Cellular localization of apolipoprotein D and lecithin:cholesterol acyltransferase mRNA in rhesus monkey tissues by in situ hybridization

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Abstract Apolipoprotein D (apoD) and lecithin:cholesterol acyl transferase (LCAT) are found on high density lipoprotein particles (HDLs) and have been postulated to form part of a complex involved in the transport of cholesterol from peripheral tissues to the liver for excretion. We have examined the sites of synthesis of the mRNAs for these two proteins in the rhesus monkey by in situ hybridization. ApoD mRNA-containing cells were widely distributed throughout peripheral tissues in interstitial and connective tissue fibroblasts often associated with blood vessels or capillaries. ApoD mRNA was also found localized in cells associated with peripheral nerves, neuroglial cells, cells in the subarachnoid space on the surface of the brain including the pial cells, perivascular cells, and scattered neurons in the brain. LCAT demonstrated a much more restricted pattern of synthesis and was found to be synthesized by hepatocytes, the basal cell layer of the epidermis, and in brain cell populations distinct from those that synthesize apoD. In the brain LCAT was synthesized by scattered neurons, neuroglial cells, ependymal cells, as well as a discrete cell layer in the cerebellum. ApoD has been shown to possess extensive homology to retinol binding protein, which has a binding pocket for vitamin A. We propose that apoD may also function to bind cholesterol or its derivatives in compartments not in direct contact with the blood. The findings of both apoD and LCAT synthesis in the brain suggest that they play a significant role in lipid transport in the brain. —**Smith, K. M., R. M. Lawn, and J. N. Wilcox.** Cellular localization of apolipoprotein D and lecithin:cholesterol acyltransferase mRNA in rhesus monkey tissues by in situ hybridization. *J. Lipid Res.* 1990. **31:** 995-1004.

Supplementary key words cholesterol transport • brain • fibroblasts • epidermis • neuroglia • neurons

Apolipoprotein D (apoD) is found on plasma high density lipoprotein (HDL) (1-3) and comprises approximately 5% of the total HDL apolipoprotein. In association with the enzyme lecithin:cholesterol acyl transferase (LCAT), apoD may form part of a complex that is active in the efflux of cholesterol from peripheral tissues and its transport to the liver for catabolism. The excess cholesterol from peripheral cells is acquired by HDL, where it is converted to insoluble cholesteryl ester by LCAT. The cholesteryl ester is then transferred to the liver for excretion, directly or following transfer to low and very low density lipoproteins (LDL, VLDL) by the cholesteryl ester transfer protein (CETP) (2, 4, 5).

The complete protein sequence of both human LCAT and apoD have been determined from cDNA sequencing (6, 7). ApoD is a glycoprotein of M_r ~33,000 comprised of 169 amino acids. Although it contains no sequence similarities to other known apolipoproteins, apoD shares extensive homology to retinol-binding protein and other membranes of the α_{2u}-globulin superfamily (8). The members of this superfamily are likely to possess similar structures and bind small, sparingly soluble or labile molecules (8, 9). ApoD, present in plasma at about 70 μg/ml (10), has been postulated to act as a carrier for cholesterol or cholesteryl ester (2, 6). Although the data suggest that apoD may play a role in cholesterol transport, its precise function remains unknown.

LCAT is a glycoprotein of 416 amino acids and M_r ~63,000 that catalyzes the transfer of acyl groups from lecithin to the 3-OH of free cholesterol. The activity of LCAT is potentiated by apolipoprotein A-I (11), the major lipoprotein component of HDL. Other apolipoproteins, including apoA-IV (12) and apoC-I (13), have also been shown to activate LCAT. Most of plasma LCAT is found associated with apoD (2) in HDL particles, while a

Abbreviations: apoD, apolipoprotein D; LCAT, lecithin:cholesterol acyltransferase; HDL, high density lipoprotein; apoA-I, apolipoprotein A-I; cDNA, complementary DNA; apoB, apolipoprotein B; PDGF, platelet-derived growth factor; CSF, cerebral spinal fluid; CNS, central nervous system; mRNA, messenger ribonucleic acid; bp, base pair; GFAP, glial fibrillary acidic protein; EDTA, ethylenediaminetetraacetic acid; apoE, apolipoprotein E; apoC-I, apolipoprotein C-I; VLDL, very low density lipoprotein; LDL, low density lipoprotein; CETP, cholesteryl ester transfer protein.

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smaller fraction is found in low density lipoproteins (14, 15). It is present in plasma at 5 μg/ml (10).

McLean et al. (16) have shown that LCAT is synthesized in the liver as well as in HepG2 hepatocarcinoma cells, but not in small intestine, pancreas, adrenal gland, or white blood cells. The synthesis of apoD is more widespread. Drayna et al. (6) have detected its message in human adrenal gland, kidney, pancreas, small intestine, and liver, while Bouma et al. (17) have detected the protein in hepatocytes and enterocytes.

In order to further elucidate the physiological roles of LCAT and apoD, we have investigated the localization of their mRNAs by in situ hybridization using a number of tissues derived from rhesus monkey.

METHODS

Tissue preparation

Rhesus tissues were collected at necropsy from an 11-year-old male. The tissues were fixed in 4% paraformaldehyde buffered with 0.1 M $NaPO_4$ (pH 7.4) for 3–4 h at 4°C, cryoprotected in 15% sucrose-PBS overnight, embedded in optimal cutting temperature compound (O.C.T., Miles Laboratories), frozen in liquid nitrogen, and stored at −70°C. Cryosections (7–10 μm) were thaw-mounted onto poly-L-lysine (Sigma)-coated slides, refrozen, and stored at −70°C with desiccant until use. Samples of human saphenous vein and internal mammary artery were obtained from patients undergoing cardiac bypass surgery and atherosclerotic plaques were obtained from carotid endarterectomy surgery. These tissues were prepared similarly.

Probes

The apoD probe was a 742 bp human cDNA and included the 5' noncoding region, the entire coding region, and part of the 3' untranslated region (6), subcloned into the SP64 vector (Promega). The probe for human apolipoprotein B (apoB) was a 3440 bp cDNA subcloned into the SP64 vector, which was linearized with Bgl II to give a 1550 bp transcript complimentary to the 3'-end of the mRNA. Both probes were kindly provided by Karen Fisher, Genentech. The human LCAT probe was a 1427 bp full length cDNA (7, provided by John McLean, Genentech) subcloned into the pGEM-3 vector (Promega). The human PDGF-A chain and human PDGF-β receptor cDNA probes were as previously described (18).

All probes were transcribed (19) using α[^{35}S]UTP (Amersham, sp act 1200 Ci/mmol). Full length transcripts were used for hybridization.

In situ hybridization

In situ hybridization was performed as previously described (18, 20). Briefly, cryosections were pretreated with paraformaldehyde, proteinase K (Sigma), and prehybridized in 100 ml hybridization buffer (50% formamide, 0.3 M NaCl, 20 mM Tris, pH 8.0, 5 mM EDTA, 0.02% polyvinylpyrrolidine, 0.02% ficoll, 0.02% bovine serum albumin, 10% dextran sulfate, and 10 mM dithiothreitol) at 42°C. Serial sections were hybridized with ^{35}S-labeled apoD, LCAT, and either PDGF-A chain and PDGF-β receptor RNA probes or with an apoB RNA probe. The hybridizations were performed using 600,000 cpm of ^{35}S-labeled riboprobe at 55°C. After hybridization, the sections were washed with 2 × SSC (1 × SSC = 150 mM NaCl, 15 mM Na citrate, pH 7.0) with 10 mM β-mercaptoethanol and 1 mM EDTA, treated with RNase (Sigma), again washed in the same buffer, followed by a high stringency wash in 0.1 × SSC with 10 mM β-mercaptoethanol and 1 mM EDTA, at 52°C. The slides were then washed in 0.5 × SSC and dehydrated in graded alcohols containing 0.3 M ammonium acetate. The sections were dried, coated with NTB2 nuclear track emulsion (Kodak), and exposed in the dark at 4°C for 4 to 10 weeks. After development, the peripheral and vascular tissue sections were counterstained with hematoxylin and eosin, while some of the brain sections were counterstained with toluidine blue to aid in cell identification.

Some serial brain sections adjacent to the hybridized slides were stained with a mouse anti-glial fibrillary acidic protein monoclonal antibody (α-GFAP, Boehringer Mannheim) to aid in identification of glial cells. This antibody was used at a concentration of 2 μg/ml and developed using an alkaline phosphatase detection kit according to the manufacturer's instructions (ABC-AP, Vector Labs).

RESULTS

The apoD probe hybridized to a broad distribution of cells of mesenchymal origin which were identified as fibroblasts and interstitial cells in most of the peripheral tissues examined. ApoD mRNA-containing cells were found in spleen, testes, liver, pancreas, skeletal muscle, kidney, jejunum, pituitary, peripheral nerve, brain, and adventitial fibroblasts associated with human internal mammary artery and saphenous vein. The expression of LCAT mRNA was restricted to hepatocytes, the basal epidermis, and to several different cell populations in the brain.

The specificity of the hybridizations was indicated by the different pattern of hybridization obtained with each probe. The apoD probe hybridized to many tissues that were negative with the LCAT probe (**Table 1**)). In tissues that demonstrated hybridization to both probes, such as the brain, different cell populations were identified as positive (compare Fig. 2A vs 2B and 2D vs 2E). The probe for apoB hybridized to hepatocytes in the liver and enterocytes of the intestinal villi, but not to any other cells

TABLE 1. Tissue distribution of apoD and LCAT mRNA in the rhesus monkey

Tissue	ApoD	LCAT
Spleen	(+)	(−)
Testes	(+)	(−)
Liver	(+)	(+)
Cardiac muscle	(+)	(−)
Pancreas	(+)	(−)
Skeletal muscle	(+)	(−)
Jejunum	(+)	(−)
Kidney	(+)	(−)
Renal artery	(+)	(−)
Pituitary	(+)	(+)
Skin	(+)	(+)
Brain	(+)	(+)

in the tissues examined. Mehrabian et al. (21) had observed apoB mRNA in both liver and intestine, but in no other tissues examined. Additionally the probes for PDGF-A chain and PDGF-B receptor each gave a unique pattern of hybridization in each of the tissues examined, which were distinguishable from each other and from the LCAT and apoD probes (data not shown). The LCAT and PDGF probes did not label cells in normal vessels, however the PDGF probes labeled many mesenchymal-appearing intimal cells of the plaque as seen previously (18).

Peripheral tissues

ApoD. In the spleen, the apoD probe hybridized weakly to scattered cells in the red pulp regions, particularly along the reticulin strands. These cells were often located near the trabeculae of the spleen, however no cells of the capsule, trabeculae, or the white pulp showed hybridization, nor could we identify any positive endothelial cells. Although the hybridizing cells in this sample are most likely connective tissue fibroblasts composing the matrix of the spleen, we cannot rule out the possibility that some of them may be macrophages.

In the testes, the apoD probe hybridized to scattered interstitial cells (**Fig. 1A**), which lay between the seminiferous tubules. Several of these cells had large pale-staining nuclei with little cytoplasm and were located in cell clusters in the interstitium. At least some of these cells are Leydig cells but some interstitial fibroblasts could also be positive. No other structure in the sample showed positive hybridization with this probe.

In the liver, the apoD probe hybridized to scattered fibroblasts of the interlobular septum surrounding the portal area as well as to cells composing the capsule of the liver. We could not detect any hepatocytes producing apoD mRNA.

ApoD mRNA was detected in interstitial connective tissue cells or fibroblasts scattered throughout the pancreas (Fig. 1B). The scattered distribution of the reactive cells suggested that the positive cells may be periductal, but this could not be definitely established. The positive cells were generally found in the layer of connective tissue covering pancreas, in the septa separating the lobules, as well as in the connective tissue surrounding large ducts. Most islets, acinar cells, and epithelial cells lining the larger ducts showed no hybridization to the apoD probe.

In both the skeletal and cardiac muscle samples, the apoD probe hybridized strongly to the interstitial cells between the muscle fibers (Fig. 1C). No hybridization was detected to any of the muscle cells in either of these samples or in the smooth muscle cells present in vessels and intestine. In the cardiac muscle sample, we could also identify strongly hybridizing cells in peripheral nerves and in cells adjacent to blood vessels between the muscle fibers.

The jejunum sample produced a complex picture when hybridized to the apoD probe. Fibroblasts in the adipose tissue surrounding the intestine gave strong hybridization signal, as did cells found in Auerbach's plexus, situated between the inner and the outer layers of the muscularis (Fig. 1D). Due to the close proximity of the positive cells in Auerbach's plexus it was not possible to determine which cell type was positive (neuronal or glial) in this tissue. Scattered cells in the lamina propria hybridized weakly to the apoD probe. No apoD mRNA-positive cells were detected in the muscularis layers or the intestinal epithelium.

Several different populations of cells in the kidney hybridized to the apoD probe. A strong positive signal was observed in the adventitial fibroblasts surrounding the vessels within the kidney as well as in the adventitial fibroblasts surrounding the attached vessels. Cells within peripheral nerves adjacent to these vascular structures also hybridized strongly but again it was not possible to discriminate between neuronal or glial cell hybridization due to the close proximity of cells in this tissue. Some apoD-positive cells in the kidney medulla were found adjacent to tubules and have the appearance of interstitial fibroblasts (Fig. 1E). A weak hybridization signal was occasionally seen in both the proximal and distal tubular epithelium as well as within occasional glomeruli.

In the anterior lobe of the pituitary, we observed a strong hybridization of the apoD probe to the fibroblasts of the capsule and to cells associated with the fenestrated capillaries laying between cords of secretory epithelial cells. These could be endothelial cells, pericytes, macrophages, or fibroblasts. However, considering the localization of apoD production to perivascular fibroblasts in other tissues we suspect that the positive cells in the pituitary are fibroblasts.

In a single sample of human saphenous vein and two samples of internal mammary artery, adventitial fibroblasts adherent to the vessel wall showed very strong hybridization to the apoD probe (Fig. 1F). We could not

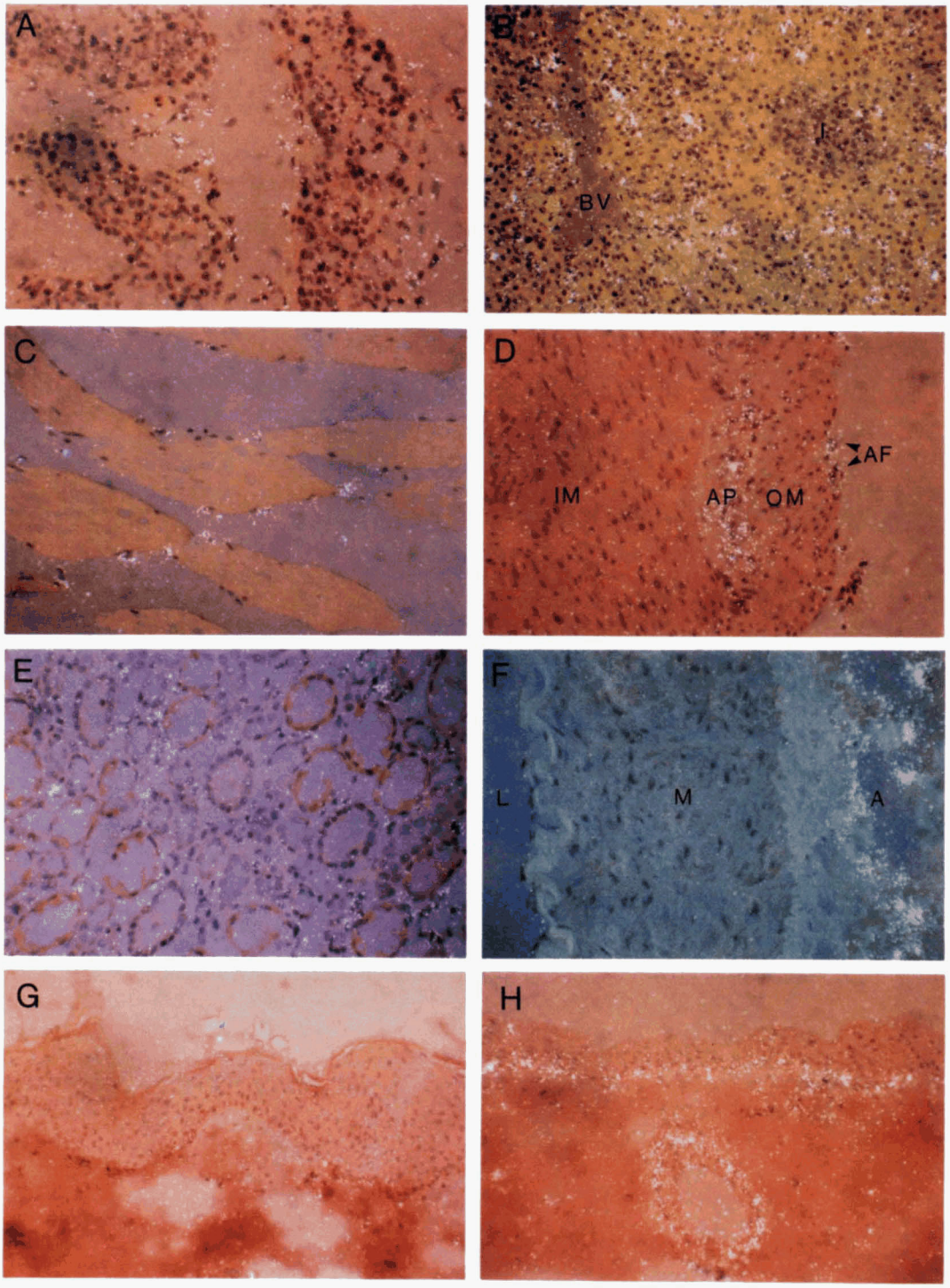

Fig. 1. ApoD mRNA production in peripheral tissues. (A) Testes: apoD mRNA was synthesized by scattered interstitial cells between the seminiferous tubules. These cells were either Leydig cells or connective tissue fibroblasts. No other cells are positive; 10-week exposure (×250). (B) Pancreas: scattered interstitial connective tissue cells or fibroblasts in the pancreas were positive. Islet cells, acinar cells, and ductal cells were negative (I, islet; BV, blood vessel); 10-week exposure (×200). (C) Skeletal muscle interstitial fibroblasts laying between the muscle fibers showed positive hybridization; 10-week exposure (×200). (D) Small intestine: cells found in Auerbach's plexus as well as fibroblasts associated with adipose tissue surrounding the intestinal wall were positive. Smooth muscle cells showed no hybridization (AP, Auerbach's plexus; AF, adipose fibroblasts; IM, inner muscularis; OM, outer muscularis); 10-week exposure (×200). (E) Kidney: scattered cells in the kidney showed positive hybridization. Many of these cells were situated between the tubules and were most likely interstitial fibroblasts; 10-week exposure (×250). (F) Human internal mammary artery: adventitial fibroblasts showed strong hybridization. Smooth muscle cells and endothelial cells were negative (L, lumen; M, media; A, adventitia); 4-week exposure (×250). (G) ApoD mRNA in rhesus skin: no cells of the epidermis or dermis showed positive hybridization; 10-week exposure (×200). (H) LCAT mRNA in rhesus skin: cells in the basal epidermis and the basal cell layer surrounding sweat glands and hair follicles contained LCAT mRNA; 10-week exposure. (×200).

detect apoD mRNA in the smooth muscle cells of the media, lumenal endothelial cells, or in any region of four human carotid atherosclerotic plaques examined obtained from endarterectomy surgery. Adventitial fibroblasts surrounding the rhesus renal artery also showed similar results.

LCAT. In contrast to the results obtained with the apoD probe, the distribution of cells containing LCAT mRNA was much more restricted. In the liver most of the hepatocytes contained low levels of LCAT mRNA while no other cell types in the liver hybridized to this probe. Scattered secretory epithelial cells in the pituitary hybridized weakly to the LCAT probe. In the skin LCAT mRNA showed a striking localization to the stratum germinativum (Fig. 1H). In contrast, the apoD probe hybridized to the cells in nerve bundles embedded in the dermis of the skin, while both the epidermis and the underlying dermis were negative (Fig. 1G).

Brain

ApoD. We hybridized four different regions of brain including the cerebral cortex, hypothalamus, cerebellum, and the brain stem. In all of the samples examined, strong hybridization was observed to a number of cells within the subarachnoid space as well as to a population of perivascular cells surrounding small vessels penetrating from the surface of the brain (**Fig. 2A**). While many of the positive cells in the subarachnoid space may be fibroblasts, distinct labeling of the pia was seen on many sections. Blood vessels originating from the pia penetrate the surface of the brain and are covered with a perivascular sheath of loose connective tissue and an investing sleeve of pia. We suspect the positive perivascular cells associated with the penetrating vessels may be either fibroblasts or a continuation of the positive hybridizations to the pial cells seen on the surface of the brain. Positive hybridizations to astrocytes closely associated with these vessels deeper within the brain cannot be excluded. Many vascular profiles were examined, but no hybridization to endothelial cells was observed.

Scattered glial cells in the white matter of the tracts hybridized moderately, but could not be identified definitively as either oligodendroglia or astrocytes. Hybridizing cells were found to be uniformly distributed throughout the gray matter. In these regions the predominant population of positive cells was neuroglial, while another smaller population of cells hybridizing to the apoD probe could be identified morphologically as neurons. Often hybridizing neuroglia were closely associated with negative neurons. Many cells that were morphologically identifiable as either neuroglia or neurons on the basis of nuclear morphology after toluidine blue staining were negative.

In the cerebellum, cells hybridizing strongly to the apoD probe were scattered throughout the sample (Fig. 2D). In the molecular layer most of these cells could be identified as neuroglia while some of these apoD-positive cells could be neurons. Foci giving strong hybridization were identified in the granular layer; however, due to the dense packing of the neuronal cell bodies in this layer, specific nuclei could not be identified as positive. A few cells adjacent to the Purkinje cell bodies that we tentatively identified as astrocytes showed strong hybridization. In the white matter of the cerebellum glia showed a less intense signal with the apoD probe than did the hybridizing cells in the white matter of the other brain samples.

In the sample containing the pons and brain stem, strongly hybridizing neurons and neuroglia were both observed. We saw no apoD hybridization to the ependymal cell layer.

No hybridization was observed to any cells of the brain with the apoB probe (Fig. 2C). The hybridization patterns observed with the PDGF-A chain and the PDGF-β receptor probes were distinct from those of either apoD or LCAT (data not shown).

LCAT. The LCAT probe showed a different pattern of hybridization than the apoD probe and gave a weaker hybridization signal throughout most of the brain samples. In all the samples the cells within the subarachnoid space including the pia and the perivascular cells associated with the penetrating blood vessels were negative. In the cerebral cortex many cells in layer 1 hybridized moderately, which were identified as either horizontal cells (neurons) or neuroglia (Fig. 2B). Few cells in this layer showed positive hybridization for apoD (Fig. 2A). In the deeper layers of the cortex, as well as in the gray matter of the rest of the samples, scattered cells were found to contain LCAT mRNA. Some of these cells were identified on the basis of nuclear morphology as neuroglia and a smaller number of neurons. These scattered cells showed a uniform distribution pattern, similar to that observed for apoD, but there were fewer LCAT-positive cells and they gave a weaker signal compared to the apoD hybridizations. In the white matter of the cerebral cortex, we observed no hybridizing cells.

The most striking result in this study was the hybridization of the LCAT probe in the cerebellum (Fig. 2E) where a discrete layer of cells in the molecular layer immediately adjacent to the Purkinje cells showed a very strong hybridization. These cells did not label with a monoclonal antibody to glial acidic fibrillary protein (GFAP) on serial sections (Fig. 2F), suggesting that they may be neurons. Alternatively, these may be a group of Bergmann glial cells showing poor staining with the anti-GFAP antibody. No hybridization to the Purkinje cells was seen. A few scattered cells in the molecular layer also hybridized strongly to the LCAT probe.

The LCAT probe hybridized weakly to scattered neu-

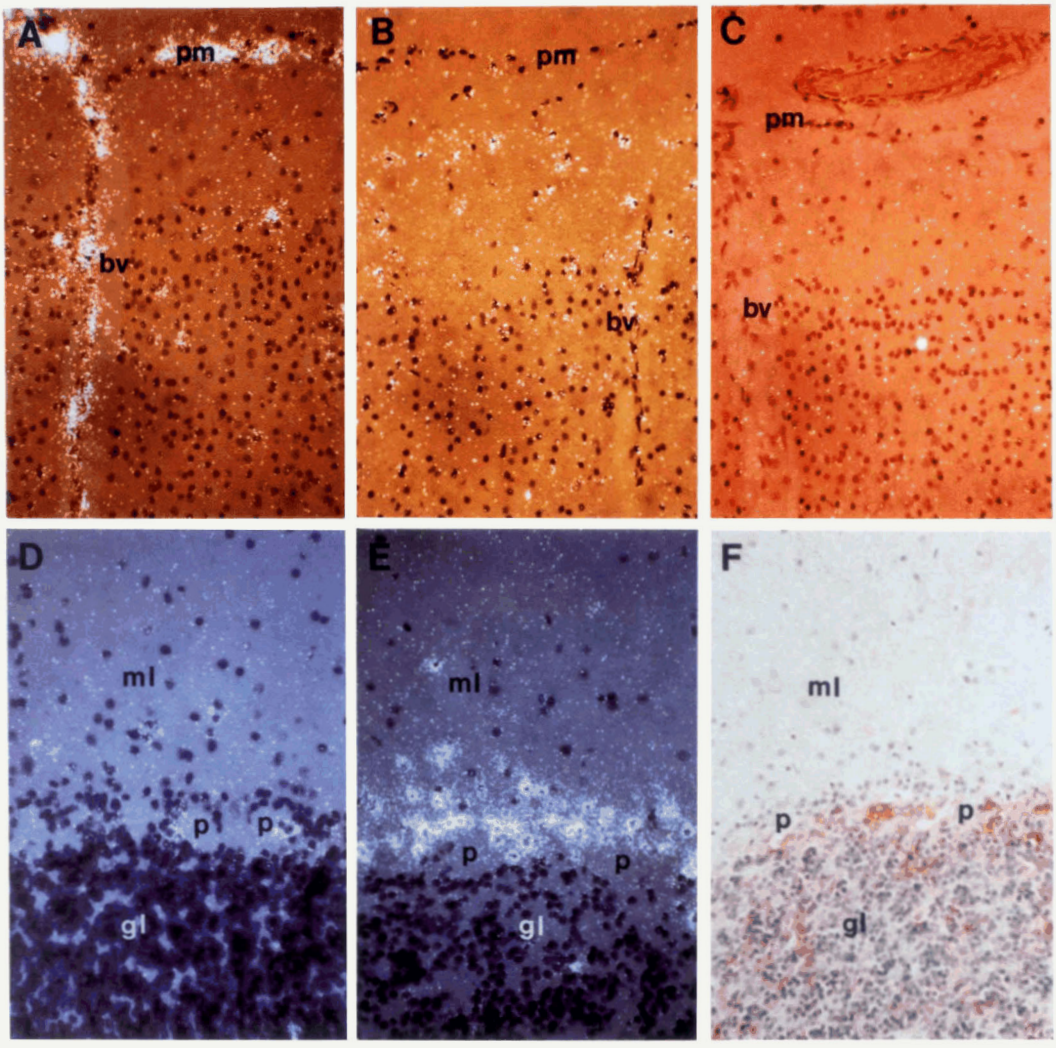

Fig. 2. Cell populations in the brain containing mRNA for apoD and LCAT. (A) ApoD hybridization in the cerebral cortex illustrating strong hybridization to cells in the subarachnoid space including the pial cells, cells associated with blood vessels, as well as scattered positive cells in the neuropile; exposure 4 weeks; magnification ×250. (B) LCAT hybridization in the cerebral cortex indicating that many cells in layer 1 of the cerebral cortex contain LCAT mRNA. On the basis of their nuclear morphology and placement some of these positive cells were identified as horizontal cells (neurons) while others were glia. Scattered LCAT positive cells were also present in deeper layers of the cortex. Pial cells and perivascular cells were negative; exposure 10 weeks; magnification ×250. (C) ApoB hybridization in the cerebral cortex shown as a negative control. This probe labeled appropriate cells in the liver and intestine; exposure 4 weeks; magnification ×250. (D) ApoD hybridization in the cerebellum, positive cells were found in the molecular and granular layers and occasionally found adjacent to Purkinje cells. Many more apoD positive cells were found on the surface of the cerebellum in the subarachnoid space and pia (not shown); exposure 10 weeks; magnification ×310. (E) LCAT hybridization in the cerebellum. In this tissue a discrete layer of cells in the molecular layer immediately adjacent to the Purkinje cells showed a very strong hybridization signal with the LCAT probe while the Purkinje cells were clearly negative; exposure 10 weeks; magnification ×310. (F) GFAP staining of the cerebellum illustrating the distribution of glial cells in this tissue. Note that the cell layer that shows positive hybridization to the LCAT probe appears to be GFAP negative; magnification ×250. Abbreviations: BV, blood vessel; PM, pia mater; L1, layer 1 of the cortex; GL, granular layer; P, Purkinje cell; ML, molecular layer.

roglia and neurons in the brain stem sample. The ependymal cell layer showed a weak, but distinct signal with this probe.

DISCUSSION

This study demonstrated the widespread synthesis of apoD mRNA in peripheral tissues by interstitial and connective tissue fibroblasts often associated with blood vessels or capillaries. ApoD mRNA was also found localized in cells associated with peripheral nerves, neuroglial cells, cells in the subarachnoid space of the surface of the brain including the pia, as well as perivascular cells and scattered neurons in the brain. LCAT mRNA was found in hepatocytes and the basal epidermis of the skin. In addition, high levels were found in brain cell populations distinct from those synthesizing apoD. These observations

suggest that the functions of apoD and LCAT extend beyond transport of cholesterol from peripheral tissues to the liver and esterification of cholesterol in plasma. Cholesterol is necessary for membrane biosynthesis and steroid hormone production. ApoD may play a direct role in cholesterol transport, but that remains to be proven. Its homology with the retinol-binding protein family is consistent with such a function.

Human apoD and plasma retinol-binding protein share 25% amino acid sequence identity that spans most of the protein (8). The crystal structure of human plasma retinol-binding protein has been determined (22). It possesses eight strands of anti-parallel beta-sheets that form a binding pocket for the labile and insoluble retinol (vitamin A). The crystal structure of bovine β-lactalbumin, which bears 25% amino acid homology to plasma retinol binding protein (23, 24), has also been solved. The crystal structures of the two proteins are nearly superimposable (25). Godovac-Zimmermann et al. (23) have proposed that bovine β-lactalbumin, an abundant protein component of milk whey, binds retinol and other hydrophobic molecules. This family of proteins with sequence similarly contains about ten known members, including two urinary globulins, α1-microglobulin and α_{2u}-globulin; insecticyanin, a pigment-containing protein of hornworm larvae; purpurin, a retina neural cell adhesion factor that binds retinol; an olfactory tissue protein postulated to bind odorants; and aphrodisin, a hamster sex attractant (26–31). It is quite possible that these related proteins represent variations of a common structure that is suited to the binding and extracellular transport of small, hydrophobic or labile molecules (9). Our finding of widespread synthesis of apoD in the tissues examined suggests that local synthesis of apoD may play a crucial role in cholesterol transport throughout the organism. It is thus postulated that apoD may function in the binding of cholesterol or its derivatives in plasma, cellular interstitia, and cerebrospinal fluid. It may also transport steroid hormones from sites of synthesis to target tissues. Leydig cells in the testis, which synthesize steroid hormones, are present in the interstitial space and are either producing apoD mRNA or in contact with cells producing apoD mRNA.

In the periphery apoD mRNA was found in interstitial cells and fibroblasts present in connective tissue associated with blood vessels, adipose tissue, and connective tissue of organ capsules. Fibroblasts are derived developmentally from the mesenchyme. They are known to synthesize and secrete collagen and other extracellular matrix macromolecules that make up connective tissue. Fibroblasts thus serve as the cells that provide the support necessary to maintain the integrity of a tissue. Interstitial cells, as discussed here, is a descriptive term referring to cells found within a tissue but not recognizable as having a function specific to that tissue. This grouping includes fibroblasts present within the matrix of a tissue. It is possible that at least some of the hybridizing cells in the spleen, kidney, and pancreas are all fibroblasts which are part of the connective tissue holding these organs together.

The detection of apoD and LCAT mRNA synthesis in the brain and apoD mRNA synthesis in peripheral nerves is an intriguing addition to the discovery of other plasma proteins of cholesterol metabolism in the brain and in regenerating nerve tissues. Skene and Shooter (32) and Ignatius and colleagues (33) demonstrated that apoE was markedly induced after injury to peripheral nerves. In this context, apoE appears to be part of the process that serves to scavenge cholesterol from degenerating cells and deliver it to growing neurons and Schwann cells. After crush injury, macrophages produce large quantities of apoE. Subsequently, as axons grow and remyelinate, high levels of LDL receptor are expressed, presumably to support new membrane biosynthesis (34). Swanson et al. (35) have reported the presence of mRNA for both the LDL receptor and 3-hydroxy-3-methylglutaryl-coenzyme A synthetase, a regulated enzyme in cholesterol biosynthesis, in both the central nervous system (CNS) and in the autonomic nervous system of rabbits. We have observed apoD mRNA in cells associated with peripheral nerves present in skin, kidney, cardiac muscle, and small intestine. These observations suggest that apoD may play an important role in the functioning and maintenance of the peripheral nervous system.

The concentration of apoE mRNA in brain is second only to its concentration in liver (36). Within the brain, astrocytes are the primary cell type expressing this apolipoprotein (37, 38). By in situ hybridization we have noted that the pattern of apoE mRNA expression in rhesus brain is distinct from those of apoD and LCAT, but shares some commonalties with apoD (J. N. Wilcox and A. J. Augustine, unpublished observations). ApoE, like apoD, is synthesized by pial cells and by cells distributed in both the white and the gray matter, mainly in cells identifiable morphologically as neuroglia. ApoE is not synthesized by cells at perivascular sites.

The lipoproteins found in the cerebral spinal fluid (CSF) are largely distinct from those present in circulating plasma (39). Unlike plasma, the CSF lacks apoB (40), the major carrier of cholesterol in human plasma. Pitas et al. (39) have shown apoE and apoA-I to be the major apolipoproteins found in human CSF, where they are present largely in lipoprotein particles of different densities. ApoE is synthesized in the brain and is present in the CSF at 3.5% of its level in plasma, where it exists in small spherical and discoidal lipoprotein particles that contain cholesterol and phospholipid (39). ApoA-I, the major protein constituent of plasma high density lipoprotein, is present in human cerebrospinal fluid at 0.5% of the level found

in plasma (40) and appears to be derived from the plasma, not the brain (36, 39). There are no data for apoD protein in the CSF, but the pial cells, which are in direct contact with the CSF, possess high levels of apoD message, which are comparable to the levels of apoE mRNA found in these same cells (J. N. Wilcox and A. J. Augustine, unpublished observations).

The high local concentrations of apoD in the brain suggest that this apolipoprotein may also play a role in brain lipid transport. The brain derives cholesterol both from the plasma, across the blood–brain barrier, as well as from de novo synthesis. Our finding of apoD synthesis at perivascular sites throughout the brain suggests that it may function to facilitate transport of cholesterol across the blood–brain barrier, assisting in either the influx or efflux of cholesterol from cells in the innermost regions of the brain. We have observed expression of apoD mRNA in sites where Pitas et al. (39) have localized LDL receptors immunocytochemically in monkey brain. Specifically, they observed LDL receptors in astrocytes abutting on the arachnoid space and the pial cells of the arachnoid itself. Relatively few receptors were present in cells of the gray matter of the cortex, while they were prominent on the astrocytes of the white matter and in the cells of the brain stem.

Recently, LCAT mRNA was detected by Northern blot hybridization in mouse brain and testes as well as in liver (41). In our studies we were only able to detect a weak hybridization to hepatocytes and no signal in the testes. It is possible that the levels of mRNA in these tissues are below our level of detection by in situ hybridization.

We have observed expression of LCAT mRNA in the basal epidermis of the skin. This finding is not readily explicable in terms of the current understanding of skin biology, but may lead one to speculate about other potential roles of LCAT. This basal, proliferating layer of the epidermis is the least differentiated layer of keratinocytes. They synthesize high levels of lipids, including cholesterol, which are packaged into lamellar granules. As differentiation proceeds, the contents of these granules are released to form an extracellular permeability barrier in the stratum corneum. Approximately 25% of the lipid in the cornified layer is cholesterol (42). Cholesteryl esters have been measured in keratinocyte cultures (43), the epidermis (44), and epidermal cysts (45), and compose up to 10% of the lipid present in differentiated keratinocyte cultures and the cornified layer of the epidermis. Mommaas-Kienhuis et al. (46) have also found that these basal keratinocytes, as well as a few suprabasal cells, possess LDL-receptor activity.

Cerebrospinal fluid (CSF) has been reported to contain LCAT (47), and a distinct enzyme with cholesterol esterification activity has been described in brain (48). Human CSF has a lower percentage of esterified cholesterol than plasma, 66% versus 75% (39). It is noteworthy that 6 of 24 patients reported with familial LCAT deficiency had central nervous system impairments (41, 49). In this study we have demonstrated the synthesis of LCAT mRNA to restricted cell populations within the brain itself. Ependymal cells, which produce this message, are in contact with the CSF. Very high levels of LCAT mRNA are also found in the cerebellum in a cell layer adjacent to the Purkinje cells. The physiological activator of LCAT in the plasma, apoA-I, is also present in the CSF. Thus, the necessary enzymatic machinery to esterify cholesterol is present in the CSF. Cholesteryl esters formed by the action of LCAT in the CSF are packaged into lipoprotein particles distinct from those found in the plasma (39) and may contain apoE as the major apolipoprotein.

In light of apoD's distribution throughout the body, the notion that it is found associated with LCAT in the plasma as part of a complex to transport cholesteryl esters from the periphery to the liver for export needs to be expanded. It is now evident that many elements of plasma lipid transport are recreated within the central nervous system to achieve cholesterol transport and homeostasis within this partially closed system. We propose that apoD plays an important physiological role in maintaining appropriate cholesterol levels in compartments not directly exposed to the blood supply.

The authors wish to thank Karen Fisher for providing the apoD and apoB riboprobes, John McLean for the LCAT cDNA clone, Andy Augustine for sharing data on apoE expression in the brain, Denny Liggitt and David Gordon for assistance in histological interpretation, Christopher Fielding and Lisa Paborsky for helpful discussions. This research was supported by Genentech, Inc.

Manuscript received 28 July 1989 and in revised form 23 January 1990.

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