Phospholipid transfer protein is present in human atherosclerotic lesions and is expressed by macrophages and foam cells

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Abstract Phospholipid transfer protein (PLTP) in plasma promotes phospholipid transfer from triglyceride-rich lipoproteins to HDL and plays a major role in HDL remodeling. Recent in vivo observations also support a key role for PLTP in cholesterol metabolism. Our immunohistochemical analysis of human carotid endarterectomy samples identified immunoreactive PLTP in areas that colocalized with CD68-positive macrophages, suggesting that PLTP could be produced locally by intimal macrophages. Using RT-PCR, Western blot analysis with a monoclonal anti-PLTP antibody, and a PLTP activity assay, we observed PLTP mRNA and protein expression in human macrophages. In adherent peripheral blood human macrophages, this PLTP expression was increased by culture with granulocyte macrophage colony-stimulating factor. Incubation of macrophages with acetylated-LDL induced an increase in PLTP mRNA and protein expression that paralleled cholesterol loading. PLTP expression was observed in elicited mouse peritoneal macrophages and in cultured Raw264.7 cells as well. Thus, this study demonstrates that PLTP is expressed by macrophages, is regulated by cholesterol loading, and is present in atherosclerotic lesions.—Desrumaux, C. M., P. A. Mak, W. A. Boisvert, D. Masson, D. Stupack, M. Jauhiainen, C. Ehnholm, and L. K. Curtiss. **Phospholipid transfer protein is present in human atherosclerotic lesions and is expressed by macrophages and foam cells. *J. Lipid Res.* 2003.** 44: **1453–1461.**

Supplementary key words atherosclerosis • lipoproteins • lipid transfer

Plasma levels of HDL cholesterol are inversely related to the risk of atherosclerosis and coronary heart disease (1–3). Although several hypotheses have been raised to explain the protective role of HDL, the most widely held hypothesis is its role in reverse cholesterol transport. This transport involves the unloading of cholesterol from peripheral cell membranes and its transfer to the liver, where it can be metabolized and excreted (4–6). HDL particles are not stable entities in vivo. They are being continuously remodeled through the action of several factors, including lipolytic enzymes, lecithin:cholesterol acyltransferase, cholesteryl ester transfer protein (CETP), and phospholipid transfer protein (PLTP) (7).

In atherosclerosis, the dysregulated endocytosis of oxidized LDL by intimal macrophages leads to their transformation into foam cells characteristic of fatty streak lesions (8–10). Efficient removal of cholesterol from these foam cells is critical to preventing disease progression; however, mechanisms governing cholesterol removal from lesion macrophages have not been completely elucidated. Macrophages express a number of lipid transport and lipid removal proteins. They secrete apolipoprotein E (apoE), apoC-I, apoC-IV, apoC-II (11, 12), lipoprotein lipase (LPL) (13), hepatic lipase (14), and CETP (15). More recently, it has been reported that scavenger receptor class B type I, an HDL receptor that can promote both influx and efflux of cholesterol from cell membranes (16), and the transmembrane protein, ATP binding cassette transporter A1 (ABCA1), a fundamental mediator of apolipoprotein-mediated cellular cholesterol efflux (17), are expressed by macrophages (18, 19). Ex-

Manuscript received 18 July 2002 and in revised form 30 April 2003.

Published, JLR Papers in Press, May 1, 2003.
DOI 10.1194/jlr.M200281-JLR200

Abbreviations: ABCA1, ATP binding cassette transporter A1; Ac-LDL, acetylated LDL; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FC, free cholesterol; GM-CSF, granulocyte macrophage colony-stimulating factor; LPDS, lipoprotein-deficient serum; LPL, lipoprotein lipase; PLTP, phospholipid transfer protein.

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pression of ABCA1 by macrophages, its up-regulation by cholesterol loading (19), and its presence in human lesions (20) suggest that the efflux of excess cholesterol by lipid-free apolipoproteins or pre-β HDL in situ is key to the prevention of foam cell formation.

Although PLTP was originally described as a mediator of phospholipid transfer between lipoprotein particles, it is now recognized as a key factor in the intravascular metabolism and remodeling of HDL (21, 22). PLTP facilitates the transfer of multiple compounds, including phospholipids (23), lipopolysaccharides (24), α-tocopherol (25, 26), and unesterified cholesterol (27) among lipoprotein classes and between lipoproteins and cells. This transfer activity enhances the formation of large, spherical HDL and pre-β HDL through HDL fusion and the release of lipid-poor apoA-I (7, 28). Recent studies conducted with PLTP transgenic and knockout mice suggest that plasma PLTP influences both HDL and pre-β HDL levels in vivo (29). PLTP deficiency is atheroprotective in different strains of hypercholesterolemic mice (30), and human PLTP transgenic mice have an increased risk of atherosclerosis (31).

In the present study, we identified PLTP in human atherosclerotic lesions that was colocalized predominantly with macrophages. We confirmed the expression of PLTP by freshly isolated as well as immortalized human and mouse macrophages. Moreover, we demonstrated that macrophage PLTP gene and protein expression was up-regulated by cholesterol loading. Taken together, these observations suggest that locally produced macrophage-derived PLTP could influence foam cell formation and the progression of atherosclerosis within the vessel wall.

EXPERIMENTAL PROCEDURES

Immunohistochemistry

Immunohistochemical analyses were performed on sections of human carotid endarterectomy samples that were fixed in formalin and embedded in paraffin. Sections on glass slides were deparaffinized using EZ-Dewax solution (Biogenex, San Ramon, CA). Antigen retrieval was carried out in a microwave using an antigen retrieval solution (Vector Laboratories) and blocked for 30 min with 10% goat serum. The sections were incubated for 16 h in a humidified chamber at room temperature with a 1:50 dilution of a mouse monoclonal antibody specific for human CD68 (clone KP1, Dako), and a 1:100 dilution of a rabbit polyclonal serum specific for human PLTP [Novus Biologicals, purchased through Abcam (http://www.abcam.com, product code ab7735)]. To detect CD68, the slides were washed and incubated for 2 h with biotinylated goat anti-mouse IgG antibody (Dako) followed by 30 min incubation in Vectastain solution (ABC, Vector Laboratories, Burlingame, CA). To enhance the sensitivity of this staining, the sections were incubated for 10 min in tyramide signal amplification solution (TSA, Perkin Elmer Life Sciences, Boston, MA) and for 30 min with a 1:500 dilution of streptavidin-fluoresceinisothiocyanate (FITC) (Perkin Elmer). To detect PLTP, the same slides were subsequently incubated for 2 h with biotinylated goat anti-rabbit IgG (Dako), Vectastain solution, tyramide signal amplification solution, and streptavidin-Texas Red. Controls were performed in which streptavidin-Texas Red was applied to the slide immediately after incubation with streptavidin-FITC. Negative control staining was achieved using normal mouse or rabbit sera in place of primary antibodies. The images were captured with a laser scanning confocal microscope (Biorad, Hercules, CA). In order to illustrate the morphology of each atherosclerotic specimen, a photograph of hematoxylin-stained sections was taken using a light microscope.

Cell culture

Mouse Raw264.7 cells and human THP-1 cells were from the American Type Culture Collection (Manassas, VA). Peritoneal macrophages were harvested from the peritoneal cavity of C57Bl/6 mice 5 days after a 1 ml injection of 3% thioglycollate. Human monocytes were purified from fresh blood by density gradient centrifugation on Histopaque 1077 (Sigma) following the manufacturer's instructions. All cell lines and the elicited peritoneal macrophages were maintained in RPMI 1640 medium (Life Technologies) containing 10% FBS, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 2 mM L-glutamine, 0.1 mM nonessential aminoacids, 1 mM sodium pyruvate (Life Technologies), and 0.01 mM β-mercaptoethanol. The peripheral blood mononuclear cells were plated at 2×10^6 cells/ml in 12-well plates and grown in RPMI 1640 medium containing 10% autologous serum. THP-1 cells were plated at 0.5×10^6 cells/ml in 12-well plates (Costar, Cambridge, MA) and differentiated into macrophages by a 24 h incubation with PMA (10^{-7} M) in serum-free RPMI containing 1% Nutridoma-Hu (Roche Diagnostics, Mannheim, Germany). For the experiments, PMA-differentiated THP-1 cells were grown in serum-free RPMI 1640 medium (Life Technologies) containing 1% Nutridoma-Hu. Elicited peritoneal macrophages were cultured for 3 days before the experiments and plated at 2×10^6 cells/ml in 12-well plates. The human peripheral blood macrophages were transferred to 10% lipoprotein-deficient serum (LPDS) (Intracel Corp., Rockville, MD) before they were used for the experiments. Nutridoma-Hu (1%) was used in place of 10% LPDS when cell culture supernatants had to be collected for PLTP activity assays and Western blot analysis.

Isolation and modification of lipoproteins

LDLs ($1.019 < d < 1.063$ g/ml) were isolated from plasma of normolipidemic volunteers by sequential ultracentrifugation (32). LDLs were acetylated by repeated additions of acetic anhydride (33).

Macrophage cholesterol loading

After culture for 10 days in 10% autologous serum with granulocyte macrophage colony-stimulating factor (GM-CSF), the human peripheral blood macrophages were washed and cholesterol-loaded by incubation with 80 μg/ml of acetylated LDL (Ac-LDL) for 24 h to 96 h in serum-free RPMI containing 10% LPDS and 100 μM mevalonic acid. As controls, cells were incubated without Ac-LDL in the same culture media supplemented with 5 μM compactin. For PLTP activity measurements and Western blot analysis of cell culture supernatants, the 10% LPDS was replaced by 1% Nutridoma-Hu. Cells were washed with PBS, and lipids were extracted into 1 ml of hexane:isopropanol. Free cholesterol (FC) and cholesteryl esters (CEs) were quantitated by thin-layer chromatography as described (33). Protein was quantitated after cell lysis (0.1 N NaOH) using the micro BCA protein assay kit (Pierce) and the results expressed as FC or CE/mg of cell protein.

RT-PCR

Cells and tissues were lysed and homogenized following the QIAshredder procedure, and total RNA was isolated by using the

RNeasy mini kit (Qiagen). cDNA was synthesized from 0.2 μg total RNA using random primers and the Superscript II Reverse Transcriptase (Life Technologies). The PCR reaction was performed using deoxynucleotide triphosphates, and 0.1 μg each of primer and the Hot Start Taq DNA polymerase (Qiagen). Analysis of primer secondary structure was performed using NetPrimer software. The primer sequences used are given below. Amplification of cDNA with primers for G3PDH served as an internal control. PCR was performed in a PTC-200 thermal cycler (MJ Research), with an initial 15 min denaturation step at 95°C, followed by 38 cycles comprising a 1 min denaturation at 95°C, 1 min annealing at 60°C, and 2 min extension at 72°C. The amplification was terminated by a 7 min final extension at 72°C. The identity of the PCR products was confirmed by sequence analyses on an automated DNA Sequencer (**Table 1**).

PLTP activity measurements

PLTP activity was measured in serum-free cell culture supernatants from peripheral blood macrophages, THP-1 cells, and elicited mouse peritoneal macrophages using a PLTP activity kit (Cardiovascular Targets, Inc., New York) according to the manufacturer's instructions. This fluorimetric assay measures the transfer (unquenching) of pyrene-labeled phosphatidylcholine from donor to acceptor synthetic liposomes.

Western blot analysis

THP-1 cells and primary human macrophages were cultured in serum-free RPMI containing 1% Nutridoma. Undiluted supernatants were electrophoresed for 50 min at 200 V in 10% Bis-Tris gels (Novex) under reducing conditions. Proteins were transferred to PVDF membranes, and the membranes were incubated for 16 h at 4°C with PLTP-specific monoclonal antibodies (from Drs. Matti Jauhiainen and Christian Ehnholm, National Public Health Institute, Helsinki, Finland). These monoclonal antibodies were obtained by injecting Balb/c mice with a full-length recombinant PLTP produced in *Escherichia coli* (34). Blots were developed with a chemiluminescent immunodetection system (WesternBreeze kit, Invitrogen).

RESULTS

Immunohistochemical analysis of human carotid endarterectomy sections

Figure 1 illustrates PLTP and CD68 immunostaining of endarterectomy sections from three different patients. The morphology of the lesions is shown in the right panels. The lesions are all advanced and contained thick smooth muscle cell layers with disseminated macrophages. Regions containing strong PLTP staining (red) were colocalized with CD68-positive cells (green), indicating the colocalization of macrophages and cells expressing PLTP (yellow appearance in the merged images). In all sections examined, PLTP was colocalized with CD68-positive macrophages; however, occasional expression by other cell types, possibly smooth muscle cells, was observed within the lesions. In all sections examined, PLTP was present in a macrophage-rich area of the human lesions, suggesting its expression by lesion macrophages.

TABLE 1. Primers used in the study

Primer Set	Primer Sequences	Product Size
Mouse PLTP	5′-ATGCTGGGACGGTGTTGCTC-3′ 5′-CTCGTTCAGCAAAGGCATCACTCC-3′	715
Human PLTP	5′-ACGCAGGGACGGTCCTGCTC-3′ 5′-CTCATTGAGCATGGGCATCACCCC-3′	715
G3PDH	5′-ACCACAGTCCATGCCATCAC-3′ 5′-TCCACCACCCTGTTGCTGTA-3′	426

Expression of PLTP by human macrophages

Macrophage expression of PLTP was confirmed by in vitro examination. A sensitive semiquantitative RT-PCR technique employing a set of primers designed to amplify a 715 bp cDNA fragment of the human PLTP gene was used to confirm expression of PLTP mRNA in freshly isolated human peripheral blood macrophages cultured in serum-free media in the presence of GM-CSF to promote differentiation. The expected 715 bp PCR product was amplified, and its identity confirmed by sequencing. The sensitivity of the RT-PCR permitted identification of PLTP mRNA within 1 day of culture in GM-CSF. Moreover, PLTP gene expression increased during the 7 days of culture (**Fig. 2A**). Semi-quantitative analysis confirmed that PLTP gene expression increased gradually during macrophage differentiation (Fig. 2B).

To make sure that the PLTP mRNA expression observed in the human peripheral blood monocyte-derived macrophages was not due to a minor blood cell contaminant, we confirmed that PMA-differentiated human THP-1 cells expressed similar amounts of human PLTP mRNA (not shown).

Human PLTP antigen was detected as an 80 kDa PLTP band by Western blot analysis (**Fig. 3**). Supernatants obtained after 24 h of culture in serum-free medium from both GM-CSF-stimulated peripheral blood macrophages and PMA-stimulated THP-1 cells contained PLTP. This confirmed that PLTP was produced and secreted by differentiated human macrophages.

Expression of PLTP gene by cultured mouse macrophages

To document that mouse macrophages also express PLTP, we studied the expression of PLTP by elicited mouse peritoneal macrophages and by mouse Raw264.7 cells. Because lungs express PLTP at a high level in mice (35), cDNA was also prepared from mouse lung tissue and was used as a positive control. Amplification with the mouse PLTP primers produced the expected 715 bp product from all samples, indicating that the PLTP gene was expressed by elicited peritoneal macrophages as well as by the immortalized Raw264.7 cells. Moreover, the use of serial dilutions of cDNA to produce similar levels of G3PDH product suggested that comparable amounts of PLTP PCR products were recovered from each of the cell sources (**Fig. 4**). This suggests that macrophage PLTP gene expression was as robust as lung PLTP expression.

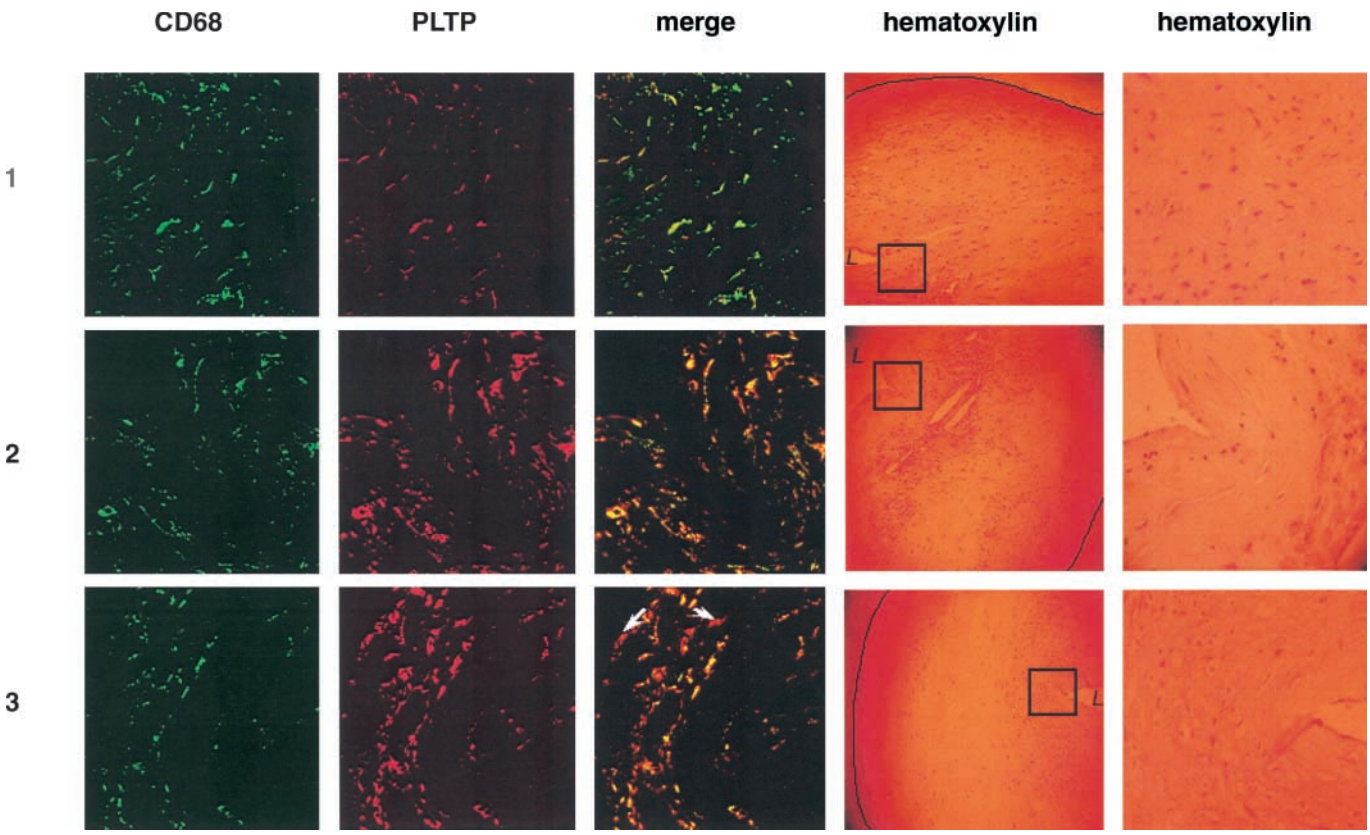

Fig. 1. Immunohistochemical analysis of phospholipid transfer protein (PLTP) expression in human carotid endarterectomy sections. Immunostaining was performed using a polyclonal human PLTP-specific antibody (1:100) and a monoclonal CD68-specific antibody (1:50). Overlaying of PLTP (red) staining areas and macrophage (CD68) (green) staining areas indicated that PLTP was colocalized (yellow) with macrophages in human atherosclerotic lesions. Macrophages staining positively for PLTP appear yellow in the merged images. In these merged images, the white arrows indicate occasional CD68-negative cells that are PLTP positive and appear red. Magnification, ×630. The right panels (magnifications, ×100 and ×630) illustrate the morphology of the atherosclerotic lesions stained with hematoxylin. Black squares correspond to the magnified panels. *L*, lumen.

Effect of cholesterol loading on PLTP expression

Because PLTP was observed in CE-rich macrophages within lesions, we examined the effect of cholesterol loading of cultured macrophages on PLTP mRNA expression. FC and esterified cholesterol levels of human peripheral blood macrophages and PMA-differentiated THP-1 cells incubated with Ac-LDL were verified by thin-layer chromatography. When FC and CEs were quantitated in monocyte-derived macrophages cultured in the absence of Ac-LDL, no significant increase in either cellular FC or esterified cholesterol content was observed over a 7 day culture period (data not shown). As expected, cellular FC and CE levels were increased by exposure of the cells to Ac-LDL for 48 h (**Fig. 5**, **top**). The effect of this cellular cholesterol loading on PLTP mRNA expression was assessed by semi-quantitative RT-PCR and is shown in Fig. 5, bottom. Cholesterol loading by incubation in the presence of Ac-LDL for only 48 h was accompanied by an increase in PLTP gene expression in both peripheral blood macrophages and THP-1 cells.

PLTP activity was recovered from cell supernatants of peripheral blood macrophages and PMA-differentiated THP-1 cells. PLTP activity was measured in cell supernatants using a commercial fluorimetric assay kit. First, a series of experiments was conducted to validate the use of this kit for PLTP activity measurements. PLTP activity was measured in the presence of various amounts (0–20 μl) of fresh human plasma after a 30 min incubation with donor and acceptor particles. As shown in **Fig. 6A**, a concentration-dependent increase in PLTP activity was observed with up to 5 μl of fresh human plasma. Figure 6B illustrates the time-dependent variations of PLTP activity in 3 μl of fresh human plasma. A progressive increase in PLTP activity occurs during the first 60 min of the incubation. To ascertain the specificity and reliability of this assay method, we compared PLTP activity levels measured in 5 μl of fresh human plasma, 5 μl of preheated (1 h at 56°C, leading to a complete loss of PLTP activity without affecting CETP activity) human plasma and 5 μl of purified human CETP (concentration, 0.464 g/l). As shown in Fig. 6C, there was no detectable PLTP activity in preheated plasma or purified CETP after a 30 min incubation with donor and acceptor particles. This observation indicated that the assay method measured the activity of PLTP. PLTP activity measured in eight fresh plasma samples using either this fluorimetric assay or the radioisotopic assay described by Damen, Regts, and Scherphof (36) were highly

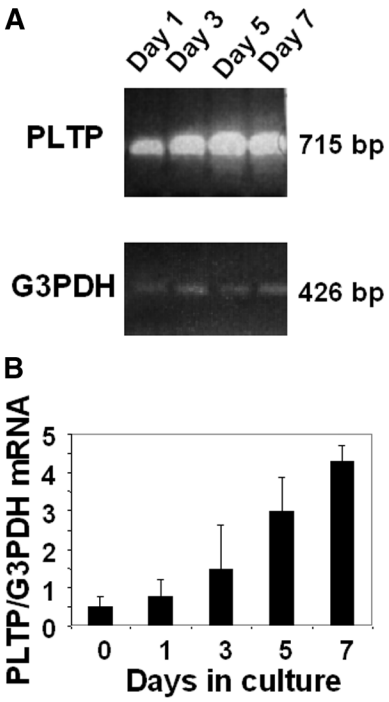

Fig. 2. PLTP gene expression in human macrophages. Human macrophages obtained from fresh peripheral blood were cultured for up to 7 days in serum-free media containing 1% Nutridoma and 1 ng/ml of granulocyte macrophage colony-stimulating factor (GM-CSF). Total RNA was isolated after 1, 3, 5, or 7 days of culture and subjected to RT-PCR analysis (A). For the semi-quantitative RT-PCR analyses (B), a standard curve was generated by using different amounts of RNA, which was used to calculate PLTP/G3PDH mRNA ratios. Data shown in B represent the mean ± SD of two independent experiments.

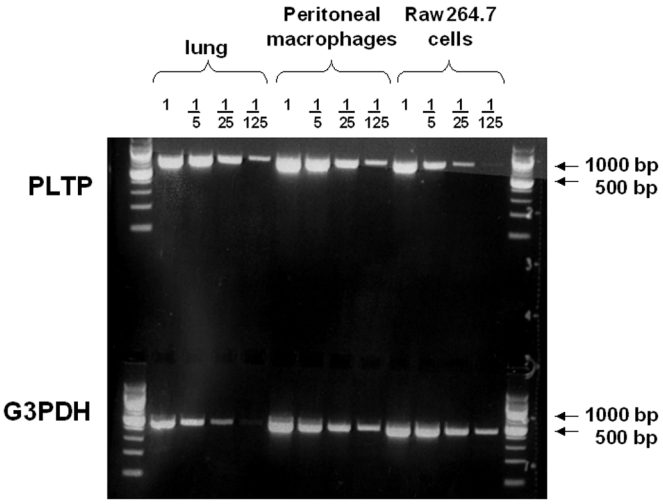

Fig. 4. RT-PCR analysis of PLTP gene expression in mouse lung tissue, elicited peritoneal macrophages, and Raw264.7 cells. Elicited peritoneal macrophages were harvested from the peritoneal cavity of mice 5 days after thioglycollate injection and cultured for 3 days in RPMI 1640 medium containing 10% FBS. Raw264.7 cells were cultured for 3 days in the same medium. Two hundred nanograms of total RNA from lung tissue, peritoneal macrophages, and Raw264.7 cells was reverse transcribed. Three microliters of cDNA (diluted as indicated) were used for PCR amplification with PLTP primers. One microliter of cDNA was used for PCR amplification with G3PDH primers. The 426 bp G3PDH products and the 715 bp PLTP products were subjected to agarose gel electrophoresis and visualized by ethidium bromide staining.

correlated ($r^2 = 0.9$) (Fig. 6D). The intra- and interassay coefficients of variation obtained with the commercial assay kit were 2.4% and 5.2%, respectively.

The PLTP activity level measured in culture media of human macrophages was 6–8% of that measured in human plasma. PLTP activity in the culture supernatants of the THP-1 cells was significantly ($P < 0.05$) increased by exposure to Ac-LDL for 48 h (**Fig. 7A**). PLTP activity in the supernatant of human peripheral blood macrophages also was increased by exposure of the cells to Ac-LDL (Fig. 7B). Likewise, increased secreted PLTP protein was detected by Western blot when THP-1 cells were cultured with Ac-LDL for 24 h and 48 h (Fig. 7C). It is noteworthy that because secreted PLTP does not remain fully active during prolonged incubation at 37°C, the increases of PLTP mass observed between T = 24 h and T = 48 h by Western blot are not paralleled by similar increases in PLTP activity.

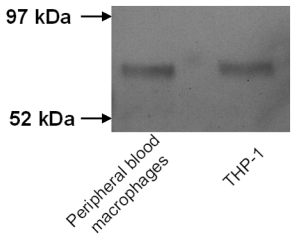

Fig. 3. Expression of PLTP protein by human macrophages. Cell supernatants (serum-free RPMI containing 1% Nutridoma) were obtained from GM-CSF-stimulated peripheral blood macrophages and PMA-stimulated THP-1 cells harvested after 24 h of culture. The proteins were separated by 10% SDS-PAGE. Western blot analysis was performed using human PLTP-specific monoclonal antibodies, mAbJH66 (1:1,000).

DISCUSSION

We demonstrated for the first time that PLTP was present in human atherosclerotic lesions. Immunohistochemical detection using antibodies specific for a macrophage antigen (CD68) and human PLTP revealed that PLTP was localized within distinct macrophage-rich areas. This suggested that PLTP could be produced by macrophages. Macrophage expression of PLTP was confirmed by RT-PCR in total RNA isolated from peripheral blood human macrophages. RT-PCR products also were obtained from RNA isolated from the established human THP-1 monocyte cell line, the murine Raw264.7 macrophage cell line, and mouse elicited peritoneal macrophages. In all cases, substantial PLTP gene expression was observed. Moreover, Western blot analysis using a mono-

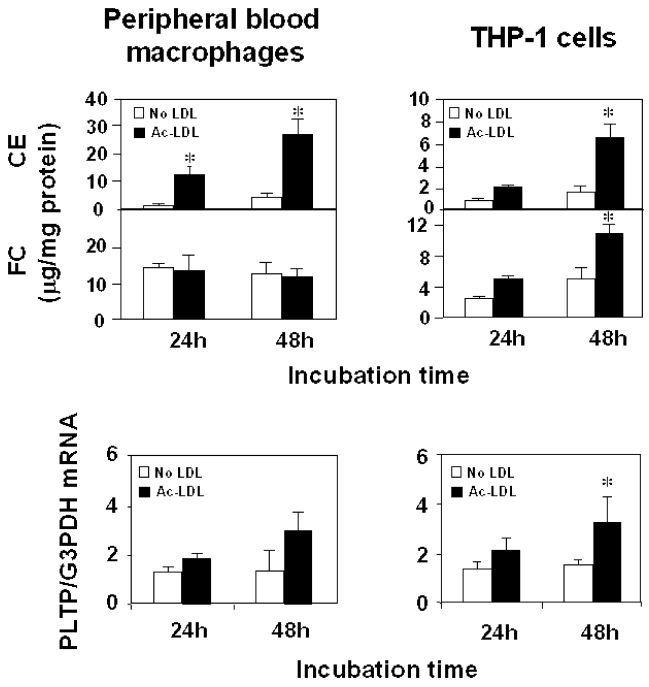

Fig. 5. Effect of cholesterol loading on PLTP gene expression by human peripheral blood macrophages and THP-1 cells. Top: Human monocyte-derived macrophages were cultured for 10 days in RPMI containing 10% autologous serum, then for 24 h or 48 h in the presence of acetylated LDL (Ac-LDL) (80 μg/ml) in serum-free medium containing 10% lipoprotein-deficient serum. PMA-differentiated THP-1 cells were incubated for 24 h or 48 h in the presence of Ac-LDL (80 μg/ml) in serum-free medium containing 1% Nutridoma-Hu. Free cholesterol and cholesteryl esters (CEs) were quantitated by thin-layer chromatography as described in Experimental Procedures. Data shown represent the mean ± SD of three determinations. Bottom: Human monocyte-derived macrophages and PMA-differentiated THP-1 cells were cultured as described above and the RNA isolated. RT-PCR was performed as described in Experimental Procedures. A standard curve was generated by using different amounts of RNA, which was used to calculate PLTP/G3PDH mRNA ratios. Data shown represent the mean ± SEM of three (THP-1 cells) or two (peripheral blood macrophages) independent experiments. $*P < 0.05$ compared with cells incubated in the absence of Ac-LDL.

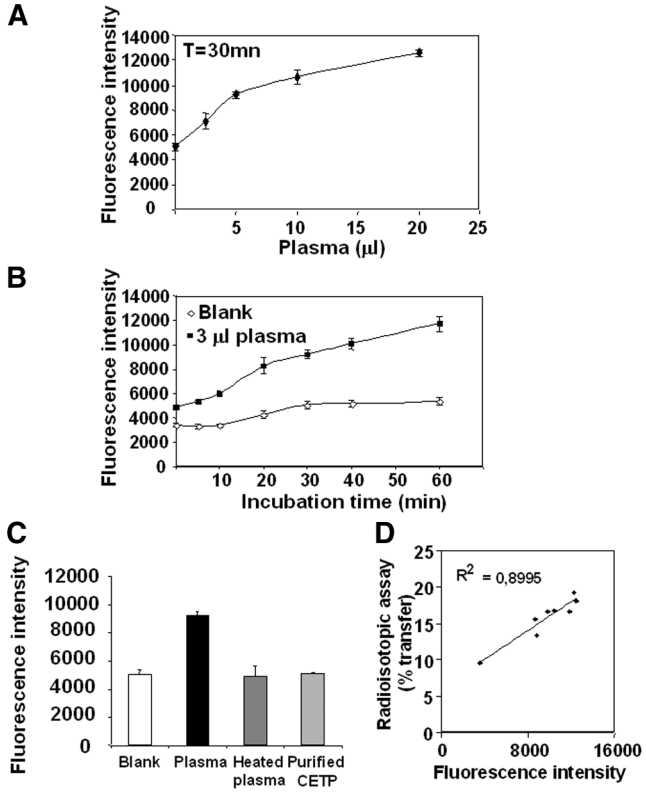

Fig. 6. Fluorimetric assay of PLTP activity. A: PLTP activity in 0, 2.5, 5, 10, and 20 μl of fresh human plasma after a 30 min incubation with donor and acceptor particles from the commercial fluorimetric assay kit (Cardiovascular Targets, Inc.). B: Time course analysis of PLTP activity in 0 μl (blank) or 3 μl of fresh human plasma incubated with donor and acceptor particles from the commercial fluorimetric assay kit. C: PLTP activity in 5 μl of fresh human plasma, 5 μl of preheated (1 h at 56°C) human plasma, and 5 μl of purified human cholesteryl ester transfer protein after a 30 min incubation using the commercial fluorimetric assay kit. All data shown represent the mean ± SD of three determinations. D: PLTP activity in 3 μl of fresh human plasma samples (n = 8) measured using the commercial fluorimetric assay kit (horizontal axis) and the radioisotopic assay described by Damen, Regts, and Scherphof (36) (vertical axis).

clonal PLTP-specific antibody and PLTP activity determinations confirmed the presence of macrophage-derived PLTP in all culture supernatants. RT-PCR analyses indicated that macrophage PLTP gene expression was up-regulated by differentiation (in the absence of any detectable increase in cellular cholesterol content) and by cholesterol loading. A similar effect of cholesterol loading on PLTP gene expression was observed in primary human macrophages as well as in PMA-differentiated THP-1 cells. These observations suggested that the differentiation of macrophages as well as their transformation into foam cells after uptake of oxidatively modified LDL is accompanied by substantial synthesis and secretion of PLTP within the subendothelial space.

The mechanisms underlying the up-regulation of human PLTP expression during macrophage differentiation and cholesterol loading warrant further investigation. In a recent report by Cao et al. (37), a liver X receptor (LXR) agonist-mediated up-regulation of mouse PLTP was observed in vivo as well as in cultured mouse macrophages. These findings identified mouse PLTP as a direct target gene of LXRs, a class of nuclear receptors thought to be activated in vivo by oxysterols (38). Together with these findings, our observations that human PLTP gene expression is increased upon macrophage cholesterol loading suggest that human PLTP might be a target gene of LXRs as well.

Studies conducted over the past decade suggest that PLTP is a multifunctional protein that plays a major role in lipoprotein metabolism (21, 22, 29). Many studies led to the demonstration that PLTP, through its ability to promote HDL fusion, plays a determinant role in the generation of pre-β HDL in plasma (28, 29). In addition to its HDL remodeling activity, PLTP promotes the transfer of numerous biologically important amphipathic molecules,

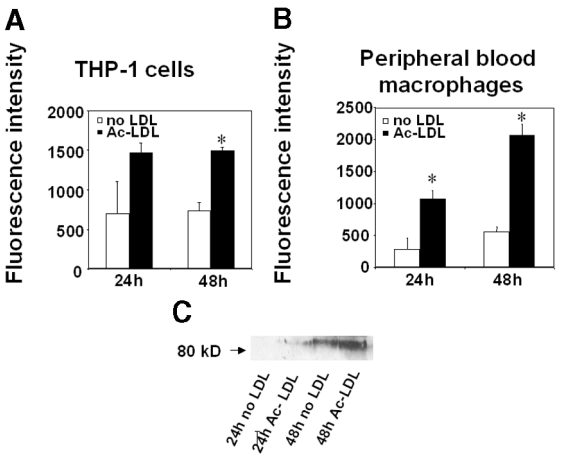

Fig. 7. Effect of cholesterol loading on PLTP secretion by human peripheral blood macrophages and THP-1 cells. A: PMA-differentiated THP-1 cells were incubated for 24 h or 48 h in the presence of Ac-LDL (80 μg/ml) in serum-free media containing 1% Nutridoma-Hu. B: Human monocyte-derived macrophages were cultured for 10 days in RPMI containing 10% autologous serum, then for 24 h or 48 h in the presence of Ac-LDL (80 μg/ml) in serum-free media containing 1% Nutridoma-Hu. Supernatants were harvested and the PLTP activity was measured at T = 30 min using a fluorimetric assay kit. Data shown represent the mean ± SEM of three determinations. $*P < 0.05$ compared with cells incubated in the absence of Ac-LDL. C: Supernatants from THP-1 cells were analyzed by Western blot using human PLTP-specific monoclonal antibodies, MabJH59 (1:2,000).

and both its phospholipid and α-tocopherol transfer activities were shown to be physiologically relevant in in vivo and ex vivo experiments (39, 40).

So far, specific studies of macrophage-produced PLTP in atherosclerosis have not been reported, although the relationship between plasma PLTP and atherosclerosis was recently examined (30, 31). PLTP deficiency has a marked protective effect against atherosclerosis in a variety of atherosclerosis-prone mouse strains, i.e., mice that are deficient for the LDL receptor ($LDLR^{-/-}$) or for apoE ($apoE^{-/-}$), or transgenic mice overexpressing the human apoB and CETP genes. Although the protective effect of the PLTP deficiency could be explained at least in part by a decreased secretion of apoB-containing lipoproteins by the liver, comparable apoB levels were measured in PLTP-deficient mice lacking the LDL receptor ($LDLR^{-/-}$), indicating that additional protective effects of PLTP deficiency should be envisioned (30). In a subsequent report, PLTP deficiency was shown to induce marked alterations in the distribution of α-tocopherol among plasma lipoproteins and tissues. In particular, a marked enrichment of VLDLs and LDL particles in α-tocopherol and a resulting increased resistance of these particles to oxidation were observed in PLTP-deficient mice (40). This could constitute an additional mechanism for the antiatherogenic effects of total PLTP deficiency (40).

Differentiated macrophages and foam cells are a hallmark of atherosclerotic lesions. The evidence presented in this study that PLTP is produced by macrophages and that its production is increased during foam cell formation indicates that we need to understand more about the role of locally produced, macrophage-derived PLTP in atherogenesis. As in the case of LPL (41), macrophage-derived and plasma PLTP could play different roles in atherogenesis. Recently, studies led to the demonstration that PLTP, through its ability to promote HDL fusion, plays a determinant role in the generation of pre-β HDLs, which are the best acceptors of cholesterol from peripheral cells (42). Efficient removal of excess cholesterol from intimal lipid-loaded macrophages is critical to preventing atherosclerosis progression. Macrophages produce a number of factors that can control lipid homeostasis, including scavenger receptors (43), apoE, apoCs (11, 12), LPL (13), hepatic lipase (14), SR-BI (18), and ABCA1 (19).

The recent discovery that the gene encoding ABCA1 is defective in Tangier disease patients (44, 45) sheds light on the mechanism controlling lipid accumulation in macrophages. ABCA1 plays a crucial role in the active apolipoprotein-mediated efflux of cholesterol and phospholipids from macrophages (17). The availability of extracellular acceptors, or the clearance of FC- and phospholipid-rich efflux products, could be envisioned as potential rate-limiting factors in the overall process of ABCA1-mediated cellular cholesterol efflux. Although pre-β HDLs have been identified in interstitial fluids in animal species and in humans (46), it is not known whether they originate from plasma pre-β HDL and cross the endothelial barrier, or whether they are generated in situ through HDL remodeling. Nanjee et al. recently studied the determinants of the level of small pre-β HDL in human tissue fluids. These authors reported that the concentration of small pre-β HDL in tissue fluids is determined only in part by the transfer of pre-β HDL across capillary endothelium from plasma. Therefore, local production of pre-β HDL by remodeling of spherical HDL in tissue fluids may contribute as well (47). The demonstration that PLTP is expressed in macrophages suggests that it could influence the remodeling of large spherical HDL to generate FC-poor pre-β HDL or FC-rich apoA-I in the intima of atherosclerotic lesions. Macrophage-derived PLTP could also promote lipid removal from intimal macrophages through its ability to enhance cell surface binding of HDL and apolipoprotein-mediated cholesterol and phospholipid efflux (48). Finally, the α-tocopherol transfer activity of PLTP is likely to affect the distribution of α-tocopherol between lipoproteins and cells within the vascular wall, thereby modulating such processes as LDL oxidation and vasomotricity. Such roles for locally produced macrophage PLTP could either promote or prevent foam cell formation. The contribution of systemic as well as local intimal foam cell-derived PLTP to atherosclerosis is currently under investigation.

The excellent technical assistance of Audrey Black and Jennifer LaVallee is greatly acknowledged. This work was supported by a Grant from the National Institutes of Health (HL-43815 to L.K.C.), an American Heart Association Predoctoral Fellowship (#0110041Y to P.A.M.), the Tobacco Related Disease Research

Program grant TRD-0161 to L.K.C., and an International HDL Research Awards Program grant to C.E.

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