PLTP deficiency improves the anti-inflammatory properties of HDL and reduces the ability of LDL to induce monocyte chemotactic activity

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Abstract **We reported that phospholipid transfer protein (PLTP) deficiency decreased atherosclerosis in mouse models. Because the decreased atherosclerosis was accompanied by a significant decrease in plasma HDL levels, we examined the properties of PLTP knockout (PLTP0) HDL and tested its ability to prevent LDL-induced monocyte chemotactic activity in human artery wall cell cocultures. We isolated HDL and LDL from LDL receptor knockout/PLTP knockout (LDLr0/PLTP0) mice and from apolipoprotein B transgenic (apoBTg)/PLTP0 mice as well as their controls. PLTP0 HDL was relatively rich in protein and depleted in phosphatidylcholine. Turnover studies revealed a 3.5- to 4.0-fold increase in the turnover of protein and cholesteryl ester in HDL from PLTP0 mice compared with control mice. The ability of HDL from LDLr0/PLTP0 and apoBTg/PLTP0 mice to prevent the induction of monocyte chemotactic activity in human artery wall cell cocultures exposed to human LDL was dramatically better than that in controls. Moreover, LDL from PLTP0 mice was markedly resistant to oxidation and induced significantly less monocyte chemotactic activity compared with that in controls. In vitro, PLTP0 HDL removed significantly more oxidized phospholipids from LDL than did control HDL. We conclude that PLTP deficiency improves the anti-inflammatory properties of HDL in mice and reduces the ability of LDL to induce monocyte chemotactic activity.**—Yan, D., M. Navab, C. Bruce, A. M. Fogelman, and X-C. Jiang. **PLTP deficiency improves the anti-inflammatory properties of HDL and reduces the ability of LDL to induce monocyte chemotactic activity. *J. Lipid Res.* 2004.** 45: **1852–1858.**

Supplementary key words high density lipoprotein • low density lipoprotein • phospholipid transfer protein • phospholipid

There is accumulating in vitro evidence indicating that plasma phospholipid transfer protein (PLTP) plays an important role in the remodeling of lipoproteins. During lipolysis of apolipoprotein B (apoB)-containing lipoproteins (BLp), partially purified PLTP was shown to mediate both the transfer and exchange of phospholipid between these particles and HDL (1–3). Furthermore, PLTP activity on HDL modulates the activities of LCAT and cholesteryl ester transfer protein (4, 5).

Genetically altered mouse models have played a crucial role in elucidating the role of PLTP in lipoprotein metabolism and atherosclerosis. PLTP-deficient mice provided in vivo evidence for PLTP-mediated lipid transfer in the maintenance of lipoprotein levels (6) and in modulating the development of atherosclerosis. PLTP deficiency resulted in markedly decreased atherosclerosis (7) attributable in part to *1*) decreased production and levels of BLp (7) and *2*) increased bioavailability of vitamin E in atherogenic lipoproteins (8). In apoB transgenic (apoBTg) and apoE-0 backgrounds, PLTP deficiency resulted in reduced production and levels of BLp and markedly decreased atherosclerosis. BLp secretion was diminished in hepatocytes from apoBTg/PLTP knockout (PLTP0) mice, a defect that was corrected when PLTP was reintroduced by adenoviral vector (7). These studies revealed a major, unexpected role of PLTP in regulating the secretion of BLp and identified PLTP as a therapeutic target. Furthermore, PLTP deficiency increased vitamin E content in BLp in four mouse models of atherosclerosis, producing a dramatic delay in the generation of conjugated dienes in BLp as well as markedly lower titers of plasma IgG autoantibodies to oxidized LDL (8). PLTP deficiency greatly reduced atherosclerotic lesions in mouse models, despite decreased HDL levels (7).

Recently, it was reported that a 2.5- to 4.5-fold increase in PLTP activity in PLTPTg mice resulted in a 30% to 40%

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Abbreviations: apoB, apolipoprotein B; BLp, apolipoprotein B-containing lipoprotein; CE, cholesteryl ester; FCR, fractional catabolic rate; FPLC, fast-protein liquid chromatography; LDLr0, LDL receptor knockout; PC, phosphatidylcholine; PLTP, phospholipid transfer protein; PLTP0, phospholipid transfer protein knockout; SM, sphingomyelin; Tg, transgenic; WT, wild-type.

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decrease in plasma HDL-cholesterol levels and the induction of atherosclerotic lesions in these mice (9, 10). Compared with wild-type (WT) mice, these mice also showed moderate stimulation of VLDL secretion (11).

Most recently, we demonstrated that a 2-fold increase in PLTP activity in mice mediated by adenovirus-associated virus infection resulted in *1*) decreased HDL cholesterol, HDL phospholipid, and apoA-I levels; *2*) decreased vitamin E content in total plasma and in individual lipoprotein fractions; *3*) increased lipoprotein oxidation as assessed by copper-induced formation of conjugated dienes; *4*) increased autoantibodies against oxidized apoB-containing particles; and *5*) increased atherosclerotic lesions in the proximal aorta (12).

Because the decreased atherosclerosis in PLTP0 mice was always accompanied by a significant decrease in HDL levels, we hypothesized that although HDL levels in PLTP-deficient mice are significantly lower than those of controls, PLTP-deficient HDL particles might have improved anti-inflammatory properties. The studies reported here support that hypothesis.

MATERIALS AND METHODS

Animals

The PLTP0 mice were backcrossed onto a C57BL/6 background (nine backcrosses) and then intercrossed with human apoBTg mice and LDL receptor knockout (LDLr0) mice, each with a C57BL/6 background. The mice were fed Purina Rodent Chow (No. 5001).

Lipid and lipoprotein measurements

Fasted blood was collected for lipoprotein isolation and lipid measurement. Total cholesterol, free cholesterol, and phospholipids in plasma and lipoprotein were assayed by enzymatic methods (Wako Pure Chemical Industries Ltd., Osaka, Japan). Cholesteryl ester (CE) concentration was calculated by subtracting the amount of free cholesterol from the total plasma cholesterol. Choline-containing phospholipids and sphingomyelin (SM) were measured as described (13). Phosphatidylcholine (PC) was calculated by subtracting the amount of SM from the choline-containing phospholipids.

In vivo turnover studies

Except as otherwise noted, HDL was isolated by ultracentrifugation ($1.063 < d < 1.21$ g/ml). For heterologous turnover studies, PLTP0 and control mice were injected intravenously in the femoral vein with WT mouse HDL labeled with [^{125}I]CE and [^{3}H]CE (1.1×10^6 and 0.95×10^6 cpm, respectively). For autologous studies, PLTP0 and control mice were injected intravenously with their own HDL that was labeled with [^{125}I]CE and [^{3}H]CE (3×10^6 and 2×10^6 cpm, respectively). After injection, blood (70 μl) was taken from the tail vein at 0.25, 0.5, 1, 2, 4, 8, and 24 h for determination of radioactivity. The fractional catabolic rates (FCRs) for protein and lipid were calculated from the decay curves of [^{125}I]CE, and [^{3}H]CE radioactivity in whole plasma was determined according to the Matthews method (14). The production rates were calculated by multiplying the FCR by the plasma pool and dividing by the body weight (15).

Human artery wall cocultures and monocyte chemotaxis assay

Human monocytes were prepared and chemotaxis assays were performed as described (16, 17). As previously reported (18) in the coculture assay, HDL isolated by fast-protein liquid chromatography (FPLC), ultracentrifugation, or gel electrophoresis gives similar results and there is no difference between the results obtained with whole HDL versus HDL_2 or HDL_3. In general, the cocultures were treated with native LDL (250 μg of LDL-cholesterol per milliliter) in the absence or presence of HDL (50 μg of HDL-cholesterol per milliliter) for 8 h. The supernatants were removed and the cocultures washed, and fresh culture medium 199 (M199; Gibco) without any additions was added and incubated for an additional 8 h. This allowed for the collection of monocyte chemotactic activity released by the cells as a result of stimulation by the oxidized LDL. At the end of incubation, the supernatants were collected from cocultures, diluted 40-fold, and assayed for monocyte chemotactic activity. Briefly, the supernatant was added to a standard Neuroprobe chamber (Neuroprobe, Cabin John, MD), with monocytes added to the top. The chamber was incubated for 60 min at 37°C. After the incubation, the chamber was disassembled and the nonmigrated monocytes were wiped off. The membrane was then air dried and fixed with 1% glutaraldehyde and stained with 0.1% Crystal Violet dye. The number of migrated monocytes was determined microscopically and expressed as the mean ± SD of 12 standardized high-power fields counted in quadruple wells.

Paraoxonase activity assay

Pooled plasma (5,300 μl) was separated by FPLC. Each FPLC fraction was assayed for paraoxonase activity using paraoxon as a substrate (19). The reaction was initiated by the addition of the FPLC fraction to a cuvette containing 1.0 mM paraoxon in 20 mM Tris-HCl, pH 8.0, and the increase in the absorbance at 405 nm was recorded over a 90 s period. Blanks were included to correct for the spontaneous hydrolysis of paraoxon. Enzymatic activity was calculated from the molar extinction coefficient 1,310 M^{-1} cm^{-1}. One unit of paraoxonase activity is defined as 1 nmol of 4-nitrophenol formed per minute under the above assay conditions (19).

Oxidized lipid transfer assay

Preparation of labeled, oxidized LDL. [9,10-^{3}H]palmitoyl phosphatidylcholine with arachidonic acid in the *sn*-2 position (20 μl; DuPont-NEN) in toluene solution was dried under N_2 gas for 10 min. The dried [^{3}H]PC was dissolved in 50 μl of ethanol and then added to 1 ml of human plasma [containing 1.5 mM 5,5-dithiobis(2-nitrobenzoic acid) to inactivate LCAT activity] slowly (drop by drop) while stirring. The plasma density was increased to 1.006 g/ml and centrifuged at 98,000 rpm for 2 h to separate labeled VLDL. Then, the bottom solution was adjusted to a density of 1.063 g/ml and spun at 98,000 rpm for 4 h to separate labeled LDL. Copper sulfate was added (5 μM final concentration) to the labeled LDL solution and incubated overnight at 37°C to oxidize.

Isolation of HDL from WT and PLTP0 mice. VLDL/LDL-depleted mouse plasma was increased to a density of 1.21 g/ml and spun at 98,000 rpm for 4 h to separate HDL. The HDL was desalted.

Oxidized lipid absorption assay. HDL (250 μg of protein, 200 μl) from WT or PLTP0 mice was incubated with labeled, oxidized LDL (100 μg of protein, 1×10^7 cpm, 30 μl) for 30 min. The LDL and HDL particles were then separated by sequential flotation, first at $d = 1.063$ g/ml and second at $d = 1.21$ g/ml. The radioactivity in the HDL fraction represents the amount of oxidized PC transferred to HDL from LDL. Alternatively, the LDL

can be precipitated by 40 μl of HDL reagent (Sigma). The radioactivity in the supernatant was measured as representing the amount of oxidized PC transferred to HDL from LDL.

Statistical analysis

Differences between groups were tested by Student's *t*-test. Data are presented as means ± SD.

RESULTS

Anti-inflammatory properties of PLTP0 HDL

HDL particles from apoBTg/PLTP0 (**Fig. 1B**) and LDLr0/PLTP0 (Fig. 1C) mice showed significant inhibition of monocyte chemotactic activity induced by human LDL compared with their controls (apoBTg vs. apoBTg/PLTP0, $P < 0.01$; LDLr0 vs. LDLr0/PLTP0, $P < 0.01$). Furthermore, HDL particles from apoBTg and LDLr0 mice showed significantly less inhibitory activity compared with normal human HDL (Fig. 1; apoBTg HDL vs. normal HDL, $P < 0.01$; LDLr0 HDL vs. normal HDL, $P < 0.01$). We also found that LDL taken from mice with PLTP deficiency induced significantly less monocyte chemotactic activity (**Fig. 2**).

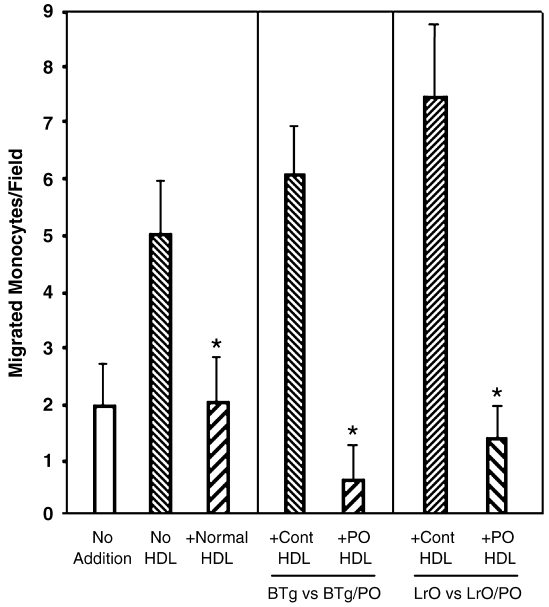

Fig. 1. HDL particles from apolipoprotein B (apoB) transgenic/phospholipid transfer protein knockout (apoBTg/PLTP0) and LDL receptor knockout (LDLr0)/PLTP0 mice prevented LDL-induced monocyte chemotactic activity in a human artery wall coculture, whereas HDL from apoBTg and LDLr0 mice did not. The cocultures were treated with native LDL (250 μg of LDL-cholesterol per milliliter) in the absence or presence of HDL (50 μg of HDL-cholesterol per milliliter) for 8 h. The supernatants were removed and the cocultures washed, and fresh culture medium 199 (M199; Gibco) without any additions was added and incubated for an additional 8 h. At the end of the incubation, the supernatant was removed to a standard Neuroprobe chamber with monocytes added to the top. The chamber was incubated for 60 min at 37°C. After the incubation, the membrane was air dried, fixed with 1% glutaraldehyde, and stained with 0.1% Crystal Violet dye. The number of migrated monocytes was determined microscopically and is expressed as the mean ± SD of 12 standardized high-power fields counted in quadruple wells, and significance was determined by Student's *t*-test. * $P < 0.01$. Normal HDL, normal human HDL; Cont, control.

Paraoxonase activity in PLTP0 mice

As an explanation for the improved anti-inflammatory properties of HDL in PLTP-deficient mice, we considered the possibility that paraoxonase activity in these animals might be higher than that of controls, because it is known that higher paraoxonase activity can effectively prevent LDL oxidation (20). However, we found that paraoxonase activity was reduced by ~2-fold and 4-fold, respectively, in apoBTg/PLTP0 and LDLr0/PLTP0 mice, relative to their controls (**Fig. 3**). These decreases are similar to the decrease in the HDL mass in PLTP0 mice of these genetic backgrounds (7). Because HDL is the carrier for paraoxonase (20), the decrease in paraoxonase activity was proportional to the decrease in HDL.

Ability of PLTP0 HDL to remove oxidized lipids from LDL

Next, we examined whether PLTP0 HDL might have a greater capacity to extract oxidized lipids from LDL, relative to WT HDL particles. We incubated oxidized, [^{3}H]PC-labeled LDL with PLTP0 HDL or control HDL, separated the LDL and HDL by ultracentrifugal flotation, and counted the radioactivity associated with HDL. We found

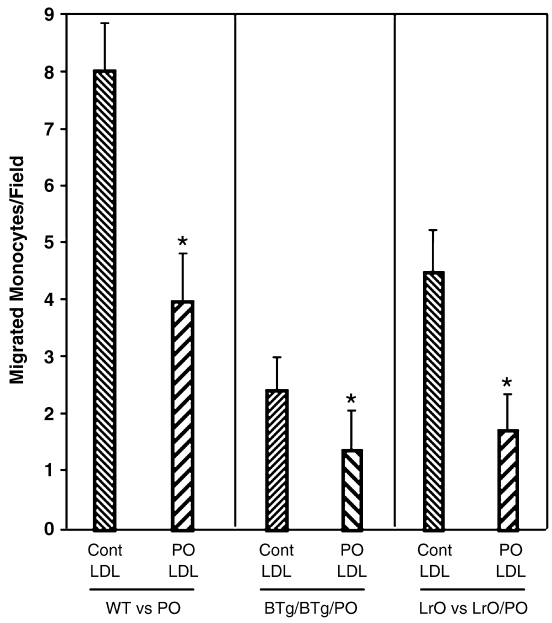

Fig. 2. LDL from apoBTg/PLTP0 and LDLr0/PLTP0 mice induce significantly less monocyte chemotactic activity than controls. The cocultures were treated with LDL (250 μg of LDL-cholesterol per milliliter) for 8 h. The supernatants were removed and the cocultures washed, and fresh culture medium 199 (M199; Gibco) without any additions was added and incubated for an additional 8 h. At the end of the incubation, the supernatant was removed to a standard Neuroprobe chamber and monocyte chemotactic activity was determined as described for Fig. 1. * $P < 0.01$. Cont, control; WT, wild-type.

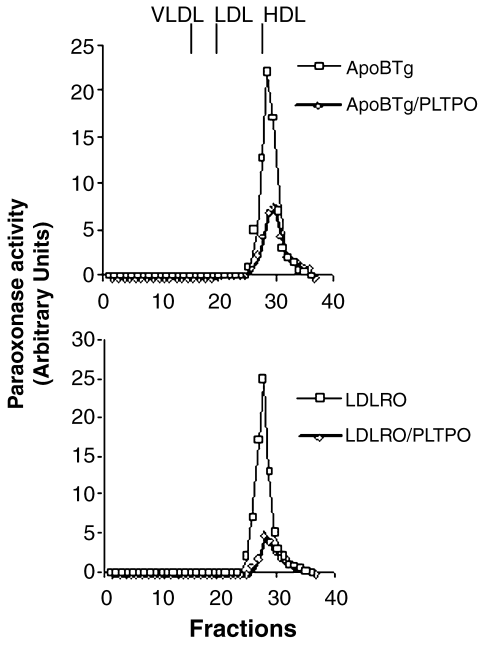

Fig. 3. Paraoxonase activity was decreased in PLTP0 mice. Pooled plasma (5,300 μl) was separated by fast-protein liquid chromatography (FPLC), and each fraction was assayed for paraoxonase activity using paraoxon as a substrate (19). The reaction was initiated by the addition of the 100 μl of FPLC fraction into a cuvette containing 100 μl of 1.0 mM paraoxon in 20 mM Tris-HCl, pH 8.0. The increase in the absorbance at 405 nm was recorded over a 90 s period. Blanks were included to correct for the spontaneous hydrolysis of paraoxon. Enzymatic activity was calculated from the molar extinction coefficient 1,310 M^{-1} cm^{-1}. One unit of paraoxonase activity is defined as 1 nmol of 4-nitrophenol formed per minute under the above assay conditions (19).

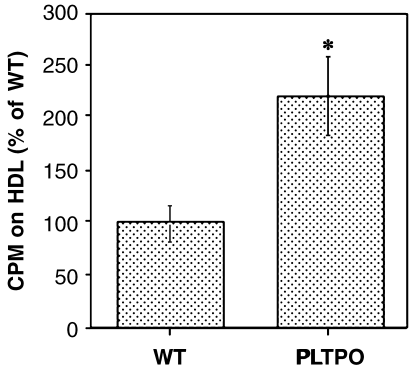

Fig. 4. PLTP0 HDL particles absorb labeled, oxidized phosphatidylcholine (PC) from LDL better than control HDL. The oxidized lipid absorption assay was done as described in Materials and Methods. Briefly, HDL (250 μg of protein, 200 μl) from WT or PLTP0 mice was incubated with labeled, oxidized LDL (100 μg of protein, 1×10^7 cpm, 30 μl) for 30 min. The LDL and HDL particles were then purified by sequential flotation, first at d = 1.063 g/ml and second at d = 1.21 g/ml. The radioactivity in the HDL fraction represents the amount of oxidized PC transferred to HDL from LDL. The data shown are representative of three independent experiments. The error bars represent the average ± SD. WT vs. PLTP0, * $P < 0.01$.

that PLTP0 HDL absorbed 2.2 times more labeled PC from LDL than did control HDL (**Fig. 4**). We confirmed these results by precipitating LDL and determining the radioactivity in the HDL-containing supernatant (data not shown).

Composition of PLTP0 HDL

We isolated HDL from apoBTg/PLTP0 and apoBTg mice and from LDLr0/PLTP0 and LDLr0 mice and determined their composition. Like HDL from PLTP0 mice (13), the particles from apoBTg/PLTP0 and LDLr0/PLTP0 mice were enriched in protein (59% and 56% of HDL mass, respectively) compared with HDL from the control mice (apoBTg and LDLr0), which contained 41% and 46% protein, respectively (**Table 1**). HDL from PLTP0 mice was deficient in PC (Table 1). VLDL and LDL from PLTP0 mice were relatively SM poor (Table 1). The PC/SM ratio was higher in VLDL and LDL and lower in HDL of PLTP0 animals.

Clearance and production of HDL in PLTP0 mice

We hypothesized that the HDL composition changes in PLTP0 mice might increase their turnover rate, thus transporting more oxidized lipid from LDL or peripheral tissue into the liver for degradation. Indeed, our previous work revealed that HDL turnover rate was significantly increased in PLTP0 mice compared with WT mice (13). To see whether this is also true in apoBTg/PLTP0 and LDLr0/PLTP0 mice, we determined the FCR using autologous and heterologous HDL in these mice and their controls. Autologous HDL was doubly labeled with [^{3}H]CE and [^{125}I]CE. In this experiment, the FCRs of [^{3}H]CE-HDL and [^{125}I]HDL in apoBTg/PLTP0 and LDLr0/PLTP0 mice were three and four times, respectively, greater than those of their controls (**Fig. 5**, **Table 2**). Based on the FCR, body weight, and pool size, we calculated the production rates of HDL-CE and HDL-protein for both PLTP0 mice and their controls and found that the difference was insignificant.

In the heterologous HDL turnover study, HDL particles from WT mice were isolated and double labeled with [^{3}H]CE and [^{125}I]CE. The doubly labeled HDL particles were then injected into WT, PLTP0, apoBTg, apoBTg/PLTP0, LDLr0, and LDLr0/PLTP0 mice. The FCRs for both [^{3}H]CE and [^{125}I]apolipoprotein in PLTP-deficient mice were approximately twice that found in control mice (**Fig. 6**, **Table 3**).

DISCUSSION

In this study, we followed up on our earlier observation that PLTP0 mice are less atherosclerosis susceptible by examining the properties of PLTP0 HDL with regard to its ability to prevent LDL-induced monocyte chemotactic activity in human artery wall cell cocultures. Circulating LDL from normal humans always contains trace amounts of oxidized lipids (17, 21). The production of monocyte chemotactic activity in the human artery wall cocultures is

TABLE 1. Lipid and protein composition of lipoproteins from PLTP0 mouse strains and their controls

Mice	SM	PC	FC	CE	TG	Protein	PC/SM
				%			
Non-HDL							
apoBTg	6.6 ± 0.3	28.4 ± 4.1	8.1 ± 2.8	28.7 ± 3.2	5.1 ± 0.8	23.1 ± 1.9	4.3 ± 0.2
apoBTg/PLTP0	4.4 ± 0.4[a]	28.1 ± 5.0	8.6 ± 1.7	29.5 ± 6.8	5.5 ± 0.9	23.9 ± 4.3	6.4 ± 0.3[b]
LDLr0	7.2 ± 0.4	27.1 ± 3.3	8.7 ± 0.4	27.2 ± 2.2	23.1 ± 4.2	6.7 ± 0.9	3.8 ± 0.2
LDLr0/PLTP0	3.3 ± 0.6[a]	31 ± 2.0	9.2 ± 1.3	27.4 ± 3.1	22.6 ± 2.7	6.5 ± 0.5	9.3 ± 0.7[a]
HDL							
apoBTg	5.8 ± 0.8	28.6 ± 2.5	4.8 ± 0.7	17.1 ± 1.9	3.7 ± 0.5	40 ± 4.4	4.9 ± 0.6
apoBTg/PLTP0	4.7 ± 1.0	13.5 ± 2.5[a]	4.4 ± 0.2	15.2 ± 2.3	3.2 ± 0.8	59 ± 6.6[a]	2.9 ± 0.3[a]
LDLr0	6.1 ± 0.7	27.3 ± 3.9	4.9 ± 0.9	16.7 ± 2.2	3.7 ± 0.8	41.3 ± 3.0	4.5 ± 0.2
LDLr0/PLTP0	5.5 ± 0.6	15.0 ± 2.1[a]	4.4 ± 0.3	15.6 ± 1.8	3.4 ± 0.6	56.1 ± 5.5[a]	2.7 ± 0.9[a]

apoBTg, apolipoprotein B transgenic; CE, cholesteryl ester; FC, free cholesterol; LDLr0, LDL receptor knockout; PC, phosphatidylcholine; PLTP0, phospholipid transfer protein knockout; SM, sphingomyelin; TC, total cholesterol; TG, triglyceride.

[a] $P < 0.01$.

[b] $P < 0.05$.

attributable to the ability of the artery wall cells to add additional oxidized lipids to LDL (17, 21). When a critical threshold of these oxidized lipids is reached in the LDL that has been added to the cocultures, phospholipids in the LDL that contain arachidonic acid in the *sn*-2 position are oxidized, producing a series of oxidized phospholipids that stimulate the artery wall cells to produce the potent monocyte chemoattractant MCP-1 (17, 21). PLTP deficiency renders HDL anti-inflammatory and significantly reduces the ability of LDL to induce monocyte chemotactic activity. Further characterization of the HDL from mice of three different genetic backgrounds with and without PLTP gene expression suggests that the changes observed in PLTP0 animals may be attributable to their greater ability to absorb oxidized phospholipids and the greater FCR of HDL in these animals.

During the development of an atherosclerotic lesion, LDL acquires oxidized phospholipids from vascular endothelial cells generated by the 12-lipoxygenase pathway. Such oxidized LDL can induce monocyte-induced chemotactic activity that leads to later stages of lesion development (21). Normal HDL, purified apoA-I, as well as purified paraoxonase can interact with oxidized LDL and inhibit this biological activity (16, 17).

Paraoxonase on HDL has been shown to have a protective effect against the biological activity of oxidized LDL (20). In our study, we compared the activity of paraoxonase from mice with and without PLTP expression. We found that on an HDL-protein weight basis, PLTP0 animals had approximately the same enzyme activity of the appropriate control strain. We thus conclude that the inhibitory effect that PLTP0 HDL particles display against

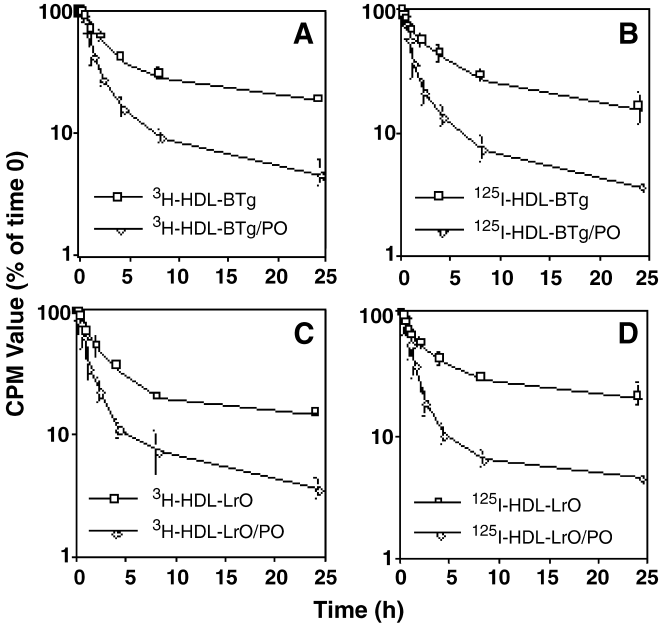

Fig. 5. Plasma fractional catabolic rates (FCRs) for [^{125}I]CE- and [^{3}H]cholesteryl ester ([^{3}H]CE)-labeled HDL in mice (autologous). PLTP0 and control mice were injected intravenously with their own HDL labeled with [^{125}I]CE and [^{3}H]CE (3×10^6 and 2×10^6 cpm, respectively). Blood (70 μl) was taken from the tail vein at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h for determination of radioactivity. A, B: [^{125}I]CE- and [^{3}H]CE-labeled HDL decay curves in apoBTg and apoBTg/PLTP0 mice. C, D: [^{125}I]CE- and [^{3}H]CE-labeled HDL decay curves in LDLr0 and LDLr0/PLTP0 mice. Values shown are means ± SD.

TABLE 2. Plasma FCRs for autologous, labeled HDL in PLTP0 mouse strains and their controls

Mice	[^{3}H]CE-HDL	[^{125}I]CE-HDL
	pools/h	
apoBTg	0.106 ± 0.017	0.099 ± 0.021
apoBTg/PLTP0	0.393 ± 0.051[a]	0.376 ± 0.065[a]
LDLr0	0.121 ± 0.033	0.118 ± 0.021
LDLr0/PLTP0	0.435 ± 0.071[a]	0.413 ± 0.052[a]

FCR, fractional catabolic rate. Mice were injected intravenously (femoral vein) with their own HDL labeled with [^{125}I]CE and [^{3}H]CE. Blood was collected periodically from mice over 24 h after injection of labeled HDL. The FCRs for protein and lipid were calculated from the decay curves of [^{125}I]CE and [^{3}H]CE radioactivity in whole plasma according to the Matthews method (14). The values shown are means ± SD, and significance was determined by Student's *t*-test.

[a] $P < 0.01$.

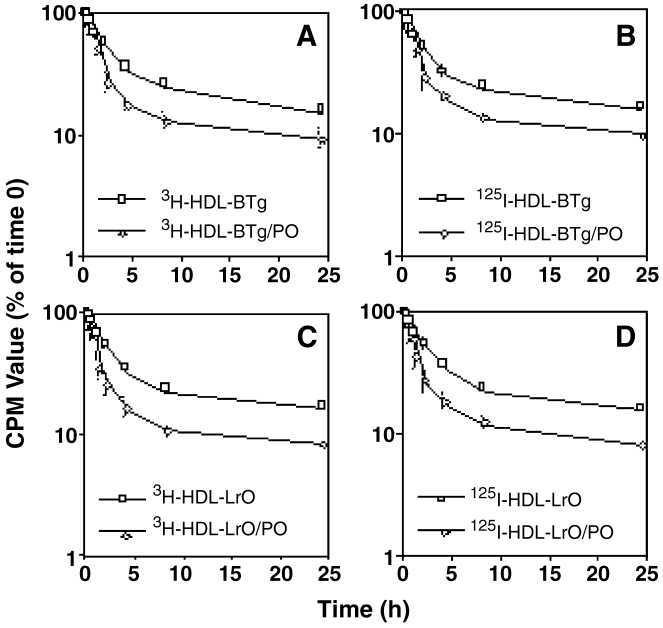

Fig. 6. Plasma FCRs for [^{125}I]CE- and [^{3}H]CE-labeled HDL from WT mice (heterologous). PLTP0 and control mice were injected intravenously (femoral vein) with WT HDL labeled with [^{125}I]CE and [^{3}H]CE (1.1×10^{6} and 0.95×10^{6} cpm, respectively). Blood (70 μl) was taken from the tail vein at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h for determination of radioactivity. A, B: [^{125}I]CE- and [^{3}H]CE-labeled HDL decay curves in apoBTg and apoBTg/PLTP0 mice. C, D: [^{125}I]CE- and [^{3}H]CE-labeled HDL decay curves in LDLr0 and LDLr0/PLTP0 mice. Values shown are means ± SD.

TABLE 3. Plasma FCRs for heterologous, labeled HDL in PLTP0 mouse strains and their controls

Mice	[^{3}H]CE-HDL	[^{125}I]CE-HDL
	pools/h	
apoBTg	0.117 ± 0.032	0.099 ± 0.026
apoBTg/PLTP0	0.251 ± 0.057[a]	0.211 ± 0.033[a]
LDLr0	0.129 ± 0.024	0.111 ± 0.011
LDLr0/PLTP0	0.277 ± 0.041[a]	0.237 ± 0.032[a]

PLTP knockout and control mice were injected intravenously (femoral vein) with [^{125}I]CE- and [^{3}H]CE-labeled HDL from wild-type mice. Blood was collected periodically from mice over 24 h after injection of labeled HDL. The FCRs for protein and lipid were calculated from the decay curves of [^{125}I]CE and [^{3}H]CE radioactivity in whole plasma according to the Matthews method (14). The values shown are means ± SD, and significance was determined by Student's *t*-test.

[a] $P < 0.01$.

the proinflammatory effects of oxidized LDL must be attributable to other factors.

Graham et al. (22) report that the phospholipid fraction of HDL inhibits LDL oxidation in a paraoxonase-independent manner. They found that phospholipid vesicles prepared from HDL_3 particles are able to inhibit LDL oxidization. Several studies have shown that in vivo, apoA-I or its homologs, infused into plasma, acquire cholesterol and phospholipids and are also able to absorb peroxidized fatty acids from LDL (17). We found PLTP0 HDL to have a 2.2-fold greater absorptive capacity for oxidized phospholipids compared with HDL from WT mice (Fig. 4) when normalized for HDL protein mass. These studies were done in the presence of DTNB, an LCAT inhibitor, and EDTA, a paraoxonase inhibitor. The form of oxidized PC used in this experiment is not a preferred substrate for LDL-associated phospholipase. Thus, the radioactivity recovered in the HDL fraction is unlikely to be an enzymatically produced derivative.

The higher affinity of PLTP0 HDL for oxidized phospholipids is likely attributable to the lipid-poor composition of these particles. HDL's lipid composition is determined by several enzymatic and lipid transport activities. HDLs of either nascent or remnant form are lipid poor relative to mature HDLs, as they consist primarily of apoA-I, phospholipids, and cholesterol. As these small HDLs are acted upon by LCAT and they absorb more cholesterol from cell membranes, their lipid ratio increases and they become rounder and larger. The absence of PLTP blocks the entry of surface remnants into the pool of small, lipid-poor HDLs. As LCAT is still able to react PC with cholesterol, the surface layer of these HDLs can be expected to incorporate hydrophobic molecules. Alternatively, the conformation of the apolipoprotein may be altered in such a way to extract phospholipids from the surface of LDL.

Turnover studies revealed that, compared with control animals, the PLTP0 mouse HDL-CE has a much greater FCR and a production rate that is not significantly different (Table 2) (13). Thus, the oxidized lipid transport capacity of plasma HDL per unit of time can be estimated to be (absorption ratio = 2.2) × (concentration ratio = 0.30) × (FCR ratio = 3.8) = 2.5-fold higher in PLTP0 mice than in WT mice. This calculation suggests that the greater oxidized lipid transport capacity of plasma HDL in PLTP-deficient animals may be an important contributor to the inhibition of atherosclerosis in these animals.

A possible explanation for the increased fractional catabolism of HDL in PLTP-deficient mice is that lipid-poor HDLs are more prone to removal from the circulation. A similar phenomenon is observed in ABCA1-deficient humans and mice. Compositional analysis of plasma lipoproteins suggests that the absence of ABCA1 leads to profound changes in the phospholipid composition of HDL that, in turn, alters its metabolic stability and maturation, leading to the virtual absence of HDL in the plasma of ABCA1-deficient humans (23) and mice (24).

PLTP deficiency leads to HDL that is protein rich and PC poor, which has an accelerated catabolism (Figs. 5, 6, Tables 2, 3). The fact that the FCR of HDL is increased by ~2-fold in PLTP0 mice when the labeled HDL is of WT origin indicates that the absence of PLTP is sufficient for the rapid removal of HDL. This enhanced removal of WT HDL could be attributable to alterations in the circulating HDL in the absence of PLTP that enhance removal, or it might indicate a general increase in removal mechanisms in the absence of PLTP. When the HDL is of autologous origin, the increase in FCR relative to control animals is even greater (i.e., ~4-fold). We can surmise that the presence of PLTP alters the composition of HDL, and conversely, HDLs with different compositions interact with

PLTP with different efficiencies, and that both processes affect HDL removal from plasma.

Although HDL levels are generally known to be a negative risk factor in human populations, we have shown that PLTP-deficient mice with low HDL have decreased levels of lesion development (7). Our results indicate that the characteristics of HDL are just as important as its plasma concentration in determining the outcome of physiologic processes that lead to atherosclerotic lesion development. Among patients with premature coronary artery disease in the Framingham Study population, ~25% did not have a detectable abnormality in their lipid profiles (25). In the original Framingham Study, ~44% of clinical events occurred in patients with normal HDL-cholesterol levels (26). Recent studies suggest that the inflammatory/anti-inflammatory properties of HDL may separate patients with coronary heart disease from normal subjects better than HDL-cholesterol levels (26). The studies reported here indicate that PLTP may play a critical role in determining the inflammatory/anti-inflammatory properties of HDL.

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