

THP-1 cells form foam cells in response to coculture with lipoproteins but not platelets

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Abstract The human monocytic leukemia cell line, THP-1, shares many properties with human monocyte-derived macrophages and might be a useful model for studying foam cell formation in vitro. Therefore, we examined the ability of THP-1 cells to accumulate cholesteryl esters, the hallmark feature of foam cells, in response to culture with native low density lipoprotein (LDL), modified LDL, and platelets. THP-1 cells stored more cholesteryl esters than macrophages in response to 200 $\mu\text{g/ml}$ of LDL. Down-regulation of LDL receptors occurred in macrophages at lower LDL concentrations than in THP-1 cells. Phorbol ester-treated THP-1 cells stored more cholesteryl esters than human macrophages in response to 25–200 $\mu\text{g/ml}$ of acetylated LDL. Because we have previously demonstrated that activated platelets enhanced macrophage cholesteryl ester storage, we examined the ability of THP-1 cells to store cholesteryl esters in response to coculture with platelets. Compared with macrophages, dividing THP-1 cells and phorbol ester-treated THP-1 cells accumulated only 50% and 33% as much cholesteryl esters, respectively. Furthermore, although platelets induced a 90% reduction in cholesterol synthesis in macrophages by day 5, cholesterol synthesis in THP-1 cells and phorbol ester-treated THP-1 cells was inhibited less than 50% by platelets. Nevertheless, both THP-1 cells and macrophages responded to platelets by increasing their secretion of apolipoprotein E. **■** Therefore, we conclude that dividing THP-1 cells and phorbol ester-treated THP-1 cells are capable of forming foam cells in response to physiologic doses of both LDL and acetylated LDL, respectively. However, they are restricted in their ability to form foam cells in response to coculture with human platelets. —Banka, C. L., A. S. Black, C. A. Dyer, and L. K. Curtiss. THP-1 cells form foam cells in response to coculture with lipoproteins but not platelets. *J. Lipid Res.* 1991. **32**: 35–43.

Supplementary key words monocyte • macrophage • LDL • acetylated LDL • phorbol ester • cholesterol

The accumulation of cholesteryl ester (CE)-laden foam cells is a striking characteristic of early atherosclerotic plaque formation. Foam cells in early lesions are thought to derive from peripheral blood monocytes that have migrated to the subendothelial space (1). Modified lipoproteins have been implicated as a major source of cholesterol for CE storage by foam cells in vivo (2). In

vitro, human monocyte-derived macrophages have been induced to store CE by exposure to both native and modified low density lipoprotein (LDL) (3–5). However, the isolation of human monocytes from whole blood is tedious, time-consuming, and expensive. Furthermore, the effects of donor variability and low levels of contamination with other cell types cannot be eliminated. Therefore, we wished to identify a human monocyte-like cell line to use as an in vitro model for macrophage foam cell formation.

The human monocytic leukemia cell line, THP-1, was chosen for study because it is a highly differentiated monocytic cell line that takes on macrophage-like characteristics when stimulated with phorbol ester (6, 7). The presence of LDL and scavenger receptors on THP-1 cells has been reported (8, 9). Very recently, Via and coworkers (10) have reported that the scavenger receptor, which binds acetyl-LDL (aLDL), is functional in delivering cholesterol to phorbol ester-stimulated THP-1 cells. However, the role of both the LDL receptors and the scavenger receptors in cholesterol delivery to the cells has not been thoroughly described. Thus, THP-1 cells were examined for their ability to store CE in response to native or modified LDL. In addition, because we have recently demonstrated that human monocyte-derived macrophages form foam cells in vitro when cultured in the presence of human platelets (11–13), we have studied the effects of platelets on THP-1 cell CE storage as well. The parameters measured to assess foam cell formation included cholesterol esterification, accumulation of CE mass, cholesterol synthesis, and secretion of apolipoprotein (apo) E.

Abbreviations: PBM cells, peripheral blood mononuclear cells; LDL, low density lipoprotein; CE, cholesteryl ester; aLDL, acetylated LDL; PRP, platelet-rich plasma; PMA, phorbol myristate acetate; apoE, apolipoprotein E; TLC, thin-layer chromatography; ACAT, acyl coenzyme A:cholesterol acyltransferase.

MATERIALS AND METHODS

Lipoproteins

LDL was isolated by density gradient ultracentrifugation in the presence of protease inhibitors, antioxidants, and anti-bacterial agents as described (11, 14). In addition, we have added 25 $\mu\text{g/ml}$ Polybrene, 20 $\mu\text{g/ml}$ lima bean trypsin inhibitor, 100 KIU/ml aprotinin (Sigma Chemical Co., St. Louis, MO), and 1–2 μM D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) (Calbiochem Corp., San Diego, CA). Density of the LDL fraction was 1.019–1.063 g/ml . Acetylated LDL was prepared by treatment of LDL with acetic anhydride as described by Basu and coworkers (15). Protein and cholesterol concentrations of LDL and aLDL were determined by a modification of the Lowry method (16) and a fluorometric enzymatic assay (17), respectively. LDL preparations were used within 1 month of isolation.

THP-1 cells

THP-1 cells were obtained from the American Type Culture Collection and maintained in suspension in T-150 culture flasks (Costar, Cambridge, MA) at a cell density of $2.0 \times 10^5/\text{ml}$ to $1.0 \times 10^6/\text{ml}$ in 100 ml of RPMI-1640 containing 7% fetal calf serum (Rehatuin F. S., Armour Pharmaceutical, Kankakee, IL), 10 mM HEPES, 1 mM glutamine, Pen-Strep (penicillin, 200 U/ml; streptomycin, 2 mg/ml; Sigma Co., St. Louis, MO), 1 mM sodium pyruvate, and 5×10^{-5} M β -mercaptoethanol. Individual experiments were conducted under serum-free conditions by washing the cells three times and culturing them in the same medium in which the 7% fetal calf serum was replaced with 1% Nutridoma-HU (Boehringer Mannheim Biochemicals, Indianapolis, IN). Cells were seeded at $2\text{--}3 \times 10^5$ cells in 1 ml per well in 24-well tissue culture dishes (Costar, Cambridge, MA). Cells cultured in the presence of phorbol myristate acetate (PMA) (10^{-7} M) (Sigma Chemical Co., St. Louis, MO) were seeded at a density of 5×10^5 cells in 1 ml per well.

Macrophage and platelet isolation

Human monocyte-derived macrophages and platelets were isolated as described (11, 18). Fresh human blood was drawn into 5 U/ml of heparin and centrifuged at 400 g for 15 min at 22°C to separate the platelet-rich plasma (PRP) from the cells. The PRP was removed and stored overnight at 22°C. The peripheral blood mononuclear (PBM) cells were isolated from the cell pellet remaining after removal of the PRP by resuspending the cells in RPMI medium and layering them over Ficoll-Hypaque. After centrifugation at 900 g for 20 min at 22°C, the PBM cells were removed from the interface, washed twice, and resuspended in RPMI containing 1% Nutridoma-HU, 10 mM HEPES, 2 mM glutamine, 1 mM sodium pyruvate, and

antibiotics. The PBM cells were incubated overnight at 37°C in 5% CO_2 –95% air at 5×10^6 cells in 1 ml/well of 12-well plastic culture plates (Costar, Cambridge, MA).

The following day platelets were sedimented from the PRP by centrifugation at 1,000 g for 20 min and were resuspended in Tyrode's buffer for two additional washes. After the final wash, the platelets were resuspended in RPMI-1640 containing 1% Nutridoma-HU, allowed to disaggregate for 2–3 h, and adjusted to a concentration of $5 \times 10^8/\text{ml}$. The nonadherent cells were removed from the PBM cultures by aspiration and several washes. The adherent cells (macrophages) were refed with 1.0 of RPMI medium containing 1% Nutridoma-HU and platelets or lipoproteins.

Metabolic labeling

Macrophages, dividing THP-1 cells, and differentiated (PMA-stimulated) THP-1 cells were cultured for 3 to 7 days in serum-free RPMI medium in the presence or absence of platelets or lipoproteins. Fourteen hours before harvest, [^3H]oleate or [^{14}C]acetate was added to the cultures. The [^3H]oleate (New England Nuclear, Boston, MA) was added as a 4.5:1.0 (oleate:albumin) molar complex at a final concentration of 200 μM oleate with a specific activity of 25 mCi/mM. Each 1.0 ml culture received 5 μCi of [^3H]oleate. The [^{14}C]acetate (New England Nuclear, Boston, MA) was added at a final concentration of 500 μM acetate with a specific activity of 5 mCi/mM. Each 1.0 ml culture received 3 μCi of [^{14}C]acetate.

Cell harvest

The culture supernatants were recovered and stored at -20°C for assay of apoE as described below. The cells were washed twice with phosphate-buffered saline and cell pellets were retained. Cell pellets and cells remaining in the culture dishes were extracted for 1 h in a total volume of 1.3 ml of absolute ethanol as described previously (11). This ethanol extraction procedure was compared with the standard hexane-isopropanol extraction (19) in experiments in which cells were heavily loaded with [^3H]oleate-labeled CE. In a representative experiment in which dividing THP-1 cells were exposed to LDL (100 $\mu\text{g/ml}$) for 3 days and labeled with [^3H]oleate during the final 24 h of culture, the recoveries of the CE standard after ethanol and hexane-isopropanol extractions were $85.5 \pm 1.6\%$ and $65.3 \pm 2.7\%$, respectively. When the rate of [^3H]oleate incorporation into CE was determined and adjusted for recovery as described below, the ethanol-extracted cells yielded 163.52 ± 13.58 pmol/24 h per μg DNA and the hexane-isopropanol-extracted cells yielded 178.27 ± 27.49 pmol/24 h per μg DNA. Thus, due to considerations of expense and toxicity, ethanol was chosen as the extraction medium. The ethanol extracts from cell

pellets and culture wells were combined and centrifuged at 8000 *g* for 2.5 min and transferred to 12 × 75 mm glass tubes for saponification of the acetate-labeled samples or to scintillation vials for drying of the oleate-labeled samples. Appropriate internal standards were added to allow for quantitation of recovery. The nonadherent cells remaining in the tube were combined with the cells remaining in the culture well and were frozen in 0.3 ml of water for assay of DNA as described below.

Time-course experiments were done twice with triplicate or quadruplicate samples in each experiment. All other experiments were done three times with triplicate or quadruplicate samples per experiment. Figures illustrate data from one representative experiment from each set.

Cholesterol esterification and CE mass

The rate of cholesterol esterification was measured as incorporation of [³H]oleate into CE during the final 14 h of culture. Ethanol extracts were separated by TLC on silica gel 60A plates (250 μm layer, 20 × 20 cm) (Whatman, Maidstone, England) using hexane-diethyl ether-glacial acetic acid 83:16:1. The CE bands were identified by autoradiography, scraped from the plates, and counted. The rate of cholesterol esterification was expressed as picomoles of [³H]oleate incorporated into CE/14 h per μg of DNA. Results were corrected for recovery based on a [¹⁴C]CE internal standard.

The CE mass was measured as described previously (11) using a fluorometric enzymatic assay (17). The results of the assay were expressed as nanograms of cholesterol/μg of DNA. Free cholesterol mass of the macrophages and THP-1 cells could not be measured due to the free cholesterol contributed by platelets.

Rate of cholesterol synthesis

Samples labeled with [¹⁴C]acetate were saponified by refluxing for 2h at 50°C in the presence of 1 N potassium hydroxide and 70% ethanol. The free cholesterol was extracted into hexane and separated by TLC as described above. Cholesterol bands were identified by autoradiography, cut, and counted. The rate of cholesterol synthesis was expressed as picomoles of [¹⁴C]acetate incorporated into cholesterol/14 h per μg of DNA. Results were corrected for recovery based on a [³H]cholesterol internal standard.

Competitive apoE immunoassay

Secreted apoE was measured with a solid-phase radioimmunoassay performed as described previously (12) using ¹²⁵I-labeled human apoE purified from plasma VLDL and a human apoE-specific monoclonal antibody (IE).

Cellular DNA

Because of the protein contribution of platelets in many experiments, all data were normalized to DNA content to eliminate variability introduced by differences in cell recoveries. The DNA of the cells was measured using a colorimetric assay as described previously (11, 20). The concentration of DNA was calculated from a standard curve prepared with calf thymus DNA (Sigma Chemical Co., St. Louis, MO). No changes in DNA values were seen to correlate with lipoprotein or platelet exposure of the cells.

RESULTS

Adaptation of THP-1 cells to serum-free medium

To study cholesterol metabolism in THP-1 cells it was necessary to eliminate lipoproteins from the culture medium. Therefore, we cultured the cells in serum-free medium with 1% Nutridoma-HU. This medium contains cholesterol but no lipoproteins. As shown in Fig. 1, THP-1 cells grew continuously in this serum-free medium for 7 days with a doubling time of approximately 48 h as evidenced by the DNA content of the cultures. These cells will be referred to as dividing or nonstimulated THP-1 cells. The DNA content of THP-1 cells treated with 10⁻⁷ M PMA did not change over the 7-day period, confirming the report that exposure to phorbol esters induces differentiation that results in the cessation of cell division (7). These cells will be referred to as stimulated or differentiated THP-1 cells.

Lipoprotein receptor function in THP-1 cells

It has been reported that dividing THP-1 cells display LDL receptors that disappear when the cells differentiate

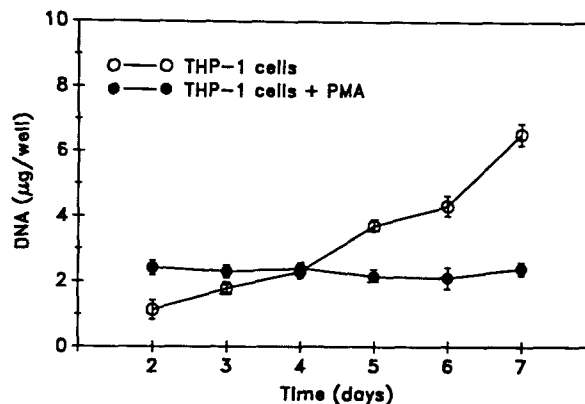


Fig. 1. Growth of THP-1 cells in serum-free medium. THP-1 cells were distributed at 2 × 10⁵/ml, or at 5 × 10⁵/ml in the presence 10⁻⁷ M PMA, and cultured for 7 days. DNA content was assayed daily beginning at day 2 to determine growth rate. Data points represent mean ± SE of triplicate cultures.

in response to phorbol ester treatment (8, 10). Scavenger receptors appear on differentiating cells concomitant with the loss of LDL receptors (8, 10). To determine whether both receptors function in the cellular uptake of cholesterol, cholesterol synthesis and esterification were measured during the final 14 h of a 3-day culture period (Fig. 2). In dividing THP-1 cells, the rate of acetate incorporation into cholesterol was high and the rate of oleate incorporation into CE was low. In the presence of LDL (100 $\mu\text{g}/\text{ml}$), the rate of cholesterol synthesis was markedly inhibited, whereas the rate of cholesterol esterification increased more than threefold (Fig. 2, upper panel). Exposure of these same dividing THP-1 cells to 100 $\mu\text{g}/\text{ml}$ of aLDL had essentially no effect on either cholesterol synthesis or cholesterol esterification.

Three days after PMA exposure the differentiated THP-1 cells had a low basal rate of cholesterol synthesis

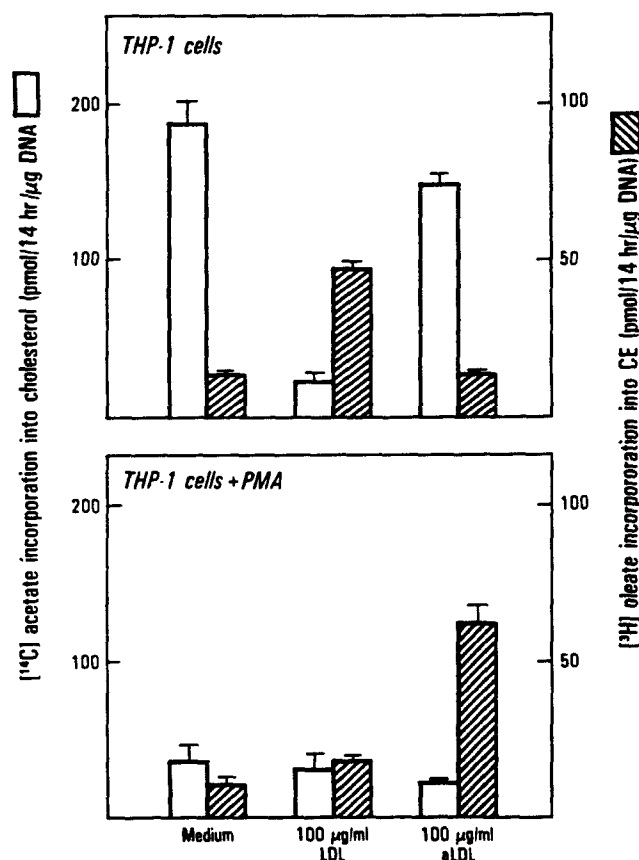


Fig. 2. THP-1 cell cholesterol synthesis and esterification rates. THP-1 cells were distributed at $3 \times 10^5/\text{ml}$, or at $5 \times 10^5/\text{ml}$ in the presence of 10^{-7} M PMA and cultured for 3 days. Cultures contained medium alone, LDL (100 $\mu\text{g}/\text{ml}$), or acetylated LDL (aLDL, 100 $\mu\text{g}/\text{ml}$). Cultures were labeled with [^3H]oleate-albumin or [^{14}C]acetate during the final 14 h and harvested for determination of rates of cholesterol synthesis or esterification. Open bars represent pmoles of [^{14}C]acetate incorporated into cholesterol/14 h per μg of DNA (mean \pm SE of triplicate cultures). Hatched bars represent pmoles of [^3H]oleate incorporated into CE/14 h per μg of DNA (mean \pm SE of triplicate cultures).

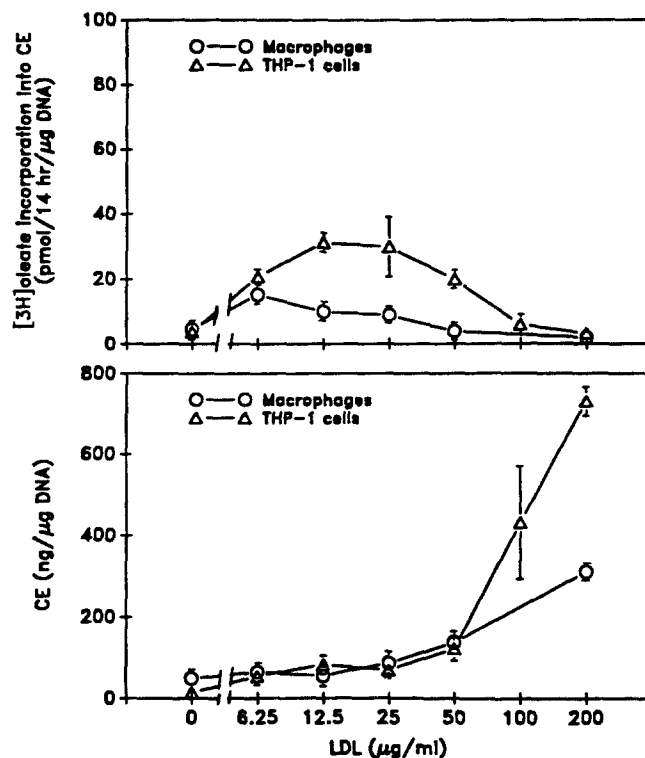


Fig. 3. THP-1 cell and macrophage response to LDL. Adherent macrophages from 5×10^6 PBM cells/ml and dividing THP-1 cells ($3 \times 10^5/\text{ml}$) were cultured for 4 days in the presence of medium alone, or increasing doses of LDL. Cultures were labeled during the final 14 h with [^3H]oleate-albumin and harvested for determination of rates of cholesterol esterification (upper panel) and CE accumulation (lower panel). Data points represent mean \pm SE of triplicate cultures.

that was unaffected by the presence of LDL (100 $\mu\text{g}/\text{ml}$) or aLDL (100 $\mu\text{g}/\text{ml}$) (Fig. 2, lower panel). However, these differentiated THP-1 cells exhibited a fourfold increase in the rate of oleate incorporation into CE in response to culture with aLDL. In contrast, the rates of cholesterol esterification did not increase when PMA-stimulated cells were exposed to LDL, supporting the observation that these cells express few, if any, LDL receptors (8).

To determine whether lipoprotein-induced increases in the rate of cholesterol esterification in THP-1 cells resulted in net storage of CE, we compared both cholesterol esterification and CE accumulation in THP-1 cells and human monocyte-derived macrophages cultured for 4 days in the presence of increasing doses of lipoproteins (Fig. 3 and Fig. 4). The rate of oleate incorporation into CE in dividing THP-1 cells during the final 14 h of culture was comparable to that of macrophages at the lowest the highest doses of LDL, but exceeded that of macrophages at doses ranging from 12.5 to 50.0 $\mu\text{g}/\text{ml}$ (Fig. 3, upper panel). At low doses of LDL, CE accumulation in dividing THP-1 cells was comparable to that of macrophages but markedly surpassed the CE storage of macro-

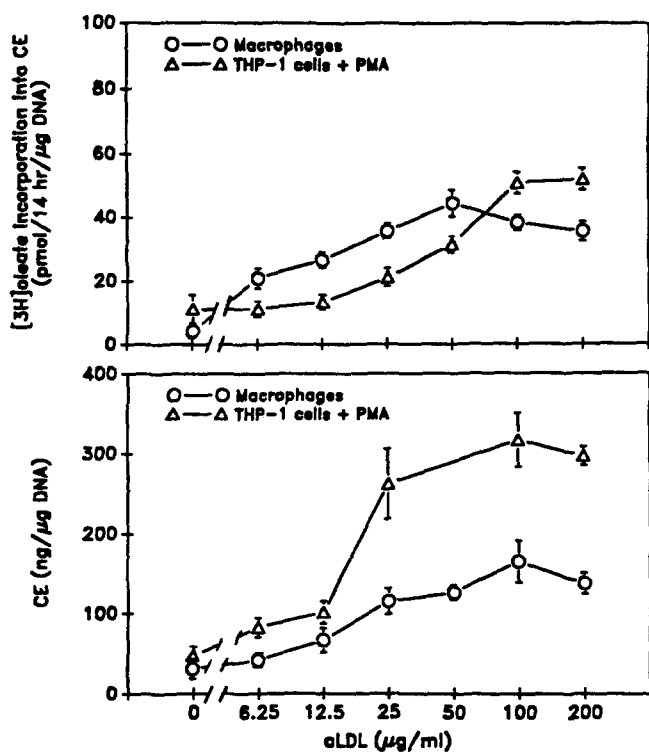


Fig. 4. THP-1 cell and macrophage response to aLDL. Adherent macrophages from 5×10^6 PBM cells/ml and PMA-stimulated THP-1 cells (5×10^5 /ml) were cultured for 4 days in the presence of medium alone or increasing doses of aLDL. Cultures were labeled during the final 14 h with [^3H]oleate-albumin and harvested for determination of rates of esterification (upper panel) and CE accumulation (lower panel). Data points represent mean \pm SE of triplicate cultures.

phages at higher doses (Fig. 3, lower panel). The apparent discordance between maximal cholesterol esterification rates and accumulated CE mass in the dividing THP-1 cells and macrophages can be explained by the fact that the esterification rate is measured over a 14 h period at a time when LDL receptors have been down-regulated on both cell types, particularly at high LDL doses; whereas, the measure of CE mass reflects accumulation during a 4-day exposure to LDL and results from high esterification rates earlier in the culture period (see Fig. 2). To rule out adsorption of LDL-associated CE or trapping of LDL-associated CE in lysosomes, we examined CE mass following the first 24 h of THP-1 cell exposure to LDL (100 $\mu\text{g/ml}$), at a time when LDL receptor number is highest, and found no differences in CE accumulation between cells exposed to LDL and those in media alone.

In the presence of aLDL the rates of cholesterol esterification in differentiated THP-1 cells 4 days after PMA stimulation and macrophages were similar over the entire dose range (Fig. 4, upper panel). However, CE accumulation by the differentiated THP-1 cells markedly exceeded that of macrophages at doses from 25 to 200 $\mu\text{g/ml}$ of aLDL (Fig. 4, lower panel).

THP-1 cell response to platelets

We have shown previously that macrophages cocultured with platelets exhibit marked increases in the rate of cholesterol esterification that result in storage of CE greater than that induced by native LDL or aLDL (11, 13). As CE accumulation is the hallmark of foam cell formation, platelet-induced CE accumulation in dividing THP-1 cells was first examined in a dose-response study that was terminated after 7 days of culture (Fig. 5). We have previously observed maximal CE accumulation in macrophages by day 3 that is maintained through day 7 of culture (11). Whereas the maximally effective platelet doses in THP-1 cultures paralleled those previously published for macrophages (i.e., 2.5×10^8 to 10^9 /ml), storage of CE in THP-1 cells in response to the most effective doses was less than 30 ng/ μg of DNA. This response was minimal compared to the previously observed response of monocyte-derived macrophages to coculture with platelets that was >200 ng of CE/ μg of DNA (11).

To determine whether the minimal CE storage observed in dividing THP-1 was a reflection of the single time point studied, we measured the kinetics of cholesterol esterification and CE accumulation. The effects of platelet treatment on the rates of oleate incorporation into CE in macrophages, dividing THP-1 cells and differentiated THP-1 cells were compared over a 7-day period using a maximally effective platelet dose of 5×10^8 /ml. Under control conditions, cholesterol esterification in macrophages was minimal but increased daily in the presence of platelets to a maximum of 184 pmol/14 h per μg of DNA on day 4 and remained high through day 7 (Fig. 6, upper panel). Coculture with platelets also caused an increase in the esterification rate in dividing THP-1 cells. The maximum rate of 109 pmol/14 h per μg of DNA was

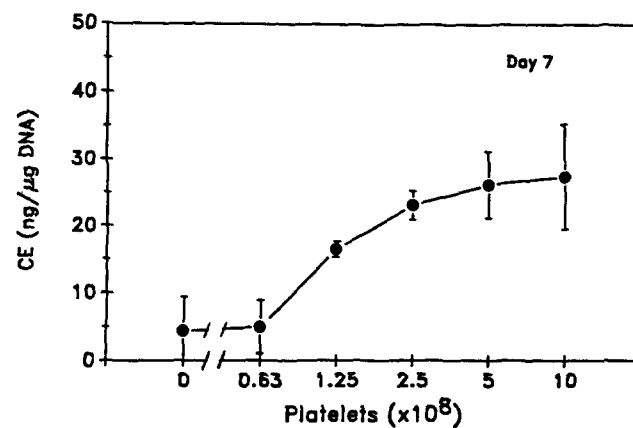


Fig. 5. THP-1 cell response to platelets. Dividing THP-1 cells (2×10^5 /ml) were cultured for 7 days in the presence of medium alone or increasing doses of human platelets and harvested for determination of CE mass. Points represent ng of CE/ μg of DNA (mean \pm SE of triplicate cultures).

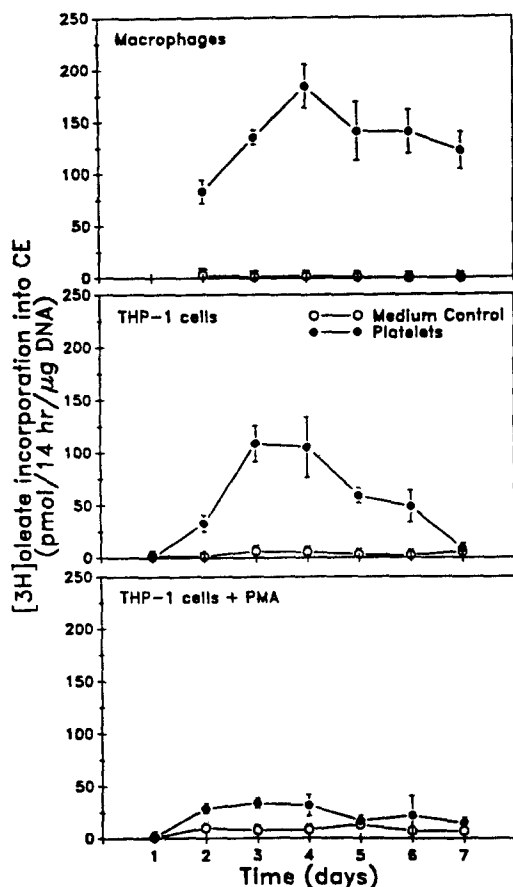


Fig. 6. Kinetics of cholesterol esterification. Adherent macrophages from 5×10^6 PBM cells/ml, dividing THP-1 cells (2×10^5 /ml) and differentiated THP-1 cells (5×10^5 /ml) were cultured for 7 days in the absence (open circles) or presence (solid circles) of 5×10^8 human platelets/ml. Cultures were harvested daily after a 14-h pulse with [³H]oleate-albumin and extracted for determination of the rate of cholesterol esterification. Points represent pmol of [³H]oleate incorporated into CE/14 h per μg of DNA (mean \pm SE of triplicate cultures).

observed on day 3. However, oleate incorporation in these cells decreased to control levels by day 7 (Fig. 6, center panel). PMA-stimulated THP-1 cells responded poorly to coculture with platelets and exhibited lower esterification rates throughout the culture period (Fig. 6, lower panel).

The kinetics of CE accumulation in the three cell types are illustrated in Fig. 7. Coculture with platelets led to measurable storage of CE in macrophages as early as day 2. Macrophage CE reached a maximum of 600 ng/μg of DNA by day 4 and remained high through day 6 (Fig. 7, upper panel). Platelet-treated dividing THP-1 cells showed no increase in CE mass until day 3 with a maximum accumulation of 230 ng/μg of DNA by day 4 (Fig. 7, center panel). This level dropped by day 7, consistent with results of the dose response at day 7 (Fig. 5). In PMA-stimulated THP-1 cells, coculture with platelets resulted in a maximum CE mass of 205 ng/μg of DNA on day 4 with little change in the level through day 7 (Fig.

7, lower panel). The fact that CE mass in these cells remains elevated late in the culture period while the esterification level drops most likely reflects the fact that these cells have ceased dividing.

To determine whether the differences in CE accumulation in the macrophages, dividing THP-1 cells, and PMA-stimulated THP-1 cells cocultured with platelets were reflected in different rates of cholesterol synthesis, we examined the effects of platelets on cholesterol synthesis on day 5 of culture (Fig. 8). The rates of cholesterol synthesis in the three cell types under control conditions were quite different. Compared with macrophages, the cholesterol synthesis in dividing THP-1 cells and in differentiated THP-1 cells in medium alone is tenfold and fourfold greater, respectively. As expected, exposure of macrophages to platelets (5×10^8 /ml), LDL (100 μg/ml), and aLDL (100 μg/ml) resulted in a dramatic down-regulation of cholesterol synthesis to less than 10% of control values. Dividing THP-1 cells responded similarly to LDL, as did

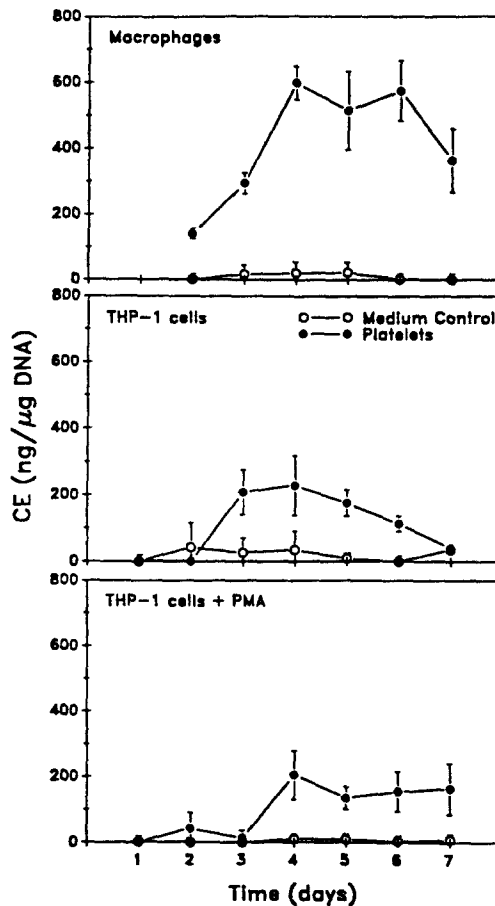


Fig. 7. Kinetics of CE accumulation. Adherent macrophages from 5×10^6 PBM cells/ml, dividing THP-1 cells (2×10^5 /ml) and PMA-stimulated THP-1 cells (5×10^5 /ml) were cultured for 7 days in the absence (open circles) or presence (solid circles) of 5×10^8 human platelets/ml. Cultures were harvested daily for determination of CE mass. Points represent ng CE/μg of DNA (mean \pm SE of triplicate cultures).

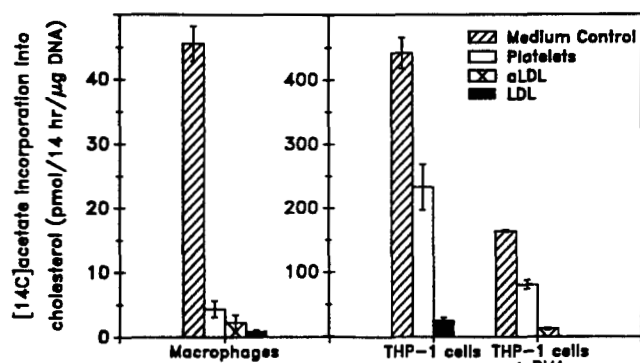


Fig. 8. Macrophage and THP-1 cell cholesterol synthesis. Adherent macrophages from 5×10^6 PBM cells/ml, dividing THP-1 cells (3×10^5 /ml), and PMA-stimulated THP-1 cells (5×10^5 /ml) were cultured for 5 days in medium alone, LDL (100 μ g/ml), aLDL (100 μ g/ml), or human platelets (5×10^8 /ml). Cultures were labeled during the final 14 h with [14 C]acetate and harvested for determination of rates of cholesterol synthesis. Bars represent pmoles of [14 C]acetate incorporated into cholesterol/14 h per μ g of DNA (mean \pm SE for triplicate cultures). Note that the scales in the two graphs are different.

PMA-stimulated THP-1 cells to aLDL, yet both cell types were less sensitive to platelets. Cholesterol synthesis in dividing THP-1 cells and PMA-stimulated THP-1 cells was decreased by only 50% in response to 5 days of coculture with platelets.

To determine whether the inability of THP-1 cells to respond fully to coculture with platelets was a generalized characteristic of the cell line, we examined another parameter of the macrophage response to coculture with platelets, apoE secretion. Mature human monocyte-derived macrophages secrete apoE and this secretion is enhanced by coculture with platelets (12). Dividing THP-1 cells also secrete apoE and this secretion increases significantly after phorbol ester stimulation (21, 22). To determine the effect of platelets on THP-1 cell apoE secretion, we compared the levels of apoE accumulated in the medium of macrophages, dividing THP-1 cells, and PMA-stimulated THP-1 cells after 4 days of culture (Table 1). Dividing THP-1 cells exhibited the lowest accumulation of apoE followed by macrophages. PMA-stimulated THP-1 cells secreted high levels of apoE. Platelet enhancement of apoE secretion was greater than threefold in macrophages and greater than fourfold in dividing THP-1 cells. Although differentiated THP-1 cells cocultured with platelets secreted large amounts of apoE, this secretion appeared to be platelet-independent as high production of apoE by PMA-stimulated cells was observed in the absence of platelets.

DISCUSSION

Much has been learned about cholesterol metabolism in the human monocyte-derived macrophage and processes leading to macrophage CE accumulation and foam

cell formation. The majority of information concerning CE loading in macrophages has come from studying macrophage handling of native and modified lipoproteins. To identify a human cell line with functional LDL and scavenger receptors to use as a model for studies of foam cell formation in vitro, we examined THP-1 cells which have been reported to display LDL and scavenger receptors on dividing and differentiated cells, respectively.

The monocytic leukemia cell line, THP-1, is characterized by distinct monocytic surface markers, enzymatic activities, the ability to phagocytize (6), and the secretion of potent cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) (21). These cells also synthesize and secrete apoE and lipoprotein lipase (22). When stimulated with phorbol ester, THP-1 cells become anchorage-dependent, cease dividing, and markedly increase their secretion of apoE and lipoprotein lipase (7, 22, 23). Dividing THP-1 cells have receptors that bind and degrade LDL through an LDL-specific pathway (8). Treatment with phorbol ester results in a transient superinduction of LDL receptor mRNA (9) followed by a gradual loss of the ability to bind and degrade LDL and the appearance of receptors that specifically bind and degrade aLDL (8, 10). Thus, the THP-1 cells appear to be quite suitable for in vitro studies of foam cell formation. However, until recently, no information concerning cholesterol metabolism in these cells was available.

In our studies, normal growth rates of THP-1 cells were achieved in the serum-free culture system used (Fig. 1) and the dividing THP-1 cells responded appropriately to the presence of LDL. The rate of cholesterol synthesis dropped dramatically, the rate of cholesterol esterification increased, and these cells stored CE more effectively at high doses of LDL than did macrophages. After 4 days of exposure to LDL, LDL receptors on the dividing THP-1 cells were down-regulated; however, these cells required higher doses of LDL for down-regulation than did macrophages, suggesting that they may be less sensitive to

TABLE 1. Apolipoprotein E accumulation

Cell Type	Culture Conditions ^a	ApoE ^b
		ng/ μ g DNA
Macrophages	Medium	12.5 \pm 1.1
Macrophages	PL	36.2 \pm 4.8
THP-1 cells	Medium	5.2 \pm 2.2
THP-1 cells	PL	20.4 \pm 2.2
THP-1 cells + PMA	Medium	40.1 \pm 6.4
THP-1 cells + PMA	PL	42.4 \pm 6.5

^aCells were cultured for 4 days as described in Materials and Methods in RPMI 1640 containing 1% Nutridoma-HU in the absence (Medium) or presence (PL) of 5×10^8 platelets/ml.

^bApoE accumulation in the culture supernatants was quantitated by solid-phase RIA. Values represent mean \pm SE of triplicate cultures.

down-regulation. This may explain the THP-1 cell's capacity to store significantly greater quantities of CE at higher LDL doses when compared to macrophages. As predicted from the reported receptor profile of these cells, undifferentiated, dividing THP-1 cells did not respond to aLDL. The observation of an early, transient induction of LDL receptor mRNA after PMA treatment of THP-1 cells (9) contrasts with our observations of the absence of a functional LDL receptor. These observations suggest a clear separation of the short-term stimulatory effects and the long-term differentiative effects of phorbol esters on THP-1 cells.

As expected, differentiated THP-1 cells responded to aLDL, but not LDL. A significant decrease in cholesterol synthesis after phorbol ester stimulation of the THP-1 cells occurred (Fig. 7) in spite of a transient increase in 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase gene transcription reported by Auwerx, Chait, and Deeb (9). These investigators also reported a significant decrease in the stability of the HMG-CoA reductase mRNA after PMA stimulation that correlates well with our observation of decreased cholesterol synthesis. Exposure of differentiated THP-1 cells to aLDL resulted in a further decrease in the rate of cholesterol synthesis that was minimal at day 3, but significant by day 5. Paralleling the decrease in cholesterol synthesis after aLDL exposure was an increase in the rate of cholesterol esterification resulting in a significant accumulation of CE. Whereas the rate of esterification paralleled that in aLDL-exposed macrophages and suggests there was no increase in acyl coenzyme A:cholesterol acyltransferase (ACAT) activity, the resulting CE accumulation in differentiated THP-1 cells surpassed that in macrophages at all doses. This may have reflected a greater number of scavenger receptors, a more efficient recycling of these receptors, or a less active cholesteryl ester hydrolase in the THP-1 cells.

LDL receptors on dividing THP-1 cells were functional in delivering cholesterol to the cells and could be down-regulated. Scavenger receptors on differentiated THP-1 cells also facilitated the internalization of cholesterol from modified LDL, but these receptors were not down-regulated. Our data regarding scavenger receptors correlates well with the recent report by Via and coworkers (10) that aLDL-specific receptors reach optimal levels on THP-1 cells after 72 h of PMA treatment and are responsible for increased cholesterol esterification and accumulation in aLDL-exposed cells. Taken together, these results suggest that both dividing and differentiated THP-1 cells are capable of hydrolyzing CE associated with lipoproteins and re-esterifying the cholesterol for storage. Furthermore, the CE storage in both cell types appears to surpass that of macrophages. The clear separation of functional LDL and scavenger receptors on distinct and synchronized differentiation stages of THP-1 cells offers a

useful model for further studies of macrophage-lipoprotein interactions.

We have recently shown that human monocyte-derived macrophages store CE and take on the appearance of foam cells when cocultured with human platelets (11, 13). This phenomenon is platelet-specific, dose-dependent, and requires platelet activation. The studies described here confirm and expand our earlier observations that in monocyte-derived macrophages, platelets were a greater stimulus for CE accumulation than were physiologic concentrations of native or acetylated LDL (11). In addition, these studies document that culture of macrophages in the presence of platelets results in a 90% decrease in endogenous cholesterol synthesis. Therefore, platelets induce macrophage CE accumulation by providing an exogenous source of cholesterol.

To further characterize the macrophage-platelet interactions leading to foam cell formation, we wished to identify a human monocyte cell line with a similar capacity to store CE in response to coculture with platelets. Kinetic studies revealed that dividing THP-1 cells stored significantly less CE than macrophages in response to platelets and this accumulation of CE was not sustained. This was consistent with the differences in cholesterol esterification rates observed in the dividing THP-1 cells in the presence of platelets. Increased CE hydrolysis rates and/or increases in free versus esterified cholesterol might explain lower CE stores in platelet-treated THP-1 cells, but these explanations are inconsistent with cholesterol synthesis data. Whereas LDL decreased cholesterol synthesis by >90% in THP-1 cells, platelets decreased this synthesis by <50%. Taken together, these results indicated that platelet induction of CE accumulation in macrophages did not involve the LDL receptor, a possibility that had been addressed in previous studies (13), but not independently confirmed.

The PMA-stimulated THP-1 cells accumulated significantly more CE in response to aLDL than to a maximal dose of platelets. The rate of cholesterol synthesis was inhibited by >90% in the presence of aLDL, but less in the presence of platelets. These observations implied that platelet-induced foam cell formation in macrophages also did not involve scavenger receptors and supported our earlier observations that neither fucoidan nor polyinosinic acid, competitive inhibitors of scavenger receptor-mediated uptake of aLDL, could inhibit platelet-induced macrophage cholesterol esterification (13).

Results presented here argue that THP-1 cells cultured with LDL and PMA-stimulated THP-1 cells cultured with aLDL are useful models for studies of lipoprotein-induced foam cell formation. In fact, these cells accumulated CE in response to the appropriate lipoprotein to a greater extent than did plasma-derived macrophages. In contrast, platelet-induced alterations in cholesterol me-

tabolism (CE accumulation and down-regulation of cholesterol synthesis) were significantly less in both dividing THP-1 cells and PMA-stimulated THP-1 cells than in macrophages. Nevertheless, dividing THP-1 cells secreted comparable amounts of apoE in response to platelets. Multiple mechanisms may be involved in cellular internalization and processing of cholesterol-containing particles (i.e., platelets and lipoproteins). It is possible that mature macrophages are competent in all aspects of these processes, whereas THP-1 cells are deficient in one or more of these mechanisms. Therefore, dissection of the differences between the macrophage and the THP-1 cell response to platelets should increase our understanding of the mechanisms involved in platelet-induced CE accumulation. Furthermore, study of the factors in THP-1 cells that protect against CE accumulation upon exposure to activated platelets should provide further insight into the mechanisms of foam cell formation. ■

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