Quantification and regulation of apolipoprotein E expression in rat Kupffer cells

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Abstract Apolipoprotein E (apoE) is synthesized by a wide variety of cells including cells of the monocyte-macrophage lineage. In order to assess the quantitative significance of apoE synthesis in a mature tissue macrophage, apoE synthesis was compared in Kupffer cells and hepatocytes isolated from rat liver. Immunoreactive apoE synthesized by both cell types exhibited identical isoform patterns when examined by high-resolution two-dimensional gel analysis. ApoE synthesis was not detected in hepatic endothelial cells. Northern blot analysis using a rat apoE cDNA probe demonstrated a single mRNA species of approximately 1200 nucleotides in freshly isolated hepatocytes and Kupffer cells. The absolute content of apoE mRNA in each cell type was determined with a DNA-excess solution hybridization assay. The apoE mRNA content (pg/μg RNA) for Kupffer cells and hepatocytes was 35.7 and 98.8, respectively. Accounting for cellular RNA content and the population size of each cell type in the liver, Kupffer cells were calculated to contain about 0.7% of liver apoE mRNA; hepatocytes account almost quantitatively for the remainder. These results suggest that Kupffer cells are not major contributors to the plasma apoE pool. After intravenous injection of bacterial endotoxin, apoE mRNA was decreased in freshly isolated Kupffer cells whereas whole liver showed no change in apoE mRNA. Endotoxin treatment had no effect on the apoE mRNA content in several peripheral tissues. These results indicate that apoE expression in vivo is differentially regulated by endotoxin in Kupffer cells as compared to hepatocytes or apoE-producing cells in peripheral tissues. — **Dawson, P. A., L. M. Lukaszewski, P. F. Ells, C. C. Malbon, and D. L. Williams.** Quantification and regulation of apolipoprotein E expression in rat Kupffer cells. *J. Lipid Res.* 1989. **30:** 403-413.

Supplementary key words hepatocytes • apoE • mRNA • apoB • cDNA

Apolipoprotein (apo) E is an important constituent of plasma lipoproteins in humans and experimental animals. ApoE binds with high affinity to receptors on the surface of liver and extrahepatic cells and thereby mediates the uptake of apoE-containing particles (1). Although most apolipoproteins are synthesized primarily by the liver and small intestine, apoE is synthesized in human adrenal gland and kidney at relative rates similar to or greater than the synthetic rate in the liver (2). Further studies have shown that apoE is synthesized at substantial rates in a wide variety of peripheral tissues in nonhuman primates and rodents (3, 4). ApoE mRNA has been identified in peripheral tissues in a number of species (3-9). Quantification of the absolute amount of apoE mRNA in monkey tissues indicated that 20-40% of the total apoE mRNA content is extrahepatic (6). Thus, the production of apoE by peripheral tissues is potentially of quantitative importance for systemic lipid transport.

ApoE is also synthesized in cells of the monocyte-macrophage lineage including mouse peritoneal macrophages (10), human monocyte-macrophages (11), and bone marrow-derived macrophages (12). Macrophage apoE synthesis is regulated by a variety of factors including cellular cholesterol content (10, 11) and the activation state of the cell (12-15). Activating agents such as bacillus Calmette-Guerin or bacterial lipopolysaccharide endotoxin markedly decrease apoE synthesis in mouse peritoneal macrophages and bone marrow-derived macrophages in culture (13, 14). Werb and Chin (13) proposed that the endotoxin-mediated decrease in macrophage apoE synthesis may be an important factor in the hyperlipidemias that accompany infections with gram-negative organisms (16) or endotoxin treatment in vivo (17).

In the present study we have examined the synthesis of apoE by freshly isolated rat Kupffer cells. Kupffer cells represent the largest macrophage population in the body, comprising almost 20% of the total macrophage content in rodents (18). An analysis of Kupffer cells may be informative about the expression of apoE by liver macrophages

Abbreviations: apo, apolipoprotein; DMEM, Dulbecco's modified Eagle's medium.

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in vivo and by mature tissue macrophages in general. Several specific questions have been asked. First, do Kupffer cells synthesize apoE, and, if so, do they contribute significantly to whole body apoE production or to apoE production by the liver? Second, is apoE expression down-regulated in Kupffer cells by endotoxin administration in vivo as occurs in peritoneal macrophage and bone marrow-derived macrophages in culture? Third, does endotoxin administration regulate apoE expression in apoE-producing cells in peripheral tissues such as kidney and brain? The results show that apoE is an abundant product of freshly isolated Kupffer cells and hepatocytes, but is not made by hepatic endothelial cells. Measurements of apoE mRNA indicate that less than 1% of liver apoE mRNA is present in Kupffer cells. These results suggest that resident liver macrophages do not contribute significantly to liver or whole body apoE synthesis. In vivo treatment with endotoxin down-regulated apoE mRNA levels in Kupffer cells but had little effect on apoE mRNA levels in liver or peripheral tissues.

MATERIALS AND METHODS

Materials

Male Sprague-Dawley rats (300–400 g) were obtained from Taconic Farms (Germantown, NY). Rats, maintained on 12 hr light/12 hr dark cycle, were fed standard rat chow and water ad libitum. Collagenase was obtained from Worthington Biochemicals. DNAase I, pronase, and lipopolysaccharide (*E. coli.* 0127:B8) were obtained from Sigma. Metrizamide was obtained from Nyegaard & Company (Norway). L-[^{35}S]methionine (1275 Ci/mmol) and [^{32}P]dCTP (800 Ci/mmol) were obtained from Amersham Corporation (Arlington Heights, IL).

Preparation of Kupffer cells

Kupffer cells were isolated by pronase perfusion and centrifugal elutriation using a modification of the procedure of Knook, Blansjaar, and Sleyster (19). Rats were fasted 10–14 hr before cell isolation. Nonparenchymal cells were harvested by centrifugation, washed in Dulbecco's modified Eagle's medium (DMEM) with 40% fetal calf serum, and in Gey's balanced salt solution. Contaminating parenchymal cells and debris, stellate cells, and red blood cells were separated from Kupffer and endothelial cells by centrifugation through an 18% metrizamide gradient (20). Kupffer cells were separated from endothelial cells by centrifugal elutriation using a Beckman JE-6 elutriator rotor in a J-21 type Beckman centrifuge (19, 20). Cell fractions were concentrated by centrifugation and used immediately for RNA isolation or cell culture. For cell culture experiments, Kupffer cells were further purified by adherence to plastic. For endotoxin treatment, *E. coli* lipopolysaccharide (seratype 0127:B8) was injected via the tail vein 24 hr before Kupffer cells were isolated. Sham-injected control animals received the saline vehicle. After in situ perfusion with Gey's balanced salt solution, a lobe of liver was removed and frozen in liquid N_2 for RNA isolation.

The purity of Kupffer cell fractions was monitored by a standard staining technique for endogenous peroxidase (21) using 3,3'-diaminebenzidine tetrahydrochloride. Hepatocyte contamination was evaluated by light microscopy and by analysis of cellular RNA via hybridization with hepatocyte-specific cDNA. Phagocytosis was assessed both in vivo and in vitro. For the in vivo studies, animals were anesthetized with ethyl ether and injected with 1 ml of colloidal carbon particles or fluorescein isothiocyanate-labeled, heat-inactivated *Staphylococcus aureus.* Approximately 30 min after injection, Kupffer cells were isolated and examined by phase contrast or epifluorescence microscopy. For in vitro studies, freshly isolated Kupffer cells were plated in DMEM for 2 hr, washed, and incubated with 1.1 μm latex microspheres for 1 hr at 37°C. The cells were washed, and phagocytically active cells were identified by phase contrast microscopy. Fc receptors were measured as described by Bianco and Pytowski (22). Freshly isolated Kupffer cells were plated onto cover slips and cultured for 24 hr to regenerate cell surface receptors prior to incubation for 1 hr at 37°C with opsinized red blood cells at 1×10^9 cells/ml. Fc receptor-mediated phagocytosis was quantified after a hypotonic wash step (22).

Isolation of hepatocytes

Hepatocytes were isolated by collagenase perfusion and differential centrifugation (23) as described (24). Cell viability was greater than 90% as monitored by trypan blue exclusion.

Labeling of cells and tissue slices

Rat liver tissue was removed, chopped into 5-mg blocks, and rinsed with DMEM minus methionine containing 50 units/ml penicillin G and 50 μg/ml streptomycin sulfate. Approximately 40 mg of tissue was incubated under an atmosphere of 95% O_2/5% CO_2 in 0.4 ml of the above medium containing 0.5 mCi/ml [^{35}S]methionine (1200 Ci/mmol) for 60 min at 37°C. Freshly isolated Kupffer cells were plated for 1 hr in DMEM and adherent cells were incubated for 2 or 4 hr in DMEM minus methionine containing 1 mg/ml D-glucose, 100 U/ml penicillin G, 0.3 mM L-ascorbic acid, 4 mU/ml insulin, and 160 μCi/ml [^{35}S]methionine. Endothelial cell-enriched fractions were labeled as described above except the selection step for adherent cells was omitted. Hepatocytes were labeled in suspension culture for 4 hr at 37°C under the conditions described above.

Tissue or cells were homogenized and centrifuged to prepare a high speed supernatant (25). Supernatant and medium were frozen in liquid nitrogen and stored at −70°C until analysis. Radiolabeled tissue or cell supernatants were analyzed with a double antibody assay using anti-rat apoE as primary antibody and goat anti-rabbit gamma-globulin as second antibody, followed by SDS-10% polyacrylamide gel analysis of the immunoprecipitate (25, 26). High resolution two-dimensional gel analysis was carried out by the method of O'Farrell (27) with previously described modifications (28). Radioactive proteins were detected by fluorography (29). For mixing experiments, rat plasma lipoproteins were isolated by ultracentrifugation (26) and mixed with radiolabeled immunoprecipitates prior to electrophoresis. ApoE was detected by silver staining (30) prior to fluorography.

Analysis of apoE, apoB, and plasminogen activator mRNAs

Total cellular mRNA was prepared from rat tissues or cells by the guanidine HCl technique or the guanidine thiocyanate-CsCl centrifugation procedure (31). RNA was electrophoresed on 1.2% agarose gels containing 2.2 M formaldehyde, transferred to nitrocellulose (32) and hybridized with rat apoE [^{32}P]cDNA that was prepared by random priming in the presence of [alpha-^{32}P]dCTP (800 Ci/mmol) (33).

For DNA-excess solution hybridization analysis, a fragment from the rat apoE cDNA clone pALE124 (34) corresponding to nucleotides 273 to 497 of the coding region was subcloned into bacteriophage M13 mp8 and a high specific activity probe was synthesized (26, 35). Template DNA or total RNA was hybridized to completion with excess probe, and S1 nuclease-resistant hybrids were precipitated, collected on glass fiber filters, and counted by scintillation spectrometry. ApoE mRNA values were determined by reference to a standard curve constructed with template DNA as the hybridization standard. Reported hybridization values are corrected for the size of the probe (224 nucleotides) compared to the size of rat apoE mRNA (1068 nucleotides). A similar assay was constructed (35) for apoB mRNA using an EcoR I fragment of rat apoB cDNA (36) cloned into the EcoRI site of M13mp19. After probe synthesis, single-stranded DNA was prepared by digestion with BamH I followed by gel isolation and elution from hydroxylapatite (35). Hybridization values were determined by reference to a standard curve and were corrected for the size of the mRNA.

Plasminogen activator mRNA was measured by dot-blot hybridization (37) using a mouse urokinase cDNA (38) labeled with [alpha-^{32}P]dCTP by random priming (33). Dots were cut from the nitrocellulose and counted by scintillation spectrometry.

RESULTS

Isolation of Kupffer cells

To obtain relatively pure Kupffer cell preparations while minimizing phenotypic alterations, the isolation procedure of Knook et al. (19) was employed with the metrizamide gradient step of Nagelkerke, Barto, and van Berkel (20). Typical yields of Kupffer cells were 5–8 × 10^6 cells/g of liver which is similar to recoveries reported previously (19, 20). This represents a recovery of 30–50% of liver Kupffer cells based on the estimate of cell number by Blumhoff et al. (39), but may represent up to 70% recovery as estimated by Knook et al. (19).

Kupffer cells were examined to assess their purity and to evaluate potential contamination with hepatocytes. More than 80% of the freshly isolated cells were positive for phagocytosis after in vivo injection of fluorescein isothiocyanate-labeled, heat-killed *S. aureus* or colloidal carbon. Phagocytically active cells were also positive for peroxidase activity as determined by cytochemical staining. The majority of those cells not containing carbon or fluorescent granules had the morphological appearance of endothelial cells and were negative for endogenous peroxidase activity. Adherent cells in culture were assayed for phagocytosis of latex beads or the presence of cell surface Fc receptors. Plated cells were greater than 85% Kupffer cells by these criteria. These results indicate that the preparations were highly enriched in Kupffer cells.

Cells with the appearance of hepatocytes were rarely seen in Kupffer cell preparations. To test quantitatively for hepatocytes, hybridization assays were carried out with cDNA probes for apoE and apoB mRNAs. ApoB is a major product of the hepatocyte, but apoB mRNA would not be expected in Kupffer cells. The results in **Table 1** show that apoB mRNA was present in Kupffer cell preparations at only 0.2% of its concentration in whole liver. In contrast, apoE mRNA was present at about 35% of its concentration in whole liver. On the basis of the apoE mRNA/apoB mRNA ratio in whole liver, contamination by hepatocytes could account for only 0.5% of the apoE mRNA in the Kupffer cell preparations.

TABLE 1. ApoB mRNA in liver and Kupffer cells

Sample	ApoE mRNA	ApoB mRNA
	pg mRNA/μg RNA	
Liver 1	73.	109.
Liver 2	119.	141.
Kupffer cell 1	40.4	0.19
Kupffer cell 2	30.2	0.30

Hybridizations were carried out with RNA isolated from two rat livers and two Kupffer cell preparations.

Detection of apoE synthesis

To determine whether apoE is synthesized by freshly isolated Kupffer cells, cells were plated for 1 hr, and adherent cells were metabolically labeled with [^{35}S]methionine. Cell extracts were reacted with antiserum against apoE, and the immunoprecipitates were analyzed by SDS-10% polyacrylamide gel electrophoresis (**Fig. 1**). The immunoprecipitate from Kupffer cells (lane 1) showed a prominent apoE band that comigrated with newly synthesized apoE from whole rat liver (lane 3). The apoE band was not observed in immunoprecipitates with preimmune serum (lanes 2 and 4). ApoE synthesized by isolated hepatocytes also comigrated with that synthesized by Kupffer cells (data not shown). Newly synthesized apoE was also secreted by Kupffer cells as judged by its presence in culture medium (data not shown). Elutriator fractions that were enriched in endothelial cells were metabolically labeled and analyzed for newly synthesized apoE. In comparison to Kupffer cells (Fig. 1, lane 5), newly synthesized apoE was not detected in endothelial cells (Fig. 1, lane 7).

Two-dimensional gel analysis

The apoE synthesized by liver, isolated hepatocytes, and isolated Kupffer cells was analyzed further by high resolution two-dimensional gel electrophoresis (**Fig. 2**). Newly synthesized liver apoE consisted of two major isoforms with pIs of 5.4 and 5.5, and a series of minor components of progressively higher molecular weight trailing to the acidic side of the isoelectric focusing dimension. This pattern is very similar to that previously described for newly synthesized rat apoE by one-dimensional isoelectric focusing (40). The major isoforms migrated slightly faster and more basic than rat plasma apoE. Using unlabeled plasma apoE as a marker to align different gels, the isoform patterns of newly synthesized apoE from isolated hepatocytes and Kupffer cells were superimposable on that of whole liver. These data indicate apparent identity between the apoE synthesized by Kupffer cells and hepatocytes.

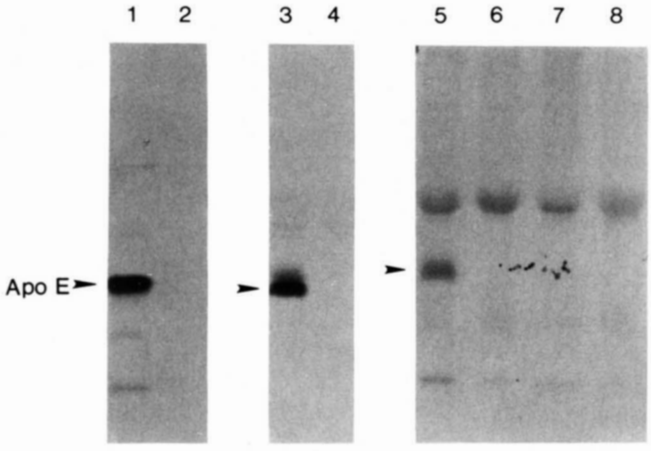

Fig. 1. ApoE synthesis in rat liver cells. Isolated Kupffer cells, endothelial cells, or liver slices were incubated with [^{35}S]methionine, and tissue or cell extracts were immunoprecipitated with anti-apoE (lanes 1, 3, 5, and 7) or preimmune serum (lanes 2, 4, 6, and 8). Immunoprecipitates from Kupffer cells (lanes 1, 2, 5, 6), liver (lanes 3 and 4) and endothelial cells (lanes 7 and 8) were analyzed by SDS-10% polyacrylamide gel electrophoresis and fluorography. Approximately 100,000 cpm (lanes 3 and 4) or 200,000 cpm (lanes 1, 2, 5–8) of protein radioactivity was analyzed. The arrows indicate the mobility of newly synthesized liver apoE.

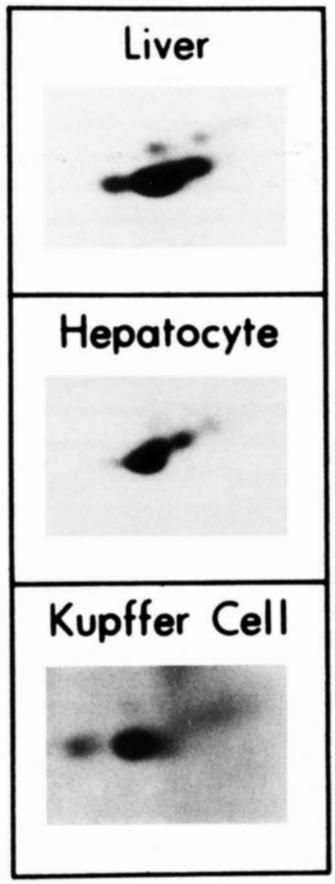

Fig. 2. Two-dimensional gel analysis of newly synthesized apoE. After in vitro incubation of cells or liver slices with [^{35}S]methionine, extracts were reacted with anti-apoE, and washed immunoprecipitates were examined by two-dimensional gel analysis and fluorography. The isoelectric focusing dimension has the basic end to the left, and the SDS-10% polyacrylamide gel electrophoresis dimension was run from top to bottom. The figure shows the fluorograms of the gels in the vicinity of apoE. The gels were aligned by virtue of the apoE staining pattern resulting from rat lipoproteins which were added to the immunoprecipitates prior to electrophoresis.

ApoE mRNA

ApoE mRNA from liver cells was analyzed by Northern blot analysis. RNA from whole liver, hepatocytes, or freshly isolated Kupffer cells was subjected to electrophoresis on agarose gels containing 2.2 M formaldehyde,

transferred to nitrocellulose, and probed with ^{32}P-labeled rat apoE cDNA. As shown in **Fig. 3,** apoE mRNAs from liver, hepatocytes, and Kupffer cells comigrated with mobilities corresponding to approximately 1200 nucleotides. The size of apoE mRNA agrees with earlier Northern blot analyses of rat apoE mRNA (3, 34). The faint band seen with RNA from the endothelial cell fraction may represent Kupffer cell contamination or may indicate that endothelial cells synthesized apoE at a rate that was too low for detection by metabolic labeling and immunoprecipitation (Fig. 1).

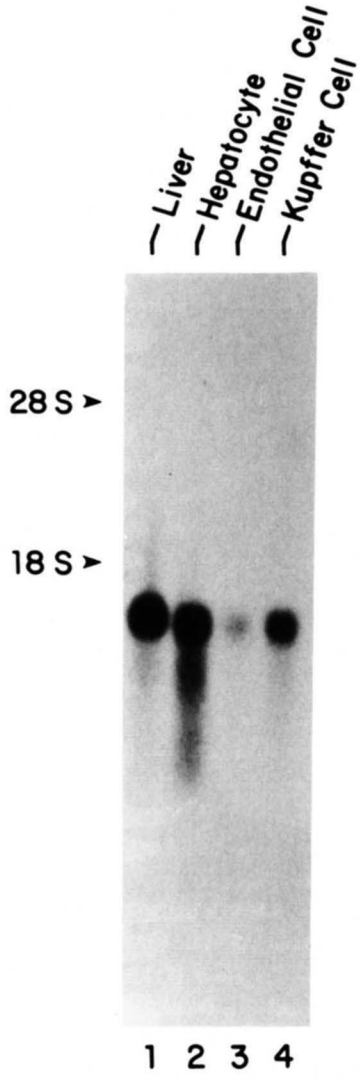

Fig. 3. Northern gel analysis of apoE mRNA. RNA (10 μg) from whole liver, from freshly isolated hepatocytes, from Kupffer cells, and from endothelial cells was electrophoresed on a 1.2% agarose gel containing 2.2 M formaldehyde, blotted to nitrocellulose, and probed with a ^{32}P-labeled rat apoE cDNA. The figure shows the autoradiogram of the nitrocellulose blot. The designations 28 S and 18 S refer to the mobilities of the major ribosomal RNA species determined by staining the gel with ethidium bromide.

Quantification of apoE mRNA

In order to determine the potential contribution of hepatocytes and Kupffer cells to total liver apoE synthesis, the absolute amount of apoE mRNA was determined for freshly isolated cells of each type using a DNA-excess solution hybridization assay specific for rat apoE. The data in **Fig. 4** show that the amount of S1 nuclease-resistant hybrids increased linearly as a function of input template DNA, liver RNA, adrenal RNA, or RNA isolated from either hepatocytes or Kupffer cells. **Table 2** summarizes the apoE mRNA values from a number of preparations. When expressed on the basis of total RNA, the apoE mRNA concentrations of Kupffer cells and hepatocytes were 35.7 pg/μg RNA and 98.8 pg/μg RNA, respectively. Taking into account the cellular content of RNA (41) and the liver content of each cell type (39), hepatocytes contain 700 ng of apoE mRNA or almost 97% of total liver apoE mRNA. Kupffer cells contain 5.2 ng of apoE mRNA or less than 1% of the total liver apoE mRNA.

In order to compare the content of apoE mRNA of Kupffer cells to extrahepatic tissues, apoE mRNA was quantified in brain, adrenal, lung, and kidney (**Table 3**). When expressed on the basis of total RNA, the concentrations of apoE mRNA in brain, adrenal, kidney, and lung were 78%, 56%, 13%, and 9% of the Kupffer cell value, respectively. On the basis of apoE mRNA content per cell, the brain and adrenal had four- to sixfold more apoE mRNA than the Kupffer cell, whereas the kidney and lung had 13–30% of the Kupffer cell value.

Endotoxin treatment

ApoE synthesis by mouse peritoneal macrophage is negatively regulated by bacterial lipopolysaccharide endotoxin (13, 14). In order to determine whether a similar regulation occurs in Kupffer cells, rats were treated with endotoxin 24 hr prior to the isolation of Kupffer cells. ApoE mRNA was measured in freshly isolated Kupffer cells to avoid metabolic changes that can be induced by plating and adherence steps or culture conditions (42). **Fig. 5** shows that Kupffer cell apoE mRNA levels decreased in response to endotoxin treatment in a dose-dependent fashion. At the high endotoxin dose (750 μg/kg body weight), freshly isolated Kupffer cells contained approximately 5.8 pg apoE mRNA/μg RNA or 15% of the value from the sham-injected controls. This decrease in apoE mRNA was not due to a general decrease in all mRNAs since a dot-blot analysis showed that urokinase mRNA levels were increased approximately fivefold in endotoxin-treated Kupffer cells (data not shown). Increased urokinase activity is a general feature of inflammatory and activated macrophages (15).

When apoE mRNA was assayed in RNA from liver wedges removed before Kupffer cells were isolated, no

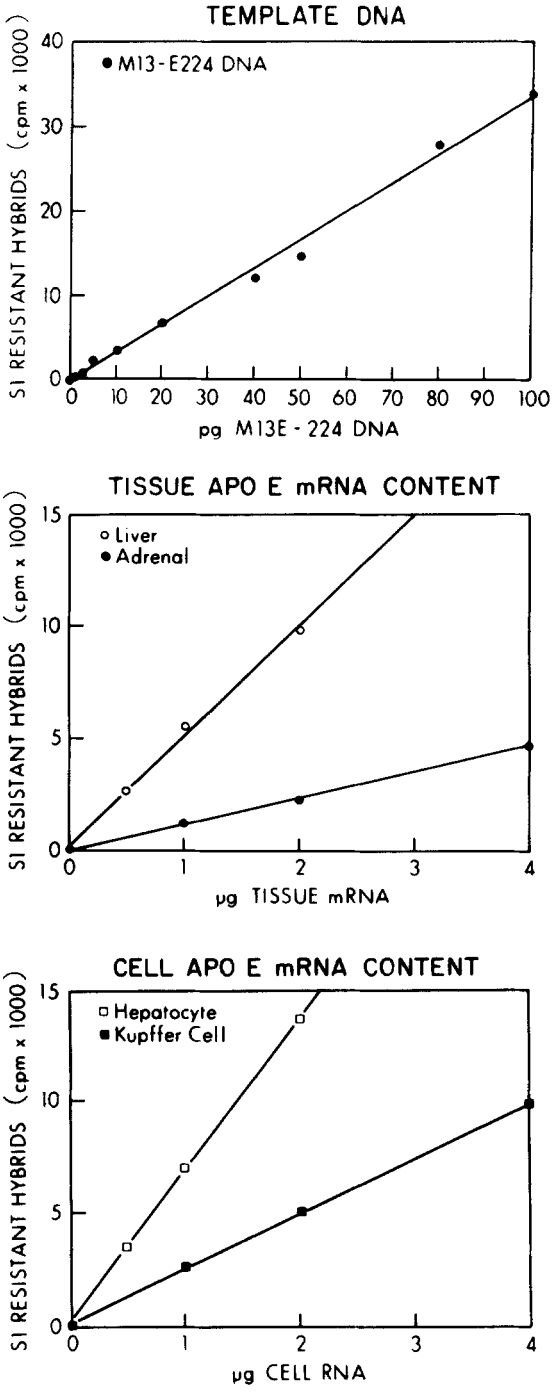

Fig. 4. Measurement of apoE mRNA in rat tissues and liver cells. RNA was prepared from the tissues or cells indicated and hybridized to completion with excess single-stranded ^{32}P-labeled apoE cDNA probe. S1 nuclease-resistant hybrids were measured by scintillation spectrometry. The panels show the probe hybridized with increasing amounts of template DNA (E224) (upper panel), liver and adrenal gland RNA (middle panel), or hepatocyte and Kupffer cell RNA (lower panel).

decrease in apoE mRNA content was observed at either the high or low dose of endotoxin. This result indicates that the apoE mRNA concentration can be selectively regulated in different cell types in the liver. **Fig. 6** shows the effect of endotoxin treatment (750 μg/kg) on apoE mRNA concentrations in liver, lung, kidney, and brain. Endotoxin treatment had no effect on the apoE mRNA concentration in the brain. Small changes in apoE mRNA concentrations were seen in the kidney and lungs, but these values were not statistically different from the controls.

DISCUSSION

Mouse peritoneal macrophages, bone marrow-derived macrophages, and human monocyte-macrophages have been shown previously to synthesize apoE (10–12). To investigate whether resident tissue macrophages also synthesize apoE, rat liver Kupffer cells were studied. Kupffer cells are the major phagocytic cell in the liver and account for almost 20% of the resident macrophages in rodent tissues (18). In addition, Kupffer cells have been implicated in the clearance of modified lipoprotein particles (20, 39).

Freshly isolated Kupffer cells were found to synthesize and secrete apoE. With the caveat that the cell isolation procedure and plating conditions could alter the pattern of proteins synthesized by Kupffer cells, the synthesis of apoE by isolated Kupffer cells suggests that Kupffer cells synthesize apoE in vivo. Comparison by either SDS-polyacrylamide gel electrophoresis or high resolution two-dimensional gel analysis suggests that Kupffer cell apoE is the same as apoE made by either hepatocytes or whole liver. These analyses, however, do not eliminate the possibility that apoE species made by hepatocytes and Kupffer cells might differ in post-translational modifications that do not alter protein charge or substantially alter electrophoretic mobility in SDS-polyacrylamide gels.

ApoE mRNA in freshly isolated Kupffer cells, hepatocytes, and whole liver was examined by Northern blot analysis. In each case, apoE mRNA co-migrated as a single band, with an apparent size of approximately 1200 nucleotides. This result suggests that apoE mRNA in Kupffer cells and hepatocytes is quite similar, but does not rule out minor heterogeneity of transcription start sites or sites of polyadenylation.

Measurements of mRNA indicate that apoE mRNA is moderately abundant in rat Kupffer cells. When expressed on the basis of total RNA, the concentration of apoE mRNA in Kupffer cells is about 35% of its concentration in hepatocytes. In order to estimate the potential of Kupffer cells to make apoE, however, the size and abundance of this cell type in the liver must be considered. Since Kupffer cells represent only 10% of liver cells and account for only a small portion of the protein and RNA content of the liver (39), the absolute amount of apoE mRNA in the Kupffer cell and in the Kupffer cell population is small. Kupffer cells contain about 160 copies

TABLE 2. ApoE mRNA in rat liver and liver cells

Sample	ApoE mRNA	RNA/DNA Ratio	Cell Number	ApoE mRNA	Percent of Liver Total	ApoE mRNA
	pg/μg RNA		*× 10⁶*	*ng/liver*	*%*	*molecules/cell*
Liver	73.6 ± 7.3	2.86	570	719.9 ± 71.3	100	2127
Hepatocyte	98.8 ± 6.0	3.14	375	697.9 ± 42.4	96.9 ± 5.9	3135
Kupffer	35.7 ± 2.9	0.43	57	5.2 ± 0.4	0.7 ± 0.06	158

ApoE mRNA was measured in total RNA extracted from the indicated tissue or cells. Each value is the mean ± SD of at least 12 determinations on tissue or cells isolated from three to eight adult Sprague-Dawley rats. The RNA/DNA ratio was obtained using published values for cell nucleic acid content (41). An organ weight for liver of 3 g per 100 g body weight was used. Total apoE mRNA is expressed as ng per organ per 100 g body weight. Calculations for molecules per cell are based on a diploid DNA content of 6 pg/cell.

of apoE mRNA per cell or only 5% of the value of the hepatocyte. Similarly, Kupffer cells contain 0.7% of the total liver apoE mRNA while hepatocytes account for about 97% of the total. As judged by the apoE mRNA content, these data suggest that Kupffer cells do not contribute significantly to the pool of plasma apoE.

On a per cell basis, the apoE mRNA content of brain and adrenal tissue is four- to sixfold greater than the Kupffer cell whereas the content of kidney and lung tissue is 13–30% as great as the Kupffer cell. The mRNA content per cell in peripheral tissues is an average value based on the diploid DNA content per cell and does not take into account the heterogeneity in apoE mRNA content that is likely to occur among various cell types. Nevertheless, it is interesting that the apoE mRNA content of the Kupffer cell falls in the middle of the range of apoE mRNA values in various tissues. As judged by the mRNA content, apoE production by Kupffer cells would not appear to be particularly remarkable in comparison to various peripheral tissues.

Previous studies have shown that apoE synthesis in peritoneal macrophage and bone marrow-derived macrophage is down-regulated by treatment with bacterial endotoxin (13, 14). The present results show that Kupffer cells exhibit a similar response to treatment with endotoxin in vivo when examined at the level of apoE mRNA. With the caveat that apoE synthesis may be subject to translational control under some circumstances, these data suggest that bacterial endotoxin down-regulates apoE synthesis in Kupffer cells. In contrast to Kupffer cells, whole liver showed no decrease in apoE mRNA in response to endotoxin, indicating that apoE mRNA concentration can be selectively regulated in different cell types in the liver. Endotoxin treatment also had no significant effect on apoE mRNA concentrations in brain, kidney, and lung. These results may indicate that macrophages in these tissues do not respond to endotoxin treatment by decreasing apoE mRNA as occurs in Kupffer cells. Alternatively, macrophages may be only a small fraction of the apoE-producing cells in these tissues such that an endotoxin effect on tissue macrophages was not detected. The latter possibility is supported by immunocytochemical studies which identified apoE-containing cells in primate peripheral tissues (43). A variety of cell types in kidney, lung, and adrenal was observed to display immunoreactive apoE in a granular intracellular pattern suggesting that such cells were sites of apoE synthesis. Also consistent with this possibility is that a number of

TABLE 3. ApoE mRNA in rat peripheral tissues

Tissue	A ApoE mRNA	B Total RNA	C ApoE mRNA	D Organ Weight	E ApoE mRNA	F Total DNA	G ApoE mRNA
	pg/μg RNA	*mg/g*	*ng/g*	*g*	*ng/organ*	*mg/g*	*molecules/cell*
Brain	27.7 ± 3.1	2.91	80.6	1	80.6	0.87	935
Adrenal	20.1 ± 1.8	4.64	93.3	0.04	3.7	1.81	520
Lung	4.6 ± 0.6	2.72	12.5	0.6	7.5	6.18	20
Kidney	3.3 ± 0.9	1.63	5.4	0.8	4.3	1.14	48

ApoE mRNA was measured in total RNA extracted from the indicated tissues as described in Materials and Methods. Each value is the mean ± SD of at least 12 determinations on tissues isolated from three to four adult Sprague-Dawley rats. Total RNA (column B) and total DNA content (column F) were measured by colorimetric procedures (35). Organ weights (column D) are expressed as g per 100 g body weight. Total apoE mRNA (column E) is expressed as ng per organ per 100 g body weight. Calculations for column G are based on a diploid DNA content of 6 pg/cell.

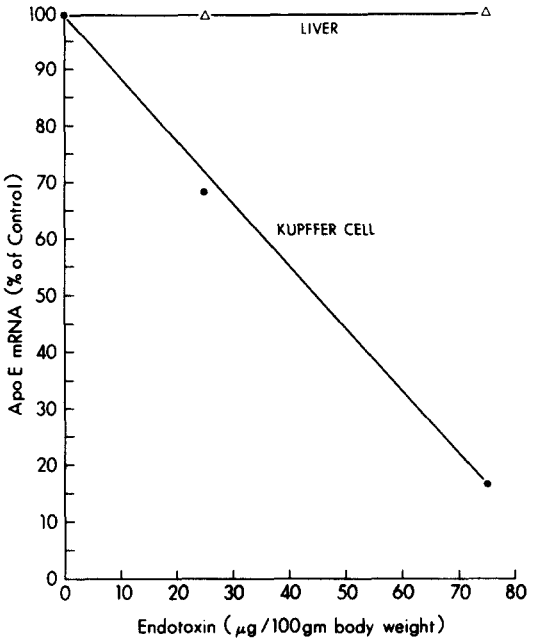

Fig. 5. Measurement of apoE mRNA in liver and Kupffer cells from endotoxin-treated rats. RNA was prepared from liver and isolated Kupffer cells of rats that received an intravenous injection of the indicated dose of endotoxin 24 hr prior to death. ApoE mRNA was measured by DNA-excess solution hybridization with single-stranded apoE cDNA. Control animals received the injection vehicle. Each value is the mean of at least 12 determinations on livers and cells from three to four rats, except for the Kupffer cell value at the 25 μg/100 g dose of endotoxin which is from cells pooled from two rats. The data are expressed as percent of the control values of liver or Kupffer cells.

non-macrophage cell types have been shown to express high levels of apoE in vivo or in culture. Such cells include ovarian granulosa cells (44), cerebral astrocytes (45), and visceral and parietal endoderm of the midgestational mouse embryo (46).

Gram-negative infections or treatment with bacterial endotoxin has profound effects on serum lipid metabolism, including a marked hypertriglyceridemia (16, 17). Werb and Chin (13, 14) proposed that the decrease in macrophage apoE production in vivo may contribute to the hypertriglyceridemia by reducing the apoE available for receptor-mediated clearance of lipoprotein particles. In order for this to be the case, macrophage-derived apoE would have to account for a substantial portion of total systemic apoE. The results of the present study suggest that at least for the Kupffer cell this is not likely. Since Kupffer cell apoE mRNA represents less than 1% of liver apoE mRNA, the endotoxin-mediated decrease in Kupffer cell apoE mRNA would not likely alter the plasma apoE concentration. Similarly, the lack of an endotoxin effect on liver apoE mRNA or on apoE mRNA in several peripheral tissues would argue against the idea that endotoxin-induced hypertriglyceridemia is due to decreased apoE production by macrophages or other cells. It should be noted, however, that changes in apoE production resulting from translational or post-translational regulation would not have been detected in the present study. Nevertheless, it seems more likely that hypertriglyceridemia results secondarily from the substantial decrease in lipoprotein lipase activity that accompanies endotoxin treatment. Kawakami and Cerami (47) have shown that adipose tissue lipoprotein lipase activity is greatly reduced in endotoxin-treated mice apparently in response to cachectin that is released by endotoxin-stimulated macrophages (48).

The results of the present study suggest that resident liver macrophages do not contribute significantly to systemic apoE production in the rat. Whether or not this conclusion can be generalized to other tissue macrophages is unclear. An estimate of the total macrophage population in the mouse has been made by Lee, Starkey, and Gordon (18) on the basis of the tissue content of the macrophage specific antigen F4/80. By this criterion, Kupffer cells are believed to represent about 20% of the total macrophage population in the mouse (18). Assuming this estimate of the macrophage population can be applied to the rat and that the apoE mRNA content of the Kupffer cell is typical of tissue macrophages, the total body macrophage population would account for only a few percent of whole body apoE mRNA. This suggestion is only speculation, however, because of the assumptions involved in estimating total macrophage (18) and the uncertainty in generalizing the Kupffer cell data to other tissue macrophages. Nevertheless, at present these are the only data available that speak to the question of the quantitative significance of apoE production in vivo by tissue macrophages.

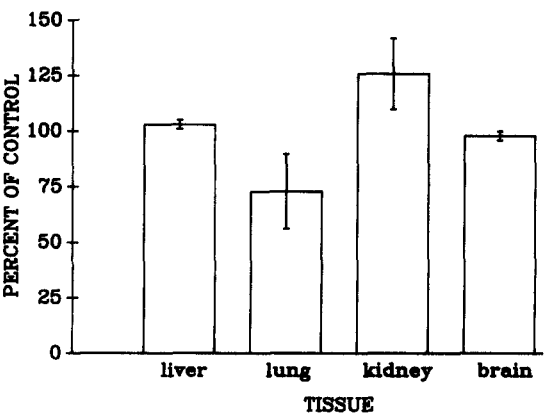

Fig. 6. Effect of endotoxin on apoE mRNA in peripheral tissues. RNA was prepared from tissues 24 hr after the administration of endotoxin (75 μg/100 g) or the injection vehicle to groups of three rats. ApoE mRNA was measured by DNA-excess solution hybridization with single-stranded apoE cDNA. Bars show the mean (± SEM) of measurements on three animals, excepting the value for the brain which shows the range of measurements on two animals. The data are expressed as percent of the control values from vehicle-injected rats.

The function of the apoE secreted by tissue macrophages or other cells in peripheral tissues is not known. Nor is it known whether the apoE species produced by tissue macrophages or other peripheral cells are functionally equivalent. Basu et al. (11) suggested that apoE-phospholipid discs secreted by macrophage may interact extracellularly with HDL to expand particle surface and facilitate the formation of larger cholesteryl ester-enriched HDL. In this way the macrophage-derived apoE could function in the early stages of the reverse cholesterol transport pathway which moves cholesterol from scavenger cells to the liver (11). Consistent with this hypothesis, apoE and cholesterol appear to leave macrophages by independent mechanisms suggesting that apoE functions at a stage subsequent to the removal of cellular cholesterol (49). Macrophage-derived apoE also appears to be functionally active as judged by its ability to interact with apoB/E receptors (50) and by its ability to promote the conversion of HDL_3 to larger cholesteryl ester-enriched HDL in vitro (50). Interestingly, in the latter in vitro studies, the source of the apoE did not appear to be important (50), perhaps indicating functional equivalence of the apoE produced by different cell types. If this is true in vivo, it may be that apoE is needed extracellularly for reverse cholesterol transport, but that the cell type from which it is derived is not particularly important. Tissue macrophage or other peripheral cells may simply serve as nonspecific sources of apoE for processes occurring in the extracellular fluids.

Another question of functional interest is why macrophage apoE expression is down-regulated in response to endotoxin. Werb and Chin (13) suggested that suppression of apoE synthesis in areas of infection may permit a more vigorous host response since apoE-containing lipoproteins suppress some lymphocyte functions (51–54). This suggestion seems less likely in view of the evidence that many cell types make apoE and that the apoE concentration in interstitial fluids is substantial (55). It remains to be established whether decreased macrophage apoE production can alter the apoE concentration encountered by lymphocytes at the site of an infection or elsewhere. Another possibility is that the down-regulation of apoE expression in macrophages is not involved with apoE function itself, but simply results from a reprogramming of the activated macrophage to perform other more essential functions. Additional studies are needed to establish the functional significance of apoE production by macrophages.

Excellent technical assistance was provided by Penelope Strockbine. This research was supported by the American Heart Association (83-849), the National Institutes of Health (HL-32868, DK-18171, and DK-25410), and a grant from the Distilled Spirits Council of United States Incorporated. P. A. D. was supported by a National Institutes of Health Training Grant (GM-07518). We thank Dr. J. Elovson for the rat apoB cDNA.

Manuscript received 1 June 1988 and in revised form 8 August 1988.

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