Increased Atherosclerosis in ApoE and LDL Receptor Gene Knock-Out Mice as a Result of Human Cholesteryl Ester Transfer Protein Transgene Expression

Andrew S. Plump, Lori Masucci-Magoulas, Can Bruce, Charles L. Bisgaier, Jan L. Breslow, Alan R. Tall

Abstract—The plasma cholesteryl ester transfer protein (CETP) plays a major role in the catabolism of HDL cholesteryl ester (CE). CETP transgenic mice have decreased HDL cholesterol levels and have been reported to have either increased or decreased early atherosclerotic lesions. To evaluate the impact of CETP expression on more advanced forms of atherosclerosis, we have cross-bred the human CETP transgene into the apoE knock-out (apoE0) background with and without concomitant expression of the human apo A-I transgene. In this model the CETP transgene is induced to produce plasma CETP levels 5 to 10 times normal human levels. CETP expression resulted in moderately reduced HDL cholesterol (34%) in apoE0 mice and markedly reduced HDL cholesterol (76%) in apoE0/apoA1 transgenic mice. After injection of radiolabeled HDL CE, the CETP transgene significantly delayed the clearance of CE radioactivity from plasma in apoE0 mice, but accelerated the clearance in apoE0/apoA1 transgenic mice. ApoE0/CETP mice displayed an increase in mean atherosclerotic lesion area on the chow diet (approximately 2-fold after 2 to 4 months, and 1.4- to 1.6-fold after 7 months) compared with apoE0 mice (P<0.02). At 7 months apoA1 transgene expression resulted in a 3-fold reduction in mean lesion area in apoE0 mice (P<0.001). In the apoE0/apoA1 background, CETP produced an insignificant 1.3- to 1.7-fold increase in lesion area. In further studies the CETP transgene was bred onto the LDL receptor knock-out background (LDLR0). After 3 months on the Western diet, the mean lesion area was increased 1.8-fold (P<0.01) in LDLR0/CETP mice, compared with LDLR0 mice. These studies indicate that CETP expression leads to a moderate increase in atherosclerosis in apoE0 and LDLR0 mice, and suggest a proatherogenic effect of CETP activity in metabolic settings in which clearance of remnants or LDL is severely impaired. However, apoA1 overexpression has more dramatic protective effects on atherosclerosis in apoE0 mice, which are not significantly reversed by concomitant expression of CETP. (Arterioscler Thromb Vasc Biol. 1999;19:1105-1110.)

Key Words: atherogenesis ■ transgenic mice ■ fractional catabolic rate ■ HDL metabolism

The plasma cholesteryl ester transfer protein (CETP) mediates the transfer of HDL cholesteryl ester (CE) to triglyceride-rich lipoproteins and thereby increases the catabolism of HDL CEs.1 The role that CETP plays in atherosclerosis appears to depend on the accompanying lipoprotein pattern and genetic background. In humans and in murine models of atherosclerosis, CETP can be either antiatherogenic or proatherogenic.

In human genetic deficiency of CETP, HDL levels are markedly elevated in homozygotes and moderately elevated in heterozygotes.2-3 The dramatic effects of human genetic CETP deficiency on HDL levels suggested the possibility that CETP inhibition by drugs or other interventions might be a therapeutic strategy to increase HDL levels and possibly to inhibit the progression of atherosclerosis.4 For subjects with CETP deficiency and HDL cholesterol >60 mg/dL, there is a low prevalence of coronary heart disease, suggesting that at high HDL levels, CETP deficiency may induce an atheroprotective state.5 However, in heterozygotes with a missense mutation in the CETP gene, who have mean HDL cholesterol levels of 55 mg/dL, there appears to be a moderate increase in coronary heart disease.5

In contrast to humans, mice do not express CETP and have negligible levels of cholesteryl ester transfer activity in plasma. In human or simian CETP transgenic mice the most prominent lipoprotein changes are decreased HDL cholesteryl and apoA1 levels and an increased proportion of apoA1 in pre-beta HDL.6-9 These CETP transgenic mice have an excess of early atherosclerotic lesions in the proximal aorta in response to a diet containing a very high cholesterol content and bile salts.10 However, in mice with hypertriglyceridemia caused by overexpression of the human apoC3 transgene, the
expression of CETP reduced the number and area of fatty streak lesions, suggesting that the impact of CETP on lesions is modified by concurrent hypertriglyceridemia.\textsuperscript{11,12} A weakness of the previous studies is that they only described changes in small, early atherosclerotic lesions, which may have limited relevance to mature atherosclerosis. The purpose of the present study was to examine the effects of CETP expression on atherosclerosis in 2 mouse models that develop more advanced lesions. Consequently, the human natural flanking region CETP transgenic mice were crossed into the apoE0 and LDLR0 backgrounds and these animals were studied on chow and Western diets, respectively.

In these hypercholesterolemic backgrounds, this CETP transgene is markedly induced, resulting in high plasma CETP levels.\textsuperscript{12} In both models we find that CETP expression leads to an increase in atherosclerosis. To further understand the atherogenic potential of CETP and its effects on HDL metabolism, we compared the development of atherosclerosis in apoE0/apoA1 and apoE0/apoA1/CETP transgenic mice. Previous studies have shown that the human CETP transgene is much more effective at lowering HDL cholesterol levels in human apoA1 transgenic mice than in wild-type mice, indicating a substrate preference for HDL containing human apoA1.\textsuperscript{13} Also, human apoA1 overexpression is potently antiatherogenic in apoE0 mice.\textsuperscript{14,15}

Methods

Animals

CETP transgenic mice were NFR-CETP transgenic mice (line 5203),\textsuperscript{16} which had been back-crossed through >5 generations with the C57BL6 background (mice obtained from The Jackson Laboratory, Bar Harbor, ME). ApoE0 mice\textsuperscript{17} and LDLR0\textsuperscript{18} mice were in mixed 129/C57BL6 backgrounds. Mice were housed at the Rockefeller University or Columbia University animal facilities on a mixed 129/C57BL6 background (mice obtained from The Jackson Laboratory, Bar Harbor, ME). ApoE0 mice\textsuperscript{17} and LDLR0\textsuperscript{18} mice were in mixed 129/C57BL6 backgrounds. Mice were housed at the Rockefeller University or Columbia University animal facilities on a 12-hour light-dark cycle (7 AM to 7 PM, light) in specific pathogen-free (SPF) caging. They were weaned at 3 to 4 weeks of age and experiments were performed on animals older than 8 weeks of age. Mice had ad libitum access to food and water except under fasting conditions. ApoE0 mice received a pelleted chow diet (4.5% wt:wt fat, 0.02% wt:wt cholesterol; PicoLab Rodent Chow 20, No. 5053), throughout the experiment. LDLR0 received a powdered high-fat Western-type diet (21% wt:wt fat, 0.15% wt:wt cholesterol; TekLad Adjusted Calories Diet, No. 88137) for 3 months. For anesthesia, either intraperitoneal injection of pentobarbital (5%) or aerosol delivery of methoxyflurane was used. Bloods were drawn from the retroorbital venous plexus or by direct transventricular catheterization into EDTA tubes, and plasma was separated by centrifugation in a microfuge at 41861 \(\times\) 4 C for 10 minutes.

Disappearance of CE Radioactivity From Plasma After Injection of Radiolabeled HDL

These studies were performed as described by Chajek-Shaul et al.\textsuperscript{19} The HDL CE core was labeled by incubating an aliquot of pooled plasma from 4 to 5 mice of similar genotype (apoE0, apoE0/CETP, apoE0/apoA1, and apoE0/apoA1/CETP) with \([\text{H}]\text{cholesteryl oleoyl ether, a nonhydrolyzable analog of cholesteryl oleate suspended in intralipid and mixed with purified CETP at 371861/2 ^{\circ} \text{C}\) for 4 hours. The \([\text{H}]\text{cholesteryl ether-labeled HDL was isolated by preparative ultracentrifugation and its specific activity determined. The labeled HDL was suspended in a volume that was <10% of the total plasma volume and injected intravenously through the femoral vein. To create a tracer appropriate for each genotype, the labeled HDL pools were injected intravenously into mice of the same genotype (ie, apoE0 HDL into apoE0 mice, apoE0/CETP HDL into apoE0/CETP mice, etc). Blood (\textsim 75 \mu L) was drawn through the retroorbital venous plexus at 10 minutes and 1.5, 3, 8, and 28 hours after injection, and radioactivity remaining in plasma was determined. Fractional catabolic rate was determined in each animal by graphical fitting of the turnover data to a 2-pool model and calculating decay by Matthew’s method.\textsuperscript{20}

Atherosclerosis

Mice were sacrificed at 2, 4, or 7 months of age and perfused with 0.9% NaCl by cardiac intraventricular canalization. The heart and proximal aorta were isolated and fixed for \(\geq 5\) days in 4% phosphate-buffered formaldehyde. After fixation, hearts were imbedded in 25% gelatin and sectioned with a cryostat at 10-\(\mu m\) thickness. Processing and staining of tissues was performed according to Paigen et al.\textsuperscript{21} Quantification of lesion area (lipid staining) was performed as previously described, using 5 sections per animal.\textsuperscript{22}

Lipoprotein Analysis

Plasma total cholesterol was determined enzymatically by the method of Allain et al.\textsuperscript{23} and plasma triglyceride was determined with a commercially available kit (Wako Pure Chemical Industries, Ltd). Cholesterol distribution among lipoproteins was determined on a Rainin HPLC by high-performance gel chromatography (HPGC) on a Superose 6 column (Pharmacia) by on-line postcolumn analysis as previously described\textsuperscript{24,25} on selected pooled plasma from each genotype. Rainin Dynamax software was used to collect and analyze data. For VLDL, the percent area under the curve (AUC) was estimated by doubling the ascending half of the VLDL peak. IDL was estimated as the percent AUC between the apex of the VLDL and LDL peaks minus the sum of half the VLDL percent AUC and half the LDL percent AUC. For LDL the percent AUC was estimated by doubling the descending half of the LDL peak. The percent AUC for HDL was directly estimated as this peak was completely resolved. The AUC of each lipoprotein multiplied by total plasma cholesterol was used to determine lipoprotein cholesterol. The distribution of lipoproteins in the representative profiles shown (n=5 mice/profile) was used to estimate the total cholesterol distribution of the larger group of mice (in which only total cholesterol was determined).

Results

Plasma Lipoprotein Changes in ApoE0 (Knock-Out) Mice Expressing CETP and ApoA1 Transgenes

After 7 months on a chow diet, there was no significant difference in total plasma cholesterol levels, comparing apoE0 (615177 \(\pm\) 243 mg/dL, n = 23), apoE0/CETP transgenic (604177 \(\pm\) 231 mg/dL, n = 33), apoE0/apoA1 transgenic (566177 \(\pm\) 137 mg/dL, n = 14), and apoE0/apoA1/CETP transgenic (497177 \(\pm\) 149 mg/dL, n = 12) mice. Fractionation of pooled plasma samples by HPGC analysis indicated that HDL cholesterol was reduced by 34% in apoE0/CETP transgenic mice, compared with apoE0 mice (Figure 1, Table 1). The presence of the apoA1 transgene caused a 3.5-fold increase in HDL cholesterol in apoE0/apoA1 transgenic mice. In apoE0/apoA1/CETP transgenic mice HDL cholesterol was reduced by 76% compared with apoE0/apoA1 transgenic mice. In apoE0 and apoE0/apoA1 backgrounds the CETP transgene also resulted in an increase in VLDL and LDL cholesterol, and a decrease in LDL cholesterol. Thus, in the apoE0 background the presence of the CETP transgene resulted in reduced HDL and LDL cholesterol and increased IDL and VLDL cholesterol. Similar to previous studies,\textsuperscript{13} apoA1 potentiated the reduction of HDL cholesterol by the CETP transgene. In addition to these effects on lipoprotein cholesterol distribution, mean plasma triglyceride levels were markedly increased in apoE0/CETP mice compared with
apoE0 mice, and in apoE0/apoA1/CETP mice compared with apoE0/apoA1 transgenic mice (Table 1).

Disappearance of CE Radioactivity From Plasma After Injection of Radiolabeled HDL

Autologous HDL containing radiolabeled cholesteryl ethers was injected intravenously and the disappearance of cholesteryl ether radioactivity from plasma was measured at several times. In apoE0/CETP transgenic mice, the decay of plasma CE radioactivity was significantly delayed compared with apoE0 mice (Figure 2, Table 2), suggesting that CETP delays the removal of plasma CE, probably by transfer to a large pool of VLDL CE that is slowly turning over. In apoE0/apoA1 transgenic mice the decay of plasma CE radioactivity appeared slower than in apoE0 mice, but this difference was not significant (Table 2). In apoE0/apoA1/CETP transgenic mice, the catabolism of plasma CE was significantly faster than in apoE0/apoA1 or apoE0/CETP transgenic mice. Thus the presence of the CETP transgene results in a significant delay in clearance of CE from plasma in apoE0 mice, but accelerates the clearance in apoE0/apoA1 transgenic mice.

Atherosclerosis Studies

Atherosclerosis was quantified in the proximal aorta of apoE0 or apoE0/CETP transgenic mice at 2, 4, and 7 months of age, and in apoE0/apoA1 transgenic and apoE0/apoA1/CETP transgenic mice at 7 months of age (Table 3). At each of the earlier times there was approximately 2.1- to 2.2-fold more atherosclerosis in apoE0/CETP mice compared with apoE0 mice, although at 7 months the increase was about 1.6-fold. The results were not statistically significant for any individual time, reflecting variability in the data and relatively small numbers of animals at the earlier times. The combined data from each of the 3 times, however, demonstrated statistically significant differences by ANOVA (P=0.016). These data indicate that CETP increases the extent of atherosclerosis in the apoE0 background, and suggest that the effect may be more pronounced at earlier times when lesions are less extensive.

The quantitative atherosclerosis data are shown for apoE0, apoE0/CETP, apoE0/apoA1, and apoE0/apoA1/CETP transgenic mice of either sex at the 7-month time in Table 4. An increase in mean lesion area (lipid staining) was apparent in both sexes of apoE0/CETP mice compared with apoE0 mice (1.6-fold, females; 1.4-fold, males), but neither difference was significant. In contrast to these findings, the presence of the apoA1 transgene resulted in an approximately 3-fold increase in mean lesion area (lipid staining) in apoE0/apoA1/CETP mice compared with apoE0 mice (1.6-fold, females; 1.4-fold, males), but neither difference was significant. In contrast to these findings, the presence of the apoA1 transgene resulted in an approximately 3-fold increase in mean lesion area (lipid staining) in apoE0/apoA1/CETP mice compared with apoE0 mice

### Table 1: Plasma Lipid and Lipoprotein Analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>VLDL-C (mg/dL)</th>
<th>IDL-C (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>Non-HDL (mg/dL)</th>
<th>HDL/Non-HDL (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E0</td>
<td>615 (n=23)</td>
<td>140</td>
<td>178</td>
<td>164</td>
<td>231</td>
<td>41</td>
<td>574</td>
<td>0.072</td>
</tr>
<tr>
<td>E0/CETP</td>
<td>604 (n=23)</td>
<td>521</td>
<td>195</td>
<td>184</td>
<td>199</td>
<td>27</td>
<td>577</td>
<td>0.046</td>
</tr>
<tr>
<td>E0/AI</td>
<td>566 (n=14)</td>
<td>228</td>
<td>132</td>
<td>100</td>
<td>189</td>
<td>144</td>
<td>422</td>
<td>0.343</td>
</tr>
<tr>
<td>E0/AI/CETP</td>
<td>497 (n=12)</td>
<td>1071</td>
<td>196</td>
<td>132</td>
<td>134</td>
<td>34</td>
<td>463</td>
<td>0.074</td>
</tr>
<tr>
<td>LDLR0*</td>
<td>283±82</td>
<td>59±14</td>
<td>3</td>
<td>11</td>
<td>223</td>
<td>45</td>
<td>237</td>
<td>0.189</td>
</tr>
<tr>
<td>LDLR0/CETP</td>
<td>373±123</td>
<td>51±4</td>
<td>4</td>
<td>19</td>
<td>313</td>
<td>41</td>
<td>336</td>
<td>0.123</td>
</tr>
</tbody>
</table>

Total cholesterol was determined in plasma from all apoE0 mice (chow diet) used in atherosclerosis studies. Triglycerides and lipoprotein cholesterol profiles (Figure 1) were determined on plasma pools obtained from 5 animals in each group. Total cholesterol values from all mice and the lipoprotein cholesterol profile (HPGC) were used to estimate average lipoprotein cholesterol.

*Mean±SD plasma lipids determined in LDLR0 and LDLR0/CETP mice (4 to 5 per group) on a Western-type diet. Plasma lipoproteins were separated by preparative ultracentrifugation of pooled plasma samples.
Differences of mean values were significant by two-tailed t test as follows: ApoE0/CETP* 11.3 ± 0.060 vs ApoE0, P < 0.01; ApoE0/CETP vs LDLR0/CETP*, P < 0.04; ApoE0/CETP vs ApoE0/apoA1/CETP, P < 0.048.

 reduction in mean lesion area, and this was highly significant (Table 4). In apoE0/apoA1/CETP transgenic mice the mean lesion area was about 1.3-fold (females) or 1.7-fold (males) greater than in apoE0/apoA1 transgenic mice, but the differences were not significant.

Atherosclerosis Studies in LDLR0 Mice With CETP Transgene

Plasma and lipoprotein lipids for LDLR0 and LDLR0/CETP mice (Western-type diet) are shown in Table 1. CETP expression was associated with a moderate decrease in HDL cholesterol, an increase in LDL cholesterol, and a decreased ratio of HDL/non-HDL cholesterol (Table 1). In LDLR0/CETP transgenic mice, the mean atherosclerotic lesion area was approximately 1.6-fold (males) and 2.0-fold (females) greater than in LDLR0 mice after 3 months on the Western diet, and the combined data for both sexes showed that the differences were significant (P < 0.01) (Table 5). An analysis of plasma CETP activity showed marked induction of activity, as reported,12 with similar levels in apoE0/CETP and apoE0/apoA1/CETP transgenic mice (not shown). These levels are 5- to 10-fold higher than normal human levels and 2 to 3 times higher than observed in human dyslipidemia.12

Discussion

The present study demonstrates that overexpression of CETP increases the extent of atherosclerosis in apoE0 and LDLR0 mice. This establishes that CETP expression has proatherogenic effects in these well-established models of murine atherosclerosis. These findings and the low prevalence of coronary heart disease in CETP-deficient humans with HDL cholesterol >60 mg/dL3 support the concept that therapeutic inhibition of CETP might be atheroprotective.

Table 2: Fractional Catabolic Rates of Cholesteryl Ether in Plasma of Transgenic Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fractional Catabolic Rate (pools h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE0 (n=4)</td>
<td>0.130±0.045</td>
</tr>
<tr>
<td>ApoE0/CETP (n=4)</td>
<td>0.066±0.015</td>
</tr>
<tr>
<td>ApoE0/apoA1 (n=3)</td>
<td>0.086±0.007</td>
</tr>
<tr>
<td>ApoE0/CETP/apoA1/CETP (n=3)</td>
<td>0.085±0.007</td>
</tr>
</tbody>
</table>

Plasma decay of [H] radioactivity after injection of HDL containing [H]cholestereryl ether (see Figure 2) was used to calculate fractional catabolic rates by graphical analysis using Matthew's method.20 Values shown are mean±SD. Differences of mean values were significant by two-tailed t test as follows: apoE0 vs apoE0/CETP, P = 0.027; apoE0/apoA1 vs apoE0/apoA1/CETP, P = 0.040; apoE0/CETP vs ApoE0/apoA1/CETP, P = 0.048.

Table 3: Mean Atherosclerotic Lesion Area (μm²×10⁻³/section) in ApoE0 and ApoE0/CETP Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE0</td>
<td>51 ± 3.7 (3)</td>
<td>23.2 ± 23 (39)</td>
</tr>
<tr>
<td>ApoE0/CETP*</td>
<td>11.3 ± 4.6 (9)</td>
<td>54.5 ± 75 (5)</td>
</tr>
</tbody>
</table>

Combined data for both sexes. Animals were fed a chow diet for the period indicated, and extent of atherosclerosis (lipid-staining area) was assessed in sections of the proximal aorta (see Methods). The number of animals are shown in parentheses. *P < 0.02 for ApoE0/CETP vs ApoE0, by ANOVA, after log transformation of data.

Table 4: Mean Atherosclerotic Lesion Area (μm²×10⁻³/section) in Different Mice at 7 Months

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE0</td>
<td>84 ± 83 (11)</td>
<td>66 ± 75 (24)</td>
</tr>
<tr>
<td>ApoE0/CETP</td>
<td>131 ± 135 (14)</td>
<td>94 ± 96 (15)</td>
</tr>
<tr>
<td>ApoE0/apoA1*</td>
<td>27 ± 23 (18)</td>
<td>22 ± 22 (33)</td>
</tr>
<tr>
<td>ApoE0/apoA1/CETP†</td>
<td>35 ± 49 (13)</td>
<td>37 ± 50 (14)</td>
</tr>
</tbody>
</table>

*P < 0.001 for apoE0/apoA1 vs apoE0. †P < 0.01 for apoE0/apoA1/CETP vs apoE0.

The increase in atherosclerosis in apoE0/CETP mice compared with apoE0 mice may be related to increased levels of VLDL and IDL cholesterol, reduced levels of HDL cholesterol, and an impairment of reverse cholesterol transport. The reduced HDL and increased VLDL and IDL cholesterol is likely secondary to the CETP-mediated transfer of HDL CE into these lipoproteins. Lacking apoE, these particles cannot be removed from the plasma by receptor-mediated uptake and hence accumulate. Consistent with this model, the expression of the CETP transgene in apoE0 mice delayed the clearance of CE from plasma after injection of cholesteryl ether-labeled HDL. This contrasts with several earlier studies,8,11,26 and with our findings here with apoE0/apoA1 transgenic mice, in which CETP expression led to an acceleration in the clearance of CE from plasma after injection of HDL. The likely explanation for delayed clearance of plasma CE in apoE0/CETP transgenic mice is that as a result of CETP activity, HDL CE is transferred into the large pool of VLDL CE that slowly turns over.

This contrasting result also correlates with a different effect on atherosclerosis in apoC3/CETP transgenic mice, in which CETP expression is associated with increased clearance of HDL CE from plasma and a decrease in fatty streak lesions.11,27 Thus, the apparently contrasting effects of CETP expression on lesions could be related to reverse cholesterol transport, with different outcomes determined by VLDL clearance mechanisms. However, it is also possible that there are additional atherogenic effects of CETP expression and the consequently reduced HDL cholesterol, related to possible anti-inflammatory, antioxidant, or other effects of HDL in the arterial wall.28–32 Also, the redistribution of HDL and LDL cholesterol into VLDL and IDL particles in apoE0 mice containing the CETP transgene could influence atherosclerosis, simply because these animals are carrying more of their total plasma cholesterol in atherogenic VLDL and IDL particles33 (Table 1).

The expression of apoA1 resulted in a no significant change in fractional catabolic rate of plasma CE in apoE0 mice. Because the pool size of HDL CE is increased in apoA1

Table 5: Mean Atherosclerotic Lesion Area (μm²×10⁻³/section) in LDLR0 and LDLR0/CETP Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR0</td>
<td>92 ± 19 (4)</td>
<td>99 ± 84 (12)</td>
</tr>
<tr>
<td>LDLR0/CETP*</td>
<td>202 ± 105 (6)</td>
<td>156 ± 55 (6)</td>
</tr>
</tbody>
</table>

Mice (6 weeks old) were placed on a Western-type diet for 4 months, then atherosclerosis was evaluated in sections of the proximal aorta (see Methods). *P < 0.01 for LDLR0/CETP vs LDLR0 by ANOVA for combined data (both sexes) after log transformation.
transgenic mice, there is likely to be an increase in net transport of HDL CE in apoA1 transgenic plasma (transport = fractional catabolic rate × pool size),34 perhaps explaining the antiatherogenic effect of apoA1 overexpression in this model. The expression of the human CETP transgene in the human apoA1 transgenic background accelerated clearance (Table 2) and resulted in a greater absolute and percentage reduction of HDL cholesterol than does expression of the CETP transgene alone, when mouse HDL is the substrate.13 (Figure 1). In contrast to the delay in catabolism in ApoE0/CETP mice, the clearance of plasma CE was accelerated in apo E0/apoA1/CETP mice compared with apoE0/apoA1 transgenic mice. A possible explanation for this finding emerges from a recent study, which suggests that in apoA1/CETP transgenic mice, CETP increases the clearance of HDL CE from plasma to the liver by enhanced remodeling of HDL, followed by increased uptake by Scavenger Receptor-B1 in the liver (X. Collet et al, unpublished results 1998); this appears to be a major pathway for clearance of HDL CE from plasma.35,36 The marked remodeling and size change of HDL produced by CETP on a human apoA1 transgenic background could enhance interaction with hepatic SR-B1 and could explain why CETP accelerates clearance of HDL CE from plasma in the apoE0/apoA1 background while delaying clearance in apoE0 mice.

The increase in atherosclerosis in apoE0/CETP transgenic mice compared with apoE0 mice was moderate and highly variable, even though plasma CETP levels are induced to 5 to 10 times normal human levels in the animal models used here.12 There may be several explanations. The first is that the effect of CETP on atherosclerosis represents an effect of limited biological potency. By contrast, in the same experiment apoA1 overexpression caused a 3-fold highly significant reduction in lesion area, and the protective effect of apoA1 was not modified by CETP expression. The effects of CETP may be limited because while decreasing HDL and increasing VLDL and LDL cholesterol levels, there may be an alteration in HDL speciation, or other effects on HDL, to increase reverse cholesterol transport.37 Moreover, it appears that reducing HDL cholesterol in mice (eg, by apoA1 gene knock-out) has limited effects on atherosclerosis.37 This could reflect multiple redundant pathways of reverse cholesterol transport in the mouse, including a role for both apoA1 and apoA1V.38,39

The present study suggests the proatherogenic potential of CETP expression in settings in which there is a marked impairment in the clearance of remnants (apoE0) or LDL (LDLR0). This contrasts with earlier studies in which the expression of CETP with either an apoC3 or lecithin-cholesterol acyltransferase transgene reduced atherosclerotic lesions.27,40 The presence of marked hypertriglyceridemia in the apoC3 transgenic mice was thought to enhance the atheroprotective properties of CETP in this model. The presence of increased triglycerides in the apoE0/CETP mice indicates that hypertriglyceridemia may not be the primary determinant of atherosclerosis susceptibility in CETP transgenic mice. Similar increases in plasma triglyceride levels were observed in the apoC3/LDLR0 mice as a result of CETP transgene expression.41 Hypertriglyceridemics effects of CETP could be related to transfer of triglycerides out of large triglyceride-rich lipoproteins into smaller VLDL, IDL, and LDL,41 which are less favorable lipolytic substrates.

The human data on the relationship of CETP to atherosclerosis are also complex. Heterozygous CETP deficiency is associated with increased coronary heart disease risk, because of an excess of coronary heart disease in subjects with HDL cholesterol <60 mg/dL.5 Individual case reports also demonstrate coronary artery disease in a few subjects with hyperalphalipoproteinemia and combined CETP and hepatic lipase deficiencies.42 Moreover, the idea that genetic CETP deficiency is a longevity factor has been called into question.43 Nonetheless, population-based studies suggest that high HDL levels associated with genetic reductions in plasma CETP levels are associated with decreased coronary heart disease.5,44,45 Finally, a recent study in cholesterol-fed rabbits using liver-directed CETP antisense oligonucleotides showed a reduction in atherosclerosis associated with decreased CETP expression.46 Together, the animal and human data suggest that therapeutic inhibition of CETP may provide a viable experimental strategy for the treatment of atherosclerosis.

Acknowledgments

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References

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