Cholesteryl Ester Transfer Protein Corrects Dysfunctional High Density Lipoproteins and Reduces Aortic Atherosclerosis in Lecithin Cholesterol Acyltransferase Transgenic Mice*

Bernhard Föger‡‡, Michael Chase‡§, Marcelo J. Amar‡, Boris L. Vaisman‡, Robert D. Shamburek‡, Beverly Paigen§, Jamila Fruchart-Najib*, Jorge A. Paiz‡, Christine A. Koch‡, Robert F. Hoyt**, H. Bryan Brewer, Jr.‡, and Silvia Santamarina-Fojo‡ ‡‡ From the %Molecular Disease Branch and *Laboratory of Animal Medicine/Surgery, NHLBI, National Institutes of Health, Bethesda, Maryland 20892, ¶The Jackson Laboratory, Bar Harbor, Maine 04609-1500, and ‡Faculte de Pharmacie-Institut, Pasteur, INSERM U325, Lille, France

Expression of human lecithin cholesterol acyltransferase (LCAT) in mice (LCAT-Tg) leads to increased high density lipoprotein (HDL) cholesterol levels but paradoxically, enhanced atherosclerosis. We have hypothesized that the absence of cholesteryl ester transfer protein (CETP) in LCAT-Tg mice facilitates the accumulation of dysfunctional HDL leading to impaired reverse cholesterol transport and the development of a pro-atherogenic state. To test this hypothesis we crossed LCAT-Tg with CETP-Tg mice. On both regular chow and high fat, high cholesterol diets, expression of CETP in LCAT-Tg mice reduced total cholesterol (−39% and −13%, respectively; p < 0.05), reflecting a decrease in HDL cholesterol levels. CETP normalized both the plasma clearance of [3H]cholesteryl esters ([3H]CE) from HDL (fractional catabolic rate in days−1: LCAT-Tg = 3.7 ± 0.34, LCATxCETP-Tg = 6.1 ± 0.16, and controls = 6.4 ± 0.16) as well as the liver uptake of [3H]CE from HDL (LCAT-Tg = 36%, LCATxCETP-Tg = 65%, and controls = 63%) in LCAT-Tg mice. On the pro-atherogenic diet the mean aortic lesion area was reduced by 41% in LCATxCETP-Tg (21.2 ± 2.0 μm² × 10²) compared with LCAT-Tg mice (35.7 ± 2.0 μm² × 10²; p < 0.001). Adenovirus-mediated expression of scavenger receptor class B (SR-BI) failed to normalize the plasma clearance and liver uptake of [3H]CE from LCAT-Tg HDL. Thus, the ability of SR-BI to facilitate the selective uptake of CE from LCAT-Tg HDL is impaired, indicating a potential mechanism leading to impaired reverse cholesterol transport and atherosclerosis in these animals. We conclude that CETP expression reduces atherosclerosis in LCAT-Tg mice by restoring the functional properties of LCAT-Tg mouse HDL and promoting the hepatic uptake of HDL-CE. These findings provide definitive in vivo evidence supporting the proposed anti-atherogenic role of CETP in facilitating HDL-mediated reverse cholesterol transport and demonstrate that CETP expression is beneficial in pro-atherogenic states that result from impaired reverse cholesterol transport.

Increased plasma levels of high density lipoproteins (HDL) are powerful indicators of low cardiovascular risk in humans (1–3). This relationship may in part be indirect, reflecting the fact that high levels of HDL are a marker for efficient clearance of pro-atherogenic remnant particles from the circulation (4). However, increased plasma HDL levels achieved by either infusion of HDL or overexpression of the apoA-I gene (5–8) protect against the development of atherosclerosis in different animal models, indicating a direct anti-atherogenic role of HDL. The mechanism underlying this relationship is poorly understood. Potential anti-atherogenic properties of HDL include antioxidant effects (9, 10) as well as the ability to prevent monocyte recruitment into the intima (11, 12), to inhibit the aggregation of atherogenic lipoproteins (13, 14), and to serve as a thrombolytic agent (15). HDL has also been proposed to play a major role in reverse cholesterol transport, a process that involves the movement of cholesterol from peripheral cells to the liver for removal from the body (16–19). Thus, raising plasma HDL by dietary, pharmacologic, or genetic interventions may be an effective strategy for the prevention of cardiovascular disease in humans. Cholesteryl ester transfer protein (CETP) and lecithin cholesterol acyltransferase (LCAT) are two key proteins that modulate the plasma concentrations of HDL. LCAT mediates the esterification of free cholesterol on plasma lipoproteins, thereby converting discoidal, nascent HDL particles into mature spherical HDL containing a central core of cholesteryl esters (CE) (16, 19). Overexpression of LCAT in transgenic rabbits (20, 21) increases the plasma HDL cholesterol levels and significantly reduces atherosclerosis. LCAT transgenic mice (LCAT-Tg) also have elevated HDL cholesterol levels (22–24). However, high plasma HDL concentrations are associated with either no change (25) or enhanced diet-induced atherosclerosis in LCAT-Tg mice (26).

CETP is another key protein that modulates the plasma levels of HDL. CETP promotes the transfer of CE from HDL to apoB-containing lipoproteins in exchange for triglyceride (19, 27–29). The absence of atherosclerosis in some of the first CETP-deficient Japanese patients to be described (30, 31) and the increase in diet-induced atherosclerosis in mice expressing human (32, 33) or simian CETP (34) provided support for the initial hypothesis that CETP was a “pro-atherogenic”

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‡ These authors contributed equally.

†† To whom correspondence should be addressed: National Institutes of Health, Molecular Disease Branch, NHLBI, Bldg. 10, Rm. 7N115, 10 Center Dr. MSC 1666, Bethesda, MD 20892-1666. Tel.: 301-496-5095; Fax: 301-402-0190.

1 The abbreviations used are: HDL, high density lipoproteins; LDL, low density lipoproteins; CE, cholesteryl ester; CETP, CE transfer protein; SR-BI, scavenger receptor class B; Bis-Tris, [bis(2-hydroxyethyl)aminol]-2-[2-hydroxyethyl]propane-1,3-diol; LCAT, lecithin cholesterol acyltransferase; LCAT-Tg, LCAT in mice; FPLC, fast protein liquid chromatography; FCR, fractional catabolic rate; r, recombinant.
factor. A recent study demonstrating that infusion of CETP antisense oligonucleotides in cholesterol-fed rabbits inhibited atherosclerosis provided further support for this concept (35). However, large population-based studies in Honolulu (36) and Japan (37) have indicated that low CETP plasma levels due to a common genetic polymorphism as well deficiency of CETP are associated with an increased incidence of coronary heart disease. In addition, Hayek et al. (32) demonstrated that expression of CETP protects against atherosclerosis in hypertriglyceremic mice. The conflicting data regarding the role of CETP in atherosclerosis that has emerged from these combined studies probably reflect the intricate balance between the pro- and anti-atherogenic properties of CETP. CETP activity leads to decreased plasma levels of HDL cholesterol (34, 38) and increased LDL cholesterol (34), a lipoprotein profile that may promote atherogenesis. However, CETP can also promote dissociation of apoA-I from HDL, generating pre-β-HDL particles (39, 40), which together with the enhanced transport of CE to triglyceride-rich lipoproteins, may facilitate reverse cholesterol transport.

We have previously reported that the increased susceptibility of LCAT-Tg mice to development of atherosclerosis is due, at least in part, to the presence of dysfunctional HDL, which leads to delayed hepatic delivery of HDL-CE (26). Thus, LCAT-Tg mice represent an animal model with enhanced atherosclerosis susceptibility due primarily to impaired reverse cholesterol transport (26). In contrast to rabbits, mice lack CETP, resulting in major differences in the metabolism of HDL between the two animal models. We have hypothesized that deficiency of CETP plays a major role in promoting a pro-atherogenic state in LCAT-Tg mice by impairing the hepatic delivery of HDL-CE. To test this hypothesis we cross-bred human LCAT-Tg mice with simian CETP-Tg mice and analyzed the lipoprotein profile, the metabolic properties of HDL, and development of aortic lesions. LCAT-Tg mice (i) have decreased plasma HDL concentrations, (ii) normalize the plasma clearance and hepatic uptake of CE from LCAT-Tg mouse HDL, and (iii) show reduced development of aortic atherosclerosis by 41%. Our findings demonstrate that CETP corrects the functional abnormalities in LCAT-Tg mouse HDL and reduces the susceptibility of this animal model for atherosclerosis. These data provide new in vivo evidence establishing the proposed role of CETP in reverse cholesterol transport and demonstrate that by facilitating hepatic cholesterol delivery, CETP can reduce atherosclerosis in this animal model. The presence or absence of CETP is a key factor in determining the atherogenic consequences of LCAT expression.

**Materials and Methods**

**Animals and Diet**—Human LCAT-Tg mice containing either 120 or 240 copies of the human transgene (22), simian CETP-Tg mice (UCTP-20) (34), LCATxCETP-Tg mice derived from cross-breeding the two groups above, and normal C57BL/6 mice were used in these studies. Human LCAT-Tg, simian CETP-Tg, and LCATxCETP-Tg mice were generated and propagated on a C57BL/6 genetic background. All mice used in the diet studies were female, 2.5–4.5 months of age, housed at the National Institutes of Health under protocols approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute, and fed a regular chow diet (NIH-07 chow diet 5% fat; Zeigler Brothers Inc., Gardners, PA). For the study, mice were maintained for 16 weeks on a high fat high cholesterol diet that contained 15% fat (ratio of polyunsaturated to saturated fatty acids = 0.69), 1.25% cholesterol, and 0.5% cholic acid. Blood samples from the retroorbital plexus were obtained from mice fasted for 4 h and subjected to anesthetization with methoxyflurane (Pitman-Moore, Mundelein, IL); the blood samples were placed into precooled tubes containing EDTA (final concentration 4 mM) and centrifuged at 2500 × g for 20 min at 4 °C, and aliquots of plasma were stored at −70 °C.

**Quantitation of Plasma Lipids, Lipoproteins, and Apolipoproteins**—Cholesterol, triglycerides, phospholipids, free cholesterol, and HDL cholesterol levels were determined previously described (41). Mouse apoA-I and apoE were quantitated by sandwich enzyme-linked immunosorbent assay utilizing polyclonal antibodies raised in rabbits against synthetic peptides of mouse apoA-I and apoE (42). Mouse apoB was quantitated by nephelometry using polyclonal antibodies raised against purified mouse apoB. Pooled mouse plasma was separated by gel filtration using two Superose 6 HR 10/30 columns connected in series (Amersham Pharmacia Biotech). Lipoproteins were eluted at a constant flow rate of 0.3 ml/min with phosphate-buffered saline containing 0.32% w/v NaCl, 0.04% sodium azide. For visualization of apolipoproteins by immunoblot, 20 μl of pooled fast protein liquid chromatography (FPLC) fractions were separated on a 4–20% Tris-glycine gel (Novex, San Diego, CA) and transferred to polyvinylidene difluoride microporous membranes (Immobilon polyvinylidene difluoride; Millipore, Bedford, MA). Mouse apoA-I, apoE, and apoB were identified by blotting with polyclonal rabbit anti-mouse IgG raised against the purified apolipoproteins and visualized by a biotinylated secondary antibody (Vectastain ABC kit; Vector Laboratories Inc., Burlingame, CA). Two-dimensional electrophoresis of plasma lipoproteins was performed as described previously (43). Plasma lipoproteins were also analyzed by native agarose gel electrophoresis on Titan Gels (Helena Laboratories, Beaumont, Texas) according to the manufacturer's instructions.

**Analysis of Plasma Lipoproteins**—Plasma lipoproteins from either individual mice or from pooled mouse plasma were separated by gel filtration using two Superose 6 HR 10/30 columns connected in series (Amersham Pharmacia Biotech). Lipoproteins were eluted at a constant flow rate of 0.3 ml/min with phosphate-buffered saline containing 0.32% w/v NaCl, 0.04% sodium azide. For visualization of apolipoproteins by immunoblot, 20 μl of pooled fast protein liquid chromatography (FPLC) fractions were separated on a 4–20% Tris-glycine gel (Novex, San Diego, CA) and transferred to polyvinylidene difluoride microporous membranes (Immobilon polyvinylidene difluoride; Millipore, Bedford, MA). Mouse apoA-I, apoE, and apoB were identified by blotting with polyclonal rabbit anti-mouse IgG raised against the purified apolipoproteins and visualized by a biotinylated secondary antibody (Vectastain ABC kit; Vector Laboratories Inc., Burlingame, CA). Two-dimensional electrophoresis of plasma lipoproteins was performed as described previously (43). Plasma lipoproteins were also analyzed by native agarose gel electrophoresis on Titan Gels (Helena Laboratories, Beaumont, Texas) according to the manufacturer's instructions.

**Preparation of Plasma Lipoproteins**—Plasma lipoproteins were fractionated by preparative ultracentrifugation at 1.063 and 1.21 g/ml density in a TLA-100.2 rotor using a TL-100 Ultracentrifuge (Beckman Instruments).

**HDL Metabolic Studies**—HDL derived from either C57BL/6, LCAT-Tg, or LCATxCETP-Tg mice were labeled with [1H]cholesteryl palmitate ether—HDL labeled with [2H]cholesteryl palmitate ether were prepared as described previously (26). [2H]Phosphatidylcholine type XI-E (Sigma), cholesteryl-1,2-[3H]hexadecyl ether (NEN Life Science Products), and butylated hydroxytoluene (Sigma) (500 μg/ml) were dried under nitrogen, 50 μl Tris, pH 7.4, EDTA 0.01% was added, and liposomes were prepared from these constituents by sonication (46). Liposomes were incubated with HDL (density, 1.063–1.21 g/ml, 3 mg of total protein) and density > 1.21 serum (30 μg of total protein) from C57BL/6, LCAT-Tg, and LCATxCETP-Tg mice for 18 h at 37 °C. Labeled HDL were isolated by sequential ultracentrifugation at 1.063 and 1.21 g/ml density in a TLA-100.2 rotor using a TL-100 Ultracentrifuge (Beckman Instruments).

**HLDR Metabolic Studies**—HDL derived from either C57BL/6, LCAT-Tg, or LCATxCETP-Tg mice were labeled with [2H]cholesteryl palmitate ether, and 1 million dpm of labeled HDL were injected into the saphenous veins of C57BL/6, LCAT-Tg, and LCATxCETP-Tg mice, respectively. Plasma decay curves were generated by dividing the plasma radioactivity at each time point by the plasma radioactivity at the initial 1-min time point, which was similar between the study groups. Fractional catabolic rate (FCR) was determined from the area under the plasma radioactivity curves using a multiplexponential curve-fitting technique on the SAAM program (47). The 120-min blood sample was collected, a subset of mice injected with [2H]cholesteryl palmitate ether-labeled HDL was perfused with cold 0.15 μl NaCl. Livers were harvested and extracted in 20 volumes of chloroform-methanol 2:1 (v/v), phases were separated by the addition of water (48), and aliquots of the lower (organic) phase were counted in a Tri-Carb 2500 TR liquid scintillation counter (Packard Instrument Co.).

**Analysis of Aortic Lesions**—Histologic analysis of aortic atherosclerosis was performed as previously described (49).

**Generation of Recombinant Adenoviruses**—The expression vector pEXV-3 containing the human SR-BI cDNA (CLA-1, a generous gift from Dr. Miguel A. Vega) (50, 51) was employed as template to amplify SR-BI cDNA by the polymerase chain reaction. Specific 5' and 3' oligonucleotides containing NotI restriction sites (5'TCGGACGGCGGGCCGCGCGCGCCAGGCGCCAGGCGCCGCGG3'-5'TGGTTGGCCTGGGGGGCCGCCTGTGGGAGG3') were synthesized on a 380B DNA synthesizer (Applied Biosystems, Foster City, CA). Polymerase chain reaction was performed in an automated DNA thermal cycler (Perkin-Elmer) using DNA polymerase from *Pyrococcus furiosus* (Stratagene, La Jolla, CA). Full-length human SR-BI was subcloned into the unique NotI site of the pAd12 (52), containing the cytomegalovirus promoter and enhancer as well as the SV40 polyadenylation signal. The recombinant adenoviruses were generated after cotransfection of pAd12 and pJM17 (Ad5 genome) in human embryonal kidney 293 cells (American Type Culture Collection, Manassas, VA) as previously reported (53, 54). The recombinant adenoviral vectors, designated rLucif-AdV and rSRBI-AdV, were stored frozen at −70 °C. Before infusion into animals, the
recombinant adenovirus was titered and diluted in 0.2% mouse albumin (Sigma). Mice were injected with either control rLucif-AdV (2 × 10⁸ plaque-forming units) or rSRBI-AdV (2 × 10⁸ plaque-forming units) via the saphenous vein. After a 4-h fast, mice were anesthetized with methoxyflurane (Metofane) (Pitman-Moore, Mundelein, IL), and blood samples were collected from the retroorbital plexus before (day 0) and after (days 2, 4, and 5) recombinant adenovirus infusion. Blood samples were centrifuged at 2500 × g for 20 min at 4 °C. The plasma was removed, divided into aliquots, immediately frozen on dry ice, and stored at −70 °C. Metabolic studies were performed by injecting [²H]cholesteryl palmitate ether-labeled HDL as described above on day 5 after rSRBI-AdV or rLucif-AdV infusion.

Quantitation of SR-BI Expression—Livers (0.5 g wet weight) from sham-injected control mice (day 0) as well as LCAT-Tg and control C57Bl mice injected with either rLucif-AdV or rSRBI-AdV (day 5) were harvested, cut into small pieces, and immediately suspended (1:10 weight/volume (g/ml) in a protease inhibitor mixture buffer containing 4-(aminoethoxy)benzenesulfonyl fluoride hydrochloride (AEBF; ICN, Aurora, OH). Mice were injected with either control rLucif-AdV (2 × 10⁸ plaque-forming units) or rSRBI-AdV (2 × 10⁸ plaque-forming units) via the saphenous vein. After a 4-h fast, mice were anesthetized with methoxyflurane (Metofane) (Pitman-Moore, Mundelein, IL), and blood samples were collected from the retroorbital plexus before (day 0) and after (days 2, 4, and 5) recombinant adenovirus infusion. Blood samples were centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatant was then re-centrifuged in a TI-60 centrifuge.

**Table I.** Compared with control mice, LCAT-Tg mice had increased plasma cholesterol, CE, phospholipid, and HDL cholesterol (+123, +122, +36, and +168%, respectively; p < 0.001), and CETP-Tg mice had decreased cholesterol, CE, phospholipid, and HDL cholesterol (−35, −29, −32, and −35%, respectively; p < 0.005) (Table I). LCATxCETP-Tg mice had intermediate levels between their founder lines with plasma cholesterol, CE, and HDL cholesterol increased (+35, +32, and +32%, respectively; p < 0.05) compared with controls but decreased (−39, −41, and −51%, respectively; p < 0.001) compared with LCAT-Tg mice (Table I). Plasma triglyceride levels were increased in CETP-Tg mice (+24%; p < 0.05) and decreased in LCATxCETP-Tg mice (−19%; p < 0.01) compared with controls. Quantitation of the apoB-containing (non-HDL) lipoprotein cholesterol by subtraction of HDL cholesterol from total cholesterol failed to reveal a significant difference (p > 0.5) between LCAT-Tg and LCATxCETP-Tg mice (data not shown). Co-expression of LCAT and CETP decreased apoA-I (−46%; p < 0.05) and apoE (−67%; p < 0.05) but did not significantly alter plasma apoB levels compared with LCAT-Tg mice (Table I). Thus, CETP expression reduced the plasma concentrations of cholesterol, CE, phospholipid, HDL-C, apoA-I, and apoE in LCAT-Tg mice.

Analysis of the plasma lipoproteins by FPLC (Fig. 1, *panels A–C*) and immunoblot analysis of pooled FPLC fractions (Fig. 2) demonstrated the presence of heterogeneous HDL (Fig. 1) consisting of large apoE-rich HDL (Fig. 1C), which eluted in the HDL₃/LDL fractions on FPLC (Fig. 1C), and apoA-I/apoA-II-containing HDL (Fig. 2) in LCAT-Tg mice. Small amounts of apoB-48 and apoB-100 could be detected in the HDL₃/LDL elution fractions of all four study animal groups (Fig. 2). Expression of CETP in LCAT-Tg mice significantly reduced HDL cholesterol, with a preferential decrease in the apoE-rich HDL₃ (Figs. 1 and 2). The cholesterol in the apoA-I/apoA-II-containing HDL was increased in LCATxCETP-Tg mice compared with controls and CETP-Tg mice (Fig. 1). Analysis of the plasma lipoproteins by native agarose gel electrophoresis (Fig. 1D) confirmed the virtual absence of β-migrating (apoB-containing lipoproteins) in all study animals as well as a preferential decrease in HDL₃ in LCATxCETP-Tg compared with CETP-Tg mice. Despite the reduction in HDL cholesterol, CETP expression led to the accumulation of pre-β-HDL absent in LCAT-Tg mouse plasma (Fig. 3).

After 16 weeks of a high fat, high cholesterol diet, the plasma cholesterol and CE levels increased in all four study groups (all p < 0.01 compared with base line; Table II). Compared with control mice, cholesterol, CE, and HDL-C were increased by +37, +44, and +291%, respectively, in LCAT-Tg, increased by
+19, +24, and +51%, respectively, in LCATxCETP-Tg mice, and decreased by −12, −13, and −41%, respectively, in CETP-Tg mice (all \( p < 0.05 \); Table II). Analysis of plasma lipoproteins by FPLC (Fig. 4) revealed that HDL cholesterol plasma levels were most increased in LCAT-Tg and most decreased in CETP-Tg mice. Thus, CETP expression reduced the plasma concentrations of total cholesterol and CE as well as HDL cholesterol (Fig. 4), indicating that CETP expression reversed the abnormal plasma clearance of CE from LCAT-Tg mouse HDL. In addition, the hepatic accumulation of \(^{3}H\)CE-HDL derived from LCATxCETP-Tg mouse HDL (65% of total counts) was similar to that of control animals (63% of total counts) and significantly increased compared with that of LCAT-Tg mice (36% of total counts). Thus, CETP corrected the dysfunctional properties of LCAT-Tg mouse HDL, resulting in the formation of HDL that functions effectively in reverse cholesterol transport.

Analysis of atherosclerotic lesion size and number in the proximal aorta was performed in mice from all four study groups (Fig. 6). The mean aortic lesion area in LCAT-Tg (increased 3.1-fold compared with controls) was reduced by 41% in LCATxCETP-Tg (increased 1.85-fold compared with controls). Interestingly, the lesion area in CETP-Tg and controls had similar values (14.1 ± 0.84 and 15 ± 1.56, respectively). Subgroup analysis revealed a protective effect of CETP against aortic lesion development in both heterozygous (−44.4%, \( p < 0.001 \))
and homozygous (−37.8%, \( p < 0.001 \)) LCAT-Tg mice (data not shown). Thus, expression of CETP in LCAT-Tg mice led to a 41% reduction in aortic atherosclerosis (\( p < 0.001 \)).

To further evaluate potential mechanism(s) leading to the normalization of LCAT-Tg HDL function and reversal of atherosclerosis in LCAT-Tg mice, we expressed the selective HDL-CE uptake receptor, SR-BI, in control and LCAT-Tg mice using recombinant adenovirus. Immunoblot analysis followed by densitometric scanning (Fig. 7, inset) demonstrated a similar 1.5-fold increase in hepatic SR-BI expression compared with base line in both control and LCAT-Tg mice compared with base line. SR-BI increased the base-line (day 0) plasma concentrations of cholesterol, phospholipids, CE, and HDL-C in both control (−62, −56, −64, and −70%, respectively; \( p < 0.001 \) all; data not shown) and LCAT-Tg mice (−54, −46, −53, and −56%; \( p < 0.002 \) all; data not shown). As previously observed with adenovirus-mediated expression of HL in LCAT-Tg mice (55), FPLC analysis revealed a preferential reduction in the cholesterol of the apoE-rich HDL(a) (data not shown). SR-BI enhanced the plasma clearance of \(^{3} \text{H} \)CE from both controls (FCR = 10.7 ± 1.1 versus 6.4 ± 0.16; \( p < 0.05 \)) and LCAT-Tg HDL (FCR in \( d^{-1} \): 6 ± 0.41 versus 3.7 ± 0.34; \( p < 0.05 \)). However, even after SR-BI expression, the plasma clearance of CE from LCAT-Tg mouse HDL remained significantly delayed (FCR = 6 ± 0.41 versus 10.7 ± 1.1; \( p < 0.006 \)) compared with controls (Fig. 7). Similarly, the hepatic accumulation of \(^{3} \text{H} \)CE HDL derived from LCAT-Tg mouse HDL (78% of total counts) was decreased compared with control animals (95% of total counts) despite similar expression of SR-BI in the liver (Fig. 7, inset). These findings indicate a reduced ability of SR-BI to promote the selective uptake of CE from LCAT-Tg mice compared with control mice HDL and are consistent with abnormal interaction between the receptor and the dysfunctional LCAT-Tg HDL.

**DISCUSSION**

We have previously reported that LCAT-Tg mice accumulate HDL that are abnormal with respect to both composition and ability to transport HDL-CE to the liver (26). The increased atherosclerosis susceptibility in LCAT-Tg mice is due primarily to impaired reverse cholesterol transport. Thus, LCAT-Tg mice represent an ideal animal model to investigate the potential effect of genes, such as CETP, proposed to modulate atherosclerosis by facilitating the hepatic delivery of HDL CE. In the present study we have investigated the proposed in vivo role of CETP in facilitating reverse cholesterol transport and atherogenesis by expressing the transfer protein in LCAT-Tg mice.

By promoting the transfer of CE from HDL to triglyceride-rich lipoproteins, CETP may facilitate hepatic cholesterol transport and, thus, modulate the development of atherosclerosis (19, 27–29). However, to date definitive in vivo data clearly demonstrating that CETP enhances reverse cholesterol transport and, more importantly, that CETP-mediated enhanced reverse cholesterol transport alters atherogenesis in vivo, is lacking. Furthermore, the elucidation of the role that CETP plays in atherogenesis has proved a major challenge. Animal and human studies have provided evidence supporting its function both as a pro-atherogenic (32, 36, 37) and anti-atherogenic (30–32, 34, 35, 56) factor. These conflicting data suggest a complex metabolic role for CETP in vivo. CETP activity can lead to decreased plasma levels of HDL cholesterol (34, 38) and increased LDL cholesterol (34), a change in the lipoprotein profile that may promote atherogenesis. However, CETP can also promote HDL remodeling with generation of pre-β-HDL particles (39, 40), which together with the CETP-mediated enhanced transfer of CE to triglyceride-rich lipoproteins, can facilitate reverse cholesterol transport. Ultimately, the effect of CETP on atherosclerosis may depend on which of these functions is most effective in altering the dynamics of cholesterol transport from HDL to the liver, which in turn may depend on the metabolic status of the animal model (i.e., the presence of hypertriglyceridemia, LDL receptor, or apoA-I deficiency) (32, 36, 37, 56).

In contrast to LCAT-Tg mice, expression of the same human LCAT transgene in New Zealand White rabbits, which have CETP, markedly reduces aortic atherosclerosis (21). We have proposed that the absence of CETP plays a major role in promoting a pro-atherogenic state in LCAT-Tg mice (26). To test this hypothesis we cross-bred mice expressing simian CETP with LCAT-Tg mice. Simian CETP exhibits a high degree of structural homology (95% at the amino acid level) with human CETP (57) and would be anticipated to have a similar effect on the metabolism of mouse lipoproteins to that of human LCAT. Previous studies have demonstrated that expression of either the simian transfer protein or human CETP leads to significant reductions in HDL cholesterol levels as well as enhanced atherosclerosis in CETP-Tg mice (34, 58). However, simian CETP and human LCAT are known to have a more pronounced effect on HDL cholesterol levels in transgenic animals expressing human apoA-I (59, 60). Thus, the interaction of simian CETP and human LCAT with endogenous mice apoA-I would be anticipated to result in less dramatic changes in HDL cholesterol levels in the LCATxCETP-Tg mice expressing mouse versus human apoA-I. Nevertheless, expression of simian CETP and human LCAT in our study mice led to the expected changes in the mouse plasma lipoproteins. Although differences in the
magnitude of HDL plasma level changes could alter the degree by which mouse atherosclerosis is changed by simian CETP and human LCAT, it would not alter the major conclusions drawn from analysis of these animals.

As reported in other animal models (32, 34, 38) CETP expression significantly decreased the total and HDL cholesterol levels in LCAT-Tg mice consistent with CETP-mediated trans-

FIG. 4. FPLC analysis of plasma from LCAT-Tg, CETP-Tg, LCATxCETP-Tg, and control mice on a high fat, high cholesterol diet. Panels A and B illustrate the cholesterol, CE, phospholipid, and free cholesterol distribution in the plasma lipoproteins of LCAT-Tg (panel A) and LCATxCETP-Tg (panel B) mice. The cholesterol distribution in the plasma lipoproteins of all four mouse study groups are shown in panel C for comparison. The elution positions of very low density lipoproteins, LDL/HDL1, and HDL are indicated.

FIG. 5. Plasma kinetics of $[^{3}H]$CE HDL from C57BL/6, LCAT-Tg, and LCATxCETP-Tg mice. Autologous HDL isolated from each study group was labeled with $[^{3}H]$cholesterylpalmityl ether and injected into control, LCAT-Tg, and LCATxCETP-Tg mice. Values indicate the percent of remaining counts compared with the 1-min value (mean ± S.E.; n = 4 mice/group). FCRs are listed in the inset. Inset, the percentage of total $[^{3}H]$cholesterylpalmityl ether counts present in the plasma and liver of control C57BL, LCAT-Tg, and LCATxCETP-Tg mice (n = 4; each group) 2 h after injection of $[^{3}H]$cholesterylpalmityl ether-labeled HDL.

FIG. 6. Aortic lesion area in control, CETP-Tg, LCAT-Tg, and LCATxCETP-Tg mice after 16 weeks on an atherogenic high fat, high cholesterol diet. Significant differences (p < 0.001; all) were present between LCAT-Tg versus controls, CETP-Tg, and LCATxCETP-Tg and between LCATxCETP-Tg versus control and CETP-Tg. Mean aortic lesion areas in controls and CETP-Tg mice were not statistically different from each other.

### TABLE II

<table>
<thead>
<tr>
<th>Mice</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>PL (mg/dl)</th>
<th>CE (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>ApoA-I (mg/dl)</th>
<th>ApoB (mg/dl)</th>
<th>ApoE (mg/dl)</th>
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<tr>
<td>LCAT-Tg (n = 49)</td>
<td>422 ± 16</td>
<td>247 ± 10</td>
<td>50 ± 6</td>
<td>192 ± 25</td>
<td>115 ± 15</td>
<td>22 ± 2</td>
<td>42 ± 3</td>
<td>19 ± 2</td>
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<td>CETP-Tg (n = 27)</td>
<td>450 ± 16</td>
<td>219 ± 9</td>
<td>44 ± 3</td>
<td>362 ± 16</td>
<td>74 ± 7</td>
<td>42 ± 1</td>
<td>24 ± 1</td>
<td>19 ± 3</td>
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<tr>
<td>LCATxCETP-Tg (n = 25)</td>
<td>335 ± 18</td>
<td>172 ± 23</td>
<td>50 ± 3</td>
<td>256 ± 14</td>
<td>29 ± 3</td>
<td>4 ± 3</td>
<td>18 ± 1</td>
<td>14 ± 2</td>
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<tr>
<td>Controls (n = 28)</td>
<td>379 ± 24</td>
<td>220 ± 19</td>
<td>47 ± 2</td>
<td>293 ± 8</td>
<td>49 ± 5</td>
<td>11 ± 2</td>
<td>101 ± 10</td>
<td>7 ± 3</td>
</tr>
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</table>

*p < 0.005 (compared with controls).

*p < 0.05 (compared with CETP-Tg).

*c p < 0.05 (compared with LCATxCETP-Tg).

*d p < 0.05 (compared with controls).
transfer of CE from HDL to triglyceride-rich lipoproteins. In addition, expression of CETP resulted in the accumulation of pre-
β-HDL, which is absent in LCAT-Tg mouse plasma. Previous
studies have demonstrated that CETP can promote the disso-
ciation of apoA-I from HDL, leading to the formation of pre-
β-HDL (39, 40), which could accelerate the transfer of cellular
cholesterol to HDL. Finally, CETPxLCAT-Tg mice have a pref-
erential reduction of the larger, apoE-rich HDL1, resulting in
the formation of more homogeneous HDL (55). Similar findings
were described in studies involving adenosine-mediated ex-
pression of hepatic lipase in LCAT-Tg mice (55). Thus, CETP
easily transfers CE from the apoE-rich HDL1 lipoproteins that
accumulate as a result of defective CE transfer from HDL to
very low density lipoproteins (61) in LCAT-Tg mice (22).

To evaluate the impact of CETP expression on the functional
properties of LCAT-Tg mouse HDL in reverse cholesterol trans-
port, we performed a kinetic analysis using [3H]CE-labeled
HDL. CETP normalized the markedly delayed clearance of
[3H]CE HDL in LCAT-Tg mice. The fractional catabolic rates
for [3H]CE HDL in LCATxCETP-Tg and control mice were
similar but significantly faster than in LCAT-Tg mice. Simi-
larly, CETP expression in LCAT-Tg mice increased the transfer
of [3H]HDL CE to the liver of LCAT-Tg mice. The accumulation
of [3H]CE derived from either LCATxCETP-Tg or control
mouse HDL was increased by 80% compared with [3H]CE
derived from LCAT-Tg mouse HDL. These findings indicate
that CETP corrects the dysfunctional properties of LCAT-Tg
HDL with respect to its function in reverse cholesterol trans-
port. Its expression results in the formation of HDL particles
that can effectively transport cholesterol to the mouse liver and
function normally in reverse cholesterol transport. Thus, the
deficiency of CETP in LCAT-Tg mice seems to be a major factor
in promoting the functional abnormality in LCAT-Tg mouse
HDL.

To evaluate whether the enhanced CETP-mediated reverse
cholesterol transport would alter the diet-induced aortic lesion
formation in LCAT-Tg mice, we placed all four study groups on
a pro-atherogenic diet. In contrast to previous reports (34) of
studies performed in CETP-Tg male mice, we did not observe
an increase in diet-induced atherosclerosis in the high expres-
sion line of simian CETP-Tg female mice (UCTP-20). It is pos-
sible that the sex difference between the two studies contrib-
uted to the discrepant results. Analysis of LCATxCETP-Tg
mice revealed that CETP expression significantly reduced the
diet-induced aortic atherosclerosis in LCAT-Tg mice by 41%,
demonstrating that by facilitating hepatic cholesterol delivery,
CETP can indeed reduce atherosclerosis in an animal model
with impaired reverse cholesterol transport.

Although CETP expression completely corrected the im-
paired hepatic transport of HDL-CE in LCAT-Tg mice, it did
not abolish all of the enhanced susceptibility to atherosclerosis
in this animal model. This suggests that in addition to im-
paired reverse cholesterol transport, other pro-atherogenic
mechanisms are enhanced by LCAT expression in mice. LCAT-
induced changes in HDL composition (26) may disrupt other
proposed anti-atherogenic properties of HDL, including its abil-
ity to protect LDL from oxidation (9, 10), to prevent monocyte
recruitment into the intima (11, 12), to inhibit the aggregation
of atherogenic lipoproteins (13, 14), and to serve as a thrombo-
lytic agent (15). LCAT may also have unknown local effects on
the vasculature. Unlike reverse cholesterol transport, these
changes might not be reversed by CETP expression.

Based on our combined findings we propose that initially,
expression of LCAT in mice stimulates cholesterol efflux from
cells (23) and cholesterol esterification (22, 23) in plasma,
which enhances reverse cholesterol transport. In this setting,
no major changes in HDL structure and function have been
reported (62). However, the limited capacity of later steps in
reverse cholesterol transport, such as the hepatic uptake of
HDL-CE mediated by SR-BI (63), may then become rate-limit-
ing. This concept is supported by our present findings, which
demonstrate that only a 1.5-fold increase in SR-BI expression
significantly enhanced the selective uptake of HDL-CE and
reduced the HDL cholesterol levels in both control and
LCAT-Tg mice. However, in the absence of CETP, the newly
synthesized HDL-CE generated by LCAT overexpression
cannot be transferred to triglyceride-rich lipoproteins, leading
to the formation of HDL with altered composition and function
(22). Our present study demonstrates that SR-BI cannot effec-
tively facilitate the selective uptake of CE from LCAT-Tg
mouse HDL. We have previously demonstrated that these par-
ticles are clearly dysfunctional in several steps of reverse cho-
lesterol transport, as evidenced by their impaired capacity for
cholesterol efflux (64), their markedly reduced interaction with
lipoprotein-modifying enzymes (55, 65), and their reduced ca-
pability to deliver CE to the liver (26). Ultimately, these
changes in HDL lead to enhanced atherosclerosis in LCAT-Tg
mice (26).

To further investigate potential mechanisms leading to im-
paired reverse cholesterol transport and enhanced atheroscle-
rosis in LCAT-Tg mice, we expressed SR-BI, the major HDL
CE-selective uptake receptor (63), in control and LCAT-Tg mice
using recombinant adenovirus. Our findings indicate that
SR-BI plays an important role in the clearance of CE not only
from control but also from LCAT-Tg HDL. SR-BI overexpres-
sion enhanced HDL-CE catabolism in both animal models,
suggesting a rate-limiting role for SR-BI in the metabolism of
HDL in both animal models. However, despite increased SR-BI
expression, the plasma clearance and hepatic uptake of CE
from LCAT-Tg HDL remained significantly less than from con-
trol HDL, indicating decreased ability of SR-BI to promote
the selective uptake of CE from LCAT-Tg HDL. These findings
are consistent with the accumulation of a dysfunctional HDL that
does not interact normally with SR-BI and provide an expla-
nation for the impaired reverse cholesterol transport and ath-
erosclerosis observed in LCAT-Tg mice.

The present study also demonstrates that by facilitating the
exchange of CE in HDL for triglycerides in apoB-containing
lipoproteins, CETP corrects the dysfunctional properties of
Cholesterol Acyltransferase Transgenic Mice

LCAT-Tg HDL, promoting reverse cholesterol transport via both HDLs and apoB-containing lipoproteins, thereby reducing atherosclerosis in LCAT-Tg mice. Similarly, LCAT-Tg rabbits, which express CETP, have reduced atherosclerosis (21). These combined data indicate that the presence or absence of CETP is a key factor in determining the atherogenic consequences of LCAT expression.

Our findings in these mouse models have a counterpart in human patients. Like LCAT-Tg mice, patients with CETP deficiency accumulate apoE-rich HDL (61) that exhibits abnormal functional properties with decreased cholesterol efflux (66) and delayed clearance of CE transported with HDL (67, 68). Furthermore, HDL isolated from CETP-deficient patients fail to protect macrophages from foam cell formation (66). As in our animal models, the consequence of CETP deficiency, in at least a subset of CETP-deficient patients (36, 37, 69), is enhanced atherosclerosis.

In summary, in the present study we demonstrate that the ability of SR-BI to facilitate the selective uptake of CE from LCAT-Tg HDL is reduced. Thus, abnormal interaction between SR-BI and LCAT-Tg mice HDL is a potential mechanism leading to impaired reverse cholesterol transport and atherosclerosis in these animals. CETP expression restores the functional properties of LCAT-Tg mouse HDL and promotes reverse cholesterol transport, reducing atherosclerosis in LCAT-Tg mice. These data confirm that the absence of CETP contributes to the functional abnormality of LCAT-Tg mouse HDL and promotes atherosclerosis in this animal model. Our studies provide definitive in vivo evidence supporting the proposed antiatherogenic role of CETP in facilitating HDL-mediated reverse cholesterol transport and demonstrate that CETP expression is beneficial in pro-atherogenic states that result from impaired reverse cholesterol transport.

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