Apolipoprotein E enhances lipid exchange between lipoproteins mediated by cholesteryl ester transfer protein

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Abstract The direct effect of apolipoprotein (apo) E on cholesteryl ester transfer protein (CETP) activity was studied using lipoproteins from a subject with apoE deficiency (*Atherosclerosis.* 1991. **88:** 15–20) as a model system. The transfer of cholesteryl ester (CE) from discoidal bilayer particles (DBP) to very low density lipoprotein (VLDL) was enhanced by incorporation of apoE into VLDL. This enhancement was induced only in the presence of CETP activity. Moreover, after incubation of CETP with VLDL, CETP activity and immunoreactivity were co-eluted with apoE-incorporated VLDL (E-VLDL) on a gel filtration column (Sephadex G-150), but there was little CETP activity and immunoreactivity with apoE-free VLDL (C-VLDL), suggesting that E-VLDL had higher affinity for CETP compared to C-VLDL. The supplementation of the apoE-deficient serum with apoE enhanced the CETP-mediated changes of amount of CE and triglyceride (TG) in the high density lipoprotein (HDL) fraction, which were completely inhibited by the addition of the monoclonal antibody against CETP that blocks CETP activity. Our results suggest that *1)* apoE enhances the cholesteryl ester and triglyceride transfer between VLDL and HDL via cholesteryl ester transfer protein, and *2)* this effect of apoE may be mediated by enhancing the affinity of CETP for VLDL.—**Kinoshita, M., H. Arai, M. Fukasawa, T. Watanabe, K. Tsukamoto, Y. Hashimoto, K. Inoue, K. Kurokawa, and T. Teramoto.** Apolipoprotein E enhances lipid exchange between lipoproteins mediated by cholesteryl ester transfer protein. *J. Lipid Res.* 1993. **34:** 261–268.

Supplementary key words lipid transfer • monoclonal antibody • VLDL • HDL

Cholesteryl ester transfer protein (CETP) plays an important role in reverse cholesterol transport system together with lecithin:cholesterol acyltransferase (LCAT) and plasma lipoproteins such as high density lipoprotein (HDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), and very low density lipoprotein (VLDL) (1, 2). The first step of reverse cholesterol transport is uptake of unesterified cholesterol of peripheral tissues by HDL particles and its esterification through the LCAT action. Then, cholesteryl ester (CE) is transferred from HDL to acceptor lipoproteins (LDL, IDL, VLDL, and perhaps chylomicrons) by plasma CETP activity (3). These lipoproteins are finally trapped and degraded in the liver by the LDL receptor-mediated pathway (1). The activity of CETP also results in the removal of surface components of lipoproteins, such as the phospholipids of VLDL and chylomicron remnants, during lipolysis and the transfer of the surface components to HDL (4, 5). Thus, CETP activity affects the plasma HDL level, which is thought to be relevant in atherogenesis (6).

As shown in other lipoprotein-metabolizing enzymes such as LCAT, apolipoproteins may regulate CETP activity. This concept was supported by the observation of Yoon et al. (7) that VLDL with apoE contained more cholesterol than VLDL without apoE in rabbit plasma, suggesting that apoE on VLDL may enhance CE transfer from other lipoproteins by the action of CETP. Therefore, it is important to test the direct effect of apolipoproteins on CETP activity for natural lipoproteins, because the mechanism by which apolipoproteins regulate CETP activity has not been elucidated. However, it is difficult to prepare lipoproteins that lack specific apolipoproteins. A discovery of an apoE-deficient patient (8) gave us an opportunity to test regulatory roles of apoE on CETP activity. We obtained apoE-free VLDL from the patient and

Abbreviations: apo, apolipoprotein; TC, total cholesterol; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid; DBP, discoidal bilayer particle; CM, chylomicron; VLDL, very low density lipoprotein; LDL, low density lipoprotein; E-VLDL, apoE-incorporated VLDL; C-VLDL, apoE-free VLDL; TG, triglyceride; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; PBS, phosphate-buffered saline; DTNB, 5,5'-dithio-bis(2-nitrobenzoic acid).

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prepared apoE-containing VLDL by incubating the apoE-free VLDL with recombinant apoE. Our results suggest that incorporation of apoE into VLDL enhanced the CETP activity by raising the affinity of VLDL for CETP.

MATERIALS AND METHODS

Materials

Recombinant human apoE (isoform E3/3) was a generous gift from Mitsubishi Kasei Co. (Tokyo, Japan). Lyophilized apoE was dissolved in 10 mM Tris-HCl (pH 7.5), 8 M urea, and the aliquots were stored at −70°C. Before use, the stored apoE was dialyzed against 20 l of phosphate-buffered saline (PBS) containing EDTA (pH 7.4) and used immediately. Monoclonal antibody (Mab) LT-A4, which inhibits CETP activity, was obtained by intraperitoneal injection of hybridomas into Pristane-primed mice, and ascites fluid was harvested after appropriate periods. LT-A4 was purified on protein A-Sepharose CL-4B.[2] Sephacryl S-300, Sephadex G-150, and protein A-Sepharose CL-4B were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). [^{14}C]cholesteryl oleate was obtained from Amersham (Buckinghamshire, England). All other chemicals used were of analytical grade and purchased from commercial sources.

Isolation of lipoproteins

ApoE-free serum was isolated from a patient with apoE deficiency (K. O.) (8) in the fasting state. Serum was separated immediately from blood cells, and 0.05% EDTA, 0.05% NaN_3, 1 mM PMSF, and 0.1 mg/ml gentamycin were added. Lipoproteins were separated by ultracentrifugation using the method of Havel, Eder, and Bragdon (9) into five fractions as follows: chylomicron ($d < 0.95$ g/ml), VLDL ($d < 1.006$ g/ml), IDL (d 1.006–1.019 g/ml), LDL (d 1.019–1.063 g/ml), and HDL (d 1.063–1.21 g/ml). After ultracentrifugation, each lipoprotein was dialyzed against PBS just before the following experiments.

Incorporation of apoE into VLDL

VLDL were incubated with apoE in PBS at 4°C for 18 h (protein ratio 1:1), after dialysis against PBS. After incubation, the mixture was applied to a Sephacryl S-300 (1 × 30 cm) column to remove unbound apoE from apoE-containing VLDL as described by Mokuno et al. (10). The latter was designated as E-VLDL. Control VLDL (C-VLDL) were obtained by incubating VLDL with PBS in the absence of apoE and prepared by the same procedure as for E-VLDL.

[2]Monoclonal antibody LT-A4 can be obtained by request from Dr. Keizo Inoue, Department of Health Chemistry, Faculty of Pharmaceutical Science, University of Tokyo, Bunkyo-ku, Tokyo, Japan.

Preparation of discoidal bilayer particle (DBP) with [^{14}C]cholesteryl ester

DBP containing [^{14}C]cholesteryl ester ([^{14}C]DBP) was prepared as described previously (11). Briefly, an ethanol solution containing 22.5 μmol of egg phosphatidylcholine, 7.5 μmol of cholesterol, and 0.3 μmol of [^{14}C]cholesteryl oleate (10 μCi) was injected into 20 ml of phosphate buffer (pH 7.4) containing EDTA. After mixing the solution for 15 min at room temperature, 1.9 ml of 200 mM sodium cholate containing 9 mg of purified apoA-I was added to the mixture while stirring. The mixture was incubated for 30 min and then dialyzed against 8 l of PBS at 4°C to remove ethanol and cholate. The solution of [^{14}C]DBP thus obtained was diluted to 25 ml with PBS.

Assay of plasma CETP activity

Plasma CETP activity was measured in terms of the radioactivity of [^{14}C]cholesteryl ester transferred from DBP to LDL as described previously (11). The reaction mixture containing 100 μl [^{14}C]CE-labeled DBP and 130 μg LDL was incubated with 2 μl of plasma for 30 min at 37°C in the presence of 1.4 mM 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), an inhibitor of LCAT activity. After incubation, 30 μl of 0.1% dextran sulfate and 30 μl of 60 mM $MgCl_2$ were added to the incubation mixture. The mixtures were kept on ice for 20 min and centrifuged at 12,000 rpm for 10 min. Supernatants were collected and the pellets were dissolved with 0.1 M NaOH. Radioactivity in the supernatants and pellets was counted using a Beckman scintillation counter. Percentage transfer of [^{14}C]cholesteryl ester was calculated as [(cpm precipitated in sample minus cpm precipitated in buffer control)/total cpm] × 100. CETP activities were expressed as percentage transfer per serum volume for a given incubation time.

Lipid exchange between C-VLDL or E-VLDL and [^{14}C]DBP

VLDL (130 μg protein) were incubated with 100 μl of [^{14}C]DBP in the presence of CETP and 1.4 mM DTNB at 37°C for 30 min. After incubation, all samples were immediately placed on ice. VLDL, DBP, and CETP were separated by density gradient ultracentrifugation by the method of Sakai et al. (12). The radioactivity of each fraction (50 μl) collected from the centrifugation tube was assayed.

Separation of VLDL and CETP by gel filtration chromatography

VLDL (135 μg) were incubated with 1 μg of CETP at 4°C for 20 min. Incubation mixtures were then applied to a Sephadex G150 column (4.5 × 450 mm) equilibrated with PBS. Eluates were collected in 200-μl fractions. The CETP activity of each fraction was assayed as described above, except that 10 μl of each fraction was added to

50 μl [^{14}C]DBP and incubated with 65 μg of LDL in the presence of 1.4 mM DTNB at 37°C for 30 min. The immunoreactive CETP was detected as follows: 50 μl of each fraction was electrophoresed on a 10% SDS polyacrylamide gel and transferred to a nitrocellulose membrane. CETP immunoreactivity of each fraction was detected by immunoblotting analysis using Mab LT-A4 as described (13).

Incubation of apoE and Mab LT-A4 with sera from a patient with apoE deficiency

Fifty μg of apoE with or without 400 μg of LT-A4, which inhibits CETP activity completely (13), was incubated with 1 ml of the patient's serum at 37°C for 18 h in the presence of 1.4 mM DTNB. After incubation, each lipoprotein (CM, VLDL, IDL, LDL, HDL) was separated by ultracentrifugation. Protein, total cholesterol (TC), free cholesterol (FC), triglyceride (TG), and phospholipid (PL) contents were measured for each lipoprotein fraction.

Other methods

CETP was purified from 2 l of human plasma as previously reported (11) with slight modifications. CM-Sepharose CL-6B column chromatography was performed at the final step instead of hydroxyapatite column chromatography. The overall purification of CETP from sera was 28,500-fold and the purified CETP appeared to have two bands on 10% SDS-polyacrylamide electrophoresis as described (13). Apolipoprotein composition of the lipoprotein fraction was analyzed by electrophoresis on an SDS 5–22.5% polyacrylamide gradient gel (SDS-PAGE) stained with Coomassie blue (14). Protein was measured by the method of Lowry et al. (15) using bovine serum albumin as standard. Apolipoproteins (A-I, B, and E) of VLDL were measured by an immunoturbidity method (16). TC and FC were measured with enzymatic kits "TC555" and "FC555" (Kyowa Medics, Tokyo, Japan), respectively. PL and TG were measured with "PL-K" and "TG-A" kits (Nippon Shouji, Tokyo, Japan), respectively. Significance of the data was analyzed by Student's *t* test.

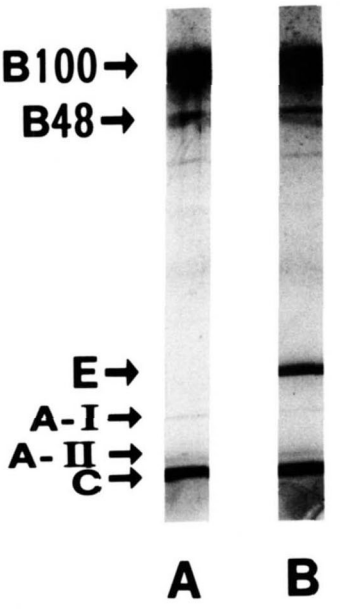

Fig. 1. SDS-PAGE apolipoprotein profiles of apoE-deficient VLDL incubated with or without apoE. A:C-VLDL; B:E-VLDL; apoE was incorporated into VLDL as described in Methods. The scale represents molecular weights of co-migrating standard proteins.

RESULTS

Apolipoprotein E incorporation into VLDL

Based on the apolipoprotein profile on 3–22.5% SDS-PAGE, a protein band that corresponded to apoE could be detected in E-VLDL, while no apoE could be detected in C-VLDL (**Fig. 1**). The conditions we used to obtain E-VLDL were reported to saturate VLDL with apoE (10, 17). Lipid and protein compositions (by weight) exhibited

TABLE 1. Lipids and protein composition of C-VLDL and E-VLDL

	TC	TG	PL	Protein
		%		
C-VLDL	25.7 ± 0.3	41.1 ± 0.7	20.3 ± 0.5	12.6 ± 0.2 (apoA-I, 2.2%) (apoB, 97.8%) (apoE, 0.0%)
E-VLDL	26.0 ± 0.2	40.9 ± 0.4	20.7 ± 0.3	12.4 ± 0.2 (apoA-I, 4.4%) (apoB, 89.6%) (apoE, 6.0%)

Results are given as mean ± SD, n = 3. C-VLDL and E-VLDL were prepared by gel filtration as described in Methods. TC, TG, PL, and VLDL protein were measured as described, and data are given as the weight percentage. Among the proteins, only apoA-I, B, and E were measured by the immunoturbidity method, and the percentages of these three apolipoproteins are indicated in the right hand column. Data were analyzed by Student's *t*-test. No significant difference ($P > 0.05$) could be observed between any figures except for apoE content.

no significant differences between C-VLDL and E-VLDL (**Table 1**). Electron microscopic study of the lipoproteins revealed that C-VLDL and E-VLDL had the same particle size before and after incubation and no aggregation of lipoprotein particles could be observed (data not shown). Apolipoprotein measurement showed that E-VLDL contained 6.3% (by weight) of apoE compared to the weight of apoB based on the immunoturbidity method (Table 1). These results indicate that the only chemical difference between E-VLDL and C-VLDL is the apoE content.

Incubation of E- or C-VLDL with [^{14}C]DBP

E-VLDL or C-VLDL were incubated with [^{14}C]DBP, which contains [^{14}C]cholesteryl ester and apoA-I but no apoE, in the presence or absence of CETP. After incubation at 37°C for 30 min, VLDL, DBP, and CETP were separated by density gradient ultracentrifugation at 4°C. Essentially no radioactivity was detected in fractions corresponding to either C-VLDL or E-VLDL after incubation in the absence of CETP (**Fig. 2B**). In the presence of CETP, significant radioactivity was transferred from DBP fractions to VLDL fractions. Significantly more radioactivity was recovered in the E-VLDL fraction compared to the C-VLDL fraction (Fig. 2A). The transfer rate of radioactivity from DBP to E-VLDL was significantly higher than to C-VLDL (13.99 ± 0.89% vs. 8.10 ± 1.86%: $P < 0.05$, n = 4). The recovery rates of [^{14}C]CE from the incubation mixture were 92.5–95.8% of the initial radioactivity. Lipid profiles of DBP incubated with E- or C-VLDL showed that TG was significantly increased in DBP incubated with E-VLDL compared with C-VLDL (**Table 2**).

TABLE 2. Lipid composition of discoidal bilayer particles after incubation with C-VLDL or E-VLDL

Incubated with	TC	TG	PL
		%	
C-VLDL	16.62 ± 0.16	0.99 ± 0.05	82.39 ± 0.20
E-VLDL	15.61 ± 0.28[a]	2.34 ± 0.01[b]	82.05 ± 0.27

Results are given as mean ± SD, n = 4.
[a] $P < 0.05$.
[b] $P < 0.001$.

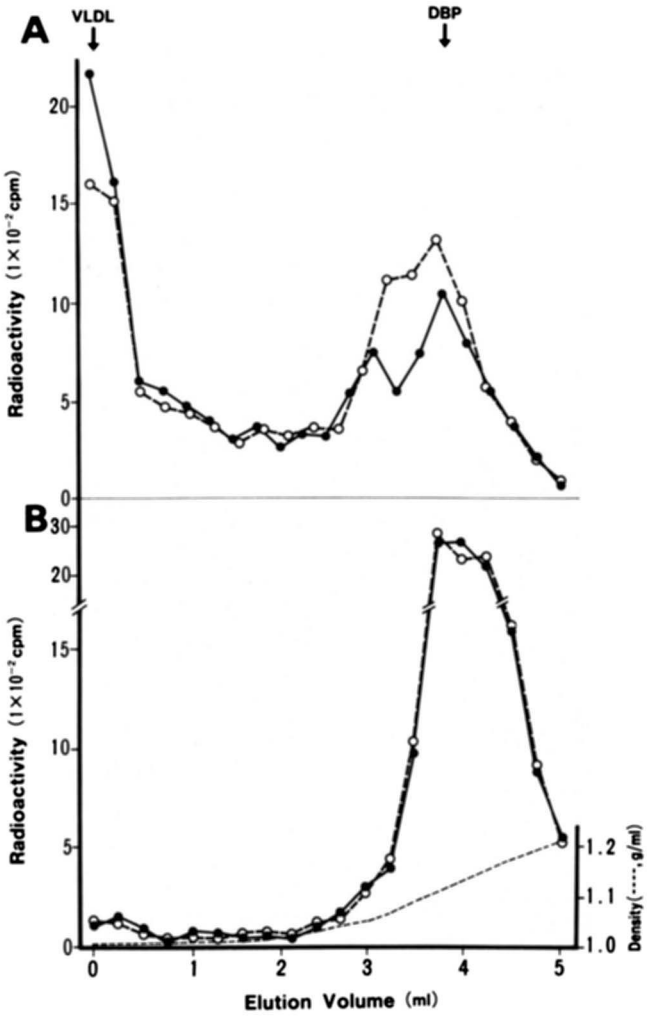

Fig. 2. The transfer of [^{14}C]cholesteryl ester from DBP to VLDL analyzed by density gradient ultracentrifugation. E-VLDL (●—●) or C-VLDL (○---○) was incubated with [^{14}C]CE-labeled DBP in the presence (A) or in the absence (B) of CETP as described in Methods. After the incubation, VLDL and DBP were separated by density gradient ultracentrifugation. The radioactivity of each fraction was measured as transfer activity of CE from DBP to VLDL.

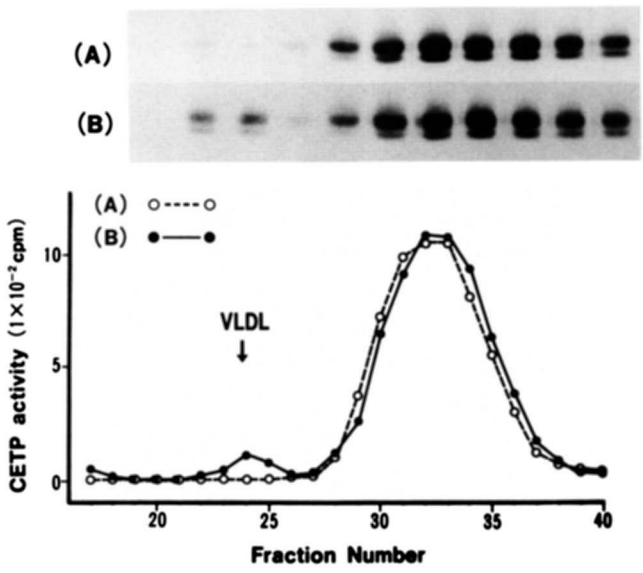

Fig. 3. The effect of apoE on the affinity of CETP to VLDL. C-VLDL (A: ○---○) or E-VLDL (B: ●—●) (135 μg of each protein) were incubated with 1 μg of CETP at 4°C for 30 min. After incubation, the incubation mixtures were applied to a Sephadex G150 column (4.5 × 450 mm) equilibrated with PBS. The eluates were collected in 200-μl fractions. The CETP activities of each fraction were assayed as described in Methods (lower panel). The immunoreactivity of CETP in 50-μl aliquots of each fraction was determined by immunoblotting with Mab LT-A4 as described in the text (upper panel).

Binding of CETP to VLDL

In order to assess the affinity of CETP for C-VLDL and E-VLDL, CETP associated with VLDL and free CETP were separated on a Sephadex G-150 column after incubation at 4°C for 30 min. Under our chromatographic conditions, 4.7% of CETP activity applied to a column was co-eluted with E-VLDL at the void volume, but no CETP activity was eluted with C-VLDL (**Fig. 3**). CETP immunoreactivity was also co-eluted with E-VLDL, but there was little immunoreactivity with C-VLDL as shown in the upper panel of Fig. 3. These results suggest that the affinity of E-VLDL for CETP is higher than that of C-VLDL.

Combination effects of apoE and Mab LT-A4 on lipid transfer activity in apoE-free whole serum

In order to clarify whether or not apoE enhances lipid transfer mediated by CETP in whole sera, we examined the changes in lipid profiles in lipoprotein fractions prepared from sera incubated in the presence of different combinations of apoE and Mab LT-A4. Based on our preliminary experiments, normal CETP activity (16.6%/h per μl; healthy volunteer 18.6 ± 1.7%/h per μl) was detected in apoE-free sera from the patient. After the incubation, each lipoprotein was separated by ultracentrifugation and the lipid composition of each lipoprotein was

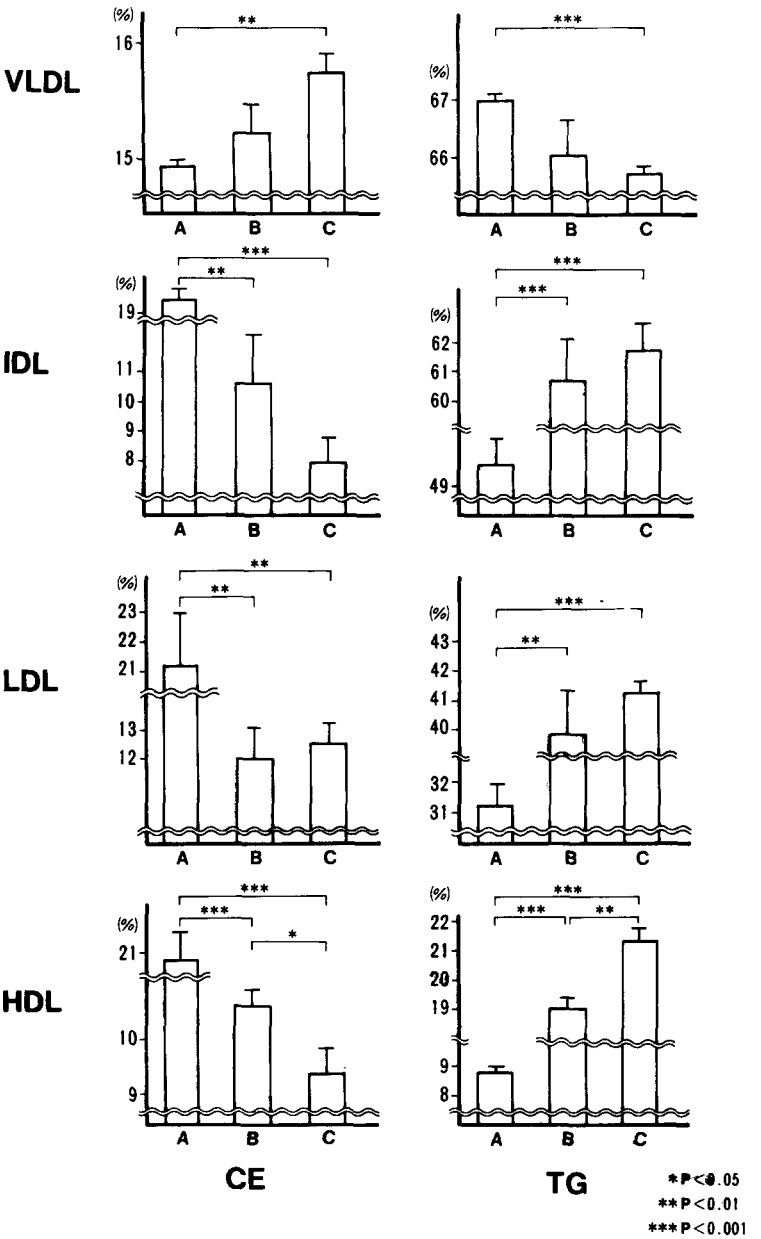

Fig. 4. The effect of apoE and CETP-blocking Mab LT-A4 on the lipid composition of each lipoprotein in serum from the apoE-deficient patient. Serum from the patient was incubated with LT-A4 (A), or with apoE (C), or without apoE and LT-A4 (B) at 37°C for 18 h in the presence of 1.4 mM DTNB and the lipoproteins were separated by ultracentrifugation. Each bar represents the percentage of CE and TG to total lipids. (mean ± SD; n = 3).

measured. The percentages of CE and TG in each lipoprotein prepared from the sera incubated in the absence of Mab LT-A4 exhibited significant differences from those incubated in the presence of Mab LT-A4, even in the presence or absence of apoE (**Fig. 4** B, C vs. A). Because the concentration of Mab LT-A4 was able to inhibit all CETP activity in the sera (13), these changes could be attributed to CETP activity in the sera. In the presence of CETP activity, the addition of apoE significantly decreased the CE content in the HDL fraction, while TG content in the HDL fraction increased, even though changes in other lipoprotein fraction were not statistically significant (Fig. 4 B, C). These changes of CE and TG in the HDL fraction induced by the addition of apoE could not be observed when CETP activity was completely blocked with Mab LT-A4 (**Table 3**). These data indicate that the effects of apoE on the changes of lipid profile in the HDL fraction are not the direct effect of apoE on lipid transfer, but are mediated by CETP activity in the serum.

DISCUSSION

It is well accepted that CETP plays a major role in the reverse cholesterol transport system, and indeed is one of the determinants of plasma HDL levels. It is therefore important to determine the factors that regulate the activities of CETP because HDL is believed to work as an antiatherogenic factor. Nishikawa et al. (18) have already reported that apolipoproteins A-I, A-II, C-II, C-III, and E equally enhanced nonpolar lipid transfer, using artificial lipid emulsions instead of physiological lipoproteins. They suggested that the effect of these apolipoproteins is caused by the stabilizing effect of apolipoprotein on lipoprotein surfaces (18). Recently, we reported a patient with a complete deficiency of apoE in the serum (8). The patient's serum free of apoE is a very useful tool for examining the role of apoE in lipoprotein metabolism because preparation of apoE-free lipoproteins from normal subjects is practically impossible. We used VLDL to test the effect of apoE on lipid transfer mediated by CETP in vitro because *1)* VLDL from normal subjects contain apoE as a major constituent but there is no apoE in VLDL from the patient; *2)* VLDL from the patient contains all other apolipoproteins (A-I, A-II, Cs, B-100, and B-48) the same as VLDL from normal subjects; and *3)* VLDL is a major acceptor of CE through the reverse cholesterol transport system.

We prepared apoE-incorporated VLDL by the incubation of apoE-free VLDL with recombinant human apoE. Lipid and protein composition and an electron microscopic study of the both VLDLs revealed no difference between E-VLDL and C-VLDL except for apoE content.

The amounts of radioactive CE transferred to E-VLDL from [^{14}C]-labeled CE DBP were significantly greater

TABLE 3. Effect of Mab LT-A4 and apoE on the changes of lipid composition of each lipoprotein induced by incubation with patient's serum

	VLDL			IDL			LDL			HDL		
	TC	TG	PL	TC	TG	PL	TC	TG	PL	TC	TG	PL
A: Serum only	34.2 ± 0.5 (24.2 ± 0.4)	42.2 ± 0.5	23.5 ± 0.5	40.3 ± 1.3 (25.6 ± 1.1)	26.8 ± 0.9	32.9 ± 0.5	44.3 ± 0.1 (35.4 ± 0.2)	23.0 ± 1.7	32.7 ± 1.7	29.6 ± 0.9 (26.5 ± 0.7)	16.9 ± 0.6	53.5 ± 0.3
B: Serum + LT-A4	29.3 ± 0.3 (18.4 ± 0.3)	48.5 ± 0.6	22.2 ± 0.9	47.7 ± 0.4 (32.1 ± 0.3)	21.1 ± 1.4	31.2 ± 1.0	50.5 ± 0.5 (41.6 ± 0.3)	14.2 ± 0.8	35.2 ± 1.0	33.0 ± 1.0 (30.0 ± 0.8)	7.8 ± 0.3	59.3 ± 1.3
C: Serum + LT-A4 + apoE	28.9 ± 0.2 (18.1 ± 0.3)	48.6 ± 1.7	22.5 ± 0.7	48.0 ± 1.9 (32.6 ± 1.0)	20.7 ± 1.1	31.3 ± 1.8	49.9 ± 0.9 (41.1 ± 0.8)	14.2 ± 1.1	35.9 ± 1.2	33.3 ± 0.8 (30.2 ± 0.6)	7.4 ± 0.6	59.4 ± 1.4

Serum from the apoE-deficient patient was incubated at 37°C for 18 h in the absence of LT-A4 or apoE (A) or in the presence of LT-A4 (B) or LT-A4 plus apoE (C). VLDL, IDL, LDL, and HDL were separated by ultracentrifugation. TC, TG, and PL contents were measured as described in Methods. Percentage of CE is indicated in parentheses. Statistical analyses were performed by Student's *t* test. No statistical change ($P > 0.05$) could be observed between (B) and (C). Because the patient has diabetes mellitus and type III hyperlipidemia, the lipid profiles of each lipoprotein varied from time to time in the clinical course. Results are given as mean ± SD, n = 3.

than to C-VLDL in the presence of CETP, while essentially no transfer could be detected in the absence of CETP (Fig. 2). In exchange, DBP accepted a greater amount of TG from E-VLDL in the same experiment than from C-VLDL (Table 2). These results indicate that incorporation of apoE into VLDL enhances CE and TG transfer between VLDL and DBP in the presence of CETP. Because apoE might be transferred from VLDL to DBP during incubation, it is also possible that apoE on DBP might enhance the CETP activity. However, in the absence of CETP, apoE on VLDL has little effect on CE transfer from DBP to VLDL. Our results clearly indicate that apoE on lipoproteins enhances the CETP-mediated lipid transfer between lipoproteins.

It has been reported that CETP has a high affinity to HDL, and that CETP can bind to LDL and VLDL (19). Swenson et al. (20) reported that after incubation of CETP with VLDL, they observed a part of VLDL bound to CETP on a Sephadex G-200 column (20). Affinity of lipoproteins to CETP may be an important regulatory factor for CETP activity in sera (21) and apoE might be a determinant for the affinity of lipoprotein for CETP. To test this possibility, we compared the affinity of CETP for E-VLDL with that for C-VLDL. A part of total CETP activity as well as CETP immunoreactivity were co-eluted with the E-VLDL fraction on a Sephadex G-150 column after incubation of E-VLDL with CETP, while little CETP activity and immunoreactivity were co-eluted with C-VLDL (Fig. 3). These data suggest that the affinity of E-VLDL for CETP is higher than that of C-VLDL. This difference of affinity may be responsible for the enhancement of CE transfer from DBP to E-VLDL.

Finally, in order to know whether apoE-enhanced lipid transfer is observed in whole serum instead of a reconstituted system, and whether this enhancement is mediated by CETP, we tested the effect of apoE and/or Mab LT-A4 on lipid transfer in serum with no apoE, but with normal CETP activity. The content of TG and CE in the HDL fraction from the serum incubated with apoE is more and less, respectively, compared to those of the serum incubated without apoE (Fig. 4). The addition of LT-A4 inhibited the changes of the lipid profile of the serum lipoproteins. ApoE had no effect on the lipid profile in the serum lipoproteins in the presence of Mab LT-A4 (Table 3), suggesting that apoE has no direct effect on lipid transfer between lipoproteins in serum. The effect of apoE may not be mediated by phospholipid transfer protein, another species of lipid transfer protein reported in serum (22), because no changes of phospholipid composition between lipoproteins could be detected during incubation as shown in Table 3.

Taken together, our results suggest that apoE incorporation in lipoproteins may potentiate CETP-mediated lipid transfer by increasing the affinity of lipoprotein to CETP. Thus, apoE may be another regulatory factor in the reverse cholesterol transfer system. The physiological significance of this apoE effect remains to be explored and is under investigation in our laboratory.

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