Dissociated regulation of macrophage LDL receptor and apolipoprotein E gene expression by sterol

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Abstract The control of apoE gene expression by sterols and the relationship between regulation of the apoE and low density lipoprotein (LDL) receptor genes were investigated in a human macrophage line. Incubation of THP1 cells in either LDL or acetylated LDL increased apoE mRNA levels 4- to 15-fold. In addition, the cellular abundance of these two mRNA species (apoE and LDL receptor) was inversely regulated by cellular cholesterol content over an identical dose–response relationship. Regulation of the LDL receptor and apoE genes could, however, be temporally dissociated in response to the accumulation or removal of lipoprotein-derived (exogenous) cholesterol and in response to perturbation of endogenous cellular cholesterol biosynthesis. In addition, we observed that the apoE gene responded more promptly to 25-hydroxycholesterol than to exogenous cholesterol. These data support the concept that the apoE gene be considered among the family of genes sensitively regulated by cellular sterol balance but suggest that the molecular mechanism accounting for the modulation of the LDL receptor and apoE genes are distinct, with the relationship between cell sterol balance and apoE gene regulation being more complex. —**Mazzone, T., and K. Basheeruddin.** Dissociated regulation of macrophage LDL receptor and apolipoprotein E gene expression by sterol. *J. Lipid Res.* 1991. **32:** 507–514.

Supplementary key words cholesterol • 25-hydroxycholesterol • mRNA • HMG-CoA reductase

Apolipoprotein E is synthesized and secreted by a wide variety of mammalian cells, and a number of hypotheses have been developed regarding the potential function(s) of apoE synthesized in extrahepatic tissues (for review see ref. 1). The macrophage was the first extrahepatic cell type in which apoE synthesis was described and a great deal of information has been reported characterizing the regulation of apoE synthesis in these cells (2, 3). Of particular importance, in view of the key role played by macrophages in the atherogenic process, is the modulation of macrophage apoE synthesis by cholesterol (2). Such modulation is accounted for by altered cell apoE mRNA levels which, in turn, can be accounted for by altered apoE gene transcription (4, 5). In previous studies we have demonstrated that macrophage apoE gene expression is sensitively modulated by macrophage cholesterol content, i.e., very small increases in total macrophage cholesterol content stimulated apoE mRNA levels and protein synthesis (4, 5). This work, delineating the relationship between macrophage cholesterol content and apoE expression, was performed using a mouse peritoneal macrophage cell model. While these cells produce large amounts of apoE, they do not express the classic LDL receptor pathway (6). Conversely, a number of macrophage cell lines exhibit typical LDL receptor activity but do not express the apoE gene (7). The recently reported information that the human monocytic cell line, THP 1, expresses both the LDL receptor and apoE genes (8, 9) provided an opportunity to *a*) examine this macrophage cell line for cholesterol responsiveness of the apoE gene and therefore determine its suitability to further dissect the molecular mechanisms by which cholesterol regulates apoE gene expression; and *b*) to compare the regulation of apoE and LDL receptor genes by sterols. In these studies, we report the results of experiments that indicate that the macrophage apoE gene displays a responsiveness to cellular sterol content which is characteristic of classic sterol-responsive genes, e.g., the LDL receptor gene. In addition, the data indicate that oxysterols previously shown to regulate the expression of sterol-responsive genes also regulate apoE gene expression. Furthermore, oxysterol derivatives of cholesterol activate apoE gene expression sooner than cholesterol itself. Interestingly, however, the responses of the apoE gene and LDL receptor gene to macrophage sterol can be dissociated *a*) in the time it takes to respond to exogenous cholesterol loading; and *b*) in their response to activation of endogenous cholesterol synthesis. These latter observations suggest that the intracellular mechanisms for the sterol regulation of these two genes are not identical.

Abbreviations: apo, apolipoprotein; LDL, low density lipoprotein; LPDS, lipoprotein-deficient serum; BSA, bovine serum albumin; HMG, 3-hydroxy-3-methylglutaryl; 25-OH, 25-hydroxy; ALDL, acetylated low density lipoprotein.

MATERIALS AND METHODS

Cell culture

The THP1 human monocytic cell line was obtained from American Type Culture Collection. The cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum. For experiments measuring mRNA abundance, 8×10^6 cells in 10% fetal bovine serum–RPMI-1640 were added into 100-mm cell cultured plates and phorbol 12-myristate 13-acetate (3.2×10^{-7} M in ethanol) was added. After 72 h the nonadherent cells were removed and the adherent cells were washed twice with 0.2% bovine serum albumin in RPMI-1640, and the experimental incubations were begun. Cells used for the measurement of free cholesterol, total cholesterol, and protein were treated identically, except that they were plated into 6-well plates. LDL was isolated from human plasma by sequential density gradient ultracentrifugation (4, 5). LDL was acetylated by the repeated addition of acetic anhydride as previously described (4, 5).

Measurement of mRNA abundance

For measurement of apoE, LDL receptor, and HMG-CoA reductase abundance, total cellular RNA was extracted in guanidine thiocyanate and purified by serial precipitation in guanidine-HCl–ethanol as previously described (4, 5). From each RNA isolate a range of RNA mass amounts was applied to nitrocellulose membranes using a slot-blot apparatus (Schleicher & Schuell). This approach allows for the measurement of a specific mRNA abundance multiple times from each isolate and for assuring linearity of the hybridization signal with respect to RNA load and autoradiographic film response (4, 5). Radiolabeled LDL receptor cDNA (10) probe was prepared by random primed synthesis using pLDL-R/HH1, which is a 1919-base pair Bam HI subfragment of a 5.3-kb cDNA clone for the human LDL receptor (generously provided by David Russell, University of Texas Southwestern Medical Center, Dallas, TX). Radiolabeled apoE cDNA probe was also prepared by random primed synthesis using pHEA4 (generously provided by C. Reardon, University of Chicago, Chicago, IL) which is a 1.1-kb Aat II-HinfI subfragment which encompasses the entire protein coding region of the human apoE cDNA subcloned into pUC. Radiolabeled HMG-CoA reductase cDNA probe was prepared by random primed synthesis using a 1.1-kb Hind III fragment of pHRed-102 (11) (generously provided by K. Luskey, University of Texas Southwestern Medical Center, Dallas, TX). Hybridization reactions were performed as previously described using 100 ng of cDNA with a minimum of 1×10^7 cpm (4, 5, 10). Autoradiograms of dried filters where quantitated by scanning densitometry and values obtained from each isolate were plotted versus mass of RNA applied to the filter. Using a linear regression method, lines were generated, the slope of which indicates relative mRNA abundance (4, 5, 10). Filters were reprobed with a labeled oligo d(T) (12) to correct for total amount of mRNA immobilized (variability of 10–30%) or were evaluated with both apoE and LDL receptor probes to eliminate the possibility that a systematic alteration in the mRNA content of total RNA contributed to our results. The results shown in each figure are representative of two to three similarly conducted experiments.

Nuclear run-off transcription assay

THP1 nuclei were isolated and run-off transcription was measured by the methods of Greenberg and Ziff with minor modifications as previously described (5, 13). Runoff reactions were started with an equal number of nuclei and newly formed transcript were purified using phenol–chloroform–isoamyl alcohol extraction and trichloroacetic acid precipitation after digestion with DNase I and proteinase K. Equal amounts of radioactivity were then hybridized to nitrocellulose membranes containing 3 μg of linearized cDNA for apoE, LDL receptor, and beta-actin (provided by E. Fuchs, University of Chicago, Chicago, IL) according to the method described by Brenner and Chojkier (14).

Preparation of lipoproteins, LPDS, and phospholipid vesicles

Human LDL (d 1.019–1.063 g/ml) was isolated and acetylated as previously described in detail (4, 5). Human LPDS was prepared by ultracentrifugation of human serum in KBr (d 1.215 g/ml) as previously described (13). Phospholipid vesicles were prepared by forcefully injecting 11 mg of phosphatidylcholine (from a 10 mg/ml stock in ethanol) into 110 ml rapidly stirring phosphate-buffered saline. This suspension was then concentrated to 5.5 ml and dialyzed against 450 ml of RPMI-1640 before being filtered through a 0.22-micron syringe filter.

Other assays

Protein was determined by the method of Lowry et al. (15) using bovine serum albumin as the standard. Free and total (after saponification) cholesterol were measured in Bligh-Dyer extracts by gas–liquid chromatography using coprostanol as an internal standard (4, 5). Cholesteryl ester mass was calculated as the difference between total and free cholesterol. Statistical significance was determined using Student's two-tailed *t*-test.

RESULTS

In contrast to the mouse peritoneal macrophage, which is the cell type in which regulation of apoE production has

been most extensively studied, the THP1 macrophage expresses the classic LDL receptor pathway, in addition to scavenger receptor activity. We therefore compared the ability of ALDL and LDL to stimulate cholesterol accumulation and apoE gene expression in these cells. **Table 1** shows that control macrophages, incubated in serum-free medium alone, contain 15.3 μg of free cholesterol per mg of cell protein and no measurable cholesteryl ester. The addition of ALDL or LDL at 100 μg/ml leads to an increase of total cellular cholesterol to 58.9 and 28.5 μg/mg, respectively; only in the case of ALDL is the accumulation of sufficient magnitude to produce substantial cholesteryl ester accumulation. As shown, in each case there is a substantial increase in apoE mRNA abundance compared to control cells. The marked potentiation of apoE gene expression by LDL, in the absence of substantial cholesteryl ester accumulation, reinforces observations in mouse peritoneal macrophages (4, 5) in which ALDL could augment apoE mRNA abundance under experimental conditions which minimize cholesteryl ester accumulation (i.e., in the presence of an inhibitor of acyl-CoA:cholesterol acyltransferase).

In mouse peritoneal macrophages, we have shown that apoE gene expression and the amount of apoE protein produced can be sensitively modulated by stepwise alteration of cellular cholesterol content (4, 5). In THP1 cells, we have the opportunity to compare the effect of increasing cellular cholesterol on apoE versus LDL receptor gene expression. The latter pathway has served as a paradigm for the study of cholesterol-responsive genes (16). As shown in **Fig. 1**, bottom panel, incubation of THP1 cells with increasing concentrations of ALDL leads to progressive enrichment of cellular cholesterol. This enrichment occurs primarily in the free portion initially, but as the concentration of ALDL is increased, most of the accumulation occurs in the esterified fraction. As shown in Fig. 1, middle panel, apoE mRNA abundance increases with increasing cholesterol content with maximal response after incubations in 10–25 μg/ml of ALDL. Fig. 1, top panel, shows that LDL receptor mRNA abundance in the same cells is regulated in the opposite direction by cholesterol over an identical dose-response relationship.

The data in Fig. 1 indicate that the apoE and LDL receptor gene responds over a similar range of cellular cholesterol content. This could mean that each gene is

TABLE 1. Induction of apoE mRNA by LDL in THP1 cells

Addition	Free Cholesterol	Cholesteryl Ester	Relative ApoE mRNA Level
	μg/mg	*μg/mg*	
None	15.3[a]	0	1.0
LDL	28.4	0.1	14.5
ALDL	40.4	18.5	15.0

Cells were plated and differentiated as described in Methods. Cells were then washed twice with 0.2% BSA in RMPI-1640 and placed in this medium along with 100 μg/ml of the indicated lipoprotein for an additional 48 h. At that time, cells were harvested for measurement of sterol and protein or apoE mRNA abundance. Values shown are the mean from duplicate plates of cells.

[a]Values are given as μg per mg of cell protein. The apoE mRNA values have been corrected for the amount of total mRNA immobilized for each condition by measuring the hybridization of a labeled oligo d(T) (12).

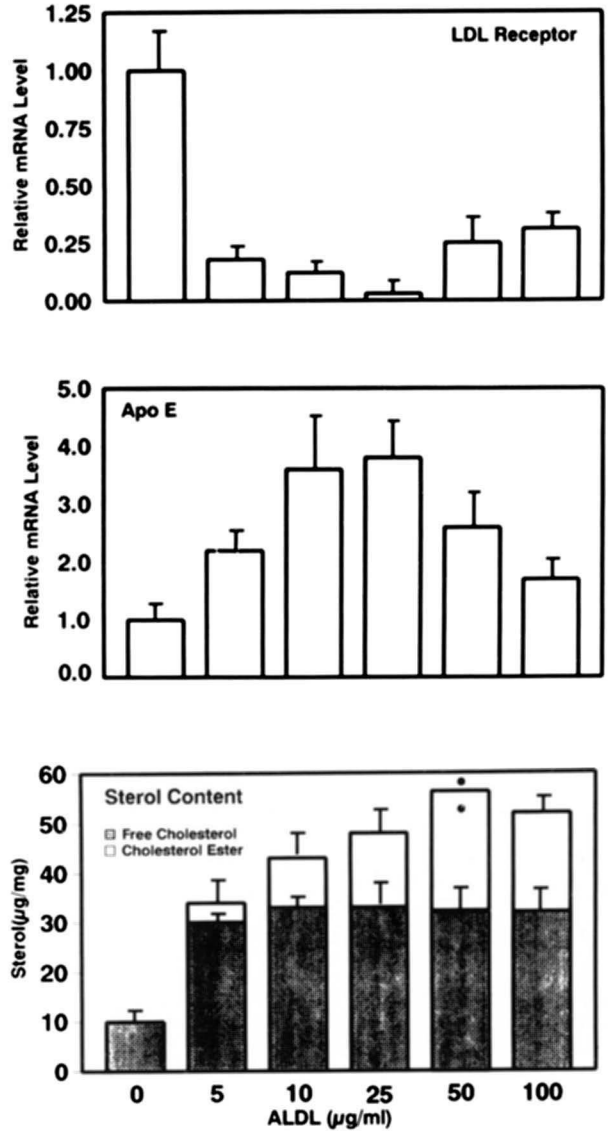

Fig. 1. Coordinate dose-response regulation of apoE and LDL receptor mRNA abundance by cell cholesterol. After differentiation, THP1 cells were washed and placed in 0.2% BSA in RPMI-1640 as described in the legend to Table 1, plus the indicated concentration of ALDL. After 48 h, cells were harvested for sterol and protein measurement (bottom) or measurement of apoE (middle) or LDL receptor (top) mRNA abundance. Values shown represent the mean ± SD of measurements from triplicate dishes except for the cholesteryl ester mass value at 50 μg/ml of ALDL which is the mean of the individually indicated duplicate determinations.

responding to the same regulatory signal, i.e., a specific sterol derivative or subcellular cholesterol pool. To examine this possibility further, we studied the time course of the response of these two genes to exogenous cholesterol loading. **Fig. 2**, bottom panel, shows the progressive enrichment of cellular cholesterol as incubation in ALDL (25 μg/ml) progresses. In Fig. 2, middle panel, it can be seen that there is no significant change in apoE mRNA abundance until 48 h after the addition of ALDL. In marked contrast, LDL receptor mRNA levels are already partially depressed by 6 h in ALDL (to 60% of control) and are significantly suppressed ($P<0.01$) by 24 h. These data indicate that the regulatory response to exogenous sterols occurs earlier for the LDL receptor gene compared to the apoE gene.

Fig. 2, top panel, shows the result of a nuclear run-off transcription assay from nuclei harvested 7 h after addition of 25 μg/ml of ALDL. Consistent with the mRNA measurements, apoE gene transcription is unchanged at 7 h while LDL receptor gene transcription is already depressed by that time. Scanning densitometry of the autoradiograph of this experiment, with correction for beta-actin gene transcription as an internal control, confirmed little change in apoE gene transcription with ALDL, (7% decrease with ALDL) while LDL receptor gene transcription was threefold higher in the absence of ALDL. In a separate and identical experiment, apoE gene transcription was 4% higher in ALDL-treated cells, while LDL receptor gene transcription was twofold higher in the absence of ALDL. The data in Fig. 2, therefore, indicate that the transcriptional response of the apoE and LDL receptor genes to exogenous cholesterol can be temporally dissociated.

In **Fig. 3** the effect of removing cholesterol from cholesterol-loaded THP1 macrophages, using phospholipid liposomes, is shown. Between 6 and 24 h after addition of liposomes there is a marked fall in total cell cholesterol, reflecting the net cholesterol efflux produced by the phospholiposomes (bottom panel). Over that same time period there is a 4.5-fold increase in LDL receptor mRNA abundance (middle panel; $P<0.02$). While there is a small decrease in apoE mRNA abundance (approximately 30%) over this same time interval (not significant), the major reduction in apoE mRNA level occurs later, between 24 and 48 h ($P<0.02$) (top panel). These data indicate that, as in the case of the exogenous cholesterol loading, apoE gene response to efflux of excess cholesterol lags behind that of the LDL receptor gene.

Further evidence of the dissociation of the regulation of the apoE and LDL receptor gene could be observed in the response of these two genes to the perturbation of endogenous cellular cholesterol synthesis (**Fig. 4**). For this experiment, fully differentiated THP1 cells were plated in 5% LPDS for 24 h in order to produce cholesterol efflux with negative cholesterol balance and thereby induce

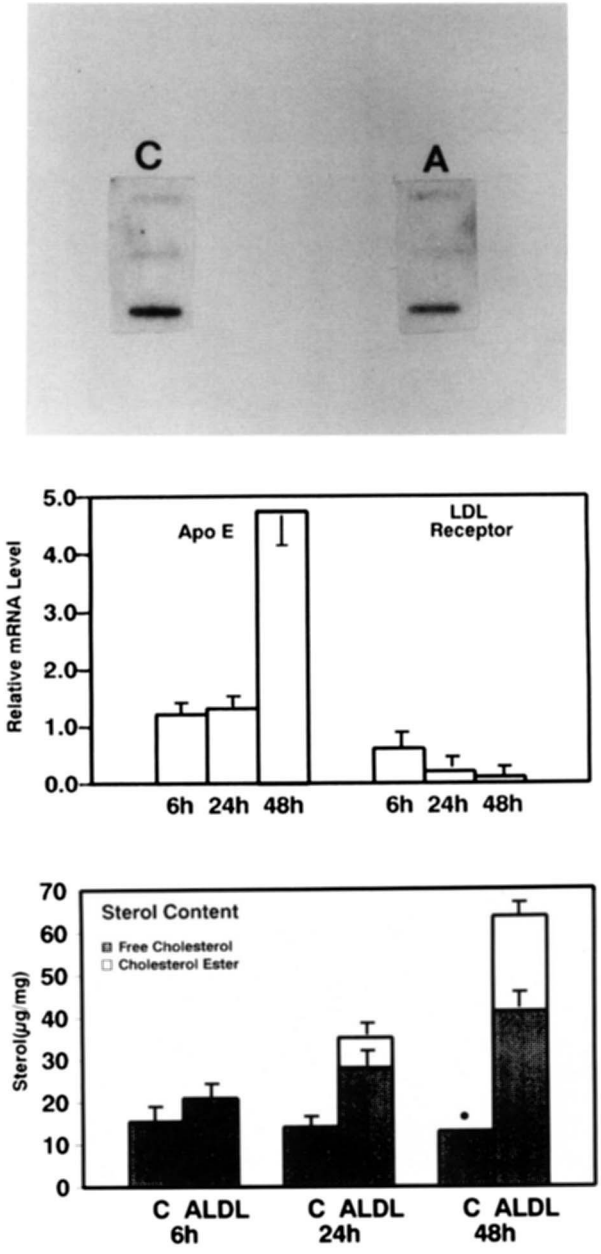

Fig. 2. Time course of sterol effects on apoE and LDL receptor gene expression. Differentiated THP1 cells were washed and placed in 0.2% BSA in RPMI-1640 alone or with 25 μg/ml of ALDL at time 0. Cultures were then harvested 6, 24, and 48 h later for measurement of sterol and protein (bottom) or mRNA abundance (middle). The mRNA abundance in cells incubated with ALDL is expressed relative to the mRNA abundance in cells without ALDL and harvested at the same time. Values shown in bottom and middle panels are mean ± SD from triplicate dishes. In the top panel, THP1 nuclei were isolated from cells 7 h after the addition of ALDL 25 μg/ml for nuclear runoff transcription measurement as described in Methods. Column labeled "C" represents control nuclei; column labeled "A" represents ALDL-incubated nuclei. Top, middle, and bottom slots show apoE and LDL receptor and β-actin gene transcription, respectively. For the run-off reaction, 15.5×10^6 nuclei were used for each condition and 8×10^6 cpm/ml were used in the hybridization reaction. For the middle panel, the apoE mRNA levels at 6 h and 24 h were not different from control levels; at 48 h the increase was significant at $P<0.01$. The LDL receptor mRNA levels at 6 h were not significantly decreased compared to control values; at 24 h and 48 h the decreases were significant at the $P<0.01$ and 0.05 levels, respectively.

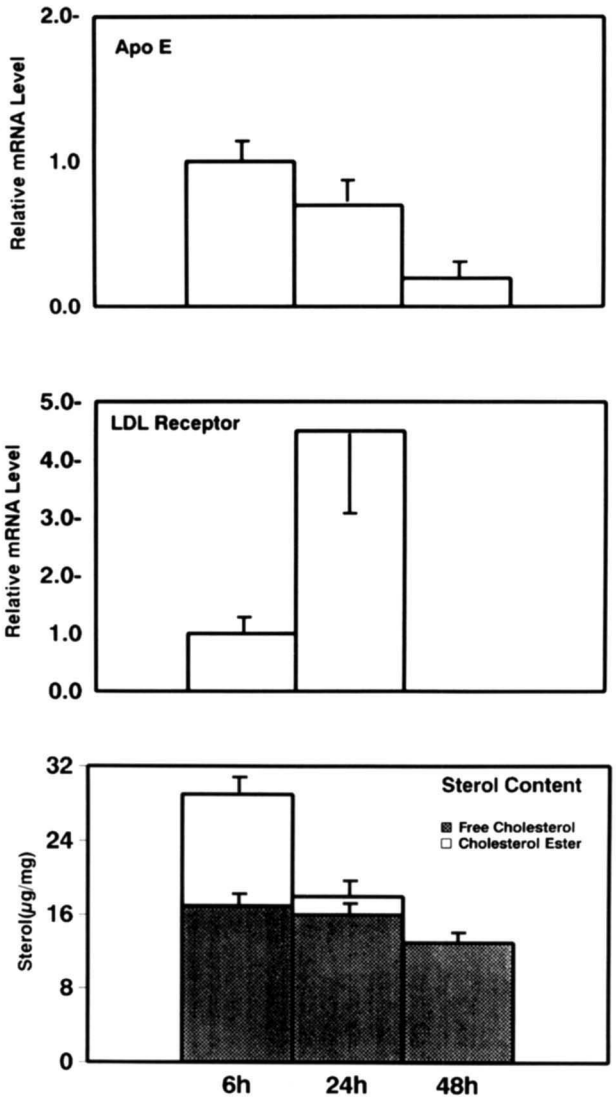

Fig. 3. Time related changes of the effect of sterol removal on apoE and LDL receptor gene expression. Differentiated cells were placed in 0.2% BSA in RPMI-1640 plus ALDL 25 μg/ml for 48 h. At that time cells were washed twice and placed in 0.2% BSA in RPMI-1640 with phospholipid vesicles at 1 mg/ml to promote cholesterol efflux. Cells were then harvested at the indicated time for measurement of sterol and protein (bottom) or LDL receptor (middle) and apoE (top) mRNA abundance. The mRNA levels at 24 and 48 h are expressed relative to the 6-h mRNA abundance. Values shown are mean ± SD of measurements from triplicate dishes. The changes in apoE mRNA abundance were not significant until 48 h ($P<0.02$). The LDL receptor mRNA increase was significant at 24 h ($P<0.02$).

cholesterol synthesis. These incubations in LPDS produced a twofold induction of HMG-CoA reductase mRNA levels (not shown) compared to cells not so incubated. After 24 h in LPDS, cells were placed in serum-free media and harvested 6, 24, and 48 h later. Cellular cholesterol was less than 10 μg/mg at 6 h, remained at this low level through 24 h, but recovered to 15 μg/mg by 48 h, reflecting the effects of endogenous cholesterol synthesis (Fig. 4, bottom). LDL receptor mRNA levels, which were elevated twofold compared to cells not incubated in LPDS (not shown), remained high and virtually unchanged throughout the experimental period (Fig. 4, middle). ApoE mRNA levels, however, rose pro-

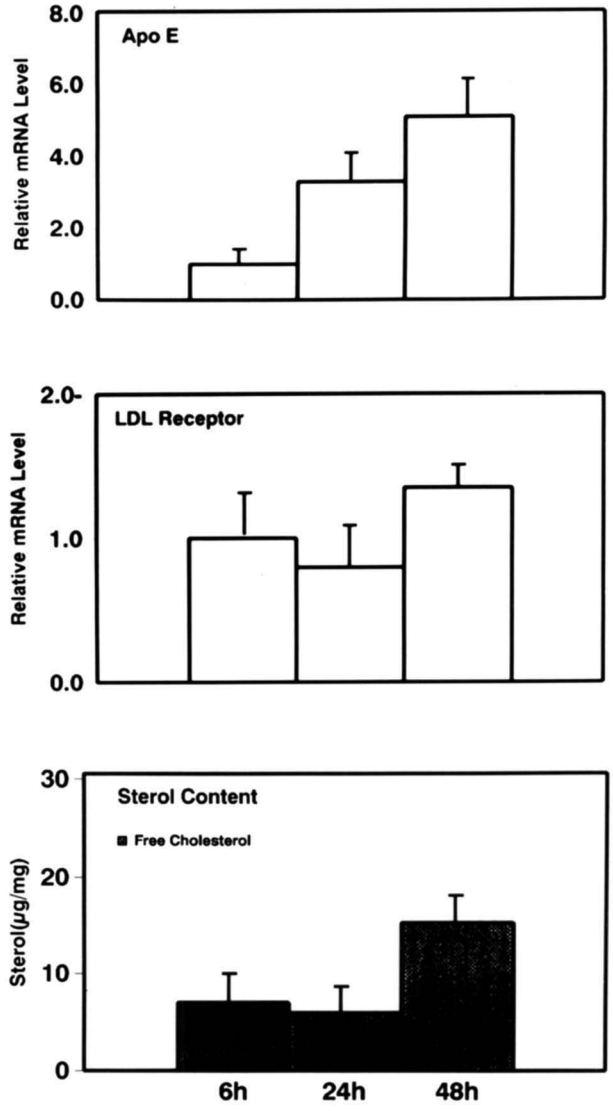

Fig. 4. Effect of LPDS-mediated modulation of endogenous cholesterol synthesis on apoE and LDL receptor gene expression. Differentiated cells were washed twice with 0.2% BSA in RPMI-1640 and placed in 5% LPDS in RPMI-1640 for 24 h. At that time, cells were washed again as described above and placed in 0.2% BSA in RPMI-1640 and harvested at the indicated times for sterol and protein measurement (bottom) or LDL receptor (middle) and apoE (top) mRNA abundance. The mRNA levels at 24 and 48 h are expressed relative to the 6-h mRNA abundance. Values shown are mean ± SD of triplicate dishes. The changes in LDL receptor mRNA abundance were not significant at any time point. The increases of apoE mRNA abundance were significant at 24 h ($P<0.05$) and 48 h ($P<0.01$).

gressively throughout the observation period eventually reaching fivefold higher levels at 48 h compared to 6 h (Fig. 4, top).

The results shown in Figs. 2–4 indicate that regulation of the apoE and LDL receptor genes by sterols can be dissociated in macrophages and therefore may be responding to distinct subcellular sterol pools or unique sterol derivatives. Oxidized sterol derivatives have been shown to be potent modulators of LDL receptor gene expression in a variety of cell types (16, 17). Oxysterols are also important regulators of the endogenous cholesterol biosynthetic pathway and for regulation of apoE protein synthesis in mouse peritoneal macrophages (4, 18). In order to further define the importance of oxysterols in the regulation of apoE gene expression in macrophages, THP1 cells were incubated in increasing concentrations of 25-hydroxycholesterol. As shown in **Table 2**, this compound results in a 3.3-fold induction of apoE mRNA abundance at the 1 μg/ml concentration. While this degree of stimulation is not as great as generally observed using ALDL, this stimulation could be observed much earlier, i.e., within 18 h of oxysterol addition.

DISCUSSION

The data in this report underscore the responsiveness of the apoE gene to small perturbations of cellular cholesterol homeostasis and reinforce the concept that this gene be considered among the family of genes that are sensitively regulated by cellular cholesterol. The data in Table 1 indicate that apoE mRNA levels are increased by exogenous cholesterol delivered via incubations in LDL. This stimulation of apoE mRNA abundance occurs notwithstanding the fact that the LDL receptor pathway represents a regulated means of cholesterol delivery which precludes significant accumulation of cholesteryl esters. The data in Fig. 1 also support the above concept by showing that, after 48-h incubations in ALDL, the apoE and LDL receptor genes respond reciprocally and within an identical dose range of macrophage cholesterol content.

TABLE 2. Regulation of apoE gene expression by 25-OH cholesterol

25-OH Cholesterol	Relative ApoE mRNA Level
μg/ml	
0	1.0 ± 0.2
0.5	1.9 ± 0.1
1.0	3.3 ± 0.8
2.5	2.6 ± 0.5
5.0	3.0 ± 0.8

Differentiated cells were incubated in 0.2% BSA in RMPI-1640 for 24 h, then the indicated concentration of 25-OH cholesterol was added for an additional 18 h. At that time cells were harvested for measurement of apoE mRNA abundance. Values shown are mean ± SD from triplicate dishes. The increases in apoE mRNA abundance at 0.5 and 5.0 μg/ml of 25-OH cholesterol are significant at the $P < 0.05$ level. The increases at 1.0 and 2.5 μg/ml are significant at $P < 0.02$. The above values have been corrected for the amount of total mRNA immobilized for each condition by measuring the hybridization of a labeled oligo d(T) (12).

Based on the above observations it would be reasonable to hypothesize that very similar, or perhaps even identical, molecular mechanism or mediators accounted for the modulation of the apoE and LDL receptor genes by sterol. This hypothesis would be further suggested by the presence of several sites in the 5' flanking and intronic portions of the apoE gene with significant homology to the sterol response element of the LDL receptor gene (5, 19). According to this hypothesis, it could by expected that apoE and LDL receptor gene response to sterol would also be temporally coordinated. The data in Figs. 2–4, however, show a clear temporal dissociation of the response of these two genes to exogenous sterol and to perturbation of endogenous cellular cholesterol biosynthesis. Under certain circumstances, differences in the kinetics of mRNA turnover could account for temporal dissociation in the response of the levels of two different mRNA species despite the fact that modulation of the transcription of the two cognate genes is coordinately regulated. This explanation, however, does not account for our observations for the following reasons. First, direct measurement of gene transcription (Fig. 2, top) documents the response of LDL receptor gene transcription within 7 h after the addition of ALDL, while apoE gene transcription rate is unchanged at this time. Second, the more prompt response of apoE mRNA levels to the stimulation of endogenous cholesterol synthesis (Fig. 4) as compared to loading the cell with exogenous cholesterol (Fig. 2) indicates that some physiological mediators can promote apoE gene expression more rapidly than others.

The data showing that apoE mRNA levels respond to 25-hydroxycholesterol provides a mechanism for our previous observation regarding the effect of this oxysterol on the amount of apoE protein synthesized by mouse peritoneal macrophages (4). In addition, it is interesting that the apoE mRNA response to 25-hydroxycholesterol occurs sooner than the response to exogenous cholesterol. An oxysterol intermediate may be an important regulator of the apoE gene as has been postulated for the LDL receptor and HMG-CoA reductase genes (16–18). Alternatively, 25-hydroxycholesterol may enter a putative regulatory pool more rapidly than lipoprotein-associated cholesterol. If oxysterols are indeed important for the regulation of apoE and LDL receptor genes, it is possible that the dissociation of the response of these two genes to experimental manipulation (Figs. 2–4) may reflect the involvement of different endogenous cellular oxysterols or of distinct subcellular pools of cholesterol.

The data reported herein indicate that the apoE gene responds to minor alterations of macrophage cholesterol balance. ApoE mRNA is increased after incubations in LDL and the apoE and LDL receptor genes respond to exogenous cholesterol loading over an identical dose range. However, the means by which these two genes are regulated by sterol cannot be identical in view of the temporal dissociation in the response to exogenous cholesterol. In addition, the modulation of apoE gene expression by cells in which endogenous cholesterol synthesis has been stimulated appears to be particularly complex. In cells depleted of cholesterol by incubation in LPDS, the endogenous cholesterol biosynthetic pathway (as monitored by HMG-CoA reductase mRNA levels) and the LDL receptor pathway (as monitored by LDL receptor mRNA levels) are induced. Total cell cholesterol rises over the period of experimental observation due solely to the production of endogenous cholesterol (as the cells are in serum-free medium). Over this same period, LDL receptor mRNA levels remain unchanged at their initially high levels, while apoE mRNA levels rise progressively from 6 to 48 h. This experimental result suggests that the temporal dissociation of LDL receptor and apoE gene regulation is not due to a greater sensitivity of the LDL receptor gene to alteration of intracellular cholesterol homeostasis.

The sensitive regulation of apoE gene expression by cellular cholesterol balance suggests that the protein product of this gene may have an important role in maintaining cell cholesterol homeostasis. This role may involve participation in *a*) the partitioning of cholesterol between the cell and extracellular cholesterol acceptors, or *b*) the disposition and movement of cholesterol between intracellular compartments. A role for apoE in intracellular cholesterol apportionment has also been suggested by the studies recently reported by Hamilton et al. (20).

In summary, our data indicate that the THP1 macrophage cell line will be a useful cell model to dissect the molecular mechanism for the sterol regulation of the apoE gene. Furthermore, the comparison of the response of LDL receptor and apoE gene confirms that, in the macrophage, the apoE gene is sensitively regulated by cell sterol content. Also supporting this notion is the 25-hydroxycholesterol modulation of apoE gene expression and the response of this gene to incubations in native LDL. The nature of the intracellular pathway for regulation of the apoE gene (positively modulated by sterol) compared to that for regulation of the LDL receptor gene (negatively modulated by sterols) will require additional investigation.

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REFERENCES

1. Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* **240:** 622–630.
2. Basu, S. K., M. S. Brown, Y. K. Ho, R. J. Havel, and J. L. Goldstein. 1981. Mouse macrophages synthesize and secrete a protein resembling apolipoprotein E. *Proc. Natl. Acad. Sci. USA.* **78:** 7545–7549.
3. Werb, Z., and J. R. Chin. 1983. Endotoxin suppresses expression of apoprotein E by mouse macrophages in vitro and in culture. *J. Biol. Chem.* **262:** 11657–11662.
4. Mazzone, T., H. Gump, P. Diller, and G. S. Getz. 1987. Macrophage free cholesterol content regulates apolipoprotein E synthesis. *J. Biol. Chem.* **262:** 11657–11662.
5. Mazzone, T., K. Basheeruddin, and C. Poulos. 1989. Regulation of macrophage apolipoprotein E gene expression by cholesterol. *J. Lipid Res.* **30:** 1055–1064.
6. Koo, C., M. E. Wernette-Hammond, and T. L. Innerarity. 1986. Uptake of canine B-very low density lipoproteins by mouse peritoneal macrophages is mediated by a low density lipoprotein receptor. *J. Biol. Chem.* **261:** 11194–11201.
7. Via, D. P., A. L. Plant, I. F. Craig, A. M. Gotto, and L. C. Smith. 1985. Metabolism of normal and modified low-density lipoproteins by macrophage cell lines of murine and human origin. *Biochim. Biophys. Acta.* **833:** 417–428.
8. Auwerx, J. H., S. Deeb, J. D. Brunzell, R. Peng, and A. Chait. 1988. Transcriptional activation of the lipoprotein lipase and apolipoprotein E genes accompanies differentiation in some human macrophage like cell lines. *Biochemistry.* **27:** 2651–2655.
9. Auwerx, J. H., A. Chait, G. Wolfbauer, and S. S. Deeb. 1989. Involvement of second messengers in regulation of the low density lipoprotein receptor gene. *Mol. Cell Biol.* **9:** 2298–2302.
10. Mazzone, T., K. Basheeruddin, L. Ping, S. Frazer, and G. S. Getz. 1989. Mechanism of the growth-related activation of the low density lipoprotein receptor pathway. *J. Biol. Chem.* **264:** 1787–1792.
11. Luskey, K. L., and B. Stevens. 1985. Human 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Biol. Chem.* **260:** 10271–10277.
12. Harley, C. B. 1987. Hybridization of oligo (dT) to RNA in nitrocellulose. *Gene. Anal. Tech.* **4:** 17–22.
13. Mazzone, T., K. Basheeruddin, and H. Duncan. 1989. Inhibitors of translation induce low density lipoprotein receptor gene expression in human skin fibroblasts. *J. Biol. Chem.* **264:** 15529–15534.
14. Brenner, D. A., and M. Chojkier. 1987. Acetaldehyde increases collagen gene transcription in cultured human fibroblasts. *J. Biol. Chem.* **262:** 17690–17695.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
16. Sudhof, T. C., D. R. Van der Westhuyzen, J. L. Goldstein, M. S. Brown, and D. W. Russel. 1987. Three direct repeats

and a TATA-like sequence are required for regulated expression of the human low density lipoprotein receptor gene. *J. Biol. Chem.* **262:** 10773–10779.
17. Takagi, K., J. G. Alvarez, M. F. Favata, J. M. Trzaskos, and J. F. Strauss. 1989. Control of low density lipoprotein receptor gene activity. *J. Biol. Chem.* **264:** 12352–12357.
18. Miller, S. C., and G. Melnykovych. 1984. Regulation of cholesterol biosynthesis and esterification by 25-hydroxycholesterol in a macrophage-like cell line: uncoupling by progesterone. *J. Lipid Res.* **25:** 991–999.
19. Smith, J. R., T. F. Osborne, J. L. Goldstein, and M. S. Brown. 1990. Identification of nucleotides responsible for enhancer activity of sterol regulatory element in low density lipoprotein receptor gene. *J. Biol. Chem.* **265:** 2306–2310.
20. Hamilton, R. L., J. S. Wong, L. S. S. Guo, S. Krisans, and R. J. Havel. 1990. Apolipoprotein E localization in rat hepatocytes by immunogold labeling of cryothin sections. *J. Lipid Res.* **31:** 1589–1603.