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Macrophage PLTP is atheroprotective in LDLr-deficient mice with systemic PLTP deficiency

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Abstract **Systemic phospholipid transfer protein (PLTP) is a recognized risk factor for coronary heart disease. In apolipoprotein E-deficient mice, systemic PLTP deficiency is atheroprotective, whereas PLTP overexpression is proatherogenic. As expected, we also observed significantly smaller lesions ($P < 0.0001$) in hypercholesterolemic double mutant low density lipoprotein receptor-deficient ($LDLr^{-/-}$) PLTP-deficient ($PLTP^{-/-}$) mice compared with $LDLr^{-/-}$ mice expressing systemic PLTP. To assess the specific contribution of only macrophage-derived PLTP to atherosclerosis progression, bone marrow transplantation was performed in $LDLr^{-/-}$ mice that also lacked systemic PLTP. Groups of double mutant $PLTP^{-/-}LDLr^{-/-}$ mice were irradiated with 1,000 rad and injected with bone marrow (BM) cells collected from either $PLTP^{-/-}$ or wild-type mice. When fed a high-fat diet, BM cell expression of PLTP decreased plasma cholesterol of $PLTP^{-/-}LDLr^{-/-}$ mice from 878 ± 220 to 617 ± 183 mg/dl and increased HDL cholesterol levels from 54 ± 11 to 117 ± 19 mg/dl. This decreased total plasma cholesterol and increased HDL cholesterol contributed to the significantly smaller atherosclerotic lesions in both aortas and heart sinus valves observed in these mice. Thus, unlike total systemic PLTP, locally produced macrophage-derived PLTP beneficially alters lipoprotein metabolism and reduces lesion progression in hyperlipidemic mice.**—Valenta, D. T., J. J. Bulgrien, D. J. Bonnet, and L. K. Curtiss. **Macrophage PLTP is atheroprotective in LDLr-deficient mice with systemic PLTP deficiency.** ***J. Lipid Res.*** **2008.** 49: **24–32.**

Supplementary key words phospholipid transfer protein • atherosclerosis • bone marrow transplant • inflammation • low density lipoprotein receptor

Increased plasma phospholipid transfer protein (PLTP) levels are a risk factor for coronary heart disease in both mice (1) and humans (2). Studies in mice show that overexpression of plasma PLTP activity decreases plasma HDL cholesterol levels (3–5). In apolipoprotein E-deficient ($apoE^{-/-}$) mice, adenovirus-mediated overexpression of systemic PLTP increases lesion development with a concomitant decrease in HDL cholesterol and apoA-I levels and reduced protection against lipoprotein oxidation (6). However, recent epidemiological studies report an inverse correlation between plasma PLTP concentrations and the incidence of coronary heart disease (7). In addition, we recently demonstrated that PLTP expression by macrophages significantly reduces atherosclerosis in hypercholesterolemic male low density lipoprotein receptor-deficient ($LDLr^{-/-}$) mice (8). This is in contrast to the findings of Vikstedt et al. (9), who reported that macrophage-derived PLTP is proatherogenic in female mice fed a Western-style diet.

Such complex and sometimes contradictory characteristics of PLTP are primarily attributed to its role in lipoprotein remodeling through its lipid transfer activities. It is particularly important in the regulation of HDL populations, which have well-described antiatherogenic properties (10, 11). PLTP can increase lipoprotein susceptibility to harmful oxidative damage by altering vitamin E distribution (12), yet it can beneficially improve cellular cholesterol efflux from peripheral tissues by increasing the rate of reverse cholesterol transport (13, 14). In the latter pathway, HDL accepts excess cholesterol from peripheral tissues and transports it to the liver for secretion into bile (15). An early event in reverse cholesterol transport involves the transfer of free cholesterol and phospholipid from cell membranes to small lipid-poor or lipid-free apoA-I (16). The lipid transfer activities of PLTP studied in vitro convert triglyceride-rich spherical HDL_3 into large (10.9 nm) and small (7.8 nm) particles with a concomitant release of the lipid-poor apoA-I needed for efflux (17, 18).

The generation of these lipid-poor, preβ-migrating molecules (preβ-HDL) is a prerequisite for the transfer of cholesterol out of cells via the cell membrane-bound ABCA1. Systemic overexpression of human PLTP in transgenic mice increases preβ-HDL generation but decreases plasma HDL (19, 20), suggesting a proatherogenic rather than an antiatherogenic function of PLTP. However, we showed that PLTP expression in peripheral tissues alters lipoprotein metabolism, even when liver expression and plasma activity remain unchanged (8). In that previous study, we examined the role of macrophage PLTP expression using bone marrow transplantation (BMT) and

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demonstrated that macrophage-derived PLTP is atheroprotective in LDLr$^{-/-}$ irradiated recipient male mice that still express systemic PLTP. This suggests that the influence of PLTP on atherogenesis is highly dependent upon its site of expression (21). The present studies extend these observations and examine the effect of macrophage-derived PLTP on lipoprotein metabolism and atherosclerotic lesion development in hypercholesterolemic LDLr$^{-/-}$ mice that lack systemic PLTP.

MATERIALS AND METHODS

Animals and facilities

All mice in this study were on a C57Bl/6 background. PLTP-deficient (PLTP$^{-/-}$) mice were a kind gift from Drs. X-C. Jiang and A. R. Tall (Columbia University). Control C57Bl/6 mice were from the Scripps rodent facility, and LDLr$^{-/-}$ mice were from a colony maintained in-house with founder mice purchased from Jackson Laboratories (Bar Harbor, ME). PLTP$^{-/-}$ mice were crossed with LDLr$^{-/-}$ mice to generate the double knockout, PLTP$^{-/-}$LDLr$^{-/-}$ mice. After weaning, all mice were fed a chow diet (diet 5015; Harlan Teklad) until 8–9 weeks of age. Blood samples were collected by retro-orbital puncture using heparin-coated capillary tubes from fasted animals anesthetized with isoflurane. Blood was transferred to EDTA-coated tubes kept on ice. All procedures were performed in accordance with institutional guidelines.

BMT

BMT was performed according to methods described previously (22). Two cohorts of male PLTP$^{-/-}$LDLr$^{-/-}$ mice at 8–9 weeks of age were irradiated with a single dose of 1,000 rad. Bone marrow (BM) cells extracted from tibias and femurs of age-matched PLTP$^{-/-}$ or C57Bl/6 mice were injected intravenously (2×10^6 cells/mouse) into recipient PLTP$^{-/-}$LDLr$^{-/-}$ mice via the tail vein. The two recipient groups with 18 mice each will hereafter be designated PLTP$^{-/-}$ BMT and PLTP$^{+/+}$ BMT mice. The diet of mice from the two BMT groups was changed at 3 weeks after BMT from a chow to an atherogenic, high-fat diet (HFD) containing 15.8% (w/w) fat, 1.25% (w/w) cholesterol, and no cholate (diet 94059; Harlan Teklad). All mice were fed the HFD for 16 weeks. Repopulation of donor BM in recipient PLTP$^{-/-}$LDLr$^{-/-}$ mice was assessed by PCR using PLTP mRNA isolated from blood.

Plasma lipids, apoA-I, PLTP activity, serum amyloid A, and tumor necrosis factor levels

Plasma was isolated from blood by centrifuging at 5,000 rpm for 5 min and stored at −80°C until use. Total plasma and HDL cholesterol levels were measured with a colorimetric kit (Thermo), with HDL cholesterol levels determined after precipitation of the VLDL and LDL fractions with phosphotungstic acid. Phospholipid and triglyceride levels were determined using commercially available kits (Wako, Raichem). Specific sandwich ELISAs were used to measure mouse apoA-I (23), serum amyloid A (SAA) (Biosource), and tumor necrosis factor-α (TNF-α) (Pharmingen) levels in plasma. Plasma PLTP activity was measured as described previously (23, 24).

Fast-protein liquid chromatography fractionation of plasma

Plasma was fractionated by fast-protein liquid chromatography (FPLC) using two Superdex 200 columns in series with 50 μl of pooled plasma from five mice per group applied to the column. Fractions (0.5 ml) were collected after elution of the columns with buffer containing 10 mM Tris, 1 mM EDTA, and 150 mM NaCl (pH 7.4). Cholesterol distribution in the different lipoprotein fractions was determined using a fluorescence detection method (22).

Lipid-poor/free apoA-I analysis

Levels of circulating lipid-poor/free apoA-I in plasma were analyzed by native polyacrylamide gel electrophoresis. Pooled (n = 5) plasma samples diluted 1:3 in sample buffer (16% sucrose and 0.01% bromophenyl blue) were electrophoresed on nondenaturing, nonreducing 4–26% polyacrylamide gels at 110 V for 18 h at 4°C. Plasma from non-BMT LDLr$^{-/-}$ and PLTP$^{-/-}$LDLr$^{-/-}$ mice that were fed either the chow diet or the

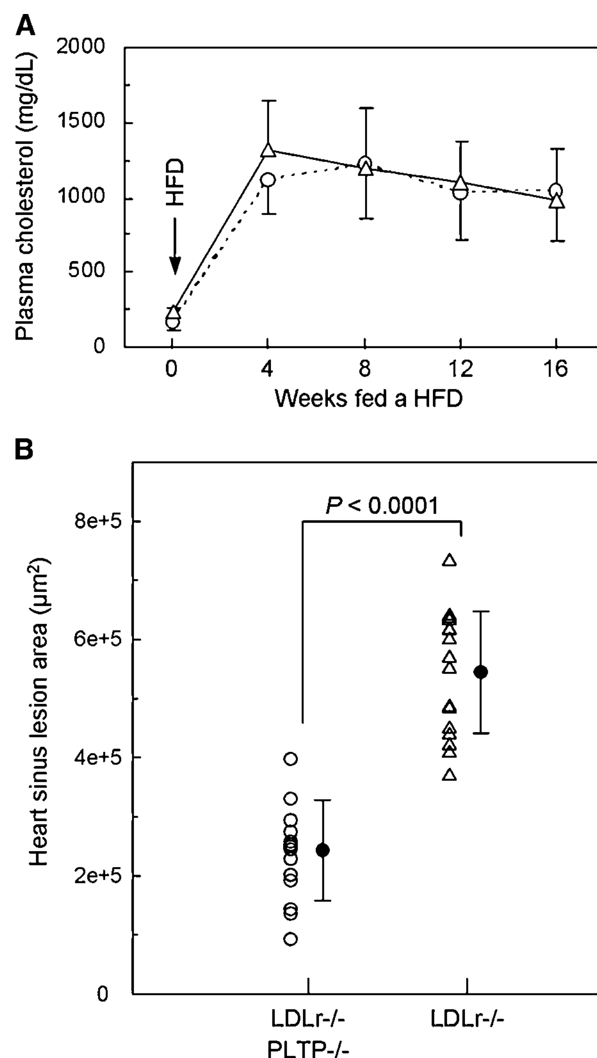

Fig. 1. Total plasma cholesterol levels and atherosclerosis in low density lipoprotein receptor-deficient (LDLr$^{-/-}$) and double mutant phospholipid transfer protein-deficient (PLTP$^{-/-}$)LDLr$^{-/-}$ mice. A: Total plasma cholesterol levels in PLTP$^{-/-}$LDLr$^{-/-}$ (circles; n = 17) and LDLr$^{-/-}$ (triangles; n = 17) mice before and after consuming the high-fat diet (HFD). Values are means ± SD. B: Lesion size in Oil Red O-stained heart sinus valves sectioned from hearts dissected from mice that had consumed the HFD for 16 weeks. Closed circles on graph B represent means and SD of each data set.

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HFD for 8 weeks was examined. In PLTP$^{-/-}$LDLr$^{-/-}$ BM recipients, lipid-poor/free apoA-I levels were compared in plasma collected from the two BMT groups before and after BMT (chow diet) and again at 8 weeks after consuming the HFD. Separated proteins were transferred to polyvinylidene difluoride membranes and blotted with rabbit anti-mouse apoA-I (Biodesign). Bound antibody was detected with an ECL chemiluminescence kit (Invitrogen). The density of the lipid-poor apoA-I fraction was determined using AlphaErase FC software (Alpha Innotech).

Assessment of atherosclerosis

Lesion size in the aortas and heart valves was measured to compare the extent of atherosclerosis in mice from the different groups according to methods described elsewhere (25, 26). After consuming the HFD for 16 weeks, the mice were euthanized and perfused with paraformaldehyde (4% paraformaldehyde and 5% sucrose in PBS) and the hearts and aortas were extracted. En face lesion areas were assessed after staining of cleaned, cut-open, and pinned aortas with Sudan IV. Frozen sections of heart valves stained with Oil Red O were examined to measure aortic valve lesion areas. Lesion quantification was achieved by averaging the area of fatty streaks in the valve cusps of four sections 40 μm apart. Statistical differences in mean heart valve lesion area and aortic en face lesion area between mice in the BMT and non-BMT study groups were calculated using the Mann-Whitney *U* test for nonparametric data.

RESULTS

PLTP deficiency in hypercholesterolemic LDLr$^{-/-}$ mice

As expected, total plasma cholesterol levels in LDLr$^{-/-}$ and double mutant PLTP$^{-/-}$LDLr$^{-/-}$ mice increased considerably after a change in diet from chow to the atherogenic HFD (**Fig. 1A**). However, despite no differences in plasma cholesterol levels between the groups, the size of atherosclerotic lesions in the heart valve cusps of mice fed the HFD for 16 weeks was significantly higher in LDLr$^{-/-}$ mice that expressed systemic PLTP compared with PLTP$^{-/-}$LDLr$^{-/-}$ mice (Fig. 1B). Similarly, mean lesion size measured in isolated en face aortas was greater in LDLr$^{-/-}$ mice (21.1 ± 5.9%) than in PLTP$^{-/-}$LDLr$^{-/-}$ mice (11.7 ± 4.3%) ($P < 0.0001$). Although HDL cholesterol levels were lower in chow-fed PLTP$^{-/-}$LDLr$^{-/-}$ mice, HDL cholesterol levels did not differ significantly between the two groups while they consumed the HFD (**Table 1**). Plasma triglyceride concentrations were consistently lower ($P < 0.01$) in LDLr$^{-/-}$ mice compared with LDLr$^{-/-}$PLTP$^{-/-}$ mice. The majority of plasma cholesterol was present in the VLDL and LDL lipoprotein fractions, as revealed by FPLC separation (**Fig. 2A**). These FPLC separations also suggested that a systemic deficiency of PLTP may lead to a wide range of large HDL. HDL analyzed by native polyacrylamide gel electrophoresis showed that circulating lipid-poor/free apoA-I was significantly greater in LDLr$^{-/-}$ mouse plasma compared with PLTP$^{-/-}$LDLr$^{-/-}$ mouse plasma after 8 weeks of consuming the HFD (Fig. 2B, C).

The lipid transfer activity of plasma PLTP is known to affect lipoprotein remodeling in vivo (3, 4), with gene expression upregulated by oxysterols through the liver X receptor (27). In LDLr$^{-/-}$ mice, plasma PLTP activity was not observed to differ from wild-type

TABLE 1. Changes in plasma lipids and apoA-I levels in the different mouse groups when fed a chow diet or HFD

Lipid	No.	Before Bone Marrow Transplantation	Weeks Fed a HFD				
			0	4	8	12	16
LDLr$^{-/-}$PLTP$^{-/-}$							
HDL C	5	—	37.9 ± 11.4[a]	52.2 ± 16.4	47.7 ± 5.1	62.2 ± 20.6	58.9 ± 13.2
Total PL	25	—	198.9 ± 38.6[a]	757.7 ± 169.0	672.0 ± 101.9	673.7 ± 54.3	733.7 ± 75.6
HDL PL	25	—	38.3 ± 6.4[a]	36.4 ± 9.8	36.8 ± 2.1[b]	29.1 ± 13.5	29.8 ± 6.7
TG	10	—	129.9 ± 33.9[b]	618.3 ± 262.2[a]	457.9 ± 131.5[a]	542.9 ± 193.6[a]	585.7 ± 179.6[a]
ApoA-I	5	—	38.3 ± 11.9	37.9 ± 10.6	22.4 ± 8.1[b]	27.1 ± 3.84	50.4 ± 7.2
LDLr$^{-/-}$							
HDL C	5	—	83.1 ± 7.9[a]	56.5 ± 16.3	51.4 ± 10.4	60.2 ± 14.2	61.7 ± 10.8
Total PL	25	—	254.4 ± 31.4[a]	705.9 ± 91.6	743.6 ± 141.1	671.1 ± 113.0	595.3 ± 81.0
HDL PL	25	—	66.6 ± 5.7[a]	37.9 ± 7.4	50.1 ± 6.6[b]	27.6 ± 2.7	32.1 ± 3.73
TG	10	—	72.4 ± 20.9[b]	165.9 ± 50.4[a]	171.8 ± 76.8[a]	181.4 ± 87.5[a]	129.6 ± 37.1[a]
ApoA-I	5	—	50.4 ± 11.5	42.9 ± 6.1	39.6 ± 7.9[b]	35.2 ± 5.2	55.1 ± 20.6
LDLr$^{-/-}$PLTP$^{-/-}$ + PLTP$^{-/-}$ BM							
HDL C	7	47.3 ± 6.9	39.8 ± 13.4[a]	51.9 ± 9.0[a]	58.6 ± 8.5[a]	52.5 ± 11.7[a]	66.1 ± 12.2[a]
Total PL	7	242.8 ± 46.2	288.2 ± 77.4	487.2 ± 93.4	566.8 ± 141.2	667.9 ± 187.0	748.3 ± 189.1
HDL PL	7	29.1 ± 3.2	29.4 ± 8.4[a]	26.2 ± 4.1[a]	29.5 ± 3.5[a]	32.2 ± 5.9[a]	40.1 ± 6.9[a]
TG	10	119.4 ± 15.0[b]	168.8 ± 55.9	205.5 ± 59.2	325.2 ± 154.4[a]	315.5 ± 96.7[a]	336.8 ± 151.6[a]
ApoA-I	5	42.9 ± 6.6	41.9 ± 8.8	39.7 ± 6.2	35.0 ± 11.2	41.2 ± 7.5	40.8 ± 6.4
LDLr$^{-/-}$PLTP$^{-/-}$ + PLTP$^{+/+}$ BM							
HDL C	7	46.8 ± 6.1	100.9 ± 18.6[a]	115.6 ± 35.6[a]	126.4 ± 13.5[a]	112.0 ± 9.8[a]	131.2 ± 18.0[a]
Total PL	7	268.2 ± 39.5	361.7 ± 68.3	520.2 ± 51.0	572.4 ± 61.0	661.8 ± 123.1	643.6 ± 148.4
HDL PL	7	29.7 ± 3.7	68.3 ± 9.8[a]	65.4 ± 12.9[a]	72.9 ± 10.0[a]	70.9 ± 6.7[a]	75.4 ± 6.3[a]
TG	10	174.3 ± 78.9[b]	161.1 ± 100.2	192.0 ± 56.6	160.7 ± 43.3[a]	164.1 ± 40.5[a]	149.9 ± 53.8[a]
ApoA-I	5	49.4 ± 7.3	45.1 ± 13.2	48.9 ± 10.3	40.6 ± 13.4	47.4 ± 10.9	43.3 ± 10.3

ApoA-I, apolipoprotein A-I; BM, bone marrow; HDL C, HDL cholesterol; HFD, high-fat diet; LDLr, low density lipoprotein receptor; PL, phospholipid; PLTP, phospholipid transfer protein; TG, triglyceride. Values are means ± SD.

[a] Statistical difference at $P < 0.01$.

[b] Statistical difference at $P < 0.05$.

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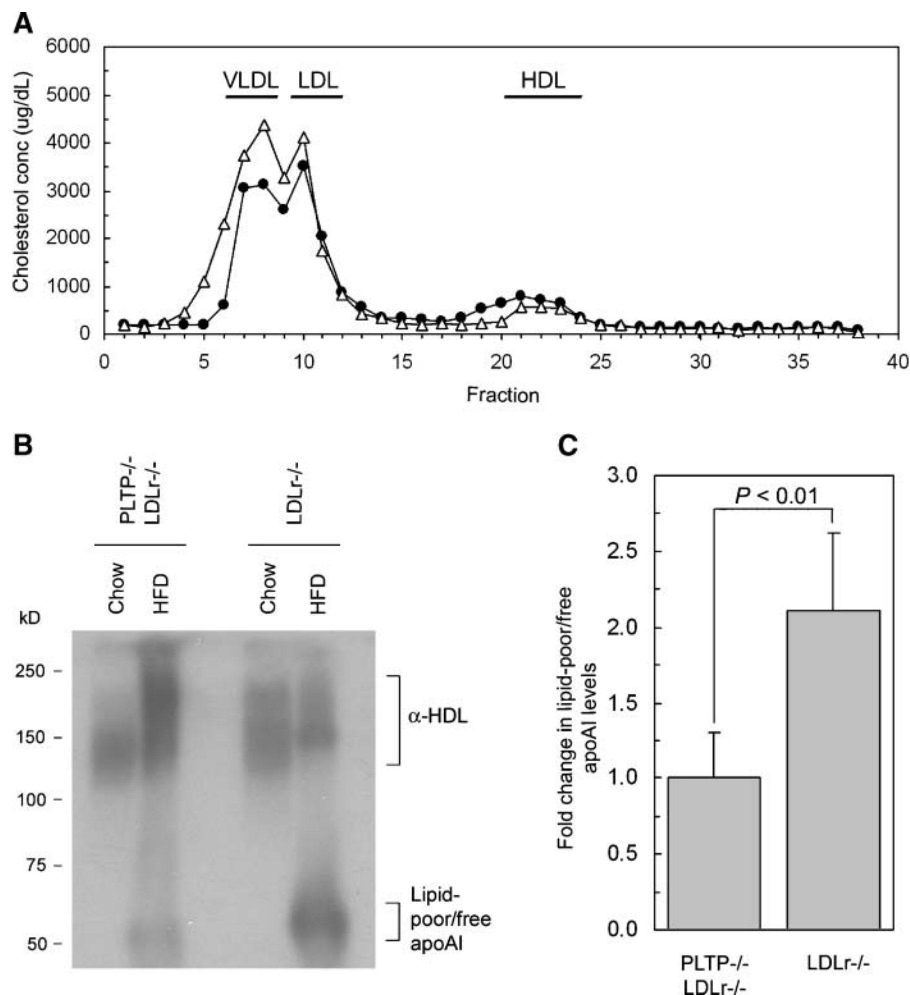

Fig. 2. Plasma lipoprotein profiles and apolipoprotein A-I (apoA-I) distribution among HDL fractions. A: Cholesterol distribution in plasma from fasted LDLr$^{-/-}$ (triangles) and PLTP$^{-/-}$LDLr$^{-/-}$ (circles) mice after size-fractionation on two Superdex 200 columns in series. The plasma was collected from mice that had been fed the HFD for 8 weeks. Elution patterns of the major lipoprotein species are indicated. B: Lipid-poor/free apoA-I revealed by native 4–26% gradient gel electrophoresis of pooled plasma (n = 5) collected from mice fed chow and after consuming the HFD for 8 weeks. Twenty microliters of plasma, diluted 1:3 in sample buffer, was loaded per well. C: Corresponding apoA-I band densities from HFD-fed mice from B assessed by scanning densitometry, expressed as fold change from densities in plasma from PLTP$^{-/-}$LDLr$^{-/-}$ mice fed the HFD. Densities are representative of four gels. Error bars indicate ± SD.

C57Bl/6 mice when they consumed the chow diet (**Fig. 3A**). However, a change in diet to the atherogenic HFD led to a significant increase in plasma PLTP activity in LDLr$^{-/-}$ mice.

Plasma SAA levels are strongly indicative of systemic inflammation (28) and have been positively correlated with hypercholesterolemia and increased atherosclerosis in mice (29). In the absence of any differences in total plasma cholesterol, LDLr$^{-/-}$ mice expressing systemic PLTP had higher plasma SAA levels than PLTP$^{-/-}$LDLr$^{-/-}$ mice fed the HFD (Fig. 3B). TNF-α levels were higher but not significantly different ($P = 0.236$) in PLTP-expressing LDLr$^{-/-}$ mice compared with double mutant PLTP$^{-/-}$LDLr$^{-/-}$ mice (20.6 ± 10.6 and 14.7 ± 6.6 pg/ml, respectively; n = 7).

Macrophage-derived PLTP in PLTP$^{-/-}$LDLr$^{-/-}$ mice

Successful reconstitution of hematopoietic cells from donor BM after total body irradiation of PLTP$^{-/-}$LDLr$^{-/-}$ recipients was confirmed by PLTP mRNA expression in blood leukocytes (**Fig. 4A**). Four weeks after BMT, PLTP expression by BM-derived cells increased total cholesterol levels in chow-fed mice compared with PLTP$^{-/-}$ BMT chimeras. However, while consuming the HFD, PLTP$^{+/+}$ BMT chimeras experienced a significantly smaller increase in total plasma cholesterol levels than PLTP$^{-/-}$ BMT chimeras (Fig. 4B), yet these same PLTP$^{+/+}$ BMT chimeras had significantly increased plasma HDL cholesterol levels (Table 1). Whereas plasma HDL phospholipid concentrations followed similar trends, plasma triglyceride levels in PLTP$^{+/+}$ BMT chimeras decreased significantly compared

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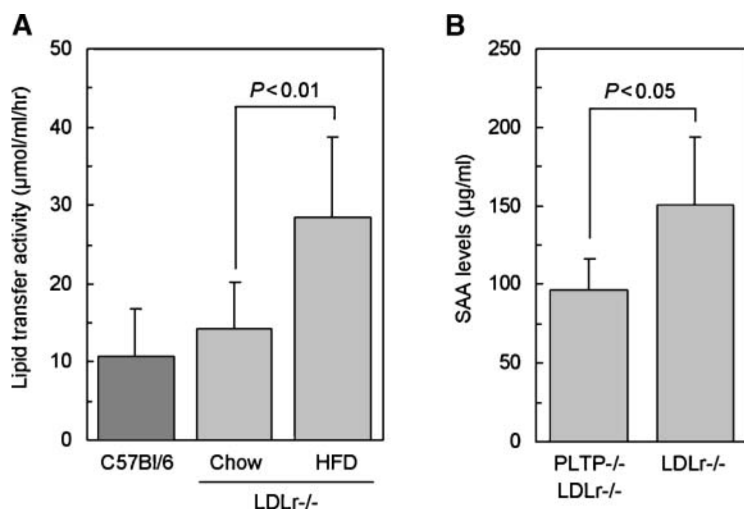

Fig. 3. Plasma PLTP activity and serum amyloid A (SAA) levels. A: Lipid transfer activity in plasma from LDLr$^{-/-}$ mice fed a chow diet and after consuming the HFD for 8 weeks, as measured by in vitro transfer of [^{14}C]1,2-dipalmitoyl-sn-glycero-3-phosphocholine from liposomes to HDL. PLTP activity in plasma from C57Bl/6 mice consuming a chow diet is shown for comparison. B: SAA levels in plasma from PLTP$^{-/-}$LDLr$^{-/-}$ and LDLr$^{-/-}$ mice that had been consuming the HFD for 12 weeks, as measured by ELISA. Error bars indicate ± SD.

with those in PLTP$^{-/-}$ BMT chimeras at 8 weeks after consuming the HFD.

Cholesterol distribution within the different lipoprotein species was influenced by BM expression of PLTP. PLTP$^{+/+}$ BMT chimeras displayed increased LDL cholesterol with a proportional decrease in VLDL cholesterol compared with PLTP$^{-/-}$ BMT mice (**Fig. 5A**). Each of the BMT groups displayed heterogeneous plasma HDL populations. In addition, apoA-I in plasma was associated primarily with spherical α-migrating HDL at the expense of lipid-poor/free apoA-I when these PLTP$^{+/+}$ BMT mice consumed the HFD (Fig. 5B, C).

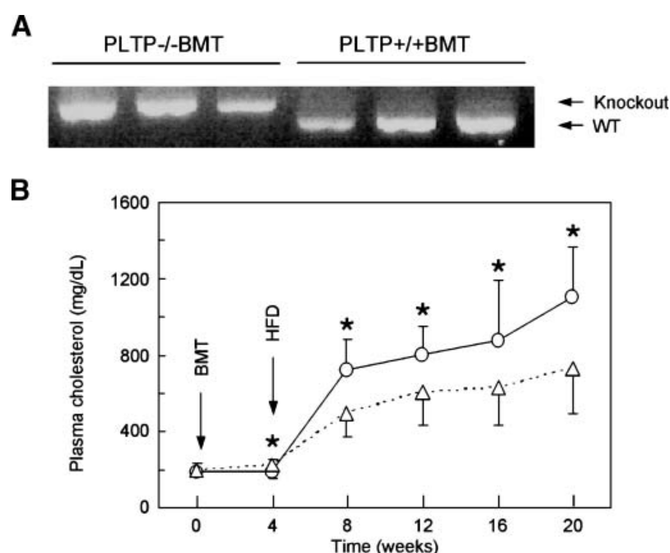

Fig. 4. Bone marrow transplantation (BMT) studies. A: Successful reconstitution of PLTP$^{-/-}$ or PLTP$^{+/+}$ donor bone marrow (BM) after BMT in PLTP$^{-/-}$LDLr$^{-/-}$ recipients assessed by PCR using blood samples. WT, wild type. B: Changes in total plasma cholesterol levels in PLTP$^{-/-}$LDLr$^{-/-}$ mice that received PLTP$^{-/-}$ BM (circles) or PLTP$^{+/+}$ BM (triangles) before BMT, 4 weeks after consuming a chow diet and while consuming the HFD for 16 weeks. Significant differences ($P < 0.05$) are indicated (asterisks). Error bars indicate ± SD.

The contribution of macrophage-derived PLTP to circulating PLTP activity in PLTP$^{+/+}$ BMT mice did not change after a change in diet (**Fig. 6A**). In addition, macrophage-specific PLTP expression in BMT recipients did not significantly alter either plasma SAA concentrations (Fig. 6B) or TNF-α levels (10.6 ± 4.0 pg/ml in PLTP$^{-/-}$ BMT mice and 8.7 ± 2.9 pg/ml in PLTP$^{+/+}$ BMT mice; $P = 0.326$, n = 7) when the mice were challenged with the atherogenic diet.

As expected from the lipoprotein changes, the influence of only PLTP expression on lesion development was remarkable: A 62.8% reduction in lesions as measured in en face aortas and a 48.0% reduction as measured in heart sinus valve lesion areas were observed (**Fig. 7A, B**). Thus, macrophage-derived PLTP significantly inhibited atherosclerosis progression in mice that lacked systemic PLTP.

DISCUSSION

Many studies have highlighted the physiologic significance of PLTP in the transfer of surface remnants from triglyceride-rich lipoproteins to HDL particles during lipolysis. PLTP$^{-/-}$ mice show a defective transfer of phospholipids into HDL that results in the depletion of plasma HDL (30) and hypoalphalipoproteinemia (31). Similarly, a decrease in HDL is observed when the PLTP gene is overexpressed in transgenic mouse strains (3, 4), which, in hypercholesterolemic apoE$^{-/-}$ mice, results in increased atherosclerosis (6). We show that endogenous PLTP expression has a comparable proatherogenic effect in LDLr$^{-/-}$ mice when they are fed a HFD (Fig. 1B). This increased disease severity in LDLr$^{-/-}$ mice occurred despite comparable plasma cholesterol and HDL cholesterol levels and the presence of higher circulating levels of lipid-poor /free apoA-I (Fig. 2B, C).

Increased plasma triglyceride levels, like those observed in LDLr$^{-/-}$ mice (Table 1), occur during an acute-phase inflammatory reaction as a result of their interaction with

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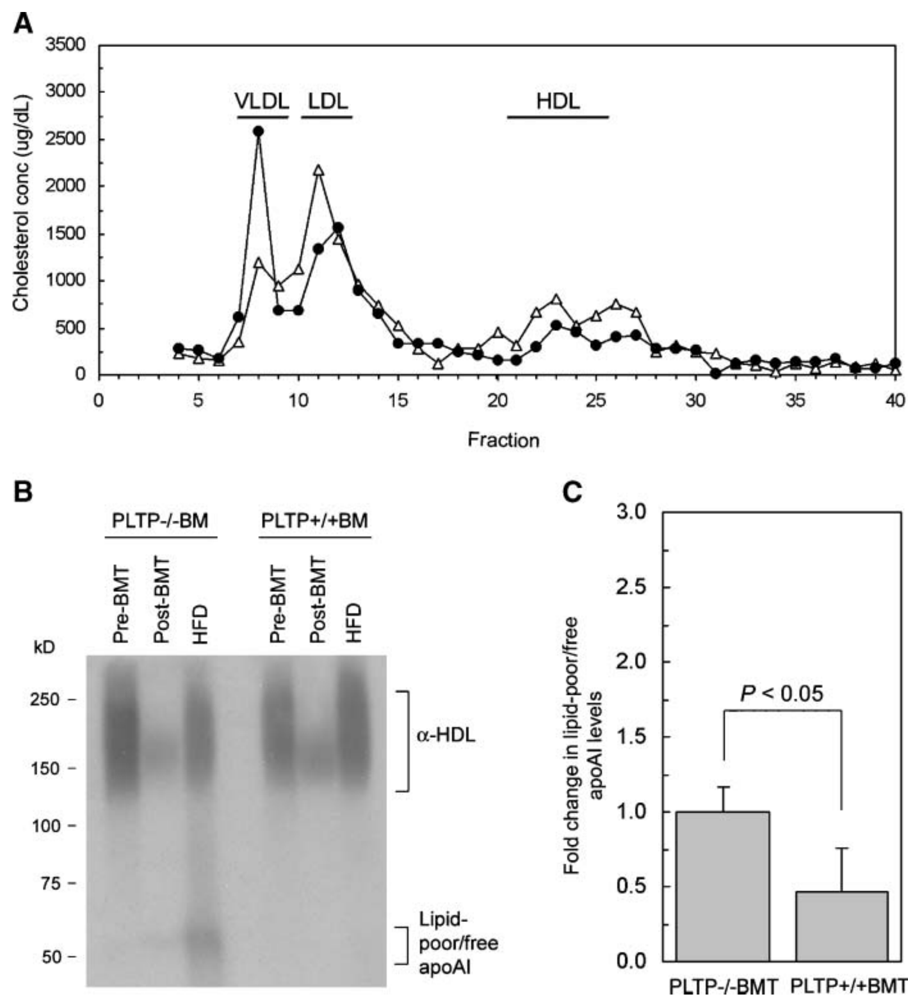

Fig. 5. Plasma lipoprotein profiles and lipid-poor apoA-I levels in PLTP$^{-/-}$LDLr$^{-/-}$ BMT recipient mice. A: Cholesterol distribution among lipoprotein fractions separated by fast-protein liquid chromatography in double mutant mice that had received either PLTP$^{-/-}$ (circles) or PLTP$^{+/+}$ (triangles) BM. B: ApoA-I size distribution in HDL fractions analyzed by 4–26% native gel electrophoresis using pooled plasma (n = 5) collected from PLTP$^{-/-}$LDLr$^{-/-}$ mice before and after BMT and after consuming the HFD for 8 weeks. Twenty microliters of plasma diluted 1:3 in sample buffer was loaded per well. C: Quantification of lipid-poor/free apoA-I levels among the two BM recipient groups by scanning densitometry, expressed as fold change from densities in plasma from PLTP$^{-/-}$ BMT mice fed the HFD. Densities are representative of four scanned gels. Error bars indicate ± SD.

several acute-phase proteins secreted by the liver (28), including SAA. LDLr$^{-/-}$ mice had higher plasma SAA protein levels than PLTP$^{-/-}$LDLr$^{-/-}$ mice (Fig. 3B); thus, the increased levels of circulating lipid-poor/free apoA-I in the LDLr$^{-/-}$ mice may have been the result of PLTP-mediated remodeling of HDL (4, 17), which occurred in plasma as a result of greater hepatic synthesis of SAA-containing HDL at the expense of apoA-I-only-containing HDL (32). Although the role of plasma PLTP in this process is poorly understood, it is known that PLTP can alter the anti-inflammatory properties of HDL, which may then affect the expression of important inflammatory cytokines (33).

Recent studies suggest that the actions of systemic PLTP on lipid metabolism may be considerably different from those regulated by PLTP expressed in peripheral tissues. We have shown previously using BMT that atheroprotection by macrophage-derived PLTP can be achieved in LDLr$^{-/-}$ mice even in the presence of systemic PLTP expression (8). In the present study, we confirm that macrophage-derived PLTP alone has the opposite effect on atherosclerosis compared with a mouse model with systemic PLTP expression (Figs. 1B, 7A, B). These anti-atherogenic properties of macrophage PLTP may be the result of enhanced cellular interaction between PLTP and the ABCA1 transporter. This association has been shown to stabilize HDL binding to ABCA1 and to increase the rate of cholesterol and phospholipid efflux (34, 35). A second possibility is that locally produced PLTP increases the rate of reverse cholesterol transport by enhancing the generation of preβ-HDL within the vessel intima (21). This hypothesis is supported by evidence that PLTP can

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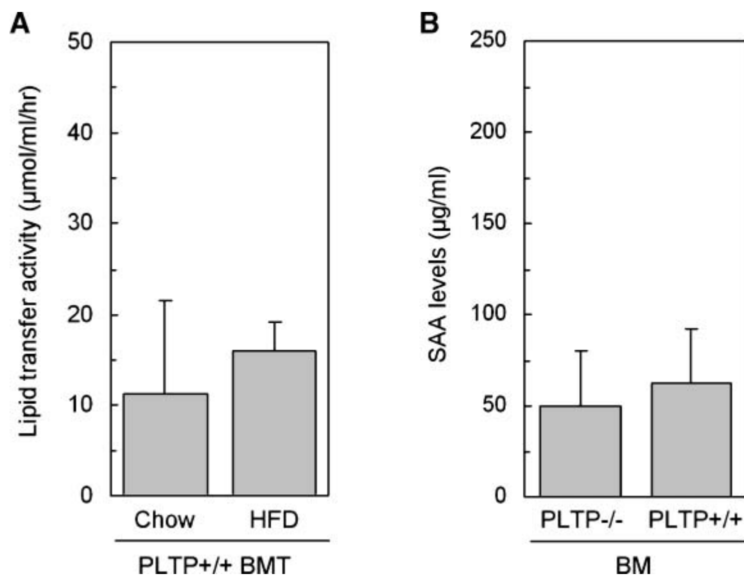

Fig. 6. Plasma PLTP activity and SAA levels in BM-transplanted $PLTP^{-/-}LDLr^{-/-}$ mice. A: Lipid transfer activity in plasma from $PLTP^{-/-}LDLr^{-/-}$ mice that had received $PLTP^{+/+}$ BM while consuming a chow diet and after consuming the HFD for 8 weeks. B: SAA levels in plasma from $PLTP^{-/-}LDLr^{-/-}$ mice that had received BM from either $PLTP^{-/-}$ or $PLTP^{+/+}$ mice and that had been consuming the HFD for 12 weeks. Error bars indicate ± SD.

generate preβ-HDL from spherical HDL in vitro (13), but it has yet to be confirmed in vivo.

It is key to point out that high levels of circulating lipid-poor/free apoA-I, similar to those observed in our $LDLr^{-/-}$ mice fed a HFD (Fig. 2B, C), may not be indicative of locally produced preβ-HDL levels. The antiatherogenic effects of macrophage-derived PLTP in $PLTP^{-/-}LDLr^{-/-}$ recipient mice were observed despite a decrease in circulating lipid-poor/free apoA-I levels (Fig. 5B, C). These observations suggest that preβ-HDL generated locally within the lesion intima by macrophage-derived PLTP need not give rise to plasma preβ-HDL. Instead, interstitial modeling of HDL near cell surfaces by PLTP and an increase in cellular cholesterol efflux through the association of lipid-poor apoA-I with ABCA1 may lead to the local generation of cholesterol and phospholipid discoidal apoA-I that in combination with ABCG1 and LCAT can give rise to spherical α-migrating HDL, which then enters the circulation to transport cholesterol back to the liver.

A recent study by Vikstedt et al. (9) using female $LDLr^{-/-}$ mice as recipients of $PLTP^{-/-}$ and $PLTP^{+/+}$ BM reported that despite an increase in plasma preβ-HDL, a deficiency of macrophage-expressed PLTP decreased atherosclerosis. In this study, a Western-style diet (containing 0.25% cholesterol) and a shorter time exposure to that diet (9 weeks) was used. Combined with known sexual dimorphism in genes that regulate lipid metabolism (36), these results highlight the complexity of PLTP action on lesion development and the need to study lesion progression over time.

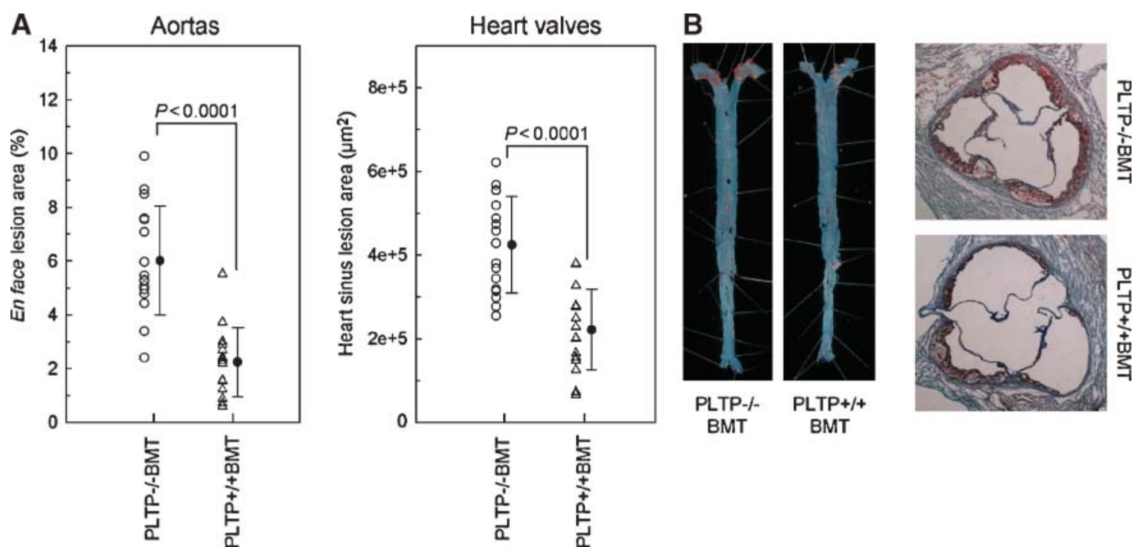

Fig. 7. Assessment of atherosclerosis. A: Lesions measured as percentage of en face aorta lesion area and lesion areas in heart valve sections in $PLTP^{-/-}LDLr^{-/-}$ mice that received either $PLTP^{-/-}$ BM or $PLTP^{+/+}$ BM after consuming the HFD for 16 weeks. Closed circles on each graph represent means and SD of each data set. B: Representative aortas and heart sinus valve sections from the two BMT recipient mouse groups. Error bars indicate ± SD.

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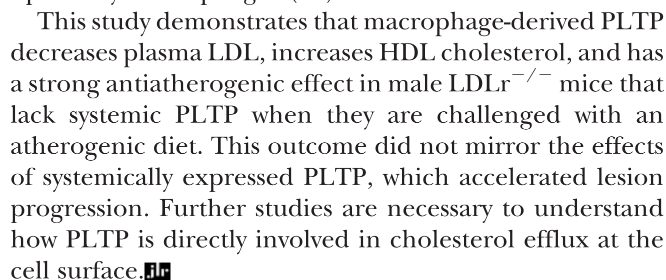
The antiatherogenic properties of macrophage-derived PLTP observed in the present study may also be the result of other associated processes. We reported that PLTP influences α-tocopherol distribution between lipoproteins and cells in the vascular wall. Cultured PLTP$^{-/-}$ BM-derived cells have lower vitamin E content than PLTP-expressing cells, resulting in higher oxidative stress and increased LDL oxidation (8). Because oxidized LDL is a key factor in the initiation of lesion development, the beneficial properties of macrophage-derived PLTP observed in PLTP$^{-/-}$LDLr$^{-/-}$ mouse recipients could also be the result of combined lower oxidative stress limiting LDL uptake by macrophages (21).



This study demonstrates that macrophage-derived PLTP decreases plasma LDL, increases HDL cholesterol, and has a strong antiatherogenic effect in male LDLr$^{-/-}$ mice that lack systemic PLTP when they are challenged with an atherogenic diet. This outcome did not mirror the effects of systemically expressed PLTP, which accelerated lesion progression. Further studies are necessary to understand how PLTP is directly involved in cholesterol efflux at the cell surface.

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