprotein receptors remain to be elucidated.

Under the binding conditions utilized in this study, a single binding site for Ac-LDL was detected at 4°C. However, after these studies were concluded, Haberland et al. (38) provided evidence for the existence of both low and high affinity binding sites for MDA-LDL on mouse peritoneal macrophages utilizing a broader ligand concentration than used in our studies. In addition, Arai et al. (39), using peritoneal macrophages, provided evidence suggesting two sites of identical affinity for Ac-LDL and both a low and a high affinity receptor for oxidized LDL. These authors concluded that peritoneal macrophages had one receptor capable of binding both ligands and two additional receptors, one specific for Ac-LDL and one specific for oxidized LDL. We, therefore, cannot rule out the presence of additional binding sites on the THP-1 cells. Further experimentation with different ligands and ligand concentration ranges will be required to assess the complexity of modified LDL binding to THP-1 cells.

The availability of a continuous human cell line in which scavenger receptor activity can be induced will be invaluable in further studies of receptor synthesis and in comparative studies with the murine receptor. In addition, it will be a useful system for studying regulation of expression of the scavenger receptor gene at the molecular level as probes for such studies become available.

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