Unique Lipoproteins Secreted by Primary Astrocytes From Wild Type, apoE (−/−), and Human apoE Transgenic Mice*

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Composition of central nervous system lipoproteins affects the metabolism of lipoprotein constituents within the brain. The e4 allele of apolipoprotein E (apoE) is a risk factor for Alzheimer’s disease via an unknown mechanism(s). As glia are the primary central nervous system cell type that synthesize apoE, we characterized lipoproteins secreted by astrocytes from wild type (WT), apoE (−/−), and apoE transgenic mice expressing human apoE3 or apoE4 in a mouse apoE (−/−) background. Nondenaturing size exclusion chromatography demonstrates that WT, apoE3, and apoE4 astrocytes secrete particles the size of plasma high density lipoprotein (HDL) composed of phospholipid, free cholesterol, and protein, primarily apoE and apoJ. However, the lipid:apoE ratio of particles containing human apoE is significantly lower than WT. ApoE localizes across HDL-like particle sizes. ApoE localizes to the smallest HDL-like particles. ApoE3 (−/−) astrocytes secrete little phospholipid or free cholesterol despite comparable apoE expression, suggesting that apoE is required for normal secretion of astrocyte lipoproteins. Further, particles were not detected in apoE (−/−) samples by electron microscopy. Nondenaturing immunoprecipitation experiments indicate that apoE and apoJ reside predominantly on distinct particles. These studies suggest that apoE expression influences the unique structure of astrocyte lipoproteins, a process further modified by apoE species.

The composition and type of lipoproteins present in the brain have implications not only for lipid delivery but also for the transport of apolipoprotein (apo) and other lipoprotein constituents within the central nervous system (CNS). Glia, in particular astrocytes, are the primary cell type in the CNS that synthesize apoE (1, 2), whereas apoJ is expressed by astrocytes and neurons (3–5). We have previously observed that primary rat astrocytes secrete discoidal particles the size of large plasma high density lipoproteins (HDLs) that contain apoE and apoJ (6). As a ligand for lipoprotein receptors, apoE helps to regulate plasma lipid and cholesterol metabolism. This process may also be operating in the parenchyma of the brain, as neural cells express a variety of receptors in the low density lipoprotein receptor family (7–10). The role of apoJ in lipid transport in both the periphery and within the CNS is less clear, and gp330 (megalin), the only known receptor for mammalian apoJ (11), appears to be expressed only by ependymal and endothelial cells in the brain (12, 13). Thus, lipoprotein secretion by isolated glial cells may provide a system in which to further dissect the role of apoE and apoJ in lipoprotein synthesis, secretion, and function in the brain.

In terms of function, several lines of evidence suggest that apoE and apoJ may be involved in neural homeostasis beyond their capacity to transport lipid. Both apoE and apoJ increase in response to different brain insults (3, 14–16). In addition, apoE and apoJ may play a role in the pathogenesis of Alzheimer’s disease (AD), as both proteins appear to interact with amyloid-β (Aβ), the primary component of senile and cerebrovascular plaques in the AD brain. ApoE and apoJ immunoreactivity is localized to senile plaques (17–19). ApoE and apoJ interact with Aβ to form a stable complex (20–24), alter the aggregation of the Aβ peptide (25–29), and affect Aβ neurotoxicity (30–34). In humans, apoE exists as three naturally occurring isoforms (apoE2, apoE3, and apoE4), and apoE4 is a risk factor for AD via an isoform-specific mechanism as yet unknown. One hypothesis is that CNS lipoproteins containing apoE and/or apoJ may provide a vehicle for clearing Aβ via lipoprotein receptors (22, 35–37).

The functional activity of apoE is affected by its conformation, and the conformation of apoE is largely determined by the size, composition, and type (disc versus sphere) of the particle with which it is associated. For example, the type of particle and ratio of apoE to lipid determine the affinity of apoE for specific receptors (38, 39). Therefore, we have characterized the particles secreted by primary astrocytes as an initial step toward understanding the function of lipoproteins unique to the CNS. Astrocytes were cultured from wild type, apoE (−/−), and apoE transgenic mice in which human apoE3 or apoE4 is immunosorbent assay; EM, electron microscopy; HDL, high density lipoprotein; IP, immunoprecipitation; PAGE, polyacrylamide gel electrophoresis; PL, phospholipid; TC, total cholesterol; TG, triglyceride; WT, wild type.
expressed under the control of the astrocyte-specific glial fibrillary acidic protein promoter on a mouse apoE (−/−) background. Our data show that expression of apoE by astrocytes is required for normal lipoprotein secretion by these cells and that apoE species appears to influence lipoprotein composition.

MATERIALS AND METHODS

Animals—Transgenic mice expressing human apoE3 (line 37) or apoE4 (line 1) by astrocytes in the brain in the absence of mouse apoE (apoE (−/−)) were generated as described (40) and mated with apoE (−/−) mice that had been backcrossed to individual neonatal (1–2 day old) mice and grown to confluence (10–14 days) in T75 flasks as described (41). Growth medium consisted of Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) containing 10% fetal bovine serum (HyClone, Logan, UT), 10% horse serum (HyClone), penicillin (100 units/ml), streptomycin (100 μg/ml), and epidermal growth factor (10 ng/ml; Sigma). Once confluent, medium was removed, cells were washed two times with sterile phosphate-buffered saline, and cultures were incubated in 5 ml of serum-free Dulbecco’s modified Eagle’s medium/Ham’s F-12 (1:1) medium containing N2 supplement (Life Technologies, Inc.) for an additional 72 h. Conditioned medium was removed, clarified by centrifugation at 800 × g for 5 min, and stored at 4°C until it was analyzed. ApoE levels in medium samples from individual cultures were quantified by Western blot or ELISA as described below. Concentrations ranged from ~5–10 μg of apoE/ml of unconcentrated medium.

Fractionation—Astrocyte-conditioned medium (ACM) containing wild type mouse apoE, no apoE (apoE (−/−)), human apoE3, or human apoE4 was concentrated 50-fold (Centriplus-10 or Centriprep-10; Millipore) or centrifuged at 100,000 × g (Beckman L-40, L-55) for 1 h. Concentrated ACM were electrophoresed on a nondenaturing 4–25% polyacrylamide gel. Proteins were transferred to nitrocellulose membrane, and probed with antibodies to human apoE (1:5000; Calbiochem, La Jolla, CA) or rat apoE (1:1000; Quidel). Immunoreactivity was visualized with enhanced chemiluminescence.

ApoE ELISA—Sandwich ELISA for human apoE was performed as described (44), except that the coating antibody was rabbit anti-sera raised against recombinant human apoE (42), and the detection antibody was a mouse monoclonal antibody (WU E-4) raised against recombinant human apoE (42). In the absence of human apoE, both antibody and substrate were nonimmune rabbit IgG (100 μg/ml) in the absence of SDS (46). Equal volumes (30 μl) of medium samples before immunoprecipitation (IP) and the supernatant after IP were subjected to SDS-PAGE (12.5% acrylamide), transferred to nitrocellulose membrane, and probed with antibodies to human apoE or rat apoJ, as described above.

RESULTS

Gel filtration chromatography was used to separate and isolate astrocyte lipoproteins. In contrast to density centrifugation, this technique has been shown to preserve the protein composition of lipoproteins during the process of fractionation (6, 47). ACM from mice expressing wild type (WT) mouse apoE, no apoE (−/−), null, human apoE3 (E3), or human apoE4 (E4) was fractionated, and selected fractions were analyzed by nonreducing SDS-PAGE and Western blotting for apoE and apoJ (Fig. 1). We were unable to detect apoAI or AII in these samples (data not shown). In all four conditions, apoE is detected as a ~80-kDa species, a covalently linked dimer of two ~40-kDa subunits. The elution pattern of apoJ, as well as the magnitude of apoJ expression, did not vary across the four conditions, suggesting that the presence of apoE does not affect apoJ secretion by mouse astrocytes. As expected, no apoE is detected in the ACM from apoE (−/−) animals. In WT- and E4-ACM, apoE is primarily detected as a ~34–36-kDa monomer, as both mouse and human E4 have no caveolae residues (Arg-112 and Arg-158). In contrast, apoE is present as both a ~35-kDa monomer and an ~80-kDa dimer in the E3-ACM, as human apoE3 has a caveeine at residue 112. In all the samples, apoE and apoJ elute in fractions consistent with lipoproteins comparable in size to plasma HDLs (−fractions 30–48, Fig. 1). The elution profiles for E3 and E4 are similar to wild type mouse apoE (peak, −fractions 37–41). In contrast to apoE, apoJ elution profiles are shifted slightly to the right (peak, −fractions 41–45), suggesting that on average, apoJ is associated with smaller particles.

Consistent with the SDS-PAGE results, profiles of the distribution of lipid (PL and TC) and apoproteins (E and J) across the size gradient demonstrate that astrocyte lipoproteins from mice expressing apoE are generally the size of plasma HDLs (Fig. 2). The broad distribution of lipid and apoproteins suggests a heterogenous population of particles ranging in size from small low density lipoprotein (−fractions 22–29) to small HDLs (−fractions 46–50). Lipid peaks in the void volume (−fractions 8–12) do not contain apoproteins, suggesting the presence of large cell membrane fragments in some samples, not the presence of large lipoproteins. In general, the PL and TC distribution encompassed −fractions 35–48, peaking at fraction 41 (Fig. 2, A and B), and is consistent with the apoprotein distribution. The apoE elution profile from the WT, null, E3, and E4 (Fig. 2C) is comparable and consistent with apoE being associated with a particle (peak, −fractions 39–49, Fig. 2C), slightly smaller than apoE, which peaks −fractions 35–45 (Fig. 2D). Again, the distribution pattern of apoJ and the amount of apoJ secreted (~1 μg/ml unconcentrated ACM) do not chromatography recognize lipoproteins (apoE3 and apoE4).

Nondenaturing Gradient Gel Electrophoresis—Samples of unconcentrated ACM were electrophoresed on a nondenaturing 4–25% polyacrylamide gel as described (37), transferred to nitrocellulose membrane, and probed with antibodies to human apoE (1:5000; Calbiochem, La Jolla, CA) or rat apoJ (1:1000; Quidel). Immunoreactivity was visualized with enhanced chemiluminescence.

Nondenaturing Immunoprecipitation—One-milliliter samples of unconcentrated ACM from apoE3 and apoE4 transgenic mice were precipitated with rabbit anti-apoE IgG (100 μg/ml) or nonimmune rabbit IgG (100 μg/ml) in the absence of SDS (46). Equal volumes (30 μl) of medium samples before immunoprecipitation (IP) and the supernatant after IP were subjected to SDS-PAGE (12.5% acrylamide), transferred to nitrocellulose membrane, and probed with antibodies to human apoE or rat apoJ, as described above.

Conclusions—Nontransfecting astrocytes do not produce apoE that is similar to recombinant human apoE. In the absence of apoE, both mouse and human apoE3 and apoE4 are expressed under the control of the astrocyte-specific glial fibrillary acidic protein promoter on a mouse apoE (−/−) background. Our data show that expression of apoE by astrocytes is required for normal lipoprotein secretion by these cells and that apoE species appears to influence lipoprotein composition.
lipoproteins. To confirm that apoE is both necessary and sufficient for the secretion of lipoproteins by cultured mouse astrocytes, we analyzed the particles secreted by apoJ/−/− mice (generously provided by M. Kindy, University of Kentucky, and B. Aronow and J. Harmony, University of Cincinnati). The lipid distribution of particles secreted by apoJ/−/− mice are virtu-
**TABLE I**
Characterization of ApoE-containing Astrocyte Lipoproteins

Fifty ml of astrocyte conditioned media was concentrated to 1 ml and fractionated using tandem Superose 6 columns. TG, TC, CE, and PL were determined (as described under “Materials and Methods”) for fraction 43 from four sample sets. Data are presented as mean µg/ml ± SE. Numbers in parentheses correspond to the percentage of each lipid component of lipoproteins in the assayed fraction.

|        | TC (µg/ml) | CE (µg/ml) | PL (µg/ml) | TG (µg/ml) |
|--------|------------|------------|------------|------------|
| WT     | 6.4 ± 4.0* | 0          | 9.3 ± 1.0* | 0          |
|        | (41%)      | (0%)       | (59%)      | (0%)       |
| ApoE (−/−) | 1.1 ± 0.06 | 0          | 1.5 ± 0.4  | 0          |
|        | (42%)      | (0%)       | (58%)      | (0%)       |
| ApoE3  | 2.6 ± 0.4  | 0          | 4.1 ± 0.4  | 0          |
|        | (39%)      | (0%)       | (61%)      | (0%)       |
| ApoE4  | 2.4 ± 0.9  | 0          | 3.9 ± 1.0  | 0          |
|        | (38%)      | (0%)       | (62%)      | (0%)       |

* Statistically different from all genotype groups; p < 0.05.

Consistent with this hypothesis, astrocytes derived from apoE knock-out (−/−) mice produce little or no detectable lipoproteins, as assessed by phospholipid and cholesterol analysis of size exclusion chromatography (Fig. 2, A and B). However, particles containing apoE and apoJ reside predominantly on different astrocyte lipoprotein fractions (Fig. 2, A and B, Table I). This difference is not due to less apoE secreted by transgenic versus WT astrocytes as analysis of apoE levels demonstrates that E3 and E4 samples actually contained ~2-fold more apoE than WT samples (Fig. 2D). Thus, the endogenous mouse apoE appears to support the production of a particle that has a greater lipid:apoE ratio than human apoE.

To directly investigate the relationship between the amount of lipid in astrocyte lipoproteins and the amount of apoE (by ELISA) within a given species, we compared ACM from mice homozygous (+/+); hemizygous (+/−) for human E4 (Fig. 3). Lipoproteins secreted from E4 hemizygotes contain more apoE (Fig. 3A) and more TC (Fig. 3B) than those secreted from E4 hemizygotes. These data suggest that apoE synthesis is one predictor of the amount of lipid secreted by cultured astrocytes. Consistent with this hypothesis, astrogocytes derived from apoE (−/−) mice produce little or no detectable lipoproteins, as assessed by phospholipid and cholesterol analysis of size exclusion chromatography (Fig. 2, A and B). In addition, compositional analysis of the peak fractions indicate that WT, E3, and E4 ACM contains greater amounts of HDL-associated lipid (PL and TC) than apoE (−/−) ACM (Table I). This analysis further suggests that, at the very least, a subset of astrocyte particles are discoidal in shape, as lipid analysis revealed no detectable TG or CE, the neutral lipids that make-up the core of a spherical particles (Table I). We have previously observed that the particles secreted by primary rat astrocytes in culture are discoidal, in contrast to the spherical particles found in human CSF and plasma (6). Thus, we used EM to visualize the particles secreted by primary mouse astrocyte cultures.

Electron micrographs suggest that astrocyte cultures expressing WT, E3, or E4 contain various types of lipoprotein particles, including those appearing as single discs, stacked discs, small spheres, and large spheres (Fig. 4). It is likely, however, that these nascent particles are actually predominantly discoidal in shape and appear as spheres because they adhered to the EM grid on their sides. This assumption is based on the observation that we could not detect any of the neutral lipids (TG or CE) that compose the core of traditional lipoproteins in any of the mouse astrocyte samples (Table I). However, synthesis of spherical particles in the absence of core lipids has been described in vitro (48). The size of mouse astrocyte particles (~11–15 nm, Table II) is consistent with the size range of particles from human CSF (~7–15 nm) and rat astrocyte cultures (~9–17 nm), as well as plasma HDLs (~5–12 nm) (6). Particles from E4 astrocytes always appear to be spherical (never as discs), and their diameters are often larger than those from WT or E3 cultures. Quantitation confirms that E4-ACM contains larger diameter particles on average than WT- or E3-ACM (Table II). Consistent with analysis of mean particle diameter, frequency distributions of individual particle diameters between samples of different apoE genotype reveal a distribution in E4 samples that is shifted slightly to the right of that for WT and E3 samples (data not shown). The astrocytes from apoE (−/−) animals do not appear to produce particles that are detectable by negative-staining EM (Fig. 4).

To further characterize the size and apoprotein composition of astrocyte lipoproteins, samples from WT, apoE (−/−), E3, and E4 were subjected to nondenaturing gradient-gel electrophoresis followed by Western blotting for apoE and apoJ (Fig. 5). Astrocyte lipoproteins containing human E3 appear similar in size to those containing E4 or mouse apoE (~10–17 nm) (Fig. 5A). Western blotting for apoJ also reveals no obvious difference between particles that contain human or mouse apoE (Fig. 5B). However, apoJ in all of these samples is found associated with a range of particles (~7.5–12 nm), smaller than those containing apoE (Fig. 5B). This pattern suggests that apoE and apoJ reside predominantly on different astrocyte lipoprotein particles, although it does not exclude the possibility that there may be a subpopulation of particles (~10–12 nm) that contains both apoproteins.

To investigate the hypothesis that apoE and apoJ reside on
distinct particles, ACM derived from E3 and E4 transgenic mice was immunoprecipitated with an antibody specific for apoE or nonimmune IgG as control. To preserve lipoprotein integrity, the IP was performed under nondenaturing conditions. ACM samples before IP and supernatant after IP were subjected to SDS-PAGE followed by Western blot analysis for apoE and apoJ. IP with nonimmune IgG depletes little apoE or apoJ from ACM samples. In contrast, IP with anti-apoE fully depletes samples of apoE, whereas the majority of apoJ remains in the supernatant (Fig. 6). Results were the same for samples containing E3 or E4 (data not shown), and IP with an antibody specific for apoJ yielded the same conclusion (data not shown).

DISCUSSION

Our results suggest that apoE expression by glial cells, specifically primary cultures of mouse astrocytes, is required for the normal secretion of HDL-like lipoprotein particles by these cells. Mouse astrocyte lipoproteins appear to be composed of two separate classes of particles that contain either apoE or apoJ, a conclusion based on the results of gel filtration chromatography, native gel analysis, and nondenaturing immunoprecipitation. The apoE-containing particles contain primarily PL and free cholesterol and are ~10–15 nm in diameter as determined by size chromatography, EM, and native gels. As analyzed here, the apoJ-containing particles are ~5–10 nm in diameter, are not associated with easily detectable lipid, and are not visible by EM, suggesting a protein-rich particle. Previous studies have identified apoE and apoJ as the primary apoproteins synthesized within the brain (49, 50). Although apoAI and AII are components of CSF lipoproteins (6, 51, 52), we were unable to detect either apoAI or AII in astrocyte-conditioned medium from either rat (6) or mouse primary cultures (data not shown). Thus, it appears that synthesis of apoE drives the production of astrocyte lipoproteins. This conclusion is further supported by the observation that lipoprotein lipid secretion is proportional to the amount of apoE protein secreted by astrocytes. The contribution of lipoprotein secretion by microglial cells, which expresses apoE (53, 54), remains to be investigated.

Our data also suggest a heterogeneity in the size and type of particle associated with a single apoE protein sequence, as well as a difference between particles that contain mouse apoE, human apoE3, or human apoE4. The ratio of lipoprotein lipid:apoE was higher in particles containing mouse apoE, human apoE3, or human apoE4. This ratio was distinctly higher in mouse apoE-containing particles than in human apoE3 or apoE4 particles. This result further supports the argument that mouse apoE is more efficient in driving lipoprotein secretion than human apoE isoforms. Our findings are consistent with previous studies that have shown differences in the structure and function of apoE isoforms (55, 56).
within particles or is somehow the result of the different promoters used to drive expression of apoE remains to be determined. At the protein level, the homology between mouse and human apoE is ~70%, including a critical substitution of threonine for arginine at residue 61 in the mouse (55). Work by Weisgraber and co-workers (56, 57) suggests that salt bridge formation involving this residue is critical for domain interactions in the human protein, an interaction that would be predicted not to occur in the mouse protein. Thus, it is reasonable to speculate that a difference in the structure of the mouse and human form of apoE could result in the secretion of nascent astrocyte lipoprotein particles with structural and composition differences. ApoE4-containing particles on average appear to be slightly larger in diameter than particles containing apoE3, and electron micrographs confirm the presence of what appear to be larger spherical particles unique to E4 samples. The preferential association of apoE4 with larger diameter particles than apoE3 may in some way be analogous to the observation that in plasma, greater quantities of 125I-apoE4 bind to very low density lipoproteins than to HDLs, whereas greater quantities of 125I-apoE2 and apoE3 bind to HDLs than to very low density lipoproteins (58). Interestingly, Guyton et al. (59) noted that CSF samples contain a novel subpopulation of large spherical particles that contain apoE, although classification by apoE isomor was not described. Although the precise significance of these observations remains unclear, it is possible that structural differences in the lipoproteins containing the three isoforms of human apoE may translate into functional differences.

ApoE-containing astrocyte particles, by virtue of interaction with the various apoE receptors expressed by both glia and neurons, may transport lipid (and other associated components) within brain parenchyma. The abundance of polar components (protein, PL, and free cholesterol) and the absence of core lipids (CE and TG) in these nascent particles makes them likely candidates for participating in the process of reverse cholesterol transport in much the same way as has been hypothesized for other interstitial fluid lipoproteins (60). In addition, the presence of apoE would allow these particles to deliver their constituents to cells through the apoE cell surface receptors known to be present on neural cells (7–10). Precise lipid trafficking is an important process in the brain as it is necessary to support the continual remodeling of the vast array of axonal and dendritic neuronal membranes. This process of membrane turnover occurs throughout the life span of the animal, accelerating under conditions of growth (during development) and following injury (traumatic or neurodegenerative disease). That apoE plays a key role in membrane maintenance is supported by the observations that apoE expression in the brain is elevated during these dynamic events (14, 61, 62), and apoE can influence neurite outgrowth in vitro (63, 64).

The proposed functions of apoJ, also known as clusterin or SP-40,40, include lipid transport, sperm maturation, regulation of ovarian follicle development, regulation of the complement cascade, apoptosis, and membrane recycling (3, 65). In terms of its role as an apoprotein, apoJ is a component of a specific class of plasma HDLs (66, 67), and a recent in vitro study demonstrated that apoJ facilitates lipid efflux from foam cells (68). However, the role of apoJ in peripheral lipid metabolism remains unclear. Cultured astrocytes from apoJ (−/−) mice offer a system to determine whether apoJ is either necessary or sufficient for the secretion of a lipoprotein particle. Astrocytes from these mice secrete apoJ-containing lipoprotein particles comparable to those secreted in the presence of both apoE and apoJ (i.e. WT), demonstrating that apoJ is not necessary for normal lipoprotein secretion by astrocytes. On the other hand, astrocytes expressing apoJ but not apoE (i.e. apoE (−/−)) do not secrete a particle containing sufficient lipid for detection by enzymatic analysis, nor do they secrete a particle visible by negative staining EM under the present culture conditions, suggesting that expression of apoJ is not sufficient for normal particle secretion by astrocytes. In preliminary work, however, we have detected putative small apoJ-containing particles utilizing in situ atomic force microscopy,2 and we have recently begun analyzing these particles. It appears clear, however, that apoJ does not play a major role in the synthesis of lipid- and apoE-containing astrocyte lipoproteins. Our data suggest that apoJ is secreted as a discrete, very lipid-poor particle. Interestingly, a subpopulation of plasma HDLs that contains apoJ has also been shown to be very lipid-poor (69). Although apoE-containing astrocyte particles may serve as ligands for neural apoE receptors, such a function for apoJ is less obvious as gp330 (megalin), the only receptor identified for mammalian apoJ (11), is not expressed by neurons or glia. Instead, cells of the choroid plexus and ependyma, as well as brain capillary endothelial cells at the blood-brain barrier (12, 13), express megalin. This receptor distribution suggests that apoJ-containing particles may be involved in the transport of lipids and associated components between the brain, blood and CSF (13, 31).

Of particular relevance to understanding the etiology of AD are the findings that apoE and apoJ co-localize to Aβ-containing senile plaques in the AD brain (17–19) and that soluble Aβ is found complexed to apoE- and apoJ-containing lipoproteins in plasma and CSF (20, 70, 71). To the extent that Aβ is complexed to either the lipid or protein components of astrocyte lipoproteins within brain parenchyma, lipoprotein trafficking would affect the metabolism (deposition and/or clearance) of this pathologic peptide, thus perhaps directly influencing AD pathogenesis itself. Recent studies suggest apoE may specifically influence the deposition of Aβ in vivo. Transgenic mice overexpressing a mutant form of the human amyloid precursor protein (APPV717F), when crossed with apoE (−/−) mice, had less Aβ deposition and no fibrillar Aβ that is normally seen in the brain in the presence of mouse apoE (72). However, recent work by Holtzman et al. (37) demonstrated that when APPV717F/apoE (−/−) mice were crossed with transgenic mice expressing human apoE by astrocytes within the brain, both apoE3 and E4 suppressed early Aβ deposition (37). Thus, compositional and/or structural differences in astrocyte lipoproteins containing the different forms of apoE may differentially affect Aβ deposition. Our data suggesting differences in the composition and size of isolated astrocyte particles containing mouse apoE, human apoE3, or human apoE4 is consistent with such a possibility. In addition, our observation that apoE and apoJ reside on predominantly distinct astrocyte particles suggests that these two populations may also subserve different functions. Further investigation will be necessary to understand the mechanism by which the various types of CNS lipoproteins influence both Aβ deposition and clearance.

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