Increased Preβ-high Density Lipoprotein, Apolipoprotein AI, and Phospholipid in Mice Expressing the Human Phospholipid Transfer Protein and Human Apolipoprotein AI Transgenes

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Abstract

Human plasma phospholipid transfer protein (PLTP) circulates bound to high density lipoprotein (HDL) and mediates both net transfer and exchange of phospholipids between different lipoproteins. However, its overall function in lipoprotein metabolism is unknown. To assess the effects of increased plasma levels of PLTP, human PLTP transgenic mice were established using the human PLTP gene driven by its natural promoter. One line of PLTP transgenic mice with moderate expression of PLTP mRNA and protein was obtained. The order of human PLTP mRNA expression in tissues was: liver, kidney, brain, small intestine > lung > spleen > heart, adipose tissue. Western blotting using a human PLTP monoclonal antibody revealed authentic human PLTP (M, 80 kD) in plasma. Plasma PLTP activity was increased by 29% in PLTP transgenic mice. However, plasma lipoprotein analysis, comparing PLTP transgenic mice to control littersmates, revealed no significant changes in the plasma lipoprotein lipids or apolipoproteins. Since previous studies have shown that human cholesteryl ester transfer protein and lecithin:cholesterol acyltransferase only function optimally in human apoAI transgenic mice, the human PLTP transgenic mice were cross-bred with human apoAI transgenic mice. In the human apoAI transgenic background, PLTP expression resulted in increased PLTP activity (47%), HDL phospholipid (26%), cholesteryl ester (24%), free cholesterol (37%), and apoAI (22%). There was a major increase of apoAI in preβ-HDL (56%) and a small increase in α-HDL (14%). The size distribution of HDL particles within α- and preβ-migrating species was not changed. The results suggest that PLTP increases the influx of phospholipid and secondarily cholesteryl into HDL, leading to an increase in potentially antiatherogenic preβ-HDL particles. (J. Clin. Invest. 1996. 96:2373–2380). Key words: transgenic mice • HDL lipoproteins • phospholipids • apolipoproteins A • carrier proteins

Introduction

Plasma high density lipoproteins (HDL) levels show an inverse relationship with atherosclerosis (1). The metabolism of HDL is regulated by lipases, lecithin:cholesterol acyltransferase (LCAT),1 and the plasma lipid transfer proteins, cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) (2). Plasma PLTP, a M, 80 kD glycoprotein, transfers phospholipids among lipoprotein particles (3, 4) and from lipid bilayers to HDL (3). There is accumulating in vitro evidence indicating that the PLTP plays an important role in the remodeling of lipoproteins. During lipolysis of apoB-containing lipoproteins, partially purified PLTP was shown to mediate both the transfer and exchange of phospholipids between these particles and HDL (5). PLTP can also cause conversion of HDL₃ to large (10.9 nm) and smaller (7.8 nm) particles in a time- and concentration-dependent fashion (6, 7). Furthermore, PLTP activity on HDL modulates the activities of LCAT and CETP (2, 4). Both human and mouse PLTP cDNA and gene have been cloned (8–10). The PLTP gene belongs to a family that includes CETP, lipopolysaccharide binding protein, and bactericidal permeability increasing protein genes (8). Mouse plasma PLTP activity is 1.5–2.0 times that of human (10). Murine PLTP activity and mRNA levels are up-regulated by a high fat/cholesterol diet and down-regulated by lipopolysaccharide administration (10). Although some information has been learned about the functions of PLTP in vitro, the overall role of the PLTP in lipoprotein metabolism is poorly understood.

Based on in vitro evidence, our working hypothesis is that in vivo PLTP mediates the net transfer of phospholipid from apoB-containing lipoproteins into HDL and perhaps from certain cell types into HDL. Thus the overexpression of human PLTP would result in increased HDL phospholipid and cholesteryl. To evaluate this hypothesis, we established lines of transgenic mice expressing the human PLTP gene. Mouse HDL is a relatively homogeneous population, while human HDL is more heterogeneous, consisting of discrete HDL₄ and HDL₃ subclasses. The difference appears to be due to the different physical properties of mouse and human apoAI (11, 12). Previous studies demonstrated that both CETP and LCAT show species-specific interaction with HDL, as the phenotypic effects of

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1. Abbreviations used in this paper: AI, apolipoprotein AI; CETP, cholesteryl ester transfer protein; FPLC, fast protein liquid chromatography; Hu, human; LCAT, lecithin:cholesterol acyltransferase; LPP, lysosomal protective protein; PC, phosphatidylcholine; PLTP, phospholipid transfer protein; Tg, transgenic.
expressing either of these human proteins in transgenic mice were enhanced when human apoAI was also expressed (13, 14). To determine whether human PLTP is also adapted to act specifically on human HDL, human PLTP transgenic mice were cross-bred with human apoAI transgenic mice.

Methods

Isolation and characterization of human PLTP genomic clones. Three P1 clones containing the entire human PLTP gene were obtained from Genome Systems Inc. (St. Louis, MO). The presence of the entire PLTP gene within these clones was confirmed by PCR amplification of the coding sequences using two sets of primers (5'-end primers: GGACTAGTCCCGGATCCCCTGAGCTGC and CGCGGATCT-GTGCGACGGCTGGG; 3'-end primers: CCACACCGTCACAGCAAGCT and GGGCTCTACAGGCTATGAACTG). Digestion of the three P1 clones with several restriction enzymes such as EcoRI, XbaI, SacI, and KpnI indicated that one of the clones (No. 2000) was different from the other two (Nos. 1999 and 2001), although all of them contained the entire PLTP gene. We chose the No. 2000 clone to create transgenic mice, since this clone contains a larger 5'-end flanking region than that of the Nos. 1999 and 2001. PCR analysis revealed that all three P1 clones contain another entire gene, lysosomal protective protein (LPP).

Creation of transgenic mice. To generate transgenic mice, the circular P1 clone (2000) was microinjected into the male pronuclei of fertilized mouse eggs taken from superovulated (C57BL/6J × CBA/J) F1 females. Injected embryos were implanted into the oviducts of surrogate females of the same genetic background (11). Human apoAI transgenic mice (line 427)(12) were crossed with F1 generation of PLTP transgenic mice. All comparisons of transgenic and nontransgenic mice are made between littermates.

DNA and RNA analysis. Tail tip DNA from 3-wk-old mice was used to screen by PCR for integration of human PLTP gene sequences within these clones. The amplification reaction using nontransgenic mouse DNA as a template yielded no amplification products. In some experiments, integration of the PLTP gene was detected by Southern blot hybridization. 10 μg genomic DNA was digested with StuI, electrophoresed in a 1% agarose gel, and transferred to a Hybond-N nylon membrane (Amersham, Arlington Heights, IL). UV was UV cross-linked to the membranes and hybridized with a 32P-radio-labeled 5'-end-labeled 200-bp PLTP cDNA as a probe.

Total RNA (30 μg) from the tissues of the transgenic and nontransgenic littermates was analyzed for PLTP and LPP mRNA by a solution hybridization–ribonuclease protection assay (15) using riboprobes highly specific for the human PLTP and human LPP mRNA, respectively. The riboprobe (220 nucleotides) for PLTP contains a portion of the vector (Bluescript KS+), and is complementary to 120 nucleotides of the 3'-untranslated region of the human PLTP mRNA.

PLTP monoclonal antibody preparation. PLTP was purified to homogeneity from human plasma using ultracentrifugation and a combination of phenyl-Sepharose, CM-cellulose, DEAE-cellulose, heparin-Sepharose, and hydroxyapatite chromato- graphy as described (4). To generate monoclonal antibodies to PLTP, BALB/c mice were immunized with purified human PLTP and hybridomas were generated by polyethylene glycol–mediated fusion. Hybridoma supernatants were screened by a solid phase radioimmunoa- nalysis using purified PLTP adsorbed to Immulon II Removawells (Dynatech, Cambridge, MA) as antigen and 125I-rabbit anti–mouse IgG to detect bound antibody. Positive hybridomas were twice recloned at 1 and 0.5 cells/well, respectively. To obtain large amounts of the monoclonal antibody, the hybridoma was injected into the peritoneal cavity of BALB/c mice and the ascitic fluid harvested about 2 wk later.

PLTP protein and activity analysis. 25 μl of plasma from Hu-PLTPtg and wild type as well as human was incubated at 37°C for 1 h with 475 μl of 20 mM Tris, pH 7.2, 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% SDS and 1% aprotinin. The human PLTP was immunoprecipitated with the anti-PLTP mAb (SD10)-agarose and dissolved in SDS loading buffer. The PLTP was analyzed by Western blotting with 125I-labeled anti-PLTP mAb (SD10), by procedures previously described for plasma CETP detection (16).

PLTP activity assay was performed as follows: 150 μl of fresh plasma was incubated with 150 μl of “H-PC vesicles (750 nmol PC), which was prepared as described previously (6), at 37°C for 30 min, then the density of the solution was adjusted to 1.063 g/ml and spun at 98,000 rpm for 4 h in an Optima TL ultracentrifuge (Beckman, Brea, CA). The tube was sliced. The top portion (800 μl) which includes VLDL, LDL, and “H-PC-vesicles was counted, while the radioactivity in the bottom portion (4.2 ml) which contains “H-PC-HDL, was taken to be the product of PLTP activity.

Plasma lipid and lipoprotein analyses. For small volumes of mouse plasma, HDL was separated from apoB-containing lipoproteins by using HDL cholesterol reagent (Sigma Chemical Co., St. Louis, MO). Using this method, an insignificant amount of mouse apoAI is precipitated (11). The total cholesterol and phospholipids in plasma, HDL, and column fractions were assayed by enzymatic methods (Wako Chemicals, Osaka, Japan). Lipoprotein profiles were obtained by means of fast protein liquid chromatography (FPLC) using a Sepharose 6B col- umn as described previously (17). A 200-μl aliquot of pooled plasma (from five animals) was loaded onto the column, and eluted with TS buffer (50 mM Tris, 0.15 M NaCl, pH 7.5) at a constant flow rate of 0.35 ml/min. An aliquot of 80 μl from each fraction (0.7 ml) was used for the determination of total cholesterol and phospholipid.

Quantitation of HDL subspecies. To determine the proportion of pre-β and α-electrophoretic mobility HDL species in the transgenic and nontransgenic mice, 20 μl plasma samples containing 1.5 mM 5,5'-dithio-bis-(2-nitrobenzoic acid) (to inactivate LCAT activity) was electrophoresed in 0.75% (wt/vol) agarose gel in 50 mM barbital buffer (pH 7.4, and then with a biotinylated goat polyclonal antibody to human apoAI (2 h at room temperature) in 2% milk in 10 mM phosphate buffer, pH 7.4, and then with a biotinylated goat polyclonal antibody to human apoAI (2 h at room temperature) in 2% milk in 10 mM phosphate buffer. ApoAI containing HDL species were visualized with 125I-Streptavidin (Amersham). Unbound 125I was washed 4 times with 1% milk in phosphate buffer and nitrocellulose membranes were exposed to Fuji XLS film at −70°C. The relative abundance of the human apoAI among the α- or preβ-HDL species was calculated by quanti- tative scanning using a Phosphorimager (Fuji, Stamford, CT). The distribution of human apoAI among the α-migrating HDL species was determined by two-dimensional gel electrophoresis. The first di- mensional gel electrophoresis was run on an agarose gel described above. The agarose strip was placed on a 3-16% polyacrylamide gradient gel (Integrated Separation Systems, Natick, MA) in 25 mM Tris-glycine buffer (pH 8.3). Electrophoresis was carried out for 4.5 h. Plasma proteins were transferred to NitroPlus transfer membranes and then immunoreacted with a goat polyclonal antibody to human apoAI as described above. The proportion of apoAI among the HDL fractions was determined by quantitative scanning using a Phosphor- imager. Each subfraction was individually quantified by a custom- ized box which isolated one fraction from another. Various exposure times were used to optimize the separation of the individual subspe- cies. Background was uniformly subtracted.

Statistical analysis. Results are expressed as mean±standard deviation. The statistical significance of the differences between the groups was estimated by the Student’s t-test. A P value less than 0.05 was considered significant.

Results

PLTP-containing P1 clone and PLTP transgenic mice. The PLTP-containing P1 clone (No. 2000) contains intact 5′ and
3' ends of the PLTP gene as determined by PCR with 5'- and 3'-specific primers, as well as by Southern blotting. Microinjection of supercoiled P1 DNA into fertilized embryos resulted in 34 offsprings, of which 2 were positive for human PLTP sequences by PCR with human-specific primers (see Methods). Southern blot analysis of these two PCR-positive mice showed the presence of multiple copies of the gene when compared with human genomic DNA and one founder had higher copy number (about 10 copies) than the other (about 3 copies). DNA from nontransgenic control mice did not hybridize with the human PLTP probe. The two founders were used to generate two lines of PLTP transgenic mice (HuPLTPTg).

**Tissue specificity of human PLTP expression.** RNase protection assay using a human-specific probe revealed that human PLTP mRNA was expressed in both transgenic lines. The order of human PLTP mRNA expression in the line with higher expression was as follows: liver, kidney, brain, small intestine > lung > spleen > heart, adipose tissue, skeletal muscle. This is similar to the distribution of PLTP mRNA in human tissues (8, 10). The PLTP mRNA abundance ranged from 10–100 pg/mg total RNA (Fig. 1), whereas the low copy number line only expressed very low levels, 1–5 pg/mg total RNA, in testis, kidney, and liver (data not shown). Mouse PLTP mRNA is expressed in a similar distribution at levels of 10–100 pg/mg total RNA (10). Analysis of murine PLTP mRNA in HuPLTPTg mice showed no alteration in pattern or levels of expression (data not shown). In order to study PLTP on a protein level, we developed a human PLTP monoclonal antibody (mAb, 5D10). This mAb, which is specific to human PLTP, immunoprecipitates 60% of human PLTP activity. Western blot using 5D10 revealed authentic human PLTP (M₉ 80 kD) expression at about 1.5–2.0-fold human plasma levels in the high expressor (Fig. 2). No human PLTP was detectable in the low expressor line. All subsequent analyses were done on the high expressor line.

Subsequent to the generation of HuPLTPTg mice we learned in a sequence database search that the 3' non-coding end of PLTP mRNA is exactly complementary to the 3' non-coding end of LPP mRNA. This indicates that PLTP and LPP which were previously localized to chromosome 20q12-q13.1 and 20q13.1, respectively (18, 19), are convergently transcribed from opposite DNA strands, with overlapping complementary 3' ends. The PLTP and LPP gene overlap exists not only in the human but also in the mouse genome. Based on the cDNA sequences, the overlapping region in human is > 57-bp and in mouse is > 64-bp. The P1 clone (#2,000) was found to contain in close proximity to the PLTP gene an intact gene for the human LPP. The HuPLTPTg mice also express LPP mRNA. RNase protection assay using a human-specific probe for LPP revealed a different pattern of LPP mRNA distribution in this animal compared to PLTP mRNA (Fig. 3). Furthermore, the low expressor HuPLTPTg line expressed the same level of LPP as the high expressor. Thus, despite their close proximity, these two genes are unlikely to be coordinately regulated.

**PLTP activity plasma lipids and lipoprotein analysis.** To measure PLTP activity, we incubated PC vesicles in whole plasma and measured transfer of PC radioactivity into HDL. PLTP activity was increased in HuPLTPTg mice at all time points.
and nontransgenic littermates under both fed and fasted conditions. There was no significant difference in plasma lipoprotein lipid concentrations between human PLTP transgenic (HuPLTPTg) and nontransgenic mice (Tables I and II). However, we observed several differences between HuPLTPTg and HuAITg mice. The phospholipid and cholesterol levels in plasma of HuAIPLTPTg mice were increased significantly under both fed (phospholipid, 22%, P < 0.02; cholesterol, 23%, P < 0.05) and, fasted (phospholipid, 17%, P < 0.05; cholesterol, 20%, P < 0.05) conditions compared to those found in HuAITg mice (Table I). These changes were due to increases in HDL-phospholipid (26%, P < 0.02, and 20%, P < 0.05, for fed and fasted conditions, respectively), HDL-cholesterol ester (24%, P < 0.01, and 22%, P < 0.05, for fed and fasted conditions, respectively), HDL–free-cholesterol (37%, P < 0.01, and 33%, P < 0.02, for fed and fasted conditions, respectively) (Table II). There were no obvious changes in VLDL and LDL lipids (Table II). FPLC gel filtration analysis using pooled plasma confirmed that there were increases of plasma HDL-phospholipid and HDL-cholesterol in HuAIPLTPTg conditions

**Table I. Plasma Lipid Composition in Wild Type, HuPLTPTg, HuAITg, and HuAIPLTPTg Mice**

<table>
<thead>
<tr>
<th>Mice</th>
<th>PL</th>
<th>TC</th>
<th>CE</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Tg</td>
<td>174±21</td>
<td>73±5</td>
<td>67±7</td>
<td>186±39</td>
</tr>
<tr>
<td>HuPLTPTg</td>
<td>182±15</td>
<td>70±3</td>
<td>71±5</td>
<td>162±41</td>
</tr>
<tr>
<td>HuAITg</td>
<td>280±26</td>
<td>135±19</td>
<td>126±19</td>
<td>282±69</td>
</tr>
<tr>
<td>HuAIPLTPTg</td>
<td>342±32</td>
<td>166±21</td>
<td>133±31</td>
<td>263±41</td>
</tr>
<tr>
<td><strong>Fasted</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Tg</td>
<td>185±31</td>
<td>75±7</td>
<td>65±8</td>
<td>58±10</td>
</tr>
<tr>
<td>HuPLTPTg</td>
<td>178±23</td>
<td>77±6</td>
<td>66±10</td>
<td>57±9</td>
</tr>
<tr>
<td>HuAITg</td>
<td>266±30</td>
<td>130±14</td>
<td>121±22</td>
<td>85±12</td>
</tr>
<tr>
<td>HuAIPLTPTg</td>
<td>312±26</td>
<td>156±18</td>
<td>130±24</td>
<td>89±15</td>
</tr>
</tbody>
</table>

5 ml of plasma was used for total cholesterol, free cholesterol, phospholipid, and triglyceride measurement using enzymatic methods kits (Wako Chemicals). *P < 0.05, HuAITg vs HuAIPLTPTg; †P < 0.02, HuAITg vs HuAIPLTPTg. Values are mean±SD of 8–9 animals/group.

**Table II. Lipoprotein Lipid Concentration in Wild Type, HuPLTPTg, HuAITg, and HuAIPLTPTg Mice**

<table>
<thead>
<tr>
<th>Mice</th>
<th>HDL-PL</th>
<th>HDL-CE</th>
<th>HDL-FC</th>
<th>V+LDL-PL</th>
<th>V+LDL-CE</th>
<th>V+LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Tg</td>
<td>154±21</td>
<td>48±4</td>
<td>6±2</td>
<td>57±8</td>
<td>15±3</td>
<td>6±2</td>
</tr>
<tr>
<td>HuPLTPTg</td>
<td>157±19</td>
<td>45±3</td>
<td>5±3</td>
<td>63±12</td>
<td>15±2</td>
<td>5±1</td>
</tr>
<tr>
<td>HuAITg</td>
<td>240±22</td>
<td>97±12</td>
<td>8±3</td>
<td>41±8</td>
<td>24±5</td>
<td>6±2</td>
</tr>
<tr>
<td>HuAI/PLTPTg</td>
<td>302±19</td>
<td>120±18</td>
<td>11±2</td>
<td>39±7</td>
<td>26±4</td>
<td>8±4</td>
</tr>
<tr>
<td><strong>Fasted</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Tg</td>
<td>165±17</td>
<td>49±9</td>
<td>6±2</td>
<td>32±11</td>
<td>14±5</td>
<td>6±3</td>
</tr>
<tr>
<td>HuPLTPTg</td>
<td>158±14</td>
<td>54±8</td>
<td>6±1</td>
<td>36±8</td>
<td>17±6</td>
<td>7±2</td>
</tr>
<tr>
<td>HuAITg</td>
<td>226±25</td>
<td>95±9</td>
<td>9±2</td>
<td>39±5</td>
<td>20±3</td>
<td>8±4</td>
</tr>
<tr>
<td>HuAI/PLTPTg</td>
<td>272±14</td>
<td>116±17</td>
<td>12±2</td>
<td>41±7</td>
<td>22±4</td>
<td>8±3</td>
</tr>
</tbody>
</table>

HDL was separated from V+LDL by using HDL cholesterol reagent (Sigma). The total cholesterol, free cholesterol, and phospholipid concentrations were determined by enzymatic methods kits (Wako Chemicals). *P < 0.05, HuAITg vs HuAIPLTPTg; †P < 0.02, HuAITg vs HuAIPLTPTg; ‡P < 0.01, HuAITg vs HuAIPLTPTg. Values are mean±SD of 8–9 animals/group.
pared with HuAITg mice (Fig. 5). These increases were coincident with the main HDL peak.

We also measured human apoAI levels in HuAITg and HuAIPLTPTg mice as well as mouse apoAI in wild type and HuPLTPTg mice. The mouse apoAI concentration was similar in wild type and HuPLTPTg mice (Table III). However, HuAIPLTPTg mice had significantly higher human apoAI concentration than HuAITg mice (Table III). As the increases in HDL phospholipid, cholesterol, cholesteryl ester, and apoAI were proportional to each other (Tables I–III), these changes indicated that the HDL particle numbers were increased in HuAIPLTPTg mice relative to HuAITg mice. SDS polyacrylamide gel electrophoresis confirmed the increase of human apoAI in HuAIPLTPTg mice compared with HuAITg mice and there were no obvious changes in the levels of other apolipoproteins (data not shown).

The effect of PLTP on HDL subclass distribution was studied by 2-dimensional non-denaturing gradient gel electrophoresis. Preβ-HDL was markedly increased (56%, $P < 0.01$)

Table III. Distribution of Human apoAI Among Preβ and α-migrating HDL in HuAITg and HuAIPLPTg Mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>ApoAI</th>
<th>Preβ-HDL</th>
<th>α-HDL</th>
<th>HDL-PL/ApoAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Tg</td>
<td>107±13</td>
<td>2.1±0.6</td>
<td>105±12</td>
<td>1.45±0.12</td>
</tr>
<tr>
<td>HuPLTPTg</td>
<td>101±9</td>
<td>1.8±0.6</td>
<td>99±8</td>
<td>1.51±0.23</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HuAITg</td>
<td>288±30</td>
<td>59±17</td>
<td>229±20</td>
<td>0.78±0.16</td>
</tr>
<tr>
<td>HuAIPLTPTg</td>
<td>352±43*</td>
<td>93±21*</td>
<td>260±36†</td>
<td>0.77±0.21</td>
</tr>
</tbody>
</table>

Lipoproteins fractions from fasted HuAITg and HuAIPLPTg mice were separated by agarose gel electrophoresis and transferred to nitrocellulose membranes as described (see Methods). Mouse apoAI was visualized with rabbit polyclonal antibody to mouse apoAI. Areas containing preβ and α-migrating HDL were visualized and quantitated using a Phosphorimager. Values shown are mean±SD, n = 8–9. *$P < 0.01$, †$P < 0.05$.

in plasma from HuAIPLPTg mice compared to HuAITg mice plasma and α-HDL levels were moderately increased (14%, $P < 0.05$) as well (Fig. 6 and Table III). Preβ-HDL are distributed into three different sized populations located in the preβ electrophoretic mobility range described for human plasma (20). The expression of the PLTP transgene did not affect the size of the preβ-HDL species.

HuAITg mice α-HDL consists of four distinct populations of particles with sizes corresponding to the human HDL2b, HDL3a, and HDL3b (12, 14). Although total α-HDL was increased in HuAIPLPTg mice by 14% compared with HuAITg mice (Table III), there were no significant changes in the proportion of its different subclasses (Table IV).

In order to eliminate the possibility that the overexpression human LPP rather than PLTP caused the phenotype observed in HuAIPLPTg mice, we crossed PLTP low-expressor mice, which expressed the same level of LPP as high-expressor mice, with HuAITg mice. There were no differences between HuAITg and HuAIPLPTP (low expressor) Tg mice comparing plasma phospholipid, cholesterol, cholesteryl ester, HDL-phospholipid, and HDL-cholesterol, indicating that human LPP overexpression did not influence plasma HDL lipid levels.

Discussion

In this study, we have utilized mice expressing human PLTP and apoAI transgenes to show that, in vivo, expression of human PLTP caused moderate increases in HDL phospholipid, free cholesterol, cholesteryl ester and apoAI levels and markedly promoted the accumulation of small HDL with preβ electrophoretic mobility. Since preβ-HDL has been shown to be the optimal mediator of initial cellular cholesterol efflux (20), these results suggest that the activity of PLTP may enhance the initial steps of the reverse cholesterol transport pathway.

Previous in vitro experiments showed that partially purified PLTP mediates both the transfer and exchange of phospholipid between triglyceride-rich lipoproteins and HDL during lipolysis (5). The hypothesis to be tested in this transgenic approach was that human PLTP overexpression would promote net phospholipid movement into HDL, and as a result,
The genomic clone used for the generation of HuPLTPtg mice has the gene for the LPP in very close proximity to the 3' end of the PLTP gene. LPP is a multifunctional protein associated with beta-galactosidase and neuraminidase in lysosomes, protecting them from degradation (21); it also has serine esterase activities (22, 23). As LPP is primarily an intracellular protein, it is unlikely to interact with plasma lipoproteins. It is also unlikely to interact intracellularly with PLTP as it does with beta-galactosidase or neuraminidase because the tissue distribution of LPP and PLTP mRNAs are dissimilar (Figs 1 and 3). We consider a close coordinate regulation of the two genes to be unlikely based on the dissimilar tissue distribution of their mRNAs and on our observation that in the low-expressor HuPLTPtg mice, the level of LPP expression is similar to that in the high-expressor HuPLTPTg mice. Furthermore, we determined the PLTP activity in galactosialidosis patients due to LPP deficiency (24) and did not find the alteration of PLTP activity in those patients compared with controls (Jiang, X.-C., and Tall, A., unpublished observation) indicating that LPP expression is not needed for PLTP activity or expression. Although a definite conclusion regarding the relevance of LPP to lipid metabolism will have to await the generation of PLTP transgenic mice not expressing LPP, the available evidence suggests that the findings reported here are PLTP specific.

Preβ-HDL are small, phospholipid rich HDL particles containing apoAI only (25) that have been observed in different species (26, 27). In human, the average concentration of preβ-HDL is about 5–10% of the plasma apoAI (28). Higher concentrations are found in lymph (29), aortic intima, (30) and plasma from hypertriglyceridemic subjects (31). Although several studies (20, 32, 33) have proposed a key role of this HDL fraction in the initial steps of cell-derived cholesterol transport,
the origin and molecular mechanisms responsible for their formation remain poorly understood.

The increase in preB particle numbers can arise from either an increase in formation or a decrease in catabolism. It has been suggested that nascent, discoidal HDL could correspond to the pool of particles that is rapidly cleared from plasma, and a portion of this apoprotein then reappears in a slowly turning-over pool that constitutes the major mass of apoAI (34). One possibility is that free apoAI or minimally lipidated apoAI, which may be either synthesized and secreted by cells, or released during the processing of HDL (35, 36), is rapidly catabolized, but that addition of phospholipids to it by PLTP leads to a longer half-life for the resulting preB particles.

Another possibility for the increase of apoAI in HuA-IPLTPTg mouse plasma may be due to changes in the preB-HDL/α-HDL cycle which is catalyzed by hepatic lipase and LCAT. Hepatic lipase reduces the lipid core of triglyceride-rich HDL and promotes the dissociation of apoAI and possibly phospholipid, inducing the formation of preB-HDL (37). LCAT provides the driving force for the formation choleryl ester which enters the core of preB-HDL and produces the mature form of HDL, α-HDL (38). Thus, PLTP expression in the human apoAI background may enhance the phospholipid enrichment in HDL, perhaps stimulate hepatic lipase activity and increase conversion of α-HDL to preB-HDL. By increasing the influx of phospholipid, PLTP expression may also prevent the decrease of phospholipid content due to LCAT action on α-HDL, thus preventing the putative fusion of preB-α particles (39), leading to an increase in preB-HDL particles.

Recent studies (6, 7, 40) have demonstrated that PLTP can promote conversion of an apparently homogenous population of HDL₂ particles into a new population with an increased average size, apparently as a result of fusion between HDL particles and a concomitant release of free apoAI (40). PreB-HDL was not measured in these studies. We did not observe a difference in HDL size between either HuPlTPtg and wild type mice or between HuA-IPLTPTg and HuA-Ig mice, suggesting that in vivo the conversion process is counteracted by other activities.

Previous studies indicated that preB-HDL participates in the initial steps of reverse cholesterol transport pathway, i.e., the centripetal movement of cholesterol from peripheral tissues or from macrophage foam cells in the arterial wall to the liver via the plasma compartment (38). Excess cholesterol in peripheral tissues may be removed by preB-HDL which in conjunction with LCAT action, provides the driving force for net cholesterol movement. However, preB particles may also be formed by cellular interactions of lipid-free apoAI that dissociates from HDL particles (32). If this is the case, the amount of preB particles in plasma may reflect an active reverse cholesterol transport pathway rather than drive it. Our results support the involvement of PLTP in the reverse cholesterol pathway. Further studies on cholesterol efflux and cholesterol esterification in HaA-IPLTPTg mice will be needed to ascertain the role of PLTP in reverse cholesterol transport and atherogenesis.

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