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Lipids and Lipoproteins

Characterization of Apolipoprotein E-containing Lipoproteins in Cerebrospinal Fluid: Effect of Phenotype on the Distribution of Apolipoprotein E

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Background: Apolipoprotein (apo) E, one of the main apolipoproteins in the central nervous system, may play an important role in lipid metabolism; however, the details of its function are poorly understood. In this study, we characterized apoE-containing lipoproteins in cerebrospinal fluid (CSF) and examined the effect of apoE phenotype on the distribution of apoE among the lipoprotein fractions.
Methods: CSF lipoproteins were fractionated by gel filtration and ultracentrifugation, and then characterized by electrophoresis, immunoblot, electron microscopy, and analysis of apoE, total cholesterol, and phospholipid concentrations.
Results: The ratio of sialylated to nonsialylated apoE was higher in CSF than in serum. However, the fundamental forms containing apoE homodimers or heterodimers [such as apo(E-AII) and apo(AII-E2-AII) complexes] were similar in CSF and serum. apoE-containing lipoproteins were fractionated at densities of <1.006, 1.063–1.125, and 1.125–1.21 kg/L. Neither apoE nor apoAI was detected in the fraction with a density range of 1.006–1.063 kg/L. The diameters of the lipoprotein particles with densities of <1.006, 1.063–1.125, and 1.125–1.21 kg/L were 16.7 ± 3.1, 14.0 ± 3.2, and 11.6 ± 2.8 nm (mean ± SD, n = 200), respectively. All of these lipoproteins exhibited a spherical structure. The distribution profile of apoE-containing lipoproteins was affected by the apoE phenotype. A relatively large amount of apoE-containing lipoproteins was isolated from the fraction with a density >1.125 kg/L obtained from CSF associated with apoE2 or apoE3. This tendency was more obvious in CSF associated with apoE2 than in CSF without apoE2. apoE-containing lipoproteins were predominantly observed in the fraction with a density of <1.006 kg/L obtained from CSF associated with apoE4.
Conclusions: The lipoproteins in CSF have a unique composition that is different from that of the lipoproteins in plasma. However, the differences in diameter between the CSF fractions were not as large as for the serum fractions. Our data suggest that the apoE phenotype may affect the distribution profile of apoE-containing lipoproteins in the CSF. This would mean that the metabolism of apoE-containing lipoproteins depends on the apoE isoform present.
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Apolipoprotein (apo)[4] E, a protein with a molecular mass of 35 kDa, is present in a number of tissues. It is synthesized primarily in the liver and is involved in cholesterol transport and metabolism *(1, 2)*. In the central nervous system (CNS), the second most active tissue in terms of apoE production *(3, 4)*, apoE is produced predominantly by astrocytes and microglia *(5, 6)*. It occurs in the cerebrospinal fluid (CSF) as one of the main components of HDL subfraction 1 *(5–7)*. In addition to apoE, the CSF also contains apoAI, apoAII, apoAVI, apoD, and apoJ *(8–10)*

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[4] Nonstandard abbreviations: apo, apolipoprotein; CNS, central nervous system; CSF, cerebrospinal fluid; AD, Alzheimer disease; TC, total cholesterol; PL, phospholipid; SDS, sodium dodecyl sulfate; and PAGE, polyacrylamide gel electrophoresis.

but not apoB (the only apolipoprotein included in the LDL fraction, and the major lipoprotein in plasma involved in cholesterol transport). This may mean that in the CSF, apoE acts as the ligand for the LDL receptor, which is expressed on glia and neurons, and thus is involved in the transportation of cholesterol and phospholipids in the CNS *(5, 7, 11)*.

Multiple lines of evidence suggest that CSF apoE is implicated in the pathogenesis of Alzheimer disease (AD) *(12–17)*; however, the precise role of apoE in the development of AD is still obscure. Characterization of apoE and apoE-containing lipoproteins in the CNS is essential to clarify its involvement in AD. Recently, CSF lipoproteins have been investigated by several groups, and their characterization has been progressing gradually *(9, 10, 18)*. However, the nature of CSF lipoproteins and their metabolic pathways are still not as clear as those of the plasma lipoproteins. One of the interesting characteristics of the plasma apoE isoforms is the preferential association of apoE3 and apoE4 with particular classes of plasma lipoproteins (apoE3 with HDL and apoE4 with triglyceride-rich lipoproteins such as VLDL) *(19–21)*.

In this study, we characterized the apoE-containing lipoproteins in the CSF and examined the effect of the apoE phenotype on the distribution of apoE among the CSF lipoprotein fractions.

Materials and Methods

SAMPLING

CSF (with no contamination by red blood cells) and sera were obtained from hospitalized patients with the apoE phenotypes apoE3/E2, apoE3/E3, and apoE4/E3, and CSF was pooled by apoE phenotype. No apoB was detected in any CSF sample by immunoblotting.

IMMUNOBLOT ANALYSIS

Samples were treated with Laemmli buffer with or without 2-mercaptoethanol *(22)* and were loaded on 8–16% polyacrylamide gradient gels. The separated proteins were electrophoretically transferred onto nitrocellulose membranes (pore size, 0.45 μm; Advantek Toyo), which were then incubated with a blocking buffer (50 mmol/L Tris-HCl, pH 8.0, containing 20 g/L skim milk) for 30 min at room temperature. After being washed three times, the membranes were incubated with the primary antibodies [rabbit anti-apoE (Dako) and mouse anti-apoAII (ICN Pharmaceuticals)], and then washed three more times. The membranes were incubated with horseradish peroxidase-conjugated anti-rabbit or -mouse IgG (goat; MBL) for 1 h at room temperature. After washing, the bands were visualized either using 3,3′-diaminobenzidine tetrahydrochloride (Dojin Chemical) and hydrogen peroxide (Wako Pure Chemicals) or by means of an enhanced chemiluminescence detection kit (Amersham Life Sciences).

ISOELECTRIC FOCUSING

Sample preparation. Serum (2 μL) or CSF (15 μL) was incubated with 5 μL of neuraminidase (20 kU/L) in 0.1 mol/L citric acid buffer containing 10 g/L Tween 20 for 12 h at 37 °C. This was followed by treatment with 5 μL of 75 mmol/L dithiothreitol (Wako Pure Chemicals) in 10 g/L Tween 20 for 1 h at room temperature.

Electrophoresis. Isoelectric focusing was carried out as described previously *(23)*. Briefly, 20 μL of the prepared sample was electrophoresed on a 4.8% polyacrylamide gel containing 8 mol/L urea and 20 g/L Ampholine® (pH 4–6; Pharmacia Biotech) using 3.3 mmol/L phosphoric acid as the anode solution and 20 mmol/L NaOH as the cathode solution. Electrophoresis was carried out overnight at 4 °C under constant voltage (200 V). After electrophoresis, immunoblot analysis was performed as described above.

LIPOPROTEIN ELECTROPHORESIS

CSF (20 μL) was applied to agarose gel (Titan Gel Lipo Kit; Helena Laboratories) and electrophoresed for 20 min under constant voltage (90 V). The separated lipoproteins were visualized by the following two method: (*a*) lipids were stained directly by Fat Red 7B (Helena Laboratories) in methanol, and (*b*) separated lipoproteins were transferred onto a nitrocellulose membrane by capillary blotting, and apoE and apoAI were developed by the immunoblot method described above.

FAST PROTEIN LIQUID CHROMATOGRAPHY

CSF (500 μL) was applied to a 130-cm Superose® 6 column (Pharmacia Biotech) equilibrated with phosphate-buffered saline, pH 7.4. Lipoprotein fractions were eluted at a flow rate of 0.5 mL/min.

ULTRACENTRIFUGATION

CSF lipoproteins were isolated by the ultracentrifugation method described by David et al. *(24)* with a small modification. CSF was centrifuged at 541 000*g* rpm for 1 h using an OptimaTM TLX Ultracentrifuge (Beckman) to isolate the fraction with a density of $<$1.006 kg/L. Solid KBr (Wako) was sequentially added to CSF after each centrifugation to adjust the density to 1.006, 1.063, 1.125, and 1.21 kg/L in sequence. The isolated fractions were dialyzed against phosphate-buffered saline.

DETERMINATION OF apoE, TOTAL CHOLESTEROL, AND PHOSPHOLIPIDS IN CSF

CSF apoE was measured using an ELISA system as described previously *(25)*. Total cholesterol (TC) and phospholipid (PL) in CSF were measured, respectively, by the cholesterol oxidase method (Kyowa Medex) and the choline oxidase method (Wako), using a Hitachi 7170 automated analyzer with a higher ratio of sample volume to reagent volume than that is routinely used for serum samples.

ELECTRON MICROSCOPY

The lipoprotein particles in each fraction isolated by ultracentrifugation were examined under a JEOL JEM1010 electron microscope. Samples were negatively stained with 20 g/L aqueous uranyl acetate. Particle diameters, measured by means of a micrometer in each photograph, were expressed as the mean ± SD nm for a random sample of 200 particles.

Results

IMMUNOBLOT ANALYSIS OF CSF apoE

The forms of apoE present in CSF, examined by nonreducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting using anti-apoE antibody, were fundamentally the same as those in serum (Fig. 1). The apoE monomer, apoE homodimer, and the apo(E-AII) complex were identified in the CSF obtained from the subject with the apoE phenotype E3/E3. In addition, the apo(AII-E2-AII) complex was also recognized in the CSF from the subject with the apoE phenotype E3/E2. These complexes were identified by reducing and nonreducing SDS-PAGE followed by immunoblotting using anti-apoAII and anti-apoE antibodies (data not shown).

Bands of slightly higher molecular size than that of the apoE monomer, which disappeared after treatment with neuraminidase, were more intense in CSF than in serum, which indicates that the apoE monomer is more highly sialylated in the CSF than in serum. As shown in Fig. 2, isoelectric focusing confirmed that CSF apoE monomers existed not only in the monosialylated form but also in the di-, tri-, or higher sialylated forms.

CHARACTERIZATION OF apoE-CONTAINING LIPOPROTEINS

Lipoprotein electrophoresis. CSF lipoproteins migrated to the α_1 and pre-α_1 position in agarose gel electrophoresis (Fig. 3A). After treatment with neuraminidase, the faint pre-α_1 band was shifted to the α_1 position (data not shown). When the gel was immunoblotted with anti-apoE antibody, a major band in α_1 region was observed with weaker reactivity extending into the pre-α_1 region (Fig. 3B). A band that reacted with anti-apoAI antibody was observed almost exclusively in the pre-α_1 region (Fig. 3C).

Ultracentrifugal analysis. The fractions isolated by ultracentrifugation were applied to SDS-PAGE using an 8–16% gradient gel followed by immunoblotting for apoE and apoAI (Fig. 4). Both apolipoproteins were contained in each of the fractions with densities of <1.006, 1.063–1.125, and 1.125–1.21 kg/L but not in the fraction with a density of 1.006–1.063 kg/L. All of the former three fractions isolated from CSF associated with apoE2 or apoE3 contained not only the apoE monomer but also apoE homo- and heterodimers. PAGE analysis for lipoproteins showed that the molecular size extended from 150 kDa to 600 kDa and also suggested that three kinds of lipoprotein particles (containing predominantly apoE, apoAI, or both apoE and apoAI, respectively) were present in each

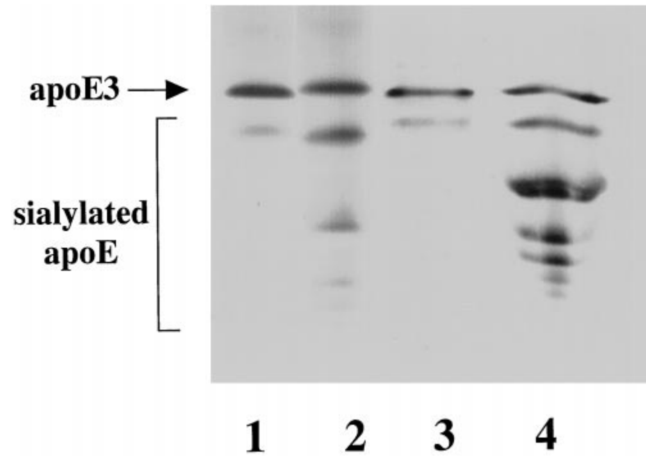


Fig. 2. Isoelectric focusing.

Two microliters of serum (*lanes 1* and *2*) and 15 μL of CSF (*lanes 3* and *4*) were treated with (*lanes 1* and *3*) or without (*lanes 2* and *4*) neuraminidase, and 20 μL of prepared sample was loaded onto a 4.8% polyacrylamide gel containing 8 mol/L urea and 20 mL/L ampholine (pH 4~6). After isoelectric focusing, immunoblotting for apoE was performed as described in *Materials and Methods*.

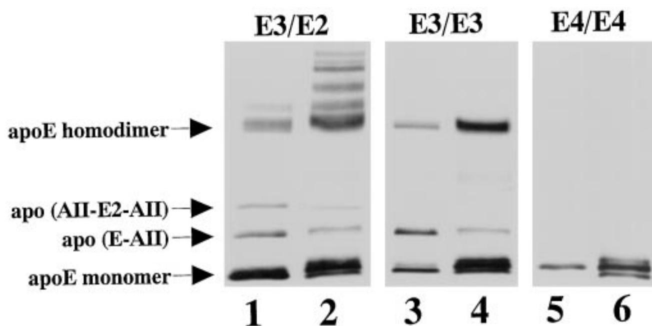


Fig. 1. Immunoblot analysis for CSF apoE.

Two microliters of serum (*lanes 1*, *3*, and *5*) and 30 μL of CSF (*lanes 2*, *4*, and *6*), both treated with Laemmli buffer (without 2-mercaptoethanol), were loaded onto an 8–16% polyacrylamide gradient gel containing 1.0 g/L SDS. After electrophoresis, separated proteins were transferred electrophoretically onto a nitrocellulose membrane. The bands containing apoE were visualized by immunoblot analysis using anti-apoE antibody as described in *Materials and Methods*.

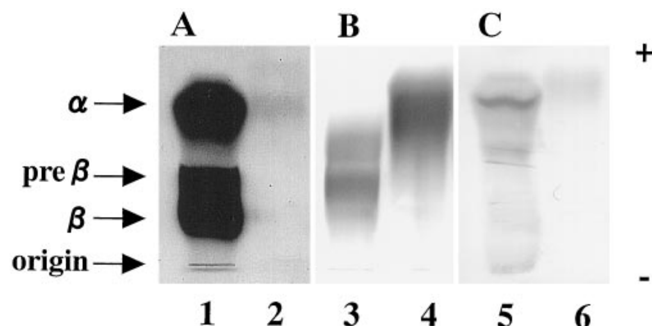


Fig. 3. Agarose gel electrophoresis.

Two microliters of serum (*lanes 1*, *3*, and *5*) and 20 μL of CSF (*lanes 2*, *4*, and *6*) with apoE phenotype E3/E3 were applied to agarose gel film electrophoresis. After electrophoresis, fractionated lipoproteins were stained with Fat Red 7B (*A*) or visualized by immunoblotting using anti-apoE (*B*) or anti-apoAI (*C*) antibodies.

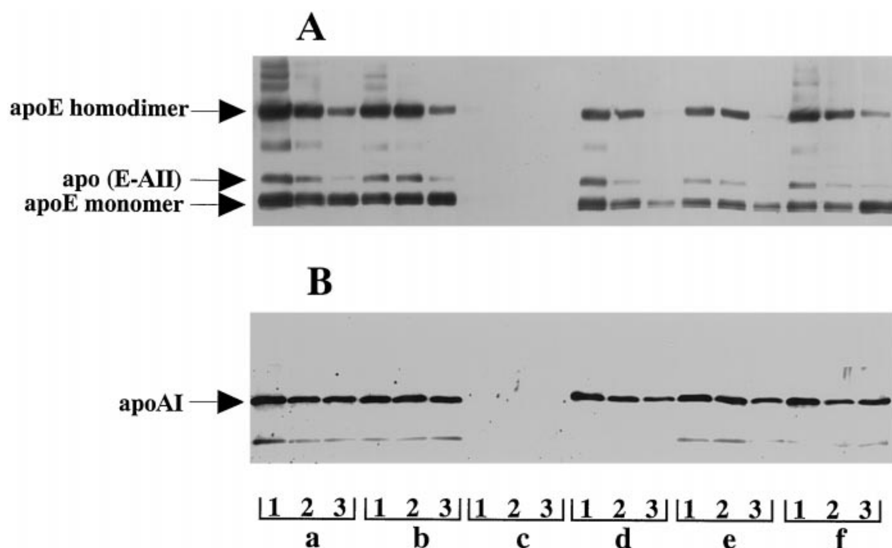


Fig. 4. Immunoblot analysis of subfractions obtained by ultracentrifugation.

Fifty microliters of CSF (*a*) and 30 μL of the ultracentrifugation subfractions with densities of <1.006 (*b*), 1.006–1.063 (*c*), 1.063–1.125 (*d*), 1.125–1.21 (*e*), or >1.21 (*f*) kg/L were analyzed. Samples were from subjects with the apoE phenotypes E3/E2 (*lane 1* for each fraction), E3/E3 (*lane 2* for each fraction), or E4/E3 (*lane 3* for each fraction). All samples were treated with nonreducing Laemmli buffer and loaded onto an 8–16% polyacrylamide gradient gel. After electrophoresis, separated proteins were transferred electrophoretically onto a nitrocellulose membrane. The bands were visualized by immunoblotting using anti-apoE (*A*) or anti-apoAI (*B*) antibodies.

fraction (Fig. 5). In the fraction with a density of <1.006 kg/L, the majority of apoAI appeared as a monomer at molecular size of 28 kDa. No apoB was detected in any fractions (data not shown).

Gel filtration analysis. As shown in Fig. 6A, the main lipoproteins in the CSF samples separated by fast protein liquid chromatography were larger than serum HDL and were eluted at a mid-position between serum HDL and LDL (fractions 26–36). The CSF lipoproteins contained much more PL than TC; therefore, the lipid composition was similar to that of the serum HDL fraction. These

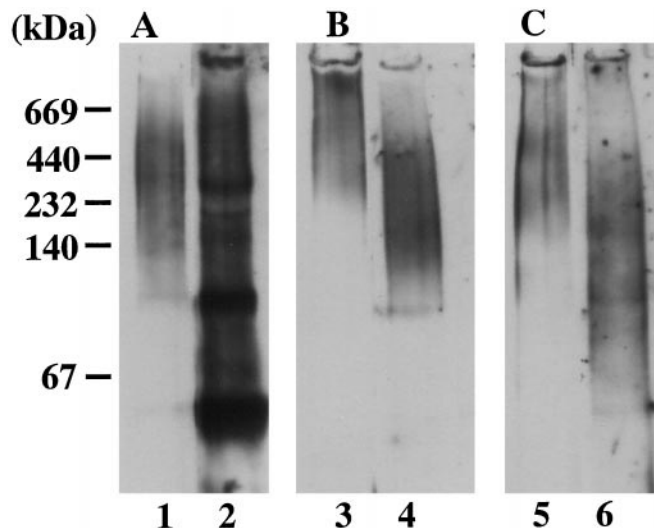


Fig. 5. Non-SDS-PAGE.

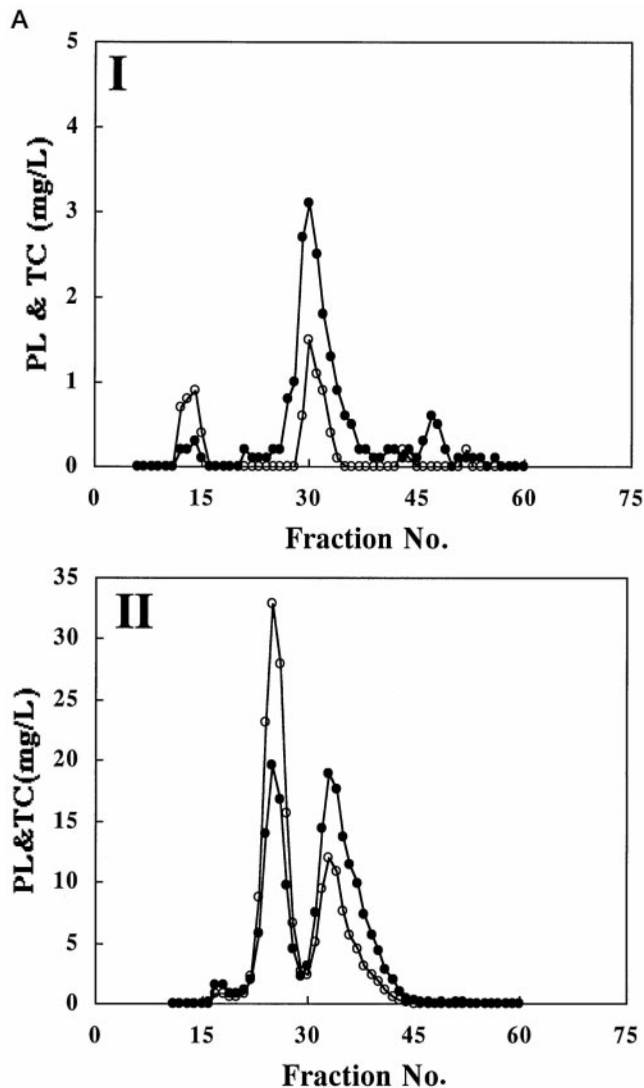
Thirty microliters of the subfractions obtained by ultracentrifugation (*A*, <1.006 kg/L; *B*, 1.063–1.125 kg/L; *C*, 1.125–1.21 kg/L) from the subject with apoE phenotype E3/E3 were loaded onto a 4∼12% gradient polyacrylamide gel. After electrophoresis, separated lipoproteins were transferred electrophoretically onto a nitrocellulose membrane. The bands containing apoE or apoAI were visualized by immunoblotting with anti-apoE (*lanes 1*, *3*, and *5*) or anti-apoAI (*lanes 2*, *4*, and *6*) antibodies. The positions of molecular mass standards are indicated on the *left*.

fractions were applied to PAGE using 8–16% gradient gel followed by immunoblotting for apoE and apoAI (Fig. 6B). The strongest immunoreactivity for apoE and apoAI was observed in fractions 28–30 and 30–32, respectively. In addition to the apoE monomer, the apoE homodimer and a small amount of the apo(E-AII) complex were detected in fractions 28–30. The lipoproteins with densities of <1.006, 1.063–1.125, and 1.125–1.21 kg/L isolated by ultracentrifugation were eluted in almost the same fractions by fast protein liquid chromatography (data not shown).

Electron microscopy. As shown by negative-staining electron microscopy, the diameters of those lipoprotein particles with densities of <1.006, 1.063–1.125, and 1.125–1.21 kg/L that were isolated from CSF by ultracentrifugation were 16.7 ± 3.1, 14.0 ± 3.2, and 11.6 ± 2.8 nm (mean ± SD, n = 200), respectively (Fig. 7). The differences in particle size among these fractions were significant; however, the differences were smaller than in the case of serum. In addition, two or more peaks were observed in the distribution of particle sizes for each of the three fractions.

EFFECT OF apoE PHENOTYPE ON THE DISTRIBUTION OF apoE-CONTAINING LIPOPROTEINS

The apoE, TC, and PL concentrations for three different pools of CSF for each of the three apoE phenotypes apoE3/E2, apoE3/E3, and apoE4/E3 are shown in Table 1. These CSFs were fractionated by ultracentrifugation, and the distribution ratios for apoE, TC, and PL were determined for the five fractions with different densities (Table 1). The total recoveries for apoE were 75.6–91.0%. In CSF associated with apoE2 or apoE3, the amount of apoE recovered in the fractions with densities of 1.063–1.125 and 1.125–1.21 kg/L was much greater than in the fraction with a density of <1.006 kg/L. In contrast, a higher ratio of apoE-containing lipoproteins was recov-



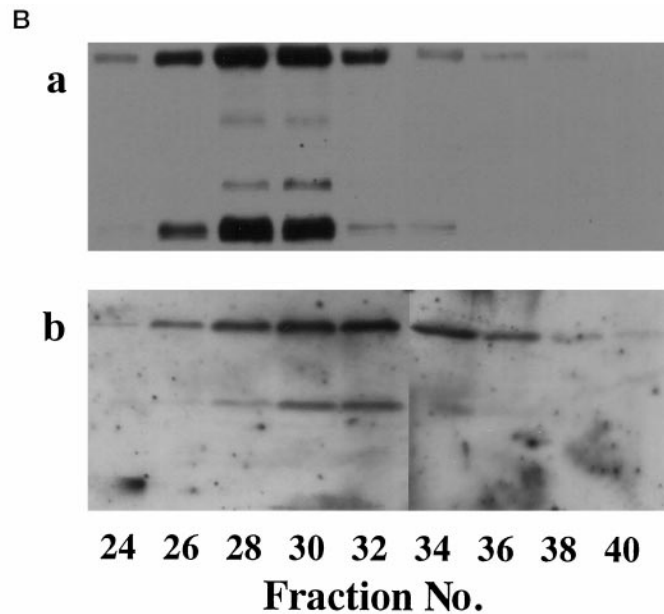


Fig. 6. Gel-filtration chromatography profile (*A*) and immunoblot analysis (*B*) of the subfractions.

(*A*), 500 μL of human CSF (*I*) and serum (*II*) from the subject with phenotype apoE3/E3 were fractionated by Superose 6 fast protein liquid chromatography, as described in *Materials and Methods*. The void volume determined by blue dextran was 6.5 mL (fraction 13). The eluted fractions were analyzed for TC (○) and PL (●), each of which is expressed in mg/L. The fraction numbers are shown on the *x-axis*. A 30-μL aliquot of each of the gel-filtration subfractions was treated with nonreducing Laemmli buffer and loaded onto an 8–16% polyacrylamide gradient gel. After electrophoresis, separated proteins were transferred electrophoretically onto nitrocellulose membranes, followed by immunoblotting using anti-apoE (*a*) or anti-apoAI (*b*) antibodies. The bands were visualized by means of an enhanced chemiluminescence kit, as described in *Materials and Methods*.

ered in the fraction with a density of <1.006 kg/L than in the other two fractions for the CSF associated with apoE4. In addition, in the subject with an E4/E3 phenotype, apoE also had a tendency to be recovered in the bottom fraction (d >1.21 kg/L), with a higher ratio than that observed for the other two phenotypes. The lipid compositions, expressed as the ratio of PL/TC, were ~2.3 for the fraction with a density of <1.006 kg/L and ~1.0 for the fractions with densities of 1.063–1.125 and 1.125–1.21 kg/L.

Discussion

It is known that apoE mRNA is abundant in the brain and adrenals as well as in the liver *(3)*. CSF apoE is synthesized and secreted mainly by astrocytes, as demonstrated by Pitas et al. *(5)*. The CSF apoE is independent of the plasma apoE; indeed, after liver transplantation, the apoE phenotype in the recipient's CSF did not change to the donor's phenotype, whereas the plasma apoE did *(26)*. This implies that plasma apoE is unable to pass through the blood-brain barrier *(25, 27)*.

As reported previously *(5)*, the CSF apoE monomer was more sialylated than the serum apoE monomer. In agarose gel electrophoresis, CSF lipoproteins showed a faint band that migrated faster than that of HDL in serum. After treatment with neuraminidase, the faint band moved to the same position as the serum HDL (data not shown). This implies that apoE affected the electrophoretic migration of the main lipoprotein in CSF. In other words, apoE is one of the main apolipoprotein components of the lipoproteins in CSF. The lipid composition of the CSF lipoproteins, in which phospholipid is more abundant than cholesterol *(25)*, might also influence the electrical charge. It is known that recently secreted apoE is more sialylated than mature apoE *(5)*. This, together with our results, suggests that almost all of the apoE in the CNS is in a more immature form than plasma apoE and that the sialylated form of apoE may be physiologically important in the CNS. However, the fundamental forms of apoE in CSF seemed to be almost the same as in plasma. Not only the apoE monomer but also the apoE

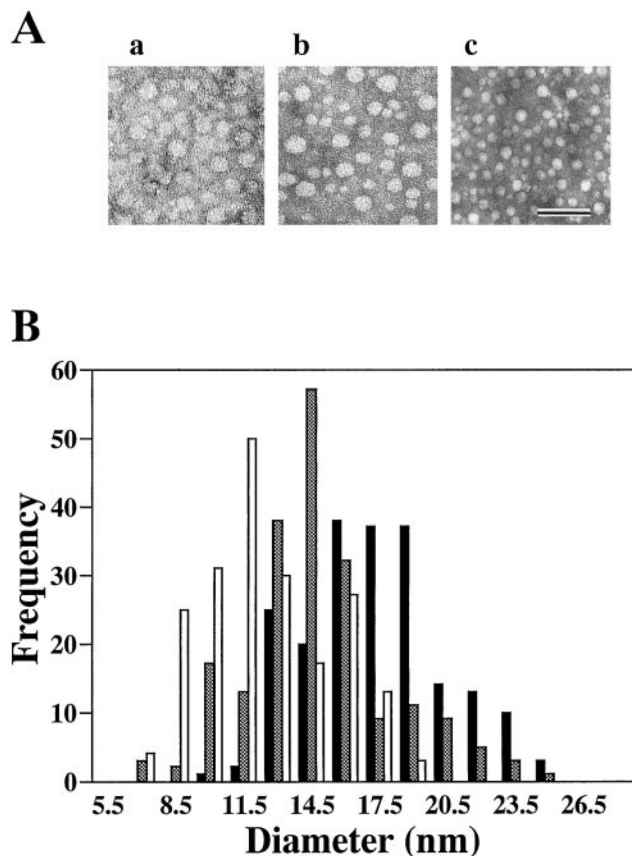


Fig. 7. Electron microscopy for lipoprotein particles from the subfractions obtained by ultracentrifugation of pooled human CSF.

(*A*), negative-staining electron microscopy. The ultracentrifugation subfractions with densities of <1.006 (*a*), 1.063–1.125 (*b*), or 1.125–1.21 (*c*) kg/L were dialyzed against phosphate-buffered saline, pH 7.4. A 20-μL sample of each was placed on a copper grid (300 mesh) and negatively stained with 40 g/L uranyl acetate. *Bar*, 50 nm. (*B*), diameter of lipoprotein particles. The lipoprotein particles were measured in enlarged photomicrographic prints using a micrometer. A histogram was constructed from the results obtained for 200 particles in each subfraction. The diameters of the particles (mean ± SD) in the three fractions were as follows: <1.006 kg/L, 16.7 ± 3.1 nm (■); 1.063–1.125 kg/L ,14.0 ± 3.2 nm (▤); and 1.125–1.21 kg/L, 11.6 ± 2.8 nm (□).

homodimer *(28)* and heterodimers such as apo(E-AII) *(29)* and apo(AII-E2-AII) complexes *(23)*, were observed in the CSF from subjects with the apoE phenotypes E3/E3 and E3/E2. Unlike apoE, the apoAII in the CSF appears to be derived from the plasma *(4)*. Therefore, the quantity of apo(E-AII) and apo(AII-E2-AII) complex in the CSF would be regulated by the quantity of apoAII passing through the blood-brain barrier. Actually, variations between individual cases were observed in the immunoreactive intensity of the apo(E-AII) complex visualized by immunoblot analysis of CSF (data not shown). This implies that the apoAII concentration in the CSF may vary among individuals.

apoE-containing lipoproteins in the CSF may be microheterogeneous in size, as suggested by LaDu et al. *(9)* and Guyton et al. *(18)*. However, our result differed from those of the above groups in two ways. First, the novel large apoE-containing lipoprotein with a density of 1.006–1.060 kg/L, which was isolated by Guyton et al. using density gradient ultracentrifugation, *(18)* seemed to us to actually be a lower density lipoprotein with a density of <1.006 kg/L or close to 1.006 kg/L. The fraction isolated under the natural density of CSF (<1.006 kg/L) contained both apoE and lipid, but we could not identify these components in the fraction with a density of 1.006–1.063 kg/L. Second, previous data *(9, 18)* suggested that apoE-containing lipoproteins exist as large particles, whereas apoAI and apoAII exist as smaller lipoprotein particles. However, our data indicate that the large lipoproteins contained both apoE and apoAI. In addition, apo(E-AII) and apo(AII-E2-AII) complexes were detected in those lipoproteins. As demonstrated previously by Pitas et al. *(7)* and Borghini et al. *(10)*, three kinds of lipoproteins predominantly containing apoAI, apoE, or both apoAI and apoE, respectively, were observed in the present study in all subfractions obtained by ultracentrifugation except for that with a density of 1.006–1.063 kg/L. Immunoblotting using non-SDS-PAGE suggested that the apoAI-containing lipoproteins are smaller than the apoE-containing lipoproteins, and there was variation in mean diameters between the fractions obtained by ultracentrifugation. Our data are similar to those of Guyton et al. *(18)* except that ours revealed a greater heterogeneity. The histogram we plotted for the size distribution of CSF lipoproteins indicated that two or more kinds of particles might exist in each subfraction obtained by ultracentrifu-

Table 1. Effect of apoE phenotype on the distribution of CSF lipoproteins.[a]

	apoE			TC			PL		
Fraction	E3/E2	E3/E3	E4/E3	E3/E2	E3/E3	E4/E3	E3/E2	E3/E3	E4/E3
<1.006 kg/L	7.4 (3.01)	7.3 (6.04)	19.6 (3.51)[b]	5.9 (2.90)	6.3 (1.43)	22.1 (7.60)[b]	15.8 (6.70)	15.6 (4.03)	40.9 (4.79)[c]
1.006–1.063 kg/L	ND[d]	ND	ND	ND	ND	ND	ND	ND	ND
1.063–1.125 kg/L	30.4 (3.12)	33.7 (3.32)	8.4 (5.01)[c]	62.5 (1.56)[b]	54.2 (4.05)	44.4 (3.80)[b]	57.4 (3.56)	48.1 (6.64)	30.1 (3.07)[b]
1.125–1.210 kg/L	29.2 (2.06)[b]	21.9 (3.24)	8.4 (2.09)[c]	31.6 (2.44)	39.5 (4.97)	33.5 (5.34)	26.8 (4.75)	36.3 (8.88)	29.0 (4.18)
Bottom	33.0 (6.81)	37.1 (1.79)	63.6 (5.66)[b]	ND	ND	ND	ND	ND	ND

[a] The distribution ratios were determined by ultracentrifugation analysis for three kinds of pooled CSF (with apoE3/E2, apoE3/E3, and apoE4/E3) and were expressed as a percentage (mean ± SD) of the total amount in each fraction.

[b,c] Statistical difference from E3/E3 was assessed by the Student *t*-test, $P < 0.05$ being considered significant: [b] $P < 0.05$; [c] $P < 0.005$.

[d] ND, not detected.

gation. These particles may differ in terms of their apolipoprotein composition, as described above.

Weisgraber *(21)* has demonstrated that the preference of apoE3 for HDL and that of apoE4 for VLDL or intermediate-density lipoproteins results from a difference in the charge at residue 112 of the apoE molecule and whether they can form an apo(E-AII) complex. Although we could not investigate CSF with subjects homozygous for apoE2 or apoE4, it is quite possible that the distribution of apoE-containing lipoproteins in CSF is affected by the apoE phenotype. The apoE in CSF associated with apoE2 or E3 preferentially existed in the fractions with densities >1.063 kg/L. This tendency was more obvious in the subject with apoE2 than in the subject without apoE2, the difference being significant for the fraction with a density range of 1.125–1.21 kg/L. In contrast, in the CSF associated with apoE4, a large amount of apoE was recovered in the fraction with a density of <1.006 kg/L. This difference might be induced by cysteine-arginine interchanges at residues 112 and/or 158 of the apoE molecules and the formation of apo(E-AII) and apo(AII-E2-AII) complexes in CSF as well as in plasma. Although the presence of apoE in the bottom fraction could be an artifact of the ultracentrifugation method, as suggested previously *(16, 30)*, it is obvious that the amount of apoE in the bottom fraction was greater in CSF associated with apoE4 than in CSF associated with the other phenotypes. One possible reason is that lipoproteins constructed with the apoE monomer would be unstable and that the formation of apoE homo- or heterodimers would be needed to stabilize these lipoproteins.

It is known that lecithin cholesterol acyl transferase *(31)* and cholesteryl ester transfer protein *(32)* are synthesized in the CNS. LaDu et al. *(9)* suggested that the lower density lipoproteins in the CSF contain much more nonesterified cholesterol than the higher density lipoproteins. This may support the operation of lecithin cholesterol acyl transferase and/or cholesteryl ester transfer protein. Although additional studies of the lipid composition of each fraction are required, the differences in lipid composition might also affect the distribution of apoE.

It has been demonstrated that subjects with apoE4 tend to show higher plasma cholesterol concentrations than those with apoE3 *(33)*. The different effects of apoE3 and apoE4 on lipoprotein distribution is one of the reasons for this tendency. apoE4 displays a preference for triglyceride-rich lipoprotein particles in plasma; therefore, the remnants would be expected to be cleared more effectively in subjects with apoE4 than in those with apoE3. Indeed, apoE homo- and heterodimers, which were formed by apoE3 and apoE2, but not by apoE4, have been assumed to be inactive forms of apoE in terms of binding to the LDL receptor. Consequently, the down-regulation of hepatic LDL receptor would be more effectively induced by apoE4, and this would raise the cholesterol concentration. In contrast, we reported previously that CSF associated with apoE4 has a lower cholesterol concentration than CSF associated with apoE3 or apoE2 *(25)*. The TC/apoE ratio in CSF is ≤1:60 of that in serum. Thus, the cholesterol concentration in CSF is unlikely to be affected by the same mechanism as that in plasma. apoE4-containing lipoproteins, which do not include apo(E-AII) and apo(AII-E2-AII) complexes, would be metabolized more effectively than apoE3- or apoE2-containing lipoproteins.

apoE in the CNS is involved in the development of AD, and the frequency is significantly higher in subjects with apoE4 *(13–15)*. Although various reasons for this correlation have been considered, such as isoform-specific binding of β-amyloid *(16, 17)*, the actual relationship between cause and effect remains to be determined. Mulder et al. *(34)* have suggested that an alteration of lipid homeostasis in the CNS might be related to AD. Our results suggest that the actual apoE isoform may influence lipid homeostasis in the CNS. If so, this would mean that the individual's phenotype plays an important role in the relationship between apoE and AD.

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