Regulation of macrophage apolipoprotein E gene expression by cholesterol

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Abstract The relationship between macrophage cholesterol content and apolipoprotein E (apoE) gene expression was investigated in cultured mouse peritoneal macrophages. Acetylated low density lipoprotein (ALDL) at 25 μg/ml led to increased macrophage messenger RNA abundance as detected by Northern blot hybridization. Incubation in a range of ALDL concentrations (0–100 μg/ml) led to graded increases in macrophage free and esterified cholesterol, and apoE mRNA detected by slot blot hybridization. After a 2-h pulse of ALDL, with or without acyl-CoA:cholesterol acyltransferase inhibition, increased apoE mRNA was not detected until after 6 h. Removal of cholesterol from upregulated macrophages, using human high density lipoprotein, resulted in a return of apoE mRNA levels toward baseline. Nuclear run-off transcription assays demonstrated increased apoE gene transcription in macrophages incubated with ALDL. It is concluded that: *a*) previously reported changes in macrophage apoE content and secretion after cholesterol enrichment or depletion can be attributed to changes in apoE mRNA levels; and *b*) cholesterol-induced changes in apoE mRNA are associated with increased apoE gene transcription. —**Mazzone, T., K. Basheeruddin, and C. Poulos.** Regulation of macrophage apolipoprotein E gene expression by cholesterol. *J. Lipid Res.* 1989. **30:** 1055–1064.

Supplementary key words atherosclerosis • modified LDL • apoE mRNA • gene transcription • high density lipoprotein

Apolipoprotein E, a surface constituent of plasma lipoproteins and a ligand for the LDL receptor, likely plays an important role in whole-body cholesterol transport (1). Unlike most other major apolipoproteins, which are synthesized primarily by liver and intestinal cells, apoE is synthesized and secreted by a wide variety of cells (2, 3). This observation has given rise to a number of hypotheses regarding potential functions of apoE synthesized outside of the entero-hepatic axis (1). In particular, apoE production by macrophages has great potential importance due to the central role these cells play in the cholesterol homeostasis of normal and diseased vessel wall (4). ApoE synthesis has been described in mouse peritoneal macrophages (5) and human monocyte-derived macrophages (6). In both of these cell types enrichment of cells with cholesterol, using a variety of methods, has been shown to augment apoE secretion (5, 6). More specifically, in mouse peritoneal macrophages small increments in cell free cholesterol have been associated with changes in apoE synthesis (7). In these cells, there is a dose-dependent enhancement of apoE synthesis in response to increased cellular cholesterol. Use of an ACAT inhibitor (S58035) (at a dose which inhibited macrophage cholesteryl ester accumulation after an 18-h incubation in 50 μg/ml of ALDL), did not prevent ALDL-induced increases in apoE synthesis. These observations led us to conclude that cholesteryl ester accumulation was not required for enhancement of apoE synthesis and that enhanced synthesis resulted from altered free cholesterol homeostasis. We have previously presented limited data indicating that increased macrophage apoE synthesis was associated with increased apoE mRNA abundance. In this study, we more completely characterize the changes in apoE mRNA after manipulation of macrophage cholesterol content and report on changes in apoE gene transcription rates in basal and cholesterol-loaded macrophages.

METHODS

Macrophages

Resident mouse peritoneal macrophages were obtained from ICR Swiss mice (Harlan-Sprague Dawley) by peritoneal lavage (8) and placed in culture with 10% fetal bovine serum in DMEM. Two hours after being placed in culture, cells were washed twice with DMEM to remove nonadherent cells and fresh 10% fetal bovine serum in DMEM was added. The next day cells were washed again

Abbreviations: apo, apolipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; ALDL, acetylated low density lipoprotein; DMEM, Dulbecco's modified Eagle's medium; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; ACAT, acyl-CoA:cholesterol acyltransferase; HMG, hydroxymethylglutaryl.

and placed in 0.2% BSA in DMEM to begin the incubations described in the figure legends. In experiments using ethanol as a solvent for S58035 (9), the amount of ethanol was always less than 0.2% (v/v) in the medium. For apoE mRNA measurements, equal numbers of cells ($5-10 \times 10^6$ cells depending on the experiment) were placed in 35-mm culture wells. Total RNA yield from each well ranged from 2 to 5 μg for all experimental groups. Purified RNA was then used for Northern blot or slot blot hybridization as described below. For sterol and protein measurements, parallel wells were established at identical density in 24-well plates ($1-2 \times 10^6$ cells per well).

Lipoproteins

Human blood was drawn into prechilled syringes containing EDTA (final concentration 1.5 mg/ml). After removal of red blood cells, phenylmethylsulfonyl fluoride (10 mM) and Trasylol (10 μg/ml) were added and LDL (d = 1.019–1.063 g/ml) and HDL_3 (d = 1.125–1.21 g/ml) were isolated by refrigerated, sequential ultracentrifugation in KBr (10). Lipoprotein fractions were dialyzed into 1 mM EDTA in PBS and stored sterilely at 4°C under nitrogen. Lipoprotein fractions were checked for purity by agarose gel electrophoresis and apoprotein composition was confirmed by SDS-PAGE (7). LDL was acetylated by the repeated addition of acetic anhydride (7). Acetylation of LDL was confirmed by agarose gel elec trophoresis (7).

ApoE mRNA measurements

Total cellular RNA was extracted in guanidine thiocyanate and isolated through a cesium chloride cushion as described (11). For slot blot hybridization, a range of cellular RNA mass, purified from each dish, was applied to nitrocellulose filters using a slot blot apparatus (Schleicher and Schuell, Keene, NH) to yield a linear response of hybridization signal with RNA load. For Northern blot hybridization, electrophoresis and transfer of RNA were performed as described by Maniatis, Fritsch, and Sambrook (12). Radiolabeled probes were prepared by nick translation (13). The cloned cDNAs used were: rat apoE, pALE 124 (14), and human B-actin (15). Hybridization reactions were performed as previously described (7). Autoradiographs of slot blots were quantitated by scanning densitometry. Film exposures of varying durations (2–5 days) were quantitated to ensure linearity of film response to hybridization signal. Regression lines were generated using values obtained from densitometry plotted versus RNA mass applied to the filter, (averaged from duplicate wells) and the slopes of these lines reflect relative changes in apoE mRNA abundance. All such lines had R values ≥ 0.94.

Nuclear run-off transcription

Mouse peritoneal macrophage nuclei were isolated and nuclear run-off transcription was performed, with minor modifications, as described by Greenberg and Ziff (16). Briefly macrophage cultures were rinsed with ice-cold PBS and then were scraped into PBS with a rubber policeman. The cells were pelleted and resuspended in lysis buffer containing NP-40 (0.5%), Tris-HCl (10 mM, pH 8.3), NaCl (10 mM) and $MgCl_2$ (3 mM). After 5 min, the nuclei were pelleted and washed again with lysis buffer. The nuclear pellet was finally resuspended in a buffer containing 40% glycerol and stored at −80°C until used (storage time < 3 months). Nuclei were pooled from different experiments and run-off transcription reactions were started with 16×10^6 nuclei per reaction. Runoff reactions were carried out in a final volume of 300 μl of a buffer containing Tris-HCl (10 mM, pH 8), KCl (140 mM), $MgCl_2$ (10 mM), $MnCl_2$ (1 mM), creatine phosphate (6.7 mM), dithiothreitol (12.5 mM), and ribonucleotides CTP and GTP (0.5 mM each) and ATP (1.0 mM), 20% glycerol, and 100 μCi of [α^{32}P]UTP (800 Ci/mM, New England Nuclear). Newly formed transcripts were purified using phenol–chloroform–isoamyl alcohol extraction and trichloroacetic acid precipitation after digestions with DNase I and proteinase K as described (16, 17). Equal amounts of radioactivity were hybridized to nitrocellulose filters containing 3 μg of linearized cDNA for apoE, β-actin, and 3μg of pBR322 DNA (the latter included to detect nonspecific hybridization) according to the method described by Brenner and Chojkier (18).

Other assays

Protein was determined by the method of Lowry et al. (19) using bovine serum albumin as a standard. Free and total (after saponification) cholesterol were measured in Bligh-Dyer cell extracts by gas–liquid chromatography using coprostanol as an internal standard (20). Sterol values are shown as averages of measurements made in duplicate wells.

RESULTS

RNA obtained from primary cultures of mouse peritoneal macrophages, incubated in serum-free medium or serum-free medium containing 25 μg/ml of ALDL, was probed with a rat apoE cDNA after Northern transfer (**Fig. 1**). ApoE message was easily detectable in control macrophages as a single band but was increased > sixfold in intensity from macrophages incubated with ALDL.

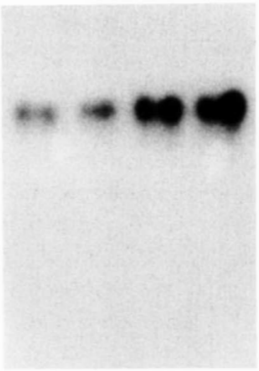

Fig. 1. Northern blot hybridization of RNA from control and cholesterol loaded macrophages. Mouse peritoneal macrophages were prepared as described in Methods and were subsequently incubated for 18 h in 0.2% BSA/DMEM (Control) or this medium plus ALDL at 25 μg/ml (ALDL). Three micrograms of RNA was added to each lane for electrophoresis, transfer, and hybridization as described in Methods. RNA isolated from duplicate wells is shown for each condition.

The mobility of the apoE mRNA band was similar in control and cholesterol loaded macrophages.

We have previously presented an extensive comparison between changes in macrophage apoE synthetic rate and cholesterol content and have also presented more limited data regarding changes in apoE mRNA levels after macrophages were incubated in ALDL at 2 and 15 μg/ml (7). **Fig. 2** shows the results of a more detailed experiment comparing apoE mRNA abundance (measured by slot blot hybridization) to macrophage cholesterol content. ALDL at 10 μg/ml leads to a large increase in apoE mRNA abundance in treated cells compared to control cells while ALDL at 2 μg/ml produces an intermediate change. There is very little change in apoE mRNA abundance when the ALDL concentration is increased from 10 μg/ml to 100 μg/ml. Over this same concentration range there is a substantial increase in cellular cholesteryl ester content, while cellular free cholesterol increases only slightly. The data in this figure represent a detailed comparison of macrophage apoE mRNA, cholesteryl ester and free cholesterol levels and demonstrate that macrophage apoE mRNA content plateaus as macrophage free cholesterol content plateaus. The approximate 25-fold increase we observed in apoE mRNA abundance demonstrated in Fig. 2b is the highest we observed in any of our experiments.

The experiments shown in Figs. 1 and 2 were performed using 18-h incubations in ALDL. **Fig. 3** and **Fig. 4** provide information regarding the time course of apoE

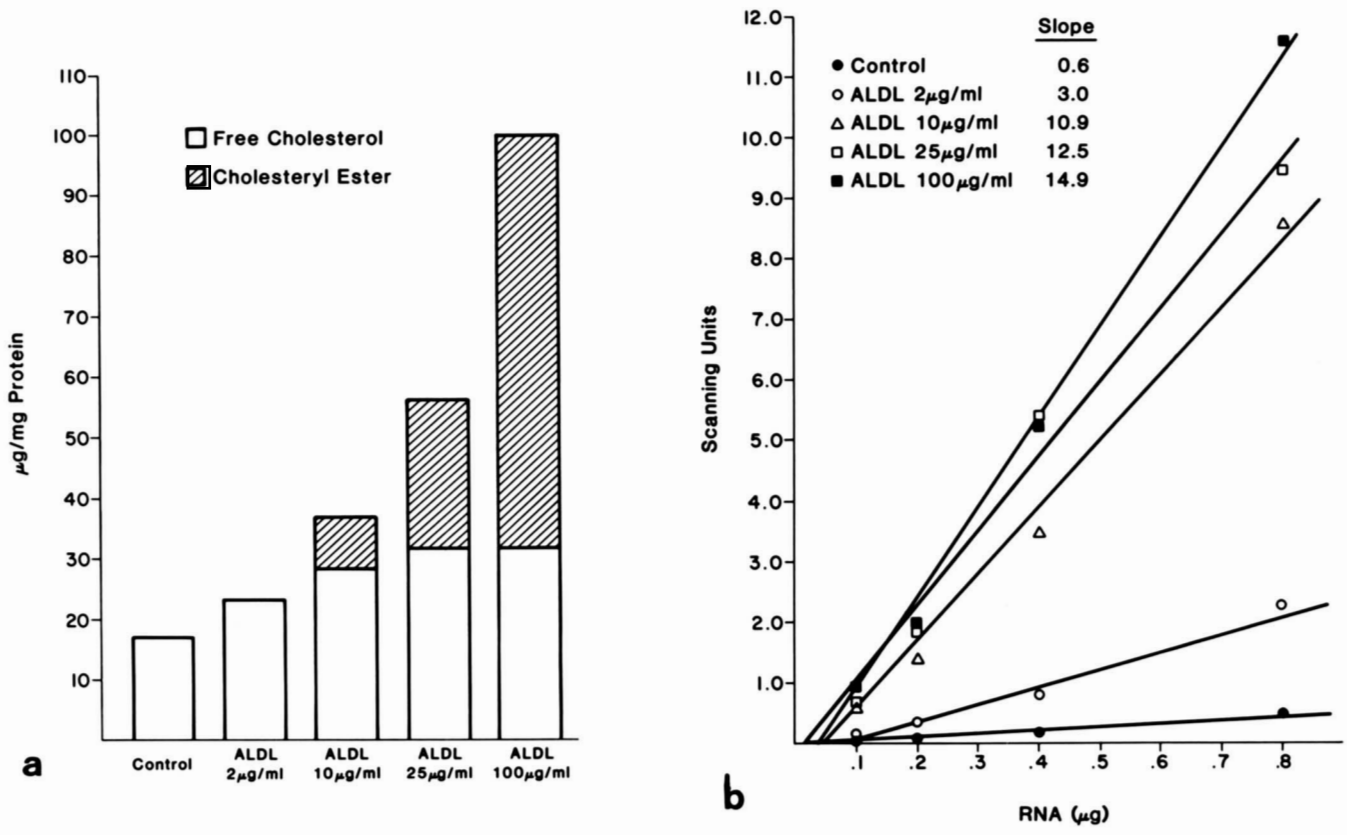

Fig. 2. Relationship of macrophage cholesterol content to apoE mRNA abundance. Mouse peritoneal macrophages were incubated for 18 h in the concentrations of ALDL shown. At that time cells were harvested for sterol and protein measurement (a) or RNA isolation for slot blot hybridization (b). Points shown in 2b represent the mean of measurements made in duplicate wells.

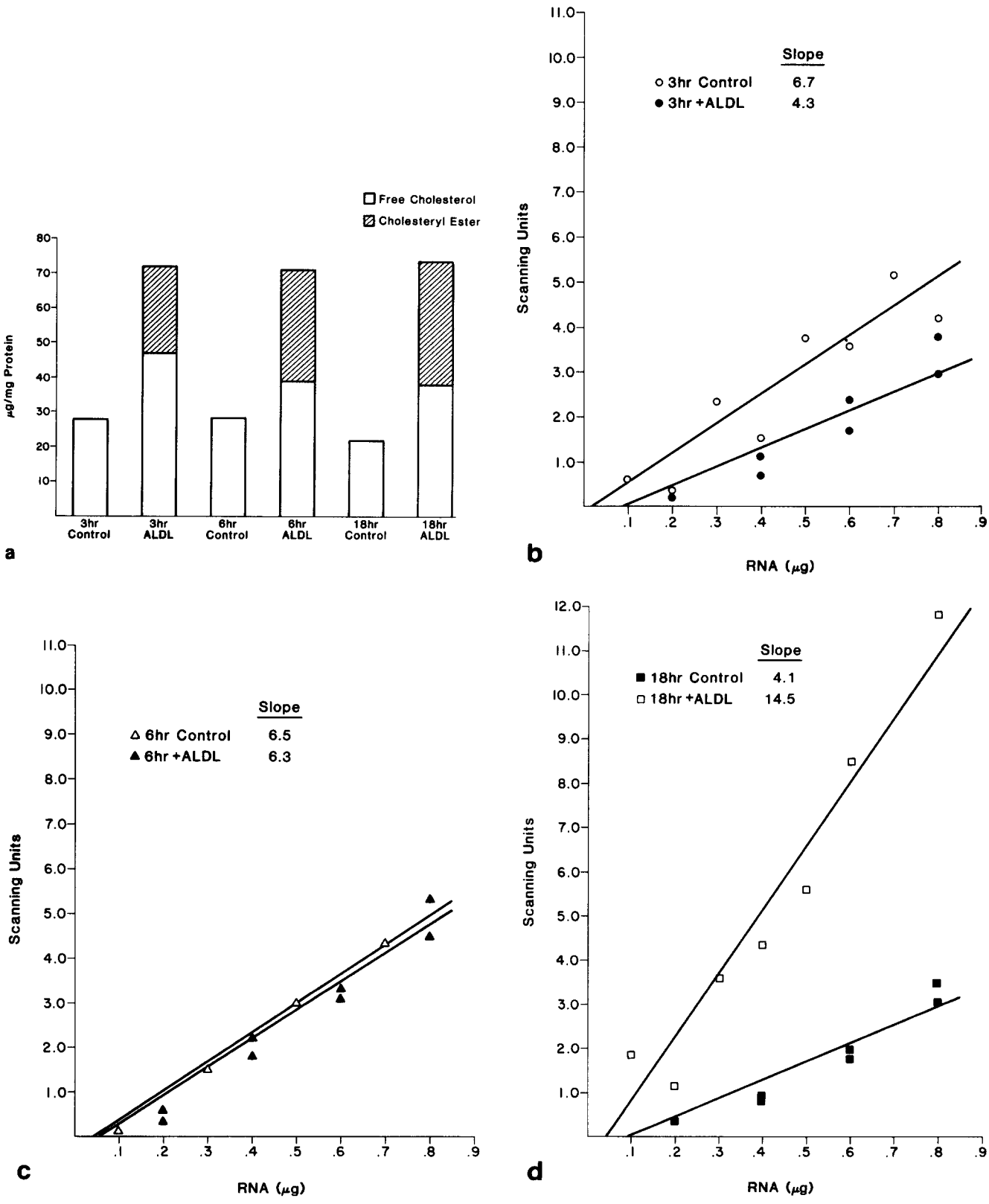

Fig. 3. Time course of apoE mRNA induction by cholesterol. Mouse peritoneal macrophages were incubated in 0.2% BSA in DMEM ± ALDL at 200 µg/ml for 2 h. Cells were then washed twice with DMEM and placed in 0.2% BSA in DMEM for harvest 3, 6, and 18 h later, to be used for sterol and protein measurement (a) or slot blot hybridization (b–d). Each line shown (b–d) represents the average of two lines derived from duplicate wells, except for the control line in c which represents a single series of measurements performed on RNA pooled from two wells. For d, RNA from one well was applied to the nitrocellulose filter at 0.2, 0.4, 0.6, and 0.8 µg; and from the duplicate well at 0.1, 0.3 and 0.5 µg.

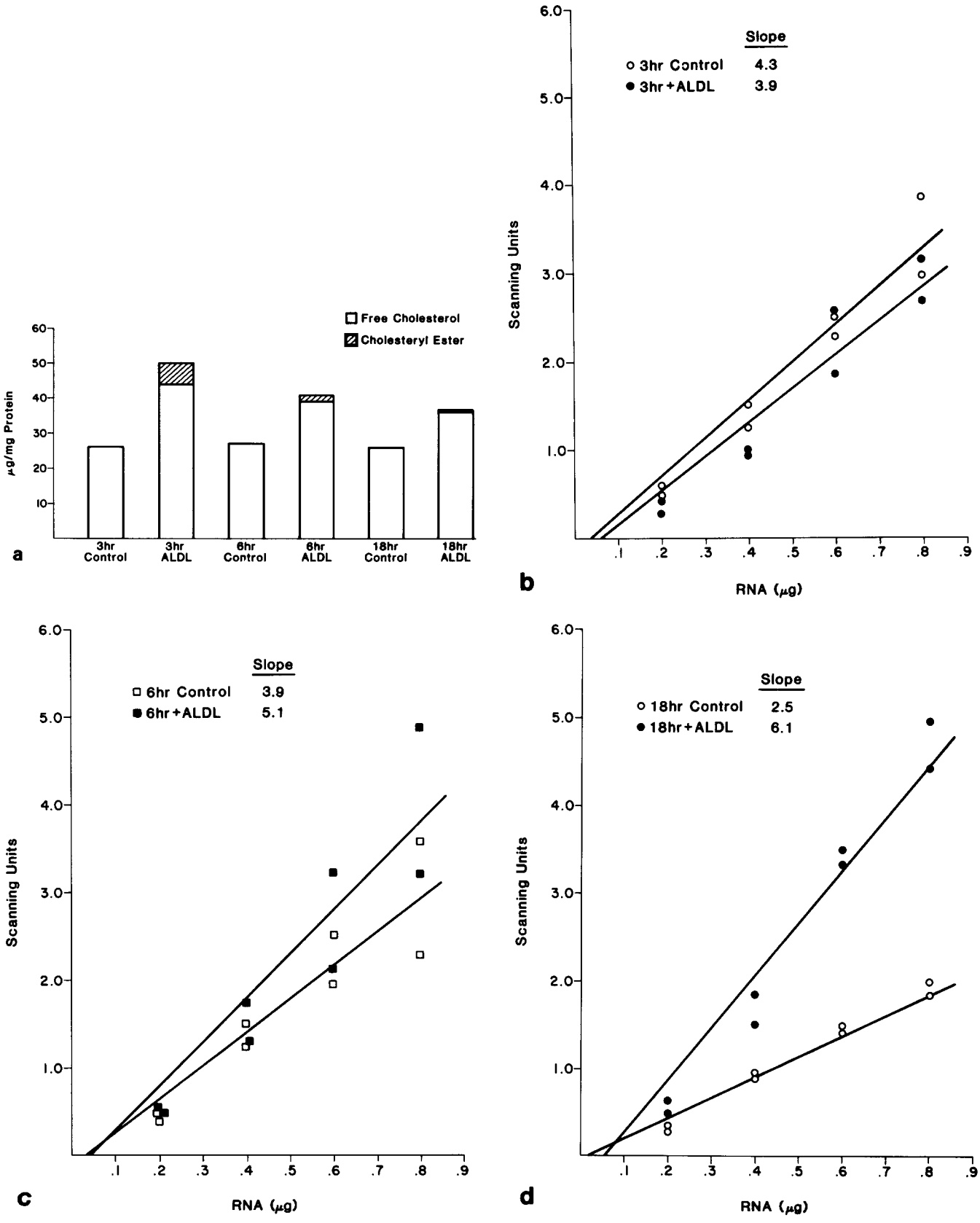

Fig. 4. Acyl-CoA:cholesterol acyltransferase inhibition and induction of apoE mRNA. Cells were incubated and harvested as described in the legend to Fig. 3 except that the ALDL concentration was 30 µg/ml and cells incubated with ALDL also received Sandoz 58035 at 5 µg/ml during the entire incubation. Control wells received S58035 alone. Each line shown (4b–4d) represents the average of two lines derived from duplicate wells.

mRNA induction by cholesterol. In preliminary experiments (not shown) we noted that macrophage free cholesterol and cholesteryl ester mass progressively increased with time during incubations in ALDL. These increased cholesterol levels would make it impossible to interpret time-course information as any observed changes in mRNA abundance could be related to the progressively increasing cholesterol level (i.e., threshold-related) and not be related to time-dependent processes. In order to deal with this, a protocol was developed in which cells were pulsed for 2 h in high concentrations of ALDL and then chased in serum-free medium. Under these conditions, total cell cholesterol remained relatively constant between 3 and 18 h after termination of the pulse, although cholesteryl ester mass increased slightly at the expense of free cholesterol (Fig. 3a). Fig. 3d shows that 18 h after completion of the ALDL pulse, a 3.5-fold increase in apoE mRNA level is detected. ApoE mRNA level was not increased in cells incubated with ALDL at 3 (Fig. 3b) and 6 h (Fig. 3c) after termination of the ALDL pulse.

In the experiment described above, cholesteryl ester increased as free cholesterol decreased during the chase period. This progressive increase in cholesteryl ester mass could be a potential explanation for the observed lag in the induction of apoE mRNA, if one assumed that a critical mass of cholesteryl ester was required before such induction occurred. We have previously demonstrated, however, that cholesteryl ester accumulation was not important for induction of apoE synthesis. In order to determine whether this delay would be observed when cholesteryl ester accumulation was inhibited, we used the ACAT inhibitor, S58035. Cells were pulsed for 2 h with 30 μg/ml ALDL with S58035 at 5 μg/ml. After washing, ALDL-treated cells were placed in chase medium containing S58035. Control wells received S58035 alone. Using these conditions, cholesteryl ester accumulation was substantially reduced at 3 h and fell progressively to the 18-h time point (Fig. 4a) compared to the experiment shown in Fig. 3. Total cholesterol also showed some decline as free cholesterol was presumably lost to the medium. Figs. 4b and 4c show that at 3 h and 6 h there is little difference between the apoE mRNA levels of control and ALDL-treated cells, while at 18 h (4d) a 2.4-fold induction by ALDL is detected. This experiment confirms the delay between induction of macrophage cholesterol mass and apoE mRNA abundance and specifically excludes the possibility that the progressive increase in macrophage cholesteryl ester content, observed in the experiment presented in Fig. 3, was responsible for the induction of apoE mRNA levels at 18 h.

The experiments presented above indicate that cholesterol loading of macrophages leads to regulation at pretranslational levels which can account for augmented apoE synthesis. We have previously shown that removal of cholesterol from cholesterol-laden macrophages, using human HDL_3, results in suppression of apoE synthesis (7). The expeiments shown in **Fig. 5** and **Fig. 6** were per-

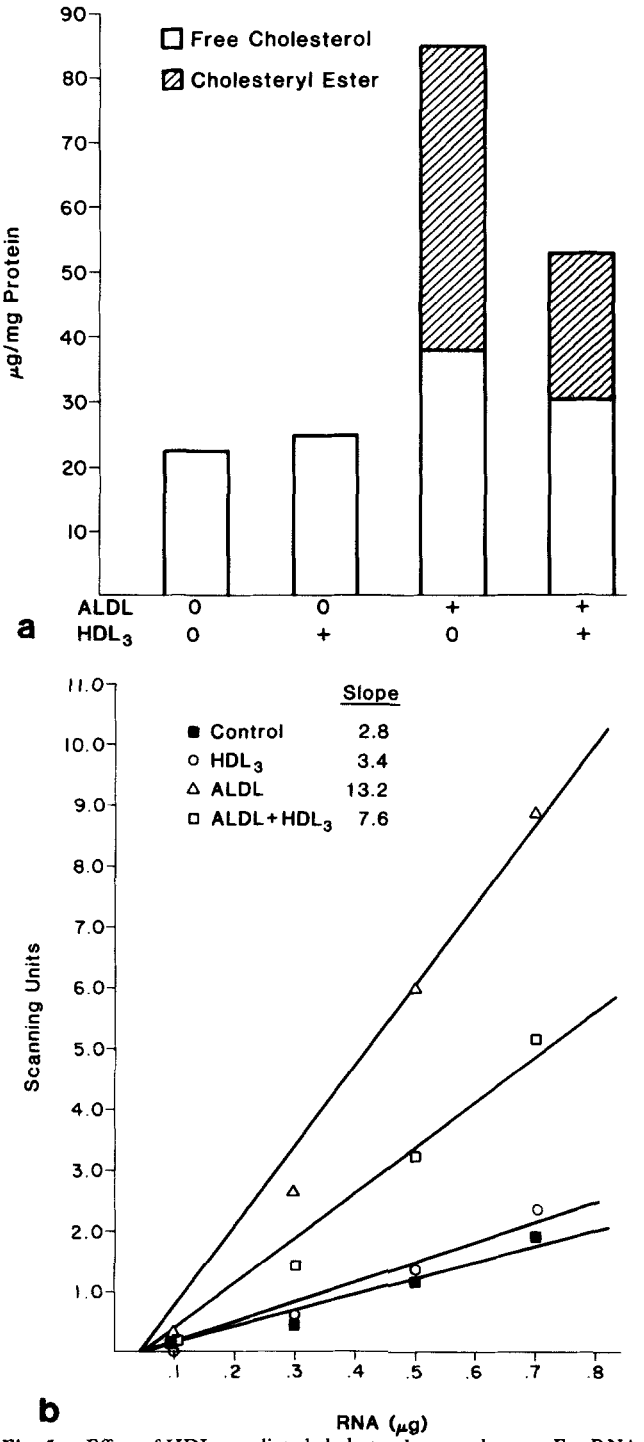

Fig. 5. Effect of HDL_3-mediated cholesterol removal on apoE mRNA levels. Cells were incubated in 0.2% BSA in DMEM ± ALDL at 15 μg/ml for 18 h. At that time cells were washed twice with DMEM and placed in fresh 0.2% BSA in DMEM. Half of the cultures from each group received 400 μg/ml HDL_3 for 24 h. Cells were then harvested for sterol and protein measurements (a) or for slot blot hybridization of RNA (b). Points shown in 5b represent means of measurements made in duplicate wells.

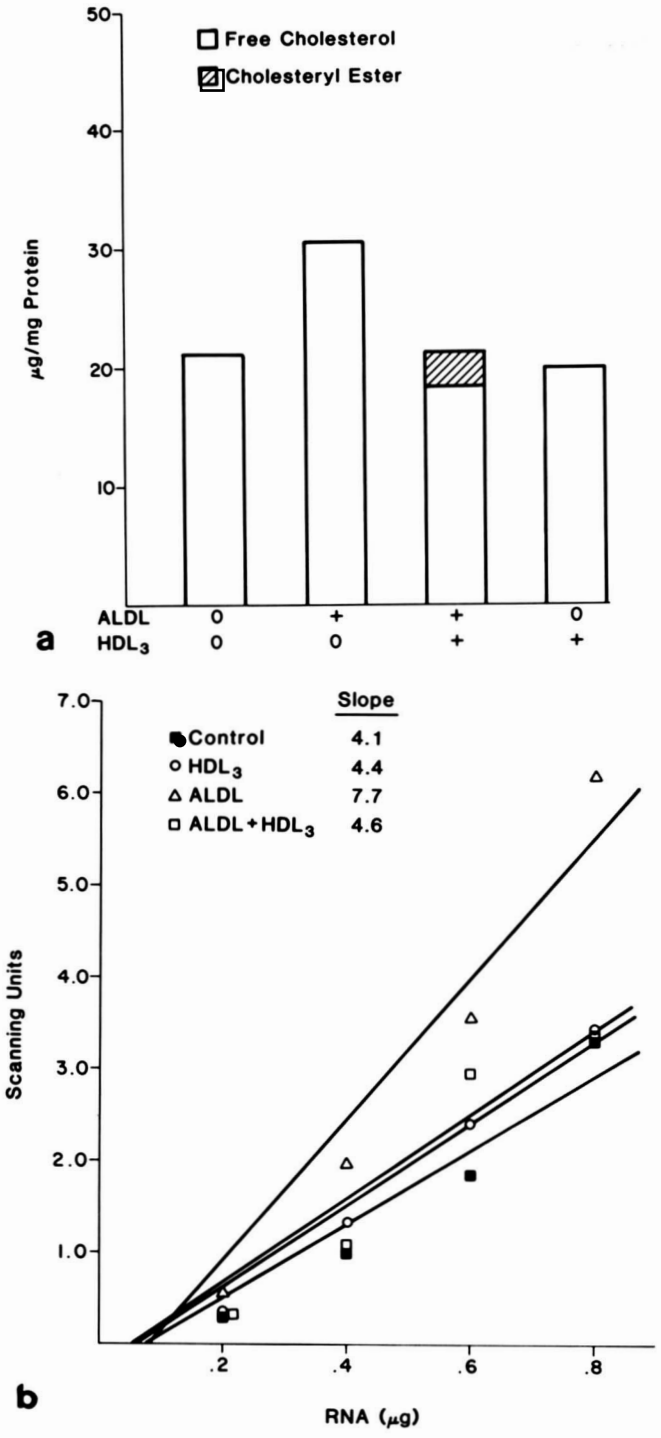

Fig. 6. Effect of ACAT inhibition on HDL_3-mediated suppression of apoE mRNA levels. The experiment shown in Fig. 5 was repeated except that Sandoz 58035 at 5 µg/ml ± ALDL at 2.5 µg/ml were present during the first 18 h of incubation. Sandoz 58035 was also included in the second incubation. Cells were harvested for sterol and protein measurements (a) or for slot blot hybridization of RNA (b). Points shown in 6b represent means of measurements made in duplicate wells.

formed to determine whether this suppression is also accomplished by regulation at pretranslational loci. Fig. 5 shows that in cells incubated for 18 h in 15 µg/ml ALDL and then for 24 h in serum-free medium, cellular free cholesterol, cholesteryl ester, and apoE mRNA levels are increased. If the ALDL incubation is followed by 24 h incubation in 400 µg/ml HDL_3, free cholesterol and cholesteryl ester fall as does apoE mRNA, although no measurement completely returns to control levels. In order to facilitate the complete removal of cholesterol from cells and thereby demonstrate the complete return of apoE mRNA to control levels, this experiment was repeated using S58035 with 2.5 µg/ml ALDL during the first 18 h incubation followed by an incubation containing S58035 plus or minus HDL_3 (Fig. 6). Under these conditions cholesterol accumulation occurs in the free cholesterol fraction which is, potentially, more immediately available for efflux (21). There is, consequently, a total return of macrophage free cholesterol to baseline levels during the incubation in HDL_3. This, in turn, allows a complete return of apoE mRNA to control levels. Additionally, this experiment again suggests that induction of apoE mRNA can occur when cholesteryl ester accumulation is inhibited.

The substantial increases in apoE mRNA abundance produced by cholesterol loading could be due to any combination of changes in messenger RNA production, processing, or degradation. The contribution of one of these potential mechanisms, changes in apoE messenger RNA production, was directly assessed by nuclear run-off transcription assays. As shown in **Fig. 7**, cholesterol loading produces a substantial increase in apoE gene transcription rate as measured by this assay. The small change in β-actin gene transcription did not lead to a detectable increase of β-actin mRNA levels after an 18 h incubation in ALDL. The values for apoE mRNA and β-actin mRNA slopes in a representative experiment after an 18-h incubation in ALDL and 24 h in serum-free medium are as follows: apoE, control 6.8, ALDL 10 µg/ml 19.1; actin, control 1.9, ALDL 10 µg/ml 2.0.

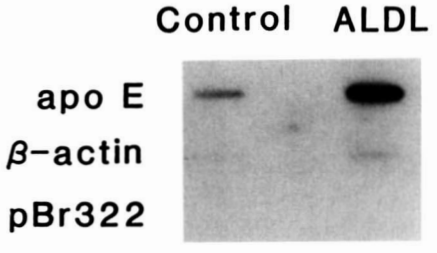

Fig. 7. ALDL induction of apoE gene transcription. Cells were incubated for 18 h in 0.2% BSA in DMEM ± ALDL at 25 µg/ml. At that time cells were harvested and purified nuclei were frozen in liquid nitrogen until processed as described in Methods. The nuclear run-off transcription assay was performed on a total of 16×10^6 pooled nuclei for each condition. In order to detect background hybridization, pBR322 DNA was included on hybridization membranes.

DISCUSSION

The data presented in this manuscript suggest that macrophage apoE gene transcription is regulated by macrophage cholesterol content or metabolism. These observations provide a mechanism for previously reported changes in macrophage apoE synthesis and secretion after cholesterol loading or removal (7). Changes in apoE mRNA levels reflect changes in cellular cholesterol content when cells are being loaded with cholesterol using incubations in ALDL, or when cells are being depleted of previously delivered cholesterol by HDL_3. These changes in apoE mRNA could readily account for changes observed in macrophage apoE synthesis after experimental manipulation of cell cholesterol content and mechanisms involving altered apoE mRNA translation efficiency or altered apoE stability need not be invoked. This situation is different from that recently described in ovarian granulosa cells, where increments in apoE synthesis versus mRNA abundance are not totally concordant (22). In these cells, choleratoxin produces a 7- to 10-fold increment in apoE accumulation but only a 2- to 3-fold increment in apoE mRNA. These observations led the authors to suggest that regulation of ovarian granulosa cell apoE production by choleratoxin is achieved at translational or post-translational, as well as transcriptional, loci. In the macrophage, we have previously detected a 2- to 8-fold induction of biosynthetically labeled, cell-lysate apoE accumulation after cholesterol loading. We believe this degree of induction is concordant with the increments of apoE mRNA described in the current study. The increments we have observed in macrophage apoE mRNA levels during cholesterol loading may result from a number of mechanisms but can be, at least partially, accounted for by corresponding changes in apoE gene transcription.

The data presented in Figs. 3 and 4 indicate that there is at least a 6-h delay from the completion of cholesterol loading to detectable increments of macrophage apoE mRNA. A number of explanations could account for this delay including a long apoE mRNA half-life relative to cholesterol-related increments of apoE gene transcription or the time required for synthesis of a new protein necessary for enhanced apoE gene expression. Alternatively, macrophage total cholesterol mass, as measured in total cell extracts, may only represent an approximate reflection of an important subcellular regulatory pool of cholesterol, and a further translocation of endocytosed cholesterol may need to occur prior to activation of apoE gene expression. With respect to this possibility, it has been established for a number of cell types that cellular free cholesterol is distributed in a number of kinetically and morphologically discrete subcellular compartments (23, 24). On the other hand, the observed delay of apoE gene expression could be explained if further metabolism of cholesterol was required. For this latter possibility, data exist that indicate that certain oxysterol derivatives of cholesterol (e.g., 25-hydroxycholesterol) are more proximate regulators of cellular cholesterol-responsive pathways (25, 26). Each of the above hypotheses will require systematic evaluation.

The observations presented in this study are consistent with the hypothesis that the apoE gene can be considered as one of a number of cholesterol-responsive genes in the macrophage. In line with this, using human monocyte-derived macrophages, we have measured a dose-related and inverse modulation of apoE mRNA (increased) and LDL receptor mRNA (decreased) levels after 18-h incubations in ALDL (T. Mazzone, unpublished observations). Regulation of apoE gene expression by cholesterol suggests that the protein product of this gene, like the protein product of the LDL receptor gene, may be involved in modulating cellular cholesterol balance. The pattern of regulation of apoE gene expression by cholesterol can be rationalized if enhanced apoE synthesis facilitated the disposition of excess cellular cholesterol. A potential mechanism by which this could occur is provided by the observation that when cells that do not normally synthesize apoE are transfected to express the apoE gene, apoE is synthesized and secreted in a particle that contains phospholipid and free cholesterol (27). With respect to this observation, the fall in macrophage free cholesterol we measured during incubations in serum-free medium with ACAT inhibition is of interest (Fig. 4a). It is possible for cells to undergo a net loss of cholesterol in serum-free medium but such losses are usually small (28). It is interesting to speculate that (enhanced or basal) apoE secretion, in the presence of a large pool of cellular free cholesterol that could not be esterified, acted to enhance the net transfer of cholesterol to serum-free medium thereby accounting for the results shown in Fig. 4a. In addition to directly influencing the efflux of free cholesterol from cells, macrophage-derived apoE could potentially modify HDL-cell interactions. Macrophage-derived apoE has been shown to associate with pericellular HDL_3 particles (29) and may thereby enrich the HDL_3 surface with free cholesterol and phospholipid. In the presence of lecithin:cholesterol acyltransferase, surface free cholesterol would diminish, leaving a phospholipid-enriched HDL_3 surface that could more potently promote cellular cholesterol efflux (30).

In our previous study of apoE synthesis (7), we related augmented apoE production to increased levels of macrophage free cholesterol. We believe the data presented in this study are consistent with this notion. In the experiment shown in Fig. 2, for example, both apoE mRNA abundance and free cholesterol content plateau while cholesteryl ester mass rises. In the experiment shown in Fig. 6, apoE mRNA levels are increased or decreased

with incubations containing an inhibitor of ACAT. There is, in addition to these observations, no obvious reason to postulate that free cholesterol is important for the modulation of apoE synthesis but not apoE mRNA given the comparable enhancement of each produced by cholesterol loading. It is important to point out, however, that experiments using ACAT inhibitors such as S58035 cannot totally exclude cholesteryl ester accumulation as a prerequisite for augmenting apoE gene expression. These accumulations could be missed if they were below assay sensitivity or if they were short-lived. Further characterization of the nature of the relationship between macrophage cholesterol (free or esterified) and apoE gene expression will require additional approaches (see below).

The molecular mechanisms accounting for sterol-mediated regulation of the LDL receptor and HMG-CoA reductase genes have been examined in great detail (31–33). For these genes, sterol repression is associated with a sterol response element. For the LDL receptor gene, the sterol response element lies adjacent to a constitutive positive transcription element that binds purified Spl. Smith et al. (34) and Paik et al. (35) have recently published detailed analyses of the 5' flanking region of the apoE gene using transient expression systems in a variety of mammalian cell types. In each case, the authors demonstrate that regulation of apoE gene expression is the result of a complex interaction of multiple regulatory elements. Identification of the *cis*-elements that are responsible for apoE gene responsiveness to cholesterol will help to clarify the mechanism by which cholesterol alters apoE gene expression. For example, cholesterol or one of its oxysterol metabolites may induce the appearance of a protein, or activate a pre-existing protein that binds to such regulatory *cis* elements and thereby augments apoE gene transcription. A computer search of the human apoE gene sequence identifies an 8-bp sequence at + 248 (from the major transcription start site) with 100% homology to the sterol response element consensus sequence. Further experimentation will be needed to determine the importance of this sequence for the sterol-responsiveness of macrophage apoE gene expression.

We are grateful to Dr. Godfrey S. Getz for support and advice regarding this work, to Dr. John Taylor for providing the apoE cDNA, to Dr. Elaine Fuchs for providing the β-actin cDNA, and to Lynne Pertum for typing the manuscript. This work was supported by grant HL 39653 from the National Institutes of Health.

Manuscript received 28 November 1988 and in revised form 30 January 1989.

REFERENCES

1. Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* **240:** 622–630.
2. Newman, T. C., P. A. Dawson, L. L. Rudel, and D. L. Williams. 1985. Quantitation of apolipoprotein E mRNA in the liver and peripheral tissues of nonhuman primates. *J. Biol. Chem.* **260:** 2452–2457.
3. Driscoll, D. M., and G. S. Getz. 1984. Extrahepatic synthesis of apoprotein E. *J. Lipid Res.* **25:** 1368–1379.
4. Ross, R. 1981. Atherosclerosis: a problem of the biology of the arterial wall cells and their interaction with blood components. *Arteriosclerosis* **1:** 293–311.
5. 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.
6. Wang-Ivenson, P., J. C. Gibson, and W. V. Brown. 1985. Plasma apolipoprotein secretion by human monocyte-derived macrophages. *Biochem. Biophys. Acta.* **834:** 256–262.
7. 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.
8. Edelson, P. J., R. Zweibel, and Z. Cohn. 1975. The pinocytic rate of activated macrophages. *J. Exp. Med.* **142:** 1150–1155.
9. Ross, A. C., K. J. Go, J. G. Heider, and G. H. Rothblat. 1984. Selective inhibition of acyl coenzyme A:cholesterol acyltransferase by compound 58-035. *J. Biol. Chem.* **259:** 815–819.
10. Oram, J. F., J. J. Albers, and E. L. Bierman. 1980. Rapid regulation of the activity of the low density lipoprotein receptor of cultured human fibroblasts. *J. Biol. Chem.* **255:** 815–819.
11. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active rebonucleic acid from sources enriched in ribonuclease. *Biochemistry.* **24:** 5294–5299.
12. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: a Laboratory Manual. 202–203.
13. Rigby, P. W. J., M. Dieckman, C. Rhodes, and P. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. Mol. Biol.* **113:** 237–251.
14. McClean, J. W., C. Fukuzawa, and J. M. Taylor. 1983. Rat apolipoprotein E mRNA. Cloning and sequencing of a double stranded cDNA. *J. Biol Chem.* **258:** 8993–9000.
15. Hanukoglu, I., N. Tanese, and E. Fuchs. 1983. Complementary DNA sequence of a human cytoplasmic actin. *J. Mol. Biol.* **163:** 673–678.
16. Greenberg, M. E., and E. B. Ziff. 1984. Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. *Nature.* **311:** 433–438.
17. Groudine, M., M. Peretz, and H. Weintraub. 1981. Transcriptional regulation of hemoglobin switching in chicken embryos. *Mol. Cell Biol.* **1:** 281–288.
18. Brenner, D. A., and M. Chojkier. 1987. Acetaldehyde increases collagen gene transcription in cultured human fibroblasts. *J. Biol. Chem.* **262:** 17690–17695.
19. 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.
20. Bates, S. R., and R. W. Wissler. 1976. Effect of hyperlipemic serum on cholesterol accumulation in monkey aortic medial cells. *Biochem. Biophys. Acta.* **450:** 78–88.
21. Brown, M. S., Y. K. Ho, and J. L. Goldstein. 1980. The cholesteryl ester cycle in macrophage foam cells. Continual hydrolysis and re-esterification of cytoplasmic esters. *J. Biol. Chem.* **255:** 9344–9352.
22. Wyne, K. L., J. R. Schreiber, A. L. Larsen, and G. S. Getz.

1989. Regulation of apolipoprotein E biosynthesis by cAMP and phorbol ester in rat ovarian granulosa cells. *J. Biol Chem.* **264:** 981-989.
23. Werb, Z., and Z. A. Cohn. 1972. Cholesterol accumulation in the macrophage. Ingestion and intracellular fate of cholesterol and cholesterol esters. *J. Exp. Med.* **135:** 21-44.
24. Lange, Y., and B. V. Ramos. 1983. Analysis of the distribution of cholesterol in the intact cell. *J. Biol. Chem.* **258:** 15130-15134.
25. Saucier, S. D. , A. A. Kandutsch, F. R. Taylor, T. A. Spencer, S. Phirwa, and A. K. Geyen. 1985. Identification of regulatory oxysterols, 24(S), 25-epoxycholesterol and 25-hydroxycholesterol, in cultured fibroblasts. *J. Biol. Chem.* **260:** 14571-14579.
26. Rudney. H., and R. C. Sexton. 1986. Regulation of cholesterol biosynthesis. *Annu. Rev. Nutr.* **6:** 245-272.
27. Herscovitz, H., M. M. Hussain, V. I. Zannis, and D. M. Small. 1988. ApoE gene expression is associated with increased lipid secretion. *Arteriosclerosis.* **8:** 546A.
28. Morton, R. E. 1988. Interaction of plasma-derived lipid transfer protein with macrophages in culture. *J. Lipid Res.* **29:** 1367-1377.
29. Gordon, V., T. L. Innerarity, and R. W. Mahley. 1983. Formation of cholesterol- and apoE-enriched high density lipoproteins in vitro. *J. Biol. Chem.* **258:** 6202-6212.
30. Johnson, W. J., M. J. Bamberger, R. A. Latta, P. E. Rapp, M. C. Phillips, and G. H. Rothblat. 1986. The bidirectional flux of cholesterol between cells and lipoproteins. *J. Biol. Chem.* **261:** 5766-5776.
31. Sudhof, T. C., D. R. Vanderwesthuyzen, 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.
32. Dawson, P. A., S. L. Hoffman, D. R. Vanderwesthuyzen, T. C. Sudhof, M. S. Brown, and J. L. Goldstein. 1988. Sterol-dependent repression of low density lipoprotein receptor promoter mediated by 16-base pair sequence adjacent to binding site for transcription factor Spl. *J. Biol. Chem.* **263:** 3372-3379.
33. Osborne, T. F., G. Gil, J. L. Goldstein, and M. S. Brown. 1988. Operator constitutive mutation of 3-hydroxy-3-methylglutaryl coenzyme A reductase promoter abolishes protein binding to sterol regulatory elements. *J. Biol. Chem.* **263:** 3380-3387.
34. Smith, J., A. Melian, T. Leff, and J. L. Breslow. 1988. Expression of the human apolipoprotein E gene is regulated by multiple positive and negative elements. *J. Biol. Chem.* **263:** 8300-8308.
35. Paik, Y., D. J. Chang, C. A. Reardon, M. D. Walker, E. Taxman, and J. M. Taylor. 1988. Identification and characterization of transcriptional regulatory regions associated with expression of the human apolipoprotein E gene. *J. Biol. Chem.* **263:** 13340-13349.