Cell density can affect cholesteryl ester accumulation in the human THP-1 macrophage

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Abstract Esterified cholesterol (EC) accumulation was induced in THP-1 macrophages after exposure to acetylated LDL (acLDL), and the extent of accumulation was dependent on cell density. EC mass was 5-fold greater in cells plated at 1.0×10^6 cells/35 mm dish compared to cells plated at density 4.0×10^6 cells/dish. In addition, [^{14}C]oleate incorporation into EC also increased with decreasing cell number, with 4-fold greater incorporation (6 h: 177 ± 0.014 vs. 45 ± 0.001 pmol/mg cell protein, $P < 0.001$; 24 h: 515 ± 0.037 vs. 120 ± 0.012 pmol/mg, $P < 0.001$) in cells plated less densely compared to cells plated at a higher density. The rate of ^{125}I-labeled acLDL degradation was about 2-fold greater in cells plated at the lower density (105 vs. 60 ng/h per mg cell protein). Northern analysis showed a 2-fold reduction in the expression of human scavenger receptor mRNA in densely plated cells, and immunoprecipitation also demonstrated a 2-fold decrease in scavenger receptor protein. Conditioned media did not differentially affect EC formation at either cell density. Fatty acid supplementation increased EC formation and the proportion of esterified sterol content only in cells plated at the higher density. The fatty acid effect was also seen when cells were exposed to β-VLDL, which induced comparable levels of EC accumulation by non-scavenger receptor-mediated processes in densely plated cells. Foam cell formation in THP-1 macrophages may depend on cell density, which appears to affect both scavenger and non-scavenger receptor activity.—**Rodriguez, A., S. D. Kafonek, A. Georgopoulos, and P. S. Bachorik.** Cell density can affect cholesteryl ester accumulation in the human THP-1 macrophage. *J. Lipid Res.* 1994. **35:** 1909–1917.

Supplementary key words foam cells • atherosclerosis • modified LDL • cellular cholesterol metabolism

Pathological specimens from atherosclerotic lesions contain cholesteryl ester-laden cells that are predominantly of smooth muscle and macrophage origin (1). The mechanism(s) by which foam cell formation results from the accumulation of excess esterified cholesterol (EC) is not clear. Both primary human monocyte-macrophage preparations and differentiated cell lines have been used in vitro to study foam cell formation. Freshly isolated human monocytes (HMM) assume macrophage-like characteristics after 1 week in culture and accumulate EC when exposed to modified low density lipoprotein (LDL) (2). The use of primary macrophage preparations, however, can pose problems including donor variability, accessibility, the limited number of cells available for study, and a finite survival period in culture. Use of a continuous cell line can help to circumvent these limitations. The THP-1 human monocyte, a transformed monocytic leukemic cell, exhibits macrophage morphology and function on exposure to phorbol esters (3). In the monocytic state, these cells express LDL receptor activity, but when transformed with phorbol esters LDL receptor activity decreases. Scavenger receptor expression, however, increases under these conditions and peaks within 48–72 h (4). After differentiation THP-1 cells secrete lipoprotein lipase and apolipoprotein E (5). To our knowledge, EC accumulation in THP-1 cells exposed to acetylated LDL has been reported in only one study and the degree of EC accumulation was apparently limited (6). The human THP-1 macrophage, however, is becoming an increasingly useful cell for in vitro studies of atherosclerosis. The aim of the present study was to establish optimal conditions for foam cell formation in the THP-1 macrophage. We found that cellular EC accumulation in response to acetylated LDL may depend upon cell density; EC mass increased 5-fold at the lowest density compared to cells plated at the higher density. This results, in part, from differences in scavenger receptor expression and acLDL degradation as a function of cell density. Both scavenger receptor activity and the

Abbreviations: acLDL, acetylated LDL; LDL, low density lipoprotein; VLDL, very low density lipoprotein; EC, esterified cholesterol; UC, unesterified cholesterol; PMA, phorbol myristate acetate; HMM, human monocyte macrophages.

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rate of ^{125}I-labeled acLDL degradation were higher in cells plated at low density.

MATERIALS AND METHODS

Materials

Tissue culture plates were purchased from Corning Inc., (Corning, NY). RPMI-1640 medium was purchased from Gibco BRL, (Grand Island, NY). Fetal calf serum was purchased from Hyclone Laboratories Inc., (Logan, UT). Phorbol myristate acetate (PMA) and [^{14}C]oleate (52.1 mCi/ml) were supplied by Sigma Chemical Co., (St. Louis, MO). ^{125}I (16.9 mCi/μg of iodine) was purchased from Amersham Life Sciences, (Arlington Heights, IL). All organic solvents were of at least reagent grade.

Cells

THP-1 monocytes were purchased from American Type Tissue Culture Collection (Camden, NJ), and maintained at 37°C in RPMI medium containing 10% fetal calf serum (FCS) plus 1% penicillin-streptomycin in 75-mm vented flasks exposed to a humidified atmosphere of 95% air-5% CO_2. For experiments, cells were plated at varying densities (1.0 to 5.0 × 10^6 cells/ml per 35-mm dish) for 48 h in 10% FCS medium containing phorbol myristate acetate (400 ng/ml) (7). The cells were then washed extensively with serum-free RPMI medium and incubated with and without lipoproteins as indicated for each experiment.

Lipoproteins

Plasma was obtained from healthy male and female volunteers; 5,5' -dithiobis(2-nitrobenzoic acid) (DTNB) (1.4 mmol/l) was added to inhibit lecithin:cholesteryl acyltransferase (LCAT) activity (8). Lipoproteins were isolated by sequential density ultracentrifugation (9). LDL was isolated at d 1.019-1.063 g/ml, dialyzed against 12 l (4 l, 3 exchanges) of 0.15 M NaCl-0.3 mM EDTA and stored at 4°C for no longer than 2 weeks prior to use. The β-VLDL-containing fraction d < 1.019 g/ml was isolated from the plasma of a fasting patient with type III dysbetalipoproteinemia. The lipoprotein fraction was dialyzed against 12 l (4 l, 3 exchanges) of 0.15 M NaCl containing 0.3 mM EDTA, stored at 4°C, and used within 1 week of isolation. LDL was acetylated as described by Frankel-Conrat (10) and the complete conversion of LDL to acetylated LDL (acLDL) was confirmed by agarose gel electrophoresis. AcLDL was generally used within 2 weeks of preparation.

Cholesterol loading

The cells were exposed for 48 h to one of the following conditions: *1)* acLDL (50-500 μg protein/ml); *2)* oleate-albumin complex (50-500 μg oleate/ml); *3)* acLDL (500 μg/ml) + oleate (360 μg/ml); *4)* linoleate (100-500 μg/ml); *5)* acLDL (500 μg/ml) + linoleate (360 μg/ml); or *6)* β-VLDL (100-400 μg protein/ml). Medium containing only bovine serum albumin (BSA) (2 mg/ml) was used as a control. PMA (400 ng/ml) was added to the medium in all conditions in order to maintain maximal expression of the scavenger receptor (7). After the 48-h loading period, the medium was removed and the monolayer was washed thrice with cold phosphate-buffered saline (PBS). Cellular lipid was extracted with hexane-isopropanol 3:2 (v/v) for 1 h at room temperature (11). Stigmasterol (1 mg/ml) was added to the extract as an internal standard. Total and unesterified cholesterol (UC) were measured by gas-liquid chromatography (Hewlett-Packard 5980), and EC was calculated as the difference between the two measurements (12). Cell protein was determined by the Markwell modification (13) of the method of Lowry et al. (14).

Cholesteryl [^{14}C]oleate incorporation

The rate of incorporation of [^{14}C]oleate into EC was determined by measuring the extent of [^{14}C]oleate incorporation into EC. After the loading period, [^{14}C]oleate (0.5 × 10^{-4} M) was added to each well for 2 h. Cellular lipids were extracted as described above. Radiolabeled sterols were separated by thin-layer chromatography on silica gel G using a solvent system of heptane-ethyl ether-glacial acetic acid 220:40:2.7 (v/v). [^{3}H]triolein and [^{3}H]cholesteryl oleate were added as internal standards. The separated lipids were visualized using iodine vapor, and the lipid fractions were scraped from the plate into scintillation vials. Radioactivity was measured using a Beckman model LS3801 liquid scintillation spectrometer.

^{125}I-labeled acLDL degradation

Freshly isolated LDL was radiolabeled to a specific activity of 128 cpm/ng protein using iodine monochloride (15), then acetylated as described above. Greater than 99% of the radioactivity was insoluble in 10% trichloroacetic acid (TCA) and <0.2% was associated with lipoprotein lipids. Cells were incubated with ^{125}I-labeled acLDL (5 μg protein/ml) in medium containing 5% lipoprotein-deficient serum in the presence and absence of excess unlabeled acLDL (50 μg protein/ml) for varying periods of time (2-6 h) at 37°C. The cells were then cooled to 4°C. The medium was removed and added to ice-cold TCA (final concentration 10%, w/v) and allowed to stand at 4°C for 30 min to precipitate undegraded acLDL. The precipitate was removed by centrifugation at 10,000 *g* for 10 min, and an aliquot of the TCA-soluble phase was treated with KI and H_2O_2, then extracted with $CHCL_3$ (16) to remove unbound ^{125}I. Radioactivity in the aqueous phase was measured by gamma scintillation spectrometry.

Northern analysis and immunoprecipitation

Northern analysis was performed from total RNA isolated from cells plated at low and high density using a RNAzol kit (Cinna/Biotecx Laboratories International Inc., Friendswood, TX). The cDNA probe for the human scavenger receptor-type 2 (plasmid hSR2, Xbal-HindIII fragment) was generously provided by Drs. A. Matsumoto and T. Kodama (Tokyo, Japan). Equal amounts of RNA (20 μg) from cells plated at 1.0 and 4.0 $\times$ 10^6 cells/dish were subjected to electrophoresis on 1% formaldehyde-agarose gels and then transferred to nitrocellulose paper (17). The paper was hybridized with random-primed [^{32}P]hSR overnight and subjected to autoradiography for 24 h at -70°C. The visualized bands were quantitated by densitometric scanning using a Discovery Series densitometer equipped with the Quantity-One software program (PDI, Inc., Huntington Station, NY). Values were normalized to random-primed frog 18S RNA. High and low density cells were solubilized, then immunoprecipitated with a monoclonal antibody to the human scavenger receptor protein-2 in a modification of the method described by Kodama et al. (18). The antibody was generously provided by Drs. A. Matsumoto and T. Kodama (Tokyo, Japan).

Statistical analysis

Analysis of statistical significance between group means was calculated by using Student's paired two-tailed *t*-test.

RESULTS

Our first aim was to induce EC accumulation in THP-1 cells exposed to acLDL. Cells were plated at density 1.0 $\times$ 10^6 cells/dish and then exposed for 48 h to acLDL plus BSA or to BSA alone. As shown in **Fig. 1,** EC mass accumulation increased with increasing acLDL concentration to a maximum level of 39.5 $\pm$ 4.6 μg/mg cell protein, (52% of total sterol) at an acLDL concentration of 500 μg protein/ml. This level of EC was greater than that reported from human monocyte-macrophages exposed to an equivalent concentration of malondialdehyde-LDL (2).

We next examined the influence of cell density on EC accumulation. It has been reported that the density of cell plating influences scavenger receptor activity in HMM; increasing the cell number increased the activity of the receptor (19). For this reason, THP-1 monocytes were plated to confluence (4.0 $\times$ 10^6 cells/35 mm dish) and maintained for 48 h in 10% fetal calf serum and PMA (400 ng/ml), then exposed to medium containing acLDL (500 μg protein/ml) and BSA (2 mg/ml) for 48 h. Control cells were plated in parallel and exposed to BSA alone. At the end of the incubation, cellular lipids were extracted and measured as described in Methods. EC increased 16-fold to 7.94 $\pm$ 1.65 μg/mg cell protein, $P < 0.001$ when cells were exposed to acLDL (500 μg protein/ml) (**Fig. 2**) compared to the controls. The UC significantly increased by 34% ($P < 0.001$) and represented 69% of the total cellular sterol mass. Thus, while EC mass increased significantly under these conditions, there was

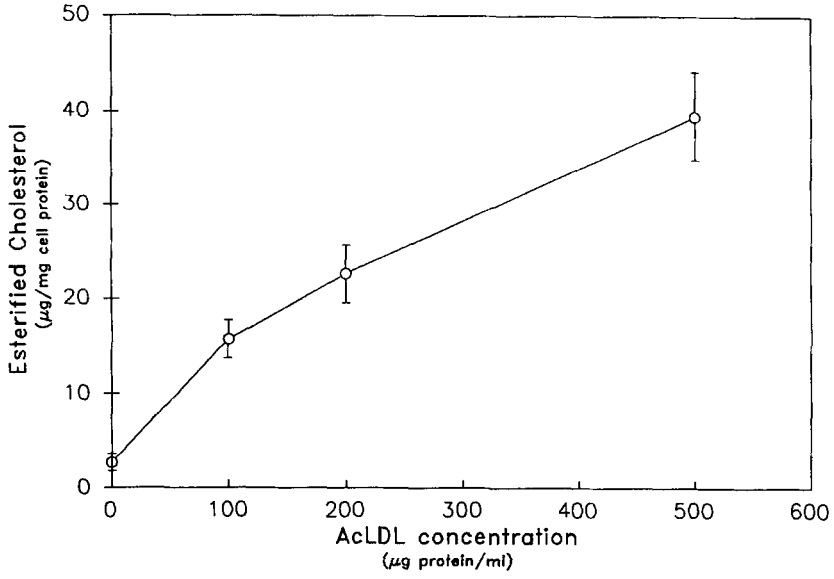

Fig. 1. Esterified cholesterol accumulation in less densely plated THP-1 cells exposed to increasing concentrations of acLDL. THP-1 cells were plated at density 1.0 $\times$ 10^6 cells/dish in 10% FCS and PMA (400 ng/ml) for 48 h. After removal of the medium, cells were exposed to acLDL (0-500 μg protein/ml) and BSA (2 mg/ml) plus PMA (400 ng/ml) for an additional 48 h. Cellular lipids were extracted and processed as described in Methods. Values represent the mean $\pm$ SE of replicate dishes (n = 9).

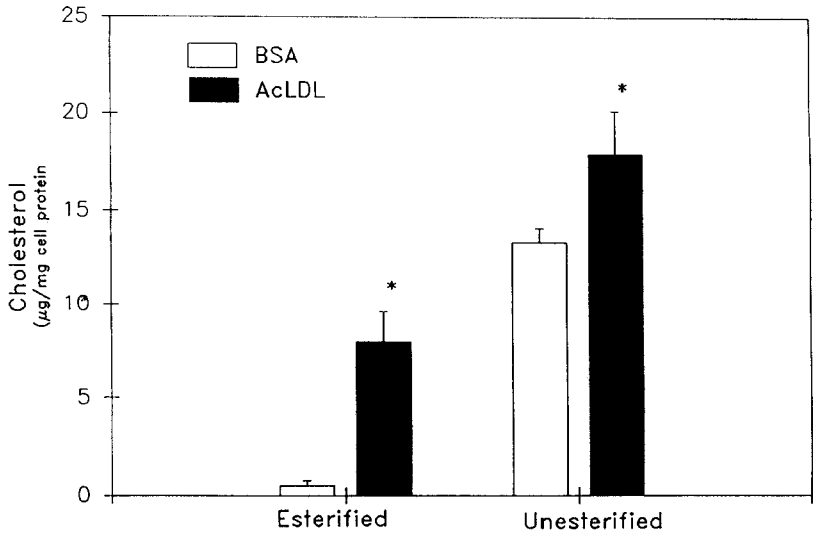

Fig. 2. Cellular sterol accumulation in THP-1 cells incubated with acLDL (500 μg protein/ml). THP-1 cells were plated at density 4.0 × 10^6 cells/dish in 10% FCS and PMA (400 ng/ml) for 48 h. The medium was removed and cells were exposed to acLDL (500 μg protein/ml) and BSA (2 mg/ml) plus PMA (400 ng/ml) for an additional 48 h. Cellular lipids were extracted and processed as described in Methods. Values are the mean ± SE of replicate dishes. *Indicates significant difference, $P < 0.001$, compared to control conditions.

quantitatively less accumulation than in cells plated at the lower density (Fig. 1) and EC constituted a lower proportion of the total cellular sterol mass. Cellular toxicity did not explain these observations as viability, measured by trypan blue exclusion, was found to be greater than 90% for cells plated at both low and high density.

We next investigated the effect of cell density on scavenger receptor function. We measured the degradation of ^{125}I-labeled acLDL at varying cell densities as a measure of scavenger receptor activity. Cells were plated at densities of 1.0 × 10^6 or 4.0 × 10^6 cells/dish, then exposed for varying periods (2–6 h) to ^{125}I-labeled acLDL (5 μg protein/ml) in the presence and absence of a 10-fold excess of unlabeled acLDL. We measured the rate of formation of TCA-soluble degradation products after first removing any unbound ^{125}I from the TCA supernatant as described in Methods. High affinity degradation, calculated as the difference between that which occurred in the presence and absence of unlabeled acLDL, is shown in **Fig. 3.** Degradation was linear with time in the cells plated at high density. In cells plated at low density, degradation increased with time and became linear with time after 2 h. The rates of degradation were calculated from the steepest portions of the time curves. Cells plated at low density degraded ^{125}I-labeled acLDL almost twice as fast (105 ng/h per mg cell protein) as those plated at high density (60 ng/h per mg cell protein). This finding indicated that cells plated at low density had internalized more labeled acLDL than those plated at high density, suggesting greater scavenger receptor activity in the sparsely plated cells.

To address this question directly, we examined the effect of cell density on the expression of the scavenger receptor mRNA and scavenger receptor protein. Cells plated at low and high densities were subjected to Northern analysis and immunoprecipitation as described in Methods. As shown in **Fig. 4A,** there was a 2-fold reduction in the ex-

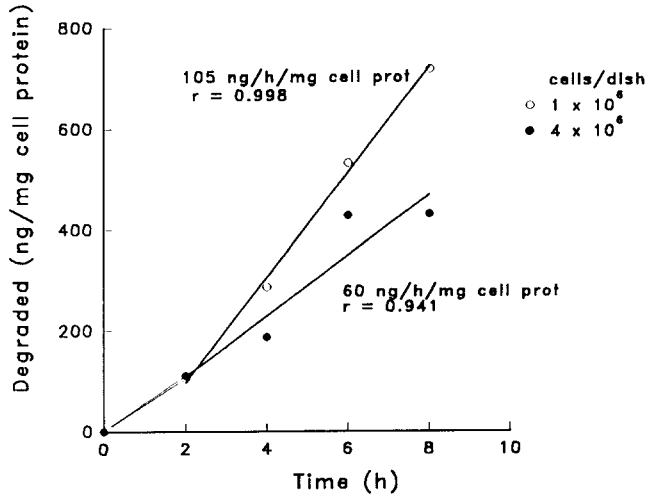

Fig. 3. The effect of cell density on high affinity degradation of ^{125}I-labeled acLDL in THP-1 cells. The cells were plated at densities 1.0 × 10^6 and 4.0 × 10^6 cells/dish in 10% FCS for 48 h. Cells were incubated with ^{125}I-labeled acLDL (5 μg protein/ml) in medium containing 5% lipoprotein-deficient serum in the presence and absence of a 10-fold excess of unlabeled acLDL (50 μg protein/ml) for varying periods of time at 37°C (see Methods). Values represent the mean of pooled triplicate samples. This figure is representative of two separate experiments.

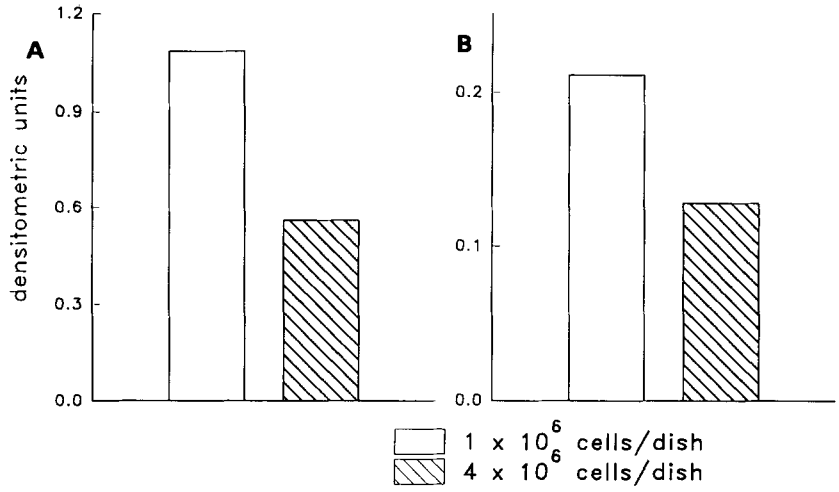

Fig. 4. Effect of cell density on the expression of the scavenger receptor. Cells were plated at 1.0 and 4.0 × 10^6 cells/dish for 48 h as described in Methods. A: Northern analysis was performed from total RNA extracted from cells and the pHSR2-probe, Xbal-HindIII fragment was used for hybridization (see Methods). Random-primed labeled frog 18S RNA was used to normalize the bands visualized after autoradiography. B: Immunoprecipitation, using rabbit anti-human scavenger receptor antibody, was performed on solubilized cells plated at 1.0 and 4.0 × 10^6 cells/dish (see Methods). Alkaline phosphatase goat anti-rabbit IgG affinity-purified was used to visualize bands.

pression of the hSR2 mRNA in cells plated at high density. In Fig. 4B, immunoprecipitation with anti-scavenger receptor antibody showed a 2-fold reduction in the quantity of scavenger receptor protein expressed in cells plated at high density.

Differences in cell density also affected endogenous EC synthesis as assessed by measuring the rate of [^{14}C]oleate incorporation into EC in cells plated at varying densities (1.0–5.0 × 10^6 cells/dish). For these experiments, the cells were not preincubated with acLDL, but rather with 10% FCS. Cellular [^{14}C]cholesteryl ester accumulation was measured in cells exposed to medium containing [^{14}C]oleate-BSA complex and incubated for 6 or 24 h. [^{14}C]oleate incorporation into EC was 4-fold greater (6 h: 177 ± 0.014 pmol/mg cell protein vs. 45 ± 0.001, $P < 0.001$; 24 h: 515 ± 0.037 pmol/mg vs. 120 ± 0.012, $P < 0.001$) in cells plated at the lower densities as compared to cells plated at the higher densities (**Fig. 5**).

It has been shown that supplementation of medium containing acLDL with fatty acid further increased EC mass in the J774 cell murine macrophage (20). We therefore examined the effect of adding fatty acid to medium containing acLDL on EC accumulation in cells plated at varying densities. THP-1 cells were plated at density 4.0 × 10^6 cells/ml and then exposed for 48 h to medium containing acLDL (500 μg protein/ml) plus oleate (50–500 μg/ml) complexed with BSA. The cells were then pulsed with [^{14}C]oleate (5.0 × 10^{-4} M) for 2 h prior to terminating the incubation. [^{14}C]oleate incorporation into EC increased with increasing oleate concentration and reached a maximum at about 360 μg oleate/ml (**Fig. 6**). When cells were plated at densities of 1.0 × 10^6 and 4.0 × 10^6 cells/ml and exposed to acLDL (500 μg protein/ml) and oleate (360 μg/ml), EC mass was 2-fold greater (14.3 ± 1.41 μg/mg cell protein, 42% of the total cellular sterol, $P < 0.001$) in cells plated at the higher density and exposed to acLDL plus oleate compared with acLDL alone (7.9 ± 1.65 μg/mg cell protein, 31% of total

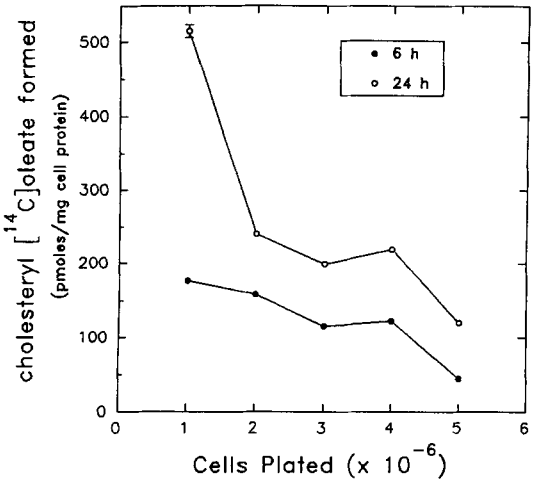

Fig. 5. Basal EC synthesis in THP-1 cells plated at varying densities. THP-1 cells were plated at varying densities (1.0–5.0 × 10^6 cells/ml) in 10% FCS for 48 h. The medium was removed and then cells were exposed to 10% FCS and [^{14}C]oleate (0.5 × 10^{-4} M) for the times indicated. Cellular lipids were extracted as described in Methods. Values represent the mean ± SE of quadruplicate dishes. Error bars not visualized are contained within the symbols.

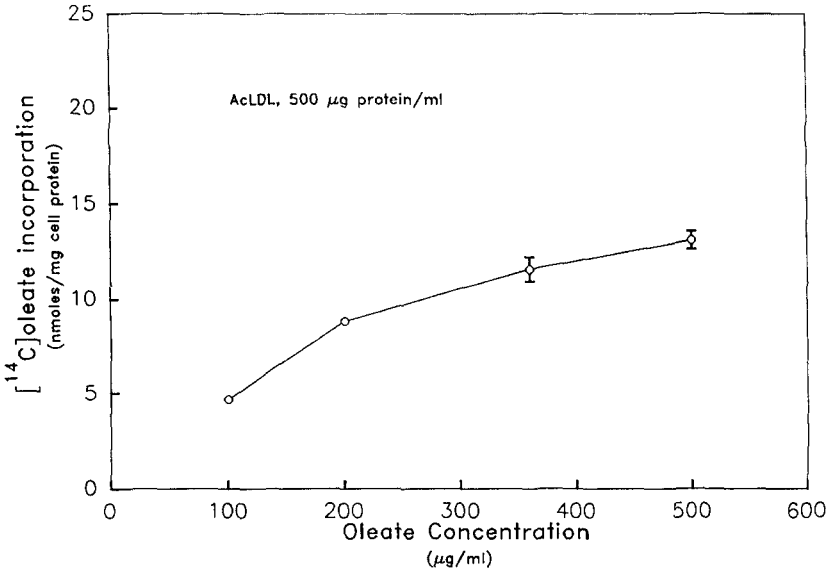

Fig. 6. [^{14}C]oleate incorporation into EC in THP-1 cells exposed to acLDL plus oleate. THP-1 cells were plated at density 4.0 × 10^6 cells/dish in 10% FCS and PMA (400 ng/ml) for 48 h. After removal of the medium, cells were exposed to acLDL (500 μg protein/ml) plus increasing concentrations of oleate (100–500 μg/ml). Cells were pulsed with [^{14}C]oleate-albumin complex (5.0 × 10^{-4} M) for 2 h prior to terminating the experiment. Cellular lipids were extracted and radiolabeled sterols were measured by thin-layer chromatography, as described in Methods. Values represent the mean ± SE of triplicate dishes. Error bars not visualized are contained within the symbol.

sterol) (**Fig. 7**). Oleate supplementation of acLDL-containing medium did not significantly increase EC accumulation in cells plated at the lower density. Thus, in confluent cells, providing cholesterol and fatty acid substrates led to greater EC accumulation than did either substrate alone. Similar results were obtained when linoleate was substituted for oleate (data not shown). There existed a slight trend toward greater accumulation of triglyceride mass in cells plated at the lower density and exposed to acLDL plus oleate as compared to densely plated cells, but the differences did not reach statistical significance (data not shown).

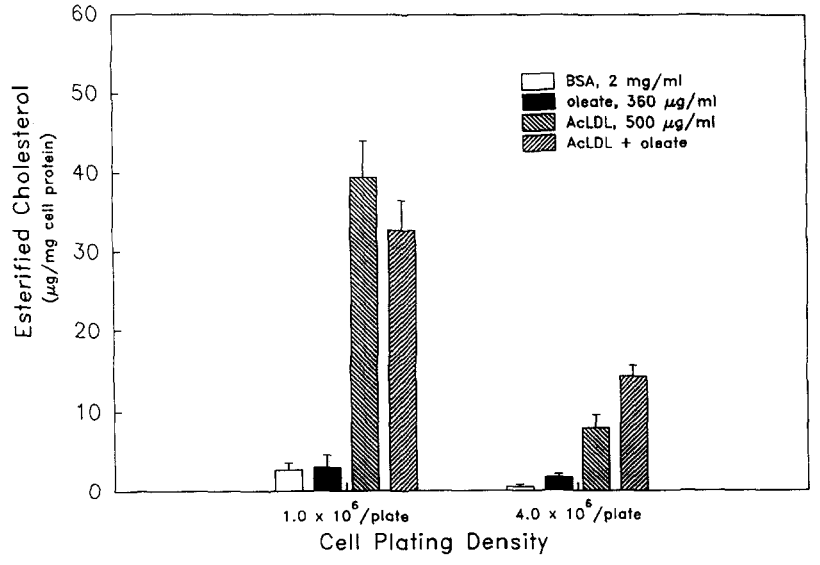

Fig. 7. Esterified cholesterol accumulation in THP-1 cells plated at varying densities and exposed to acLDL in the presence and absence of oleate. THP-1 cells were plated at densities 1.0 × 10^6 and 4.0 × 10^6 cells/dish in 10% FCS and PMA (400 ng/ml) for 48 h. After the medium was removed the cells were incubated with acLDL (500 μg protein/ml) and BSA (2 mg/ml) in the presence and absence of oleate-albumin complex (360 μg/ml). Cellular lipids were extracted and processed as described in Methods. Values represent the mean ± SE of replicate dishes ($5 \leq n \leq 11$).

The requirement for fatty acid in densely plated cells was also suggested in experiments in which cells were exposed to β-VLDL (400 μg protein/ml) from an untreated type III hyperlipoproteinemic patient. The rationale for its use is that β-VLDL is a lipoprotein enriched in both cholesterol and triglyceride (21). This particle would also provide a source of fatty acids after undergoing lipolysis. As seen in **Fig. 8,** EC mass accumulated to the same extent (13.3 ± 1.58 μg protein/ml, 47% of total sterol) when cells were exposed to β-VLDL compared to acLDL plus oleate (14.3 μg/ml, 42% of total sterol). These observations suggested that in densely plated THP-1 cells the addition of fatty acid to medium containing acLDL is required for maximal EC accumulation when acLDL is used as the source of cholesterol. In contrast, acLDL used in conjunction with cholesterol-phospholipid dispersions (using phosphatidylserine or phosphatidylcholine as the phospholipid), cholesterol in ethanol dispersions, or malondialdehyde-modified LDL did not significantly increase EC mass as compared to acLDL alone (data not shown). Other investigators have reported that such lipid dispersions enhance cholesterol accumulation in murine macrophages (22) or human fibroblasts (23), but this did not occur in THP-1 cells.

We considered the possibility that a secreted factor(s) in the densely plated cells might depress EC accumulation in the low density cells. Cell-conditioned medium was used to address this question. Cells were plated at densities of 1.0 and 4.0 × 10^6 cells/dish, for 48 h in 10% FCS and PMA 400 ng/ml. The media were removed and centrifuged to remove nonadherent cells. The cells were rinsed extensively with fresh serum-free medium. Conditioned medium from sparsely plated cells was then added to the densely plated cells, and vice versa. AcLDL, 500 μg protein/ml, was then added and the cells were incubated for an additional 48 h. Cellular lipids were extracted and processed as described in Methods. Esterified cholesterol decreased 13% in the low density and 9% in high density cells exposed to the conditioned media compared to control cells (data not shown). We attributed this decrease to the depletion of essential nutrients in the conditioned media. There did not appear to be secretory factor(s) that differentially affected either cell density.

DISCUSSION

Our studies showed that accumulation of EC within the THP-1 macrophage may be dependent on cell density. AcLDL induced a 5-fold greater accumulation of EC mass in sparsely plated cells compared to densely plated cells. UC mass was also increased 2.2-fold in cells plated at the lower density compared with only about 1.3-fold in densely plated cells. The difference in cellular sterol accumulation at differing densities appears to be related, in part, to the activity of the scavenger receptor. Three lines of evidence indicate that scavenger receptor activity may be affected by increasing cell density. First, the rate of ^{125}I-labeled acLDL degradation was increased in cells plated less densely; second, the expression of hSR mRNA was 2-fold higher in sparsely plated cells; and third, scavenger receptor protein, as determined by immuno-

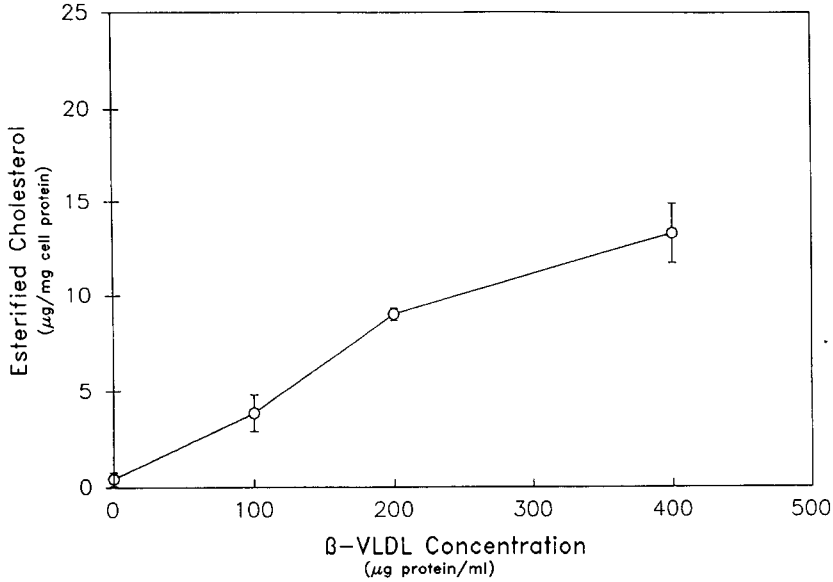

Fig. 8. Esterified cholesterol accumulation in THP-1 cells exposed to varying concentrations of β-VLDL obtained from an untreated fasting dysbetalipoproteinemic (type III) male patient. THP-1 cells were plated at density 4.0 × 10^6 cells/dish in 10% FCS and PMA (400 ng/ml) for 48 h. The medium was removed and cells were exposed to β-VLDL (0–400 μg protein/ml) for an additional 48 h. Cellular lipids were extracted and processed as described in Methods. Values represent the mean ± SE of triplicate dishes.

precipitation, was also 2-fold higher in sparsely plated cells. The mechanism for the apparent down-regulation of the scavenger receptor at higher cell densities is presently unclear. Incubation of densely plated cells with media from sparsely plated cells, and vice versa, gave no evidence of a secretory factor(s) that might regulate scavenger receptor activity. Recently, Bottalico et al. (24) reported that the addition of TGF-β to control media can down-regulate scavenger receptor mRNA expression in the THP-1 macrophage. It would appear that in addition to TGF-β and phorbol ester, cell density may also regulate the expression of the scavenger receptor. In contrast to our findings in the THP-1 cells, Fogelman et al. (19) reported that scavenger receptor activity in HMM increased with increasing cell number. One difference between the two studies was the use of a transformed human leukemic cell line in the present investigation compared with primary cells obtained from healthy human donors in the earlier study (19). The state of differentiation of the cells and differences in cell culture conditions might also have contributed to the apparent difference in cell density-related scavenger receptor activity between the HMM and THP-1 macrophages (19). Alternatively, cell-to-cell contact may have reduced scavenger receptor activity through an unknown mechanism(s). We found that cell density also affected the rate of cholesterol esterification. In experiments examining non-receptor-mediated processing of [^{14}C]oleate into EC, synthesis of EC was 4-fold higher in cells plated less densely compared to densely plated cells. It is likely that the uptake and processing of lipid in densely plated cells was diminished, thereby limiting the substrate available for esterification by acylCoA cholesterol acyltransferase (ACAT). The requirement for fatty acid supplementation to medium containing acLDL for maximal EC mass accumulation was observed only in confluent cells. In densely plated cells the addition of oleate to medium containing acLDL significantly increased EC mass compared to cells exposed to acLDL alone. The requirement for fatty acid was also suggested when cells were exposed to β-VLDL, a lipoprotein enriched in both cholesterol and triglyceride, and thought to be processed through non-scavenger receptor-mediated pathways (21). In densely plated cells, β-VLDL increased EC mass to levels similar to those obtained when cells were exposed to acLDL and oleate. It should be noted, however, that triglyceride mass accumulation was not significantly different in cells plated at varying densities and exposed to acLDL plus oleate. The possibility therefore exists that ACAT activity itself may have been directly influenced by cell density.

We conclude that cell density can have an affect on foam cell formation in the THP-1 macrophage. This effect appears to result, at least in part, in regulation of the scavenger receptor. The effect of cell density may have physiologic relevance during the early stages of atherosclerosis development in vivo. Scavenger receptor activity may be normally up-regulated, and may decrease as macrophage number increases in the progressing atherosclerotic lesion. Evidence for this possibility was provided recently by Naito et al. (25). They examined the expression of human scavenger receptor protein in tissue with varying stages of atherosclerosis. Using immunohistochemical techniques with monoclonal antibodies specific for the human scavenger receptor protein, these investigators observed less reactivity to the antibody in advanced lesions compared to fatty streaks (25).

Our findings indicate that under appropriate conditions, the human THP-1 macrophage can be a useful surrogate for primary human monocyte-macrophages in the study of foam cell formation.

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