Absence of endogenous phospholipid transfer protein impairs ABCA1-dependent efflux of cholesterol from macrophage foam cells

Miriam Lee-Rueckert,[1,*] Riikka Vikstedt,[1,†] Jari Metso,[†] Christian Ehnholm,[†] Petri T. Kovanen,[2,*] and Matti Jauhiainen[†]

Wihuri Research Institute,* Helsinki, Finland; and Department of Molecular Medicine,† National Public Health Institute, Biomedicum, Helsinki, Finland

Abstract In vitro experiments have demonstrated that exogenous phospholipid transfer protein (PLTP), i.e. purified PLTP added to macrophage cultures, influences ABCA1-mediated cholesterol efflux from macrophages to HDL. To investigate whether PLTP produced by the macrophages (i.e., endogenous PLTP) is also part of this process, we used peritoneal macrophages derived from PLTP-knockout (KO) and wild-type (WT) mice. The macrophages were transformed to foam cells by cholesterol loading, and this resulted in the upregulation of ABCA1. Such macrophage foam cells from PLTP-KO mice released less cholesterol to lipid-free apolipoprotein A-I (apoA-I) and to HDL than did the corresponding WT foam cells. Also, when plasma from either WT or PLTP-KO mice was used as an acceptor, cholesterol efflux from PLTP-KO foam cells was less efficient than that from WT foam cells. After cAMP treatment, which upregulated the expression of ABCA1, cholesterol efflux from PLTP-KO foam cells to apoA-I increased markedly and reached a level similar to that observed in cAMP-treated WT foam cells, restoring the decreased cholesterol efflux associated with PLTP deficiency. These results indicate that endogenous PLTP produced by macrophages contributes to the optimal function of the ABCA1-mediated cholesterol efflux-promoting machinery in these cells. Whether macrophage PLTP acts at the plasma membrane or intracellularly or shuttles between these compartments needs further study.—Lee-Rueckert, M., R. Vikstedt, J. Metso, C. Ehnholm, P. T. Kovanen, and M. Jauhiainen. **Absence of endogenous phospholipid transfer protein impairs ABCA1-dependent efflux of cholesterol from macrophage foam cells. *J. Lipid Res.* 2006.** 47: **1725–1732.**

Supplementary key words cholesterol loading • cholesterol efflux • ATP binding cassette transporter protein-1 upregulation • cyclic AMP • PLTP deficiency

Antiatherogenic HDLs are under continuous remodeling in the circulation by several plasma factors, and one actively studied among them is phospholipid transfer protein (PLTP). PLTP was initially described as a plasma component that transfers phospholipids to HDL (1). At present, PLTP has been assigned a key role in the intravascular remodeling of HDL (2, 3). PLTP promotes the transfer of VLDL postlipolytic surface remnants, mainly composed of phospholipids, to HDL subclasses, but the transfer mechanism is not known in detail. Studies conducted in vitro have demonstrated that plasma PLTP promotes the remodeling of HDL through the formation of large and fused HDL particles with concomitant generation of preβ-migrating HDL (preβ-HDL) (4). Furthermore, mice expressing high levels of PLTP have increased potential for preβ-HDL formation and accelerated hepatic uptake of HDL lipids in vivo (5).

There is compelling evidence that a low level of HDL cholesterol is a strong predictor of coronary heart disease and that HDL can protect against atherosclerosis by diverse mechanisms. One of them is related to the ability of HDL to promote the efflux of cholesterol from macrophage foam cells and thus inhibit the progression of atherosclerosis. HDL particles also mediate the transport of cholesterol to the liver for excretion along the pathway known as reverse cholesterol transport (RCT) (6). An important step of RCT in the arterial intima is the release of cholesterol from macrophages via ATP binding cassette transporter protein-1 (ABCA1), for which the major physiological acceptors are lipid-poor apolipoprotein A-I (apoA-I) and preβ-HDL particles (7). Cholesteryl ester transfer protein (CETP), the other plasma lipid transfer protein, also generates preβ-HDL (8). However, transgenic mouse models have demonstrated that PLTP, rather than CETP, is the main contributor to the generation of preβ-HDL (9). Other studies have clearly shown that overexpression of PLTP increases the antiatherogenic potential of HDL (5, 10, 11). However, the finding that overexpres-

Manuscript received 31 January 2006 and in revised form 13 April 2006.
Published, JLR Papers in Press, May 10, 2006.
DOI 10.1194/jlr.M600051-JLR200

[1] M. Lee-Rueckert and R. Vikstedt contributed equally to this work.
[2] To whom correspondence should be addressed.
e-mail: petri.kovanen@wri.fi

sion of PLTP in mice increased atherosclerotic lesion formation, mainly by increasing the production of apoB lipoproteins (12, 13), has challenged the suggested antiatherogenic role for PLTP.

PLTP is widely distributed among tissues and body fluids (14) and is also present in human atherosclerotic lesions (15, 16). The observations that PLTP can be secreted by intimal macrophages (15), and that cholesterol loading increases PLTP expression in cultured macrophages (16), suggest that PLTP could play an antiatherogenic role by generating lipid-poor preβ-HDL in the macrophage-rich areas of atherosclerotic plaques. More direct involvement of PLTP in the removal of cellular cholesterol and phospholipids via the ABCA1 pathway was reported recently, suggesting that pericellular PLTP could have an intermediary role in RCT (17). In addition, macrophage foam cells in human plaques contain abundant PLTP, which is associated with intracellular lipid droplets and is also located at the plasma membrane (16). These facts raise the possibility that endogenous PLTP might also have some intracellular functions. Indeed, recent data have demonstrated that PLTP activity and protein are abundant in the Golgi apparatus of hepatocytes, suggesting that intracellular PLTP might participate in apolipoprotein lipidation steps via its activity in these organelles (18).

Whether endogenous PLTP plays an intracellular role in ABCA1-dependent efflux from macrophages is unknown. To address this question, we studied the ABCA1-dependent efflux of cholesterol from peritoneal macrophages derived from PLTP-deficient mice and compared it with cholesterol efflux from wild-type (WT) macrophages. We found that cholesterol efflux from PLTP-deficient macrophage foam cells is defective and that the defect can be corrected by robust stimulation of the ABCA1-dependent pathway. These results support an intracellular role for endogenous macrophage PLTP in ABCA1-mediated cholesterol efflux from macrophage foam cells.

EXPERIMENTAL PROCEDURES

Animals

PLTP knockout (KO) (19) and C57BL/6J WT mice (8–12 weeks old) were matched for sex and age. Mice were provided with a standard chow diet and water ad libitum. Fasting blood was collected in precooled plastic tubes with EDTA (1 mg/ml) and centrifuged at low speed at 4°C to separate plasma. Plasma was placed on ice and used immediately or stored at −70°C until use.

Human plasma lipoproteins

LDL (1.019–1.050 g/ml) and HDL_3 (1.125–1.210 g/ml) were isolated from normolipidemic human plasma by sequential ultracentrifugation using KBr for density adjustments. The quantities of the lipoproteins are expressed in terms of their protein content. As reported previously (20, 21), the ultracentrifugally isolated HDL_3 fraction contained a minor component of preβ-HDL. LDL was acetylated in the presence of acetic anhydride (22), and the acetyl-LDL was radiolabeled by treatment with [1,2-^{3}H]cholesteryl linoleate (Amersham Biosciences, Piscataway, NJ) dissolved in dimethyl sulfoxide (23). The specific activities of the [^{3}H]cholesteryl ester (CE)-acetyl-LDL preparations ranged from 50 to 90 dpm/ng protein. Lipoprotein preparations were endotoxin-free based on the Limulus Amebocyte Lysate assay (Cambrex Bio Science, Walkersville, MD).

Cell cultures and loading of mouse peritoneal macrophages with CEs

Peritoneal cells from nonstimulated PLTP-KO and WT mice were harvested into PBS containing 1 mg/ml BSA. The cells were recovered after centrifugation, resuspended in DMEM (Invitrogen, Carlsbad, CA) containing 100 U/ml penicillin and 100 μg/ml streptomycin, and plated onto 24-well plates (BD Biosciences, Franklin Lakes, NJ). After incubation at 37°C for 2 h in a humidified CO_2 incubator, nonadherent cells were removed by washing the wells with PBS. The adherent cells (i.e., macrophages) were loaded with cholesterol by incubation for 18 h in the presence of 20 μg/ml [^{3}H]CE-acetyl-LDL in DMEM supplemented with 20% fetal calf serum. Macrophage morphology was monitored by microscopy before and after cholesterol loading. Viability of the cells was determined by lactate dehydrogenase activity assay using a commercial kit (Cytotoxicity Detection Kit, No. 1644793; Roche). No differences in cell viability were observed between the WT and PLTP-KO cells with or without acetyl-LDL loading (lactate dehydrogenase in culture medium ranged from 10.8% to 12.6%). For neutral lipid visualization, cells were treated with Oil Red O (Sigma-Aldrich, St. Louis, MO) and nuclei were stained with hematoxylin. To compare the ability of PLTP-KO and WT macrophages to take up acetyl-LDL, macrophages were incubated for 18 h in medium containing 10, 20, or 30 μg/ml acetyl-LDL, and cellular lipids were extracted by the addition of hexane-isopropanol (3:2, v/v). CE and free cholesterol (FC) from the lipid extracts were separated by high-performance TLC, and their quantities were determined with an automatic plate scanner (CAMAG TLC). Cellular lipids in the nonloaded macrophages were also quantified by this method. For efflux experiments using nonloaded macrophages, the cells were merely radiolabeled with cholesterol by incubating them for 24 h in DMEM containing 4 μCi/ml [1,2-^{3}H]cholesterol in ethanol (vehicle $< 0.1\%$ in medium) and 2 μg/ml of the ACAT inhibitor Sandoz 58-035 dissolved in DMSO (vehicle $< 0.5\%$ in medium) (a generous gift from Novartis, Basel, Switzerland) to prevent the esterification of radiolabeled cholesterol (24).

cAMP stimulation of mouse peritoneal macrophages

[^{3}H]CE-cholesterol-loaded (foam cells) and [^{3}H]FC-labeled (basal) macrophages were washed with PBS and incubated for 18 h in DMEM in the presence or absence of 0.3 mM 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (Sigma-Aldrich).

Cholesterol efflux assay

Purified human lipid-free apoA-I was kindly provided by Dr. Peter Lerch (Swiss Red Cross, Bern, Switzerland). The time course (2, 4, and 6 h) of efflux to apoA-I demonstrated that, under the conditions used, [^{3}H]cholesterol efflux from either WT or PLTP-KO macrophage foam cells was linear up to 4 h of incubation (data not shown). Human HDL_3 and mouse plasma were also used as cholesterol acceptors. [^{3}H]CE-cholesterol-loaded and [^{3}H]FC-labeled nonloaded macrophages were incubated with DMEM in the presence or absence of cholesterol acceptors at different concentrations, as shown in the figures. In the experiments using mouse plasma as cholesterol acceptor, 10 IU/ml recombinant hirudin (CibaGeigy, Dover Township, NJ) as anticoagulant was added to the culture medium. After 4 h

of incubation, cells were solubilized by treatment with 0.2 M NaOH and protein concentrations of the cell lysates were determined. Media were collected and centrifuged at 200 *g* for 5 min, and the radioactivity of each supernatant was evaluated by liquid scintillation counting and normalized for cellular protein mass. Specific efflux values to different acceptors were calculated by subtracting efflux values obtained with the medium only.

Other assays

ApoA-I was determined by a direct ELISA procedure (25). PLTP activity was measured by a radiometric assay, after phospholipid transfer from radiolabeled donor phospholipid liposomes to acceptor HDL_3 particles (26).

Statistical analysis

Results are reported as means ± SD of triplicate incubations. Statistical significance ($P < 0.05$) was determined by two-tailed Student's *t*-test. The software Prism (GraphPad, Inc., San Diego, CA) was used to calculate K_m values. Values shown in the figures are representative of triplicate runs of at least two or three experiments.

RESULTS

We studied the efflux of [^{3}H]cholesterol from non-elicited peritoneal macrophages derived from the PLTP-KO mouse under various conditions that upregulate ABCA1-dependent efflux. After treatment of the macrophages with acetyl-LDL, we observed that the morphological features of PLTP-KO and WT foam cells were very similar (**Fig. 1A**), as was the intensity of the stained intracellular lipid droplets (Fig. 1B). Next, we investigated the efflux of cholesterol to HDL_3 from PLTP-KO macrophages both in their basal state (i.e., when not loaded with CE) and after their conversion to foam cells (i.e., when loaded with CE) (**Fig. 2**). As reported previously, cholesterol loading of macrophages with acetyl-LDL leads to the formation of macrophage foam cells, with subsequent

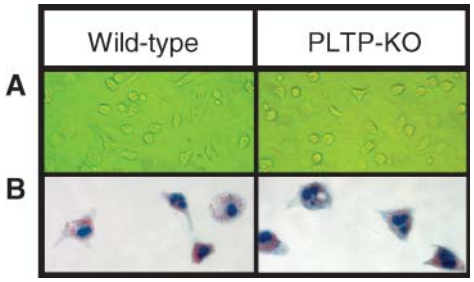

Fig. 1. Comparison of the cellular morphology of foam cells derived from wild-type (WT) and phospholipid transfer protein knockout (PLTP-KO) macrophages. Peritoneal macrophages were isolated from nonstimulated PLTP and WT mice and incubated for 18 h at 37°C in the presence of 20 μg/ml [^{3}H]cholesteryl ester (CE)-acetyl-LDL (100,000 dpm/well) in DMEM supplemented with 20% fetal calf serum. A: Morphology of WT and PLTP-KO macrophage foam cells was evaluated by light microscopy after cholesterol loading. B: Neutral lipids in the cytoplasm of foam cells derived from WT and PLTP-KO mice were stained with Oil Red O and visualized with light microscopy. Original magnifications, ×40 (A) and ×100 (B).

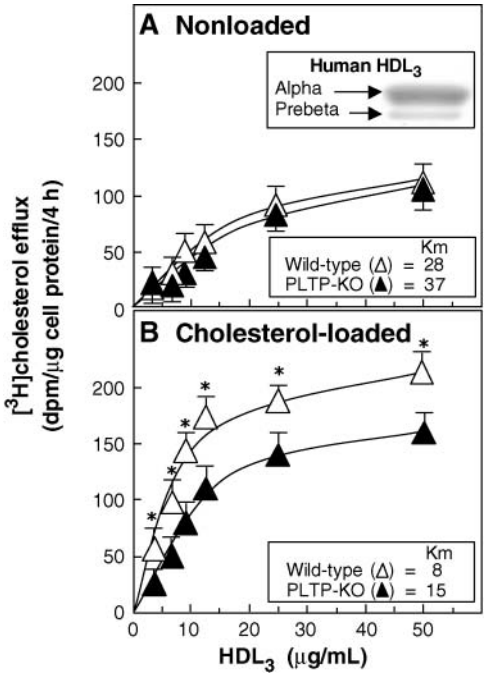

Fig. 2. [^{3}H]Cholesterol efflux from PLTP-KO and WT macrophages to HDL_3. Mouse peritoneal macrophages were labeled by exposing the cells for 24 h to [^{3}H]free cholesterol (FC; 4 μCi/ml) in the presence of Sandoz 58-035 compound (2 μg/ml) (A) or cholesterol-loaded by incubation for 18 h with 20 μg/ml [^{3}H]CE-acetyl-LDL (B). After washing the cells, increasing concentrations of HDL_3 were added to fresh medium. After incubation for 4 h, the medium was removed and centrifuged at low speed to eliminate cell debris. NaOH (0.2 M) was added to the wells to solubilize the cells and measure their protein contents. Efflux of cholesterol was expressed by determination of ^{3}H radioactivity in medium and normalizing the values for the cell protein. Parallel wells of macrophages were incubated in medium free of HDL_3 to subtract the background efflux (blanks). Each value is the mean of triplicate wells ± SD. Statistically significant differences between PLTP-KO and WT macrophages are indicated (* $P < 0.05$ by *t*-test). K_m values (Prism software) are shown in the lower insets in both panels. The upper inset in A shows apolipoprotein A-I (apoA-I) immunoblotting of HDL_3 after agarose electrophoresis, demonstrating the presence of both α and preβ bands. The content of preβ-migrating HDL (preβ-HDL) in the HDL_3 preparation was 8% (percentage of the sum of α- and preβ-HDL).

overexpression of ABCA1 and ensuing stimulation of cholesterol efflux (27), its magnitude being related to the content of lipid-poor apoA-I-containing particles (preβ-HDL) in the HDL_3 preparation used as a cholesterol acceptor (28). In three different HDL_3 preparations, preβ-HDL ranged from 6% to 8% as calculated from the sum of α- and preβ-HDL, as described previously (10, 11). The presence of preβ-HDL particles in one of the HDL_3 preparations used in this study is shown in Fig. 2A (upper inset). The nonloaded mouse peritoneal macrophages, in which ABCA1 activity is very low, were labeled with [^{3}H]FC in the presence of compound 58-035, an ACAT inhibitor. Addition of increasing concentrations of HDL_3 showed that the nonloaded macrophages released cholesterol with low efficiency, probably reflecting diffusional efflux of cholesterol, which was likely independent of the intracellular

availability of PLTP (Fig. 2A, K_m values in lower inset). In contrast, when ABCA1-mediated efflux was upregulated by cholesterol loading of the cells, high-affinity and saturable efflux of cholesterol was observed in a concentration-dependent manner (Fig. 2B). More importantly, cholesterol efflux was markedly reduced from PLTP-KO cholesterol-loaded macrophages, and the K_m value increased by 2-fold compared with that of WT macrophages (15.6 vs. 8.2 μg/ml, respectively) (Fig. 2B, lower inset). After efflux, no PLTP activity could be measured in the culture media of WT cells. Because the preβ-HDL-dependent efflux was affected, these findings suggest a functional role for endogenous macrophage PLTP in ABCA1-mediated cholesterol efflux. To further evaluate this hypothesis, we studied cholesterol efflux from [^{3}H]CE-loaded macrophages in the presence of low concentrations of human lipid-free apoA-I that specifically involves the ABCA1-mediated pathway. Time-dependent (2 and 4 h) and concentration-dependent (0 to 25 μg/ml apoA-I in medium) experiments demonstrated that the rate of cholesterol efflux to apoA-I was higher from WT than from PLTP-KO foam cells (**Fig. 3**). With higher apoA-I concentration (50 μg/ml), the differences in efflux between the two cells remained the same (data not shown). These results indicate that impaired ABCA1-mediated efflux was associated with the absence of endogenous PLTP.

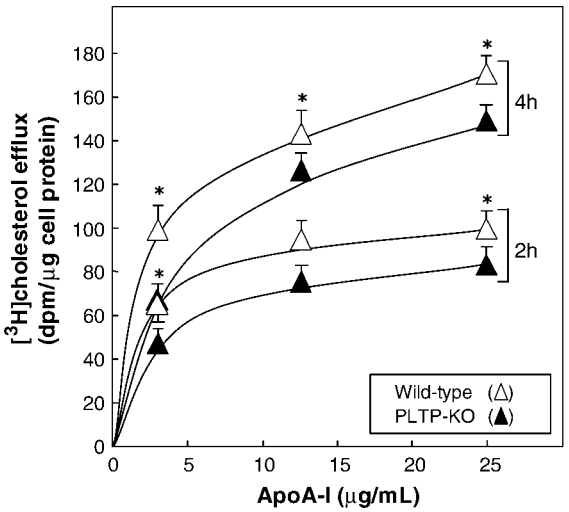

Fig. 3. [^{3}H]Cholesterol efflux from PLTP-KO and WT macrophage foam cells to apoA-I. Mouse peritoneal macrophages were converted to foam cells by incubation for 18 h at 37°C in the presence of 20 μg/ml [^{3}H]CE-acetyl-LDL in DMEM supplemented with 20% fetal calf serum. After incubation for 2 and 4 h with increasing amounts of lipid-free apoA-I, the medium was removed and centrifuged at low speed to eliminate cell debris. NaOH (0.2 M) was added to the wells to solubilize the macrophage foam cells and measure their protein contents. Efflux of cholesterol was expressed by determination of ^{3}H radioactivity in medium and normalizing the values as dpm in medium/μg cellular protein. Parallel wells of macrophages were incubated in medium free of apoA-I to subtract the background efflux (blanks). Each value is the mean of triplicate wells ± SD. Statistically significant differences between PLTP-KO and WT macrophages are indicated (* $P < 0.05$ by t-test).

To verify that the observed reduction in cholesterol efflux from PLTP-deficient macrophages was not a consequence of reduced cholesterol loading, we compared the cellular lipid contents of PLTP-KO and WT macrophages incubated for 18 h in the presence of 20 μg/ml [^{3}H]CE-acetyl-LDL. In the basal state (i.e., before cholesterol loading of the cells with acetyl-LDL), we analyzed cellular lipids in the nonloaded cells. Free cholesterol was slightly increased in WT macrophages, whereas no differences in cholesterol fatty acyl esters were observed between the two types of macrophages (**Table 1**). Acetyl-LDL treatment led to abundant formation of foam cells from both WT and PLTP-KO macrophages (Fig. 1B). Furthermore, a similar degree of lipid loading was observed when 20 μg/ml acetyl-LDL (100,000 dpm/well) was added to the cells. Thus, similar values were observed when data from triplicate wells (means ± SD) were expressed as specific radioactivity of CE (924 ± 245 and 852 ± 231 dpm/μg, respectively; $P = 0.209$) or as total radioactivity relative to total sterol mass (2,242 ± 275 and 2,134 ± 321 dpm/μg, respectively; $P = 0.140$). More importantly, when macrophages were loaded with 10, 20, or 30 μg of acetyl-LDL per milliliter of medium, quantification of intracellular FC and CE revealed no differences in lipid loading between the PLTP-KO and WT cells (Table 1). Overall, the data demonstrate that, under the conditions used, genetic deficiency of PLTP in mouse peritoneal macrophages does not impair their conversion into cholesterol-loaded foam cells upon treatment with acetyl LDL, thus excluding the possibility that reduced efflux of cholesterol is secondary to any effect of PLTP deficiency on cholesterol uptake itself.

We next assessed the effect of endogenous PLTP on cholesterol efflux from macrophage foam cells to plasma, which represents a more physiological acceptor (29). To this end, plasma isolated from both PLTP-KO and WT mice was added to either PLTP-KO or WT macrophage foam cells (**Fig. 4**). As reported previously (30), the concentration of apoA-I in plasma was significantly lower in mice lacking

TABLE 1. Cellular contents of free and esterified cholesterol in macrophage foam cells from PLTP-KO and WT mice after cholesterol loading by different levels of exposure to acetyl-LDL

	FC			CE		
Acetyl-LDL	PLTP-KO	WT	*P*	PLTP-KO	WT	*P*
μg/ml	*μg/mg cell protein*			*μg/mg cell protein*		
0	25 ± 0.3	27 ± 0.4	0.01	11 ± 0.2	14 ± 1.6	NS
10	48 ± 9.0	50 ± 8.1	NS	75 ± 17.5	81 ± 9.2	NS
20	65 ± 0.3	65 ± 6.5	NS	114 ± 17.9	123 ± 6.7	NS
30	71 ± 6.2	72 ± 3.8	NS	134 ± 19.2	149 ± 17.9	NS

CE, cholesteryl ester; FC, free cholesterol; PLTP-KO, phospholipid transfer protein knockout; WT, wild-type. Cultures of peritoneal macrophages from PLTP-KO and WT mice were converted to cholesterol-loaded macrophage foam cells by incubation with 10, 20, or 30 μg/ml acetyl-LDL, as described in Experimental Procedures. After incubation for 18 h, the cells were treated with hexane-isopropanol for extraction of lipids. CE and FC from the lipid extracts were separated by high-performance TLC, and their quantities were determined by quantitative scanning. Cell protein was determined after solubilization of the cell residue with 0.2 M NaOH. Results are means ± SD of triplicate wells. Statistical differences were analyzed by Student's t-test ($P > 0.05$).

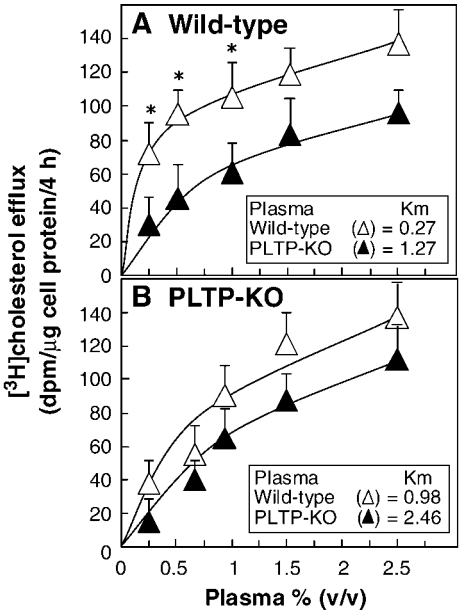

Fig. 4. [^{3}H]Cholesterol efflux from PLTP-KO and WT macrophage foam cells to plasma isolated from PLTP-KO or WT mice. Mouse peritoneal macrophages were converted to foam cells by incubation for 18 h at 37°C in the presence of 20 μg/ml [^{3}H]CE-acetyl-LDL in DMEM supplemented with 20% fetal calf serum. PLTP-KO and WT macrophage foam cells were incubated in medium containing plasma from PLTP-KO or WT mice and 10 IU/ml hirudin. After 4 h of incubation, the concentration-dependent efflux of cellular cholesterol to the plasmas was measured from WT (A) and PLTP-KO (B) macrophages. The medium was removed and centrifuged at low speed, and 0.2 M NaOH was added to the wells to solubilize the cells and measure their protein contents. Efflux of cholesterol was expressed by determination of ^{3}H radioactivity in medium and normalizing the values as dpm in medium/μg cellular protein. Parallel wells of macrophages were incubated in medium free of plasma to subtract the background efflux (blanks). Each value is the mean of triplicate wells ± SD. Statistically significant differences between PLTP-KO and WT macrophages are indicated (* $P < 0.05$ by *t*-test). K_m values (Prism software) for each type of macrophage and each type of plasma are shown in the insets.

PLTP (641 ± 26 vs. 930 ± 52 μg/ml). The plasma preβ-HDL levels were also lower in the PLTP-KO plasma (data not shown). Furthermore, in the PLTP-KO mice, no PLTP activity was observed in the plasma, whereas in the WT litter mice, PLTP activity varied between 12 and 15 μmol/ml/h. As shown in Fig. 4A, within the low concentration range in which high-affinity efflux from mouse peritoneal macrophages is apparent (31), PLTP-containing WT plasma induced more efficient cholesterol efflux from WT macrophages than did PLTP-KO plasma. More importantly, WT plasma promoted less efficient efflux of cholesterol from PLTP-KO than from WT macrophages (Fig. 4A, B, K_m values). Because the concentration of apoA-I in PLTP-KO plasma was lower than in WT plasma, we next normalized the efflux values for the concentration of apoA-I in both plasmas. Upon this analysis, as expected, the efflux curves approached each other (data not shown); however, efflux promoted by the two types of plasma still remained as depicted in Fig. 3. These results confirm that endogenous PLTP was necessary for proper cholesterol release from the macrophages to both types of mouse plasma.

Because stimulation with cAMP leads to the upregulation of expression and activity of ABCA1 in mouse peritoneal macrophages (32), the type of cell studied here, we next evaluated whether cAMP treatment could induce differences between PLTP-KO and WT macrophages in the cholesterol efflux induced by apoA-I. For this purpose, nonloaded macrophages labeled with [^{3}H]FC in the presence of ACAT inhibitor were stimulated with cAMP (0.3 mM), and efflux of cholesterol to apoA-I (25 μg/ml) was determined. As shown in **Fig. 5A**, the magnitude of basal cholesterol efflux was similar from WT and PLTP-KO nonstimulated macrophages (left). As expected, when macrophages were stimulated with cAMP, the efflux rate of cholesterol was accelerated, reflecting upregulation of the ABCA1-dependent pathway (right). However, in contrast to the cholesterol-loaded cells (Fig. 3), the efflux increased to a similar degree (~35%) in both types of cell. This finding prompted us to investigate whether a further induction of ABCA1 with cAMP, beyond its induction by cholesterol loading, would overcome the impaired ABCA1-dependent cholesterol efflux from PLTP-deficient macro-

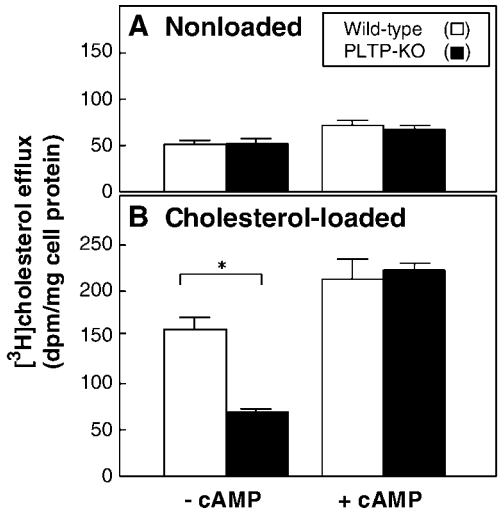

Fig. 5. [^{3}H]Cholesterol efflux from PLTP-KO and WT macrophages under the stimulation of ABCA1-dependent efflux by cholesterol loading or activation of the ABCA1 pathway with cAMP. Basal [^{3}H]FC-labeled macrophage or [^{3}H]CE-loaded macrophage foam cells from PLTP-KO (closed bars) and WT (open bars) mice were washed with PBS and incubated for 18 h in DMEM with (+cAMP) or without (−cAMP) 0.3 mM 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate. After further incubation for 4 h with lipid-free apoA-I (25 μg/ml), the medium was removed and centrifuged at low speed, and 0.2 M NaOH was added to the wells to solubilize the cells and measure their protein contents. Efflux of cholesterol was expressed by determination of ^{3}H radioactivity in medium and normalizing the values as dpm in medium/μg cellular protein. Parallel wells of macrophages were incubated in medium free of apoA-I to subtract the background efflux (blanks). Each value is the mean of triplicate wells ± SD. Statistically significant differences between PLTP-KO and WT macrophages are indicated (* $P < 0.05$ by *t*-test).

phage foam cells. For this purpose, macrophages were first loaded with cholesterol and then incubated in the presence of 0.3 mM cAMP for 18 h. After the stimulation period, apoA-I (25 μg/ml) was added, and cholesterol efflux was measured after 4 h of incubation (Fig. 5B). In agreement with the results described above (Fig. 3), cholesterol-loaded macrophages deficient in PLTP delivered cholesterol to apoA-I with significantly lower efficiency (Fig. 5B, left). Interestingly, the combined effect of cholesterol loading and cAMP treatment (Fig. 5B, right) resulted in strong stimulation of cholesterol efflux and restoration of the efflux from PLTP-KO macrophages to the level observed in cAMP-stimulated WT foam cells. This result indicates that PLTP deficiency was compensated by robust stimulation of ABCA1-dependent cholesterol efflux. Similar results were obtained when higher apoA-I amounts (up to 50 μg/ml) were added to the cell cultures as a cholesterol acceptor (data not shown).

DISCUSSION

This study demonstrates that complete absence of endogenous PLTP in cholesterol-loaded but not in nonloaded macrophages is associated with impaired ABCA1-dependent cholesterol efflux. Accordingly, the functional consequences of PLTP deficiency were restricted to the cholesterol-loaded macrophage foam cell phenotype. Interestingly, cAMP, a compound that effectively induces ABCA1-mediated lipid efflux, restored the efflux of cholesterol from the PLTP-KO macrophage foam cells.

The accumulation of cholesterol in macrophages of the arterial intima is the key early feature of atherosclerosis (33), reflecting an imbalance between cholesterol influx into and efflux from macrophages (34). In those macrophages, ABCA1 mRNA and protein levels are under positive sterol regulation by a mechanism that involves the activation of the liver X receptor (LXR) (27). Our current results revealed that a complete deficiency of PLTP did not affect the uptake of acetyl-LDL by the scavenger receptor SR-A that mediates cholesterol accumulation in macrophages (35) or the subsequent formation of cytoplasmic CE droplets. Thus, the ability of macrophages to be converted into foam cells was not impaired by the genetic absence of PLTP. In sharp contrast, cholesterol efflux from PLTP-deficient macrophage foam cells was defective. Data on the participation of PLTP in cholesterol efflux from cells are available in some detail for exogenously added PLTP (10, 17, 36–38). Although the addition of PLTP alone to cell cultures does not seem to promote cholesterol efflux (37, 38), PLTP is able to interact with the cell surface in the presence of HDL, improving the efficiency of ABCA1-mediated efflux (37). More recently, it was reported that extracellular PLTP may aid apoA-I in the stabilization of ABCA1 (17), suggesting a functional cross-talk between apoA-I, exogenous PLTP, and ABCA1 in the cholesterol efflux process.

Although the molecular mechanism by which ABCA1 governs cholesterol efflux is not completely understood, several regulatory factors that affect its expression and activity have been identified (reviewed in 39). Among these is vesicular trafficking of cholesterol from the Golgi region to the plasma membrane (40). Indeed, ABCA1 is present in intracellular compartments (41) and has been found to traffic along the endocytic pathway to late endocytic compartments (42). This suggests a possible interaction between ABCA1 and apoA-I in a recycling pathway that allows the mobilization of cholesterol from late endocytic pools to other pools from which it can be released from the cell (43). It was recently demonstrated that in hepatocytes, ABCA1 mediates early intracellular phospholipidation of apoA-I (44), thus promoting its acquisition of phospholipids rather than cholesterol (45). A previous observation also suggests a role for ABCA1 in the intracellular routing of phospholipids (46). Overall, these findings are compatible with the view that PLTP, given its major role in the transfer of phospholipids, may participate in intracellular recycling of ABCA1 by increasing the transfer of phospholipids to ABCA1, which may then be translocated to phospholipid acceptors, such as apoA-I, ultimately leading to the formation of apoA-I-phospholipid complexes (47). This could explain the defective cholesterol efflux consistently found in PLTP-KO foam cells (Figs. 2B, 3, 4, 5B).

Treatment of various macrophage cell lines with cAMP stimulates ABCA1-dependent cholesterol efflux (48, 49). We found that, after cAMP treatment, cholesterol efflux from nonloaded PLTP-KO and WT macrophages labeled with FC in the presence of ACAT inhibitor (which ensures that cholesterol released originates from the unesterified cholesterol pool) did not differ. Consistent with the data obtained when labeling the CE pool in foam cells, this result suggests that the absence of intracellular PLTP perturbs the steps of the ABCA1 pathway that involve active cholesterol mobilization from late endosomes (50), rather than those involved in the efflux of FC at the cell surface. Importantly, it has been demonstrated that cAMP treatment of mouse macrophages specifically increases the expression of ABCA1 and has no effect on the expression of ABCG1 (51). Therefore, restoration of cholesterol efflux from the PLTP-deficient foam cells when ABCA1 (and not ABCG1) is further upregulated by cAMP treatment further supports the view that PLTP is required for the optimal functionality of the ABCA1-mediated efflux. Recently, it was shown that cAMP triggers cholesterol efflux in mouse peritoneal macrophages predominantly by activation of protein kinase A rather than by induction of ABCA1 expression alone (32) (i.e., via a posttranslational upregulation of ABCA1 activity by its protein kinase A-mediated phosphorylation) (52, 53). Whether the upregulation of ABCA1-mediated efflux by LXR activation during foam cell formation or via phosphorylation mediated by protein kinase A has separate requirements for endogenous PLTP deserves further study. A previous report in elicited peritoneal macrophages from PLTP-KO mice showed an additive effect on cholesterol efflux by the combination of cholesterol loading and exposure to LXR agonists (54). However, in spite of PLTP being strongly upregulated by LXR agonists in macrophages, only a minimal failure was found in the efflux capacity of PLTP-KO relative to the WT

macrophage foam cells before or after LXR stimulation. Although we cannot clearly assert the reason for this contradiction regarding these results, possible responses induced by the inflammatory challenge via thioglycollate exposure of elicited macrophages (55) may partially account for the observed differences.

Because of its multifaceted effects on lipoprotein metabolism (3, 10, 18, 56), PLTP has been considered to promote either antiatherogenic or proatherogenic processes. Decreased atherosclerosis in the PLTP-KO mouse has been suggested to be attributable to a decreased production of apoB-containing lipoproteins (18). Thus, responses to the genetic deficiency in the animal model may mask perturbations in particular cellular responses confined to specific body compartments, such as the arterial intima. Indeed, discrepant results were observed for the effects of ABCA1 deficiency on atherosclerosis when the experimental animals had a complete absence of ABCA1 (ABCA1-KO mice) and when ABCA1 was selectively inactivated only in macrophages (57).

In this study, we have identified a novel intracellular effect of PLTP on ABCA1-mediated cholesterol efflux. Although no PLTP activity could be detected in the culture medium of WT macrophages after the 4 h efflux period, it is possible that minute amounts of macrophage-derived PLTP may also act pericellularly/extracellularly. Of note, the expression of both ABCA1 and PLTP is upregulated by LXR activators, which promote cholesterol efflux (58, 59). Thus, endogenous PLTP, upon its coordinated induction with ABCA1, seems to play an antiatherogenic role by enhancing the initial step of RCT, and so tends to prevent the formation of foam cells. Finally, our findings in cultured macrophage foam cells are consistent with the recently suggested atheroprotective role for macrophage-derived PLTP in vivo in mice (60).

The authors thank Ms. Mari Jokinen for excellent technical assistance. The Wihuri Research Institute is maintained by the Jenny and Antti Wihuri Foundation. This study was also supported by grants from the Sigrid Juselius Foundation (M.L-R. and M.J.) and the Finnish Foundation for Cardiovascular Research (M.J.).

REFERENCES

1. Tall, A. R., E. Abreu, and J. Shuman. 1983. Separation of a plasma phospholipid transfer protein from cholesterol ester/phospholipid exchange protein. *J. Biol. Chem.* **258:** 2174–2180.
2. Huuskonen, J., V. M. Olkkonen, M. Jauhiainen, and C. Ehnholm. 2001. The impact of phospholipid transfer protein (PLTP) on HDL metabolism. *Atherosclerosis.* **155:** 269–281.
3. Albers, J. J., and M. C. Cheung. 2004. Emerging roles for phospholipid transfer protein in lipid and lipoprotein metabolism. *Curr. Opin. Lipidol.* **15:** 255–260.
4. Settasatian, N., M. Duong, L. K. Curtiss, C. Ehnholm, M. Jauhiainen, J. Huuskonen, and K. A. Rye. 2001. The mechanism of the remodeling of high density lipoproteins by phospholipid transfer protein. *J. Biol. Chem.* **276:** 26898–26905.
5. Foger, B., S. Santamarina-Fojo, R. D. Shamburek, C. L. Parrot, G. D. Talley, and H. B. Brewer, Jr. 1997. Plasma phospholipid transfer protein. Adenovirus-mediated overexpression in mice leads to decreased plasma high density lipoprotein (HDL) and enhanced hepatic uptake of phospholipids and cholesteryl esters from HDL. *J. Biol. Chem.* **272:** 27393–27400.
6. Brewer, H. B., Jr., A. T. Remaley, E. B. Neufeld, F. Basso, and C. Joyce. 2004. Regulation of plasma high-density lipoprotein levels by the ABCA1 transporter and the emerging role of high-density lipoprotein in the treatment of cardiovascular disease. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1755–1760.
7. Fielding, C. J., and P. E. Fielding. 2001. Cellular cholesterol efflux. *Biochim. Biophys. Acta.* **1533:** 175–189.
8. Liang, H. Q., K. A. Rye, and P. J. Barter. 1994. Dissociation of lipid-free apolipoprotein A-I from high density lipoproteins. *J. Lipid Res.* **35:** 1187–1199.
9. Lie, J., R. de Crom, M. Jauhiainen, T. van Gent, R. van Haperen, L. Scheek, H. Jansen, C. Ehnholm, and A. van Tol. 2001. Evaluation of phospholipid transfer protein and cholesteryl ester transfer protein as contributors to the generation of pre beta-high-density lipoproteins. *Biochem. J.* **360:** 379–385.
10. van Haperen, R., A. van Tol, P. Vermeulen, M. Jauhiainen, T. van Gent, P. van den Berg, S. Ehnholm, F. Grosveld, A. van der Kamp, and R. de Crom. 2000. Human plasma phospholipid transfer protein increases the antiatherogenic potential of high density lipoproteins in transgenic mice. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1082–1088.
11. Jaari, S., K. W. van Dijk, V. M. Olkkonen, A. van der Zee, J. Metso, L. Havekes, M. Jauhiainen, and C. Ehnholm. 2001. Dynamic changes in mouse lipoproteins induced by transiently expressed human phospholipid transfer protein (PLTP): importance of PLTP in prebeta-HDL generation. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **128:** 781–792.
12. van Haperen, R., A. van Tol, T. van Gent, L. Scheek, P. Visser, A. van der Kamp, F. Grosveld, and R. de Crom. 2002. Increased risk of atherosclerosis by elevated plasma levels of phospholipid transfer protein. *J. Biol. Chem.* **277:** 48938–48943.
13. Yang, X. P., D. Yan, C. Qiao, R. J. Liu, J. G. Chen, J. Li, M. Schneider, L. Lagrost, X. Xiao, and X. C. Jiang. 2003. Increased atherosclerotic lesions in apoE mice with plasma phospholipid transfer protein overexpression. *Arterioscler. Thromb. Vasc. Biol.* **23:** 1601–1607.
14. Albers, J. J., G. Wolfbauer, M. C. Cheung, J. R. Day, A. F. Ching, S. Lok, and A. Y. Tu. 1995. Functional expression of human and mouse plasma phospholipid transfer protein: effect of recombinant and plasma PLTP on HDL subspecies. *Biochim. Biophys. Acta.* **1258:** 27–34.
15. Desrumaux, C. M., P. A. Mak, W. A. Boisvert, D. Masson, D. Stupack, M. Jauhiainen, C. Ehnholm, and L. K. Curtiss. 2003. Phospholipid transfer protein is present in human atherosclerotic lesions and is expressed by macrophages and foam cells. *J. Lipid Res.* **44:** 1453–1461.
16. O'Brien, K. D., S. Vuletic, T. O. McDonald, G. Wolfbauer, K. Lewis, A. Y. Tu, S. Marcovina, T. N. Wight, A. Chait, and J. J. Albers. 2003. Cell-associated and extracellular phospholipid transfer protein in human coronary atherosclerosis. *Circulation.* **108:** 270–274.
17. Oram, J. F., G. Wolfbauer, A. M. Vaughan, C. Tang, and J. J. Albers. 2003. Phospholipid transfer protein interacts with and stabilizes ATP-binding cassette transporter A1 and enhances cholesterol efflux from cells. *J. Biol. Chem.* **278:** 52379–52385.
18. Jiang, X. C., S. Qin, C. Qiao, K. Kawano, M. Lin, A. Skold, X. Xiao, and A. R. Tall. 2001. Apolipoprotein B secretion and atherosclerosis are decreased in mice with phospholipid-transfer protein deficiency. *Nat. Med.* **7:** 847–852.
19. Qin, S., K. Kawano, C. Bruce, M. Lin, C. Bisgaier, A. R. Tall, and X. Jiang. 2000. Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins. *J. Lipid Res.* **41:** 269–276.
20. Lee, M., A. von Eckardstein, L. Lindstedt, G. Assmann, and P. T. Kovanen. 1999. Depletion of preβ-LpA1 and LpA4 particles by mast cell chymase reduces cholesterol efflux from macrophage foam cells induced by plasma. *Arterioscler. Thromb. Vasc. Biol.* **19:** 1066–1074.
21. Lee, M., C. P. Sommerhoff, A. von Eckardstein, F. Zettl, H. Fritz, and P. T. Kovanen. 2002. Mast cell tryptase degrades HDL and blocks its function as an acceptor of cellular cholesterol. *Arterioscler. Thromb. Vasc. Biol.* **22:** 2086–2091.
22. Basu, S. K., J. L. Goldstein, R. G. W. Anderson, and M. S. Brown. 1976. Degradation of cationized low density lipoprotein and regulation of cholesterol metabolism in homozygous familial

hypercholesterolemia fibroblasts. *Proc. Natl. Acad. Sci. USA.* **73:** 3178–3182.
23. Brown, M. S., S. E. Dana, and J. L. Goldstein. 1975. Receptor-dependent hydrolysis of cholesterol esters contained in plasma low density lipoprotein. *Proc. Natl. Acad. Sci. USA.* **72:** 2925–2929.
24. Ross, A. C., K. J. Go, J. G. Heider, and G. H. Rothblat. 1984. Selective inhibition of acyl coenzyme A:cholesterol acyltransferase by compound 58-035. *J. Biol. Chem.* **259:** 815–819.
25. Siggins, S., M. Jauhiainen, V. M. Olkkonen, J. Tenhunen, and C. Ehnholm. 2003. PLTP secreted by HepG2 cells resembles the high-activity PLTP form in human plasma. *J. Lipid Res.* **44:** 1698–1704.
26. Jauhiainen, M., J. Metso, R. Pahlman, S. Blomqvist, A. van Tol, and C. Ehnholm. 1993. Human phospholipid transfer protein causes high density lipoprotein conversion. *J. Biol. Chem.* **268:** 4032–4036.
27. Langmann, T., J. Klucken, M. Reil, G. Liebisch, M-F. Luciani, G. Chimini, W. E. Kaminski, and G. Schmitz. 1999. Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence of sterol-dependent regulation in macrophages. *Biochem. Biophys. Res. Commun.* **257:** 29–33.
28. Favari, E., M. Lee, L. Calabresi, G. Franceschini, F. Zimetti, F. Bernini, and P. T. Kovanen. 2004. Depletion of prebeta-HDL by human chymase impairs ATP-binding cassette transporter A1-but not SR-B1-mediated lipid efflux to HDL. *J. Biol. Chem.* **279:** 9930–9936.
29. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
30. Jiang, X. C., C. Bruce, J. Mar, M. Lin, Y. Ji, O. L. Francone, and A. R. Tall. 1999. Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. *J. Clin. Invest.* **103:** 907–914.
31. Lee, M., L. Calabresi, G. Chiesa, G. Franceschini, and P. T. Kovanen. 2002. Mast cell chymase degrades apoE and apoA-II in apoA-I-knockout mouse plasma and reduces its ability to promote cellular cholesterol efflux. *Arterioscler. Thromb. Vasc. Biol.* **22:** 1475–1481.
32. Kiss, R. S., J. Maric, and Y. L. Marcel. 2005. Lipid efflux in human and mouse macrophagic cells: evidence for differential regulation of phospholipid and cholesterol efflux. *J. Lipid Res.* **46:** 1877–1887.
33. Ross, R. 1999. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **340:** 115–126.
34. Kovanen, P. T. 1990. Atheroma formation: defective control in the intimal round-trip of cholesterol. *Eur. Heart J.* **11 (Suppl. E):** 238–246.
35. Babaev, V. R., L. A. Gleaves, K. J. Carter, H. Suzuki, T. Kodama, S. Fazio, and M. F. Linton. 2000. Reduced atherosclerotic lesions in mice deficient for total or macrophage-specific expression of scavenger receptor-A. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2593–2599.
36. von Eckardstein, A., M. Jauhiainen, Y. Huang, J. Metso, C. Langer, P. Pussinen, S. Wu, C. Ehnholm, and G. Assmann. 1996. Phospholipid transfer protein mediated conversion of high density lipoproteins generates pre beta 1-HDL. *Biochim. Biophys. Acta.* **1301:** 255–262.
37. Wolfbauer, G., J. J. Albers, and J. F. Oram. 1999. Phospholipid transfer protein enhances removal of cellular cholesterol and phospholipids by high density lipoprotein apolipoproteins. *Biochim. Biophys. Acta.* **1439:** 65–76.
38. Lee, M., J. Metso, M. Jauhiainen, and P. T. Kovanen. 2003. Degradation of phospholipid transfer protein (PLTP) and PLTP-generated pre-beta-high density lipoprotein by mast cell chymase impairs high affinity efflux of cholesterol from macrophage foam cells. *J. Biol. Chem.* **278:** 13539–13545.
39. Oram, J. F., and S. Yokoyama. 1996. Apolipoprotein-mediated removal of cellular cholesterol and phospholipids. *J. Lipid Res.* **37:** 2473–2491.
40. Zha, X., A. Gauthier, J. Genest, and R. McPherson. 2003. Secretory vesicular transport from the Golgi is altered during ATP-binding cassette protein A1 (ABCA1)-mediated cholesterol efflux. *J. Biol. Chem.* **278:** 10002–10005.
41. Orso, E., C. Broccardo, W. E. Kaminski, A. Bottcher, G. Liebisch, W. Drobnik, A. Gotz, O. Chambenoit, W. Diederich, T. Langmann, et al. 2000. Transport of lipids from Golgi to plasma membrane is defective in Tangier disease patients and Abc1-deficient mice. *Nat. Genet.* **24:** 192–196.
42. Neufeld, E. B., A. T. Remaley, S. J. Demosky, J. A. Stonik, A. M. Cooney, M. Comly, N. K. Dwyer, M. Zhang, J. Blanchette-Mackie, S. Santamarina-Fojo, et al. 2001. Cellular localization and trafficking of the human ABCA1 transporter. *J. Biol. Chem.* **276:** 27584–27590.
43. Neufeld, E. B., J. A. Stonik, S. J. Demosky, Jr., C. L. Knapper, C. A. Combs, A. Cooney, M. Comly, N. Dwyer, J. Blanchette-Mackie, A. T. Remaley, et al. 2004. The ABCA1 transporter modulates late endocytic trafficking: insights from the correction of the genetic defect in Tangier disease. *J. Biol. Chem.* **279:** 15571–15578.
44. Maric, J., R. S. Kiss, V. Franklin, and Y. L. Marcel. 2005. Intracellular lipidation of newly synthesized apolipoprotein A-I in primary murine hepatocytes. *J. Biol. Chem.* **280:** 39942–39949.
45. Zheng, H., R. S. Kiss, V. Franklin, M. D. Wang, B. Haidar, and Y. L. Marcel. 2005. ApoA-I lipidation in primary mouse hepatocytes. Separate controls for phospholipid and cholesterol transfers. *J. Biol. Chem.* **280:** 21612–21621.
46. McNeish, J., R. J. Aiello, D. Guyot, T. Turi, C. Gabel, C. Aldinger, K. L. Hoppe, M. L. Roach, L. J. Royer, J. de Wet, et al. 2000. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc. Natl. Acad. Sci.USA.* **97:** 4245–4250.
47. Wang, N., D. L. Silver, C. Thiele, and A. R. Tall. 2001. ATP-binding cassette transporter A1 (ABCA1) functions as a cholesterol efflux regulatory protein. *J. Biol. Chem.* **276:** 23742–23747.
48. Bortnick, A. E., G. H. Rothblat, G. Stoudt, K. L. Hoppe, L. J. Royer, J. McNeish, and O. L. Francone. 2000. The correlation of ATP-binding cassette 1 mRNA levels with cholesterol efflux from various cell lines. *J. Biol. Chem.* **275:** 28634–28640.
49. Oram, J. F., R. M. Lawn, M. R. Garvin, and D. P. Wade. 2000. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. *J. Biol. Chem.* **275:** 34508–34511.
50. Chen, W., Y. Sun, C. Welch, A. Gorelik, A. R. Leventhal, I. Tabas, and A. R. Tall. 2001. Preferential ATP-binding cassette transporter A1-mediated cholesterol efflux from late endosomes/lysosomes. *J. Biol. Chem.* **276:** 43564–43569.
51. Khovidhunkit, W., A. H. Moser, J. K. Shigenaga, C. Grunfeld, and K. R. Feingold. 2003. Endotoxin down-regulates ABCG5 and ABCG8 in mouse liver and ABCA1 and ABCG1 in J774 murine macrophages: differential role of LXR. *J. Lipid Res.* **44:** 1728–1736.
52. See, R. H., R. A. Caday-Malcolm, R. R. Singaraja, S. Zhou, A. Silverston, M. T. Huber, J. Moran, E. R. James, R. Janoo, J. M. Savill, et al. 2002. Protein kinase A site-specific phosphorylation regulates ATP-binding cassette A1 (ABCA1)-mediated phospholipid efflux. *J. Biol. Chem.* **277:** 41835–41842.
53. Haidar, B., M. Denis, L. Krimbou, M. Marcil, and J. Genest, Jr. 2002. cAMP induces ABCA1 phosphorylation activity and promotes cholesterol efflux from fibroblasts. *J. Lipid Res.* **43:** 2087–2094.
54. Cao, G., T. P. Beyer, X. P. Yang, R. J. Schmidt, Y. Zhang, W. R. Bensch, R. F. Kauffman, H. Gao, T. P. Ryan, Y. Liang, et al. 2002. Phospholipid transfer protein is regulated by liver X receptors in vivo. *J. Biol. Chem.* **277:** 39561–39565.
55. Cook, A. D., E. L. Braine, and J. A. Hamilton. 2003. The phenotype of inflammatory macrophages is stimulus dependent: implications for the nature of the inflammatory response. *J. Immunol.* **171:** 4816–4823.
56. Jiang, X. C., A. R. Tall, S. Qin, M. Lin, M. Schneider, F. Lalanne, V. Deckert, C. Desrumaux, A. Athias, J. L. Witztum, et al. 2002. Phospholipid transfer protein deficiency protects circulating lipoproteins from oxidation due to the enhanced accumulation of vitamin E. *J. Biol. Chem.* **277:** 31850–31856.
57. Aiello, R. J., D. Brees, P. A. Bourassa, L. Royer, S. Lindsey, T. Coskran, M. Haghpassand, and O. L. Francone. 2002. Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. *Arterioscler. Thromb. Vasc. Biol.* **22:** 630–637.
58. Costet, P., Y. Luo, N. Wang, and A. R. Tall. 2000. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J. Biol. Chem.* **275:** 28240–28245.
59. Laffitte, B. A., S. B. Joseph, M. Chen, A. Castrillo, J. Repa, D. Wilpitz, D. Mangelsdorf, and P. Tontonoz. 2003. The phospholipid transfer protein gene is a liver X receptor target expressed by macrophages in atherosclerotic lesions. *Mol. Cell. Biol.* **23:** 2182–2191.
60. Curtiss, L. K., D. T. Valenta, N. J. Hime, and K-A. Rye. 2006. What is so special about apolipoprotein A-I in reverse cholesterol transport? *Arterioscler. Thromb. Vasc. Biol.* **26:** 12–19.