Phospholipid transfer protein interacts with and stabilizes ABCA1 and enhances cholesterol efflux from cells*

John F. Oram#, Gertrud Wolfbauer, Ashley M. Vaughan, Chongren Tang, and John J. Albers#.

Department of Medicine, Box 356426, University of Washington, Seattle, WA 98195-6426

#Address correspondence to either John F. Oram, joram@u.washington.edu, phone 206-543-3470, fax 206-685-3781, or John J. Albers, jja@u.washington.edu, phone 206-685-3330, fax 206-685-3279.
Running title: PLTP interacts with ABCA1
SUMMARY

Phospholipid lipid transfer protein (PLTP) is ubiquitously expressed in animal tissues and plays multiple roles in lipoprotein metabolism, but the function of peripheral PLTP is still poorly understood. Here we show that one of its possible functions is to transport cholesterol and phospholipids from cells to lipoprotein particles by a process involving PLTP interactions with cellular ABCA1. When ABCA1 was induced in murine macrophages or ABCA1-transfected BHK cells, PLTP gained the ability to promote cholesterol and phospholipid efflux from cells. Although PLTP alone had lipid efflux activity, its maximum activity was observed in the presence of HDL particles. Pulse-chase studies showed that the interaction of PLTP with ABCA1-expressing cells played a role in promoting lipid efflux. Overexpression of ABCA1 dramatically increased binding of both PLTP and apoA-I to common sites on the cell surface. Both PLTP and apoA-I were covalently cross-linked to ABCA1, each protein blocked cross-linking of the other, and both PLTP and apoA-I stabilized ABCA1 protein. These results are consistent with PLTP and apoA-I binding to ABCA1 at the same or closely related sites. Thus, PLTP mimics apolipoproteins in removing cellular lipids by the ABCA1 pathway, except that PLTP acts more as an intermediary in the transfer of cellular lipids to lipoprotein particles.
INTRODUCTION

Phospholipid transfer protein (PLTP)\(^1\) plays important and diverse roles in lipoprotein metabolism (1,2). Plasma PLTP transfers phospholipids between lipoproteins and remodels HDL to generate lipid-poor particles (3-7), and hepatic PLTP facilitates the production of triglyceride-rich apoB-containing particles in mice (8,9). PLTP is expressed ubiquitously in human and mouse tissues (5,10,11), suggesting that it may locally modulate lipid metabolism in peripheral tissues. Little is known, however, about the interactions of PLTP with peripheral cells and its effects on cellular lipid metabolism.

One possible function of peripheral PLTP is to transport lipids from cells to lipoproteins. Cholesterol efflux from cells occurs by at least two distinct mechanisms mediated by different components in high-density lipoproteins (HDLs) (12,13). HDL phospholipids sequester cholesterol that desorbs from the plasma membrane, and lipid-poor HDL apolipoproteins remove both cholesterol and phospholipids by an active transport process. A cell-surface receptor called scavenger receptor B1 facilitates cholesterol transport from cells to HDL phospholipids (13), while a sterol-inducible membrane transporter called ABCA1 (ATP-binding cassette transporter A1) mediates the removal of cholesterol and phospholipids by lipid-poor apolipoproteins (14). It is possible that PLTP enhances one or both of these lipid efflux pathways in peripheral tissues.

We reported previously that PLTP enhanced cholesterol and phospholipid efflux from cholesterol-loaded human fibroblasts in the presence of HDL particles (15). In contrast, PLTP had no effect on lipid efflux from fibroblasts isolated from a patient with...
Tangier disease, an HDL deficiency syndrome discovered later to be caused by mutations in ABCA1 (16-19). These findings suggested that PLTP enhanced lipid efflux only by ABCA1-dependent mechanisms. The discovery of ABCA1 has allowed us to address in more detail the role of ABCA1 in this PLTP-enhanced lipid efflux. Our current findings confirm that ABCA1 expression is required for PLTP lipid efflux activity and that this likely involves direct interactions of PLTP with ABCA1. These studies suggest a model whereby PLTP mimics apolipoproteins in removing cellular lipids by the ABCA1 pathway, except that PLTP requires the presence of lipoprotein acceptor particles for optimum lipid transport activity.
EXPERIMENTAL PROCEDURES

Lipoproteins, ApoA-I, Phospholipid Vesicles—LDL and HDL were prepared by sequential ultracentrifugation in the density range 1.019-1.063 and 1.125-1.21 g/ml, respectively, and HDL was depleted of apoE and apoB by heparin-agarose chromatography (20). Trypsinized HDL was prepared as previously described (21) by treating HDL with trypsin for 30 min at 37 °C at an HDL:trypsin protein ratio of 40:1. This procedure digests approximately 20% of the total HDL protein content of HDL without disturbing its lipid composition. ApoA-I was purified from HDL, delipidated, and labeled with $^{125}\text{I}$ as described previously (20). LDL was acetylated by the method of Goldstein et al. (22). Phospholipid vesicles were prepared with phosphatidylcholine by sonication as described (23).

Recombinant PLTP—Recombinant wild-type and mutant PLTP was isolated by Ni NTA resin column chromatography from serum-free conditioned culture media collected from BHK cells transfected with a His-tagged human PLTP cDNA using methotrexate as selection agent (5). The isolated PLTP fractions were assayed for phospholipid transfer activity and for purity by SDS polyacrylamide gel electrophoresis (SDS PAGE). Mutant PLTP contained four amino acid substitutions (L16R, L89P, Q270R, and S387P) as described previously (24). PLTP was labeled with $^{125}\text{I}$ using the Iodo-Beads iodination reagent (Pierce) and Na$^{125}\text{I}$ to a specific activity of 400 to 500 cpm/µg protein, and the integrity of the $^{125}\text{I}$-PLTP was verified by autoradiography of SDS PAGE gels.
Cell Culture and Lipid Efflux-- Murine J774 macrophages and baby hamster kidney (BHK) cells were obtained from ATCC (Rockville, MD). BHK cells expressing human ABCA1 were generated using the mifepristone-inducible GeneSwitch System (Invitrogen, Carlsbad, CA) as described (25). Control (mock) BHK cells were derived from the same clonal line transfected with plasmids lacking the ABCA1 cDNA insert. All cells were grown and maintained in DMEM containing 10% fetal bovine serum until experimental treatments. To induce ABCA1 expression, J774 macrophages were incubated for 24 h with DMEM containing 1 mg/ml bovine serum albumin (DMEM/BSA) plus 50 µg/ml acetylated LDL followed by 20-24 h incubations with DMEM/BSA plus 0.5 mM 8-Br-cAMP (26), and ABCA1-transfected BHK cells were incubated for 24h in DMEM/BSA plus 10 nM mifepristone (25). Control macrophages were treated identically except without 8-Br-cAMP, and control BHK cells were mock transfectants treated identically.

To radiolabel cellular cholesterol, 0.5-1 µCi/ml [3H]cholesterol (NEN Life Science Products) was added to the acetylated LDL medium (macrophages) or to the growth medium 24 h prior to mifepristone treatment (BHK cells) (26). To label phospholipids, 1 µCi/ml [Methyl-3H]choline chloride (NEN Life Science Products) was added to the induction media followed by two washes of cells (27).

To measure lipid efflux, cells were incubated with DMEM/BSA with or without the indicated acceptor particles for 2-6 h at 37°C and chilled on ice, and the medium was collected and centrifuged to remove detached cells. For cholesterol efflux, the medium was counted for [3H] and the cells were assayed for free and esterified [3H]cholesterol after isolation by thin-layer chromatography (27). For phospholipid efflux, medium and
cellular choline-labeled phospholipids were extracted in chloroform methanol and assayed for 3H radioactivity (27). Lipid efflux was calculated as the percent free [3H]cholesterol or [3H]choline-labeled phospholipids released into the medium.

**Cell-surface and ABCA1 Binding of ApoA-I and PLTP**—For the competitive sequential binding assay, BHK cells were first incubated for 4 h at 37°C with DMEM/BSA containing the indicated concentrations of either apoA-I or PLTP, chilled to 0°C, washed twice with ice-cold PBS/BSA, and then incubated for 2 h with ice-cold HEPES-buffered DMEM/BSA containing 2 µg/ml 125I-apoA-I or 125I-PLTP plus or minus 300 µg/ml unlabeled apoA-I or PLTP, respectively. Cells were washed twice at 0°C with PBS/BSA and twice with PBS, and cell-associated radioactivity and cell protein were measured after digestion in 0.1 N NaOH (26). Results are expressed as ng of 125I-apoA-I or 125I-PLTP per mg of cell protein after subtraction of values in the presence of unlabeled apoA-I and PLTP, respectively.

For the ABCA1 binding assay, BHK cells were incubated for 2 h at 37°C with 2 µg/ml 125I-PLTP or 125I-apoA-I without or with 200 µg/ml of either PLTP or apoA-I, washed twice with ice-cold PBS, incubated for 30 min at 0°C with PBS containing 1 mg/ml DSS (cross-linking agent), washed twice with cold PBS containing 20 mM glycine (to quench cross-linker), and extracted with detergent. ABCA1 was isolated by immunoprecipitation and SDS-PAGE, and 125I-labeled ABCA1 was detected by PhosphorImaging (26).

**Metabolic labeling of ABCA1**—Cells were incubated for 15 min at 37°C with DMEM/BSA containing 100 µCi/ml [35S] Methionine (Amersham Pharmacia Biotech, Inc.). Cells were washed and dislodged from the dish at 0°C in buffer containing
protease inhibitors. ABCA1 was isolated by immunoprecipitation and SDS-PAGE, and 
$^{35}$S-labeled ABCA1 was detected by PhosphorImaging.

**ABCA1 stabilization**—Cholesterol-loaded J774 macrophages were incubated with 
DMEM/BSA plus 0.5 mM 8-Br-cAMP for 20 h, washed twice with PBS/BSA, and then 
incubated for 6 h with DMEM/BSA with or without 8-Br-cAMP plus or minus either 10 
µg/ml apoA-I or PLTP. Microsomal membranes were isolated from homogenized cells 
by ultracentrifugation, membrane proteins were solubilized in SDS buffer and resolved 
by SDS-PAGE, and ABCA1 was identified by immunoblot analysis (26). Equal amounts 
of membrane protein were added per gel lane.
RESULTS

Our previous studies showed that PLTP enhanced the ability of HDL to promote cholesterol efflux from normal but not Tangier disease fibroblasts (15), implicating the involvement of ABCA1. To further test this idea, we examined the effects of PLTP on cholesterol and phospholipid efflux from J774 murine macrophages expressing low and high levels of ABCA1. To distinguish between different mechanisms of lipid efflux, we incubated cells for 6h with or without PLTP in the presence of either albumin alone (controls) or albumin plus HDL, trypsinized HDL (TrHDL), or purified apoA-I. HDL phospholipids promote efflux of cholesterol and, to a lesser extent, phospholipids by ABCA1-independent diffusional mechanisms, whereas HDL apolipoproteins remove cellular cholesterol and phospholipids by the ABCA1 pathway (14). Thus, apolipoprotein-digested TrHDL promotes lipid efflux by diffusion only, purified apoA-I by the ABCA1 pathway only, and HDL by both mechanisms (21,28).

In the basal state, where ABCA1 levels are very low, HDL and TrHDL promoted cholesterol efflux from macrophages to a greater extent than apoA-I did (Fig. 1A) and had small stimulatory effects on phospholipid efflux (Fig. 1C). Addition of PLTP to these media slightly increased or had no effect on lipid efflux. Thus, when lipid efflux occurs largely by diffusional mechanisms, PLTP has very little lipid efflux activity.

When macrophage ABCA1 was induced by 8-Br-cAMP (26,29,30), apoA-I-mediated cholesterol and phospholipid efflux was markedly increased and exceeded that promoted by either HDL or TrHDL (Fig. 1B and D). With these cells, addition of PLTP greatly enhanced cholesterol and phospholipid efflux in the presence of HDL or TrHDL.
but had little effect in the absence of lipid acceptors or in the presence of apoA-I. These results show that PLTP stimulates cholesterol and phospholipid efflux from J774 macrophages by an ABCA1-dependent process, but this occurs only in the presence of phospholipid-rich HDL particles.

To confirm that ABCA1 was involved in the PLTP-enhanced lipid efflux, we measured the effects of PLTP on cholesterol and phospholipid efflux from BHK cells transfected with an inducible ABCA1 gene (25). With mock-transfected cells, where ABCA1 protein is barely detectable (25), apoA-I had no ability to promote cholesterol or phospholipid efflux above control levels (Fig. 2A and C). In contrast, both HDL and TrHDL promoted significant cholesterol efflux and small levels of phospholipid efflux. Addition of PLTP to each of these media had no effect on lipid efflux.

Inducing ABCA1 expression in these transfected cells led to an increase in HDL-mediated cholesterol and phospholipid efflux and activated apoA-I-mediated lipid transport (Fig. 2B and D). As with J774 macrophages, PLTP had little effect on cholesterol or phospholipid efflux when added to the apoA-I-containing medium but markedly enhanced efflux of these lipids in the presence of HDL or TrHDL. With these ABCA1-expressing cells, addition of PLTP to the control medium also significantly increased cholesterol and phospholipid efflux.

The studies with ABCA1 transfectants suggest that PLTP has the ability to stimulate lipid efflux even in the absence of other lipid acceptors. To confirm this, we measured cholesterol efflux from mock- and ABCA1-transfected BHK cells during incubations with increasing concentrations of PLTP added to medium containing no acceptors, including albumin. Results showed a concentration-dependent increase in
cholesterol efflux only from the ABCA1 transfectants (Fig. 3). These results indicate that PLTP alone is capable of removing cholesterol from cells expressing high levels of ABCA1.

We also measured cholesterol efflux in the presence of the same concentrations of heat-inactivated PLTP or a mutant form of PLTP having approximately 10% normal phospholipid transfer activity (24). Heat-inactivated PLTP completely lacked cholesterol efflux activity, and mutant PLTP had a markedly reduced activity (Fig. 3B). These results show that the PLTP-stimulated cholesterol efflux from ABCA1 transfectants was not simply due to an increase in protein mass but required some structural determinants associated with its phospholipid transfer activity.

We examined the effects of PLTP on ABCA1-mediated cholesterol efflux in the presence of non-HDL phospholipid particles. LDL and phospholipid vesicles stimulated cholesterol efflux from both mock- and ABCA1-transfected BHK cells (Fig. 4). Addition of PLTP to these media enhanced cholesterol efflux from ABCA1-transfected cells (Fig. 4B) but not from mock-transfected cells (Fig. 4A), but the incremental increase was similar to that seen when PLTP was added to control medium alone. Thus, the PLTP effect on ABCA1-dependent lipid efflux appears to have some selectivity for HDL particles.

The above results suggest that the direct interaction of PLTP with ABCA1-expressing cells plays a role in promoting cholesterol efflux. To test this possibility further, we first incubated cells for 4 h with PLTP and then measured cholesterol efflux from washed cells during 2 h incubations without PLTP but with different acceptors. Pretreatment of ABCA1-transfected cells with PLTP significantly increased subsequent
cholesterol efflux in the presence of albumin alone (control) or albumin plus either HDL, TrHDL, LDL, or phospholipid vesicles (Fig. 5A). The relative increase was smaller than when PLTP was added directly to the lipoprotein-containing medium, as would be expected if only the small amount of pre-bound PLTP was enhancing cholesterol efflux. This pretreatment had no effect when ABCA1-transfected cells were subsequently incubated with apoA-I or when mock-transfected cells were incubated with any of these acceptors (Fig. 5B). These results support the concept that the interaction of PLTP with cells rather than with lipoproteins in solution is primarily responsible for the enhanced lipid efflux.

Induction of ABCA1 increases the number of high-affinity cell-surface binding sites for apoA-I (26, 31). We performed cross-competitive binding studies to determine whether PLTP also interacts with these same sites. ABCA1 induction in BHK cells increased high-affinity binding of $^{125}$I-PLTP and $^{125}$I-apoA-I 14 and 12 fold, respectively (Fig. 6). For the cross-competition studies, we pretreated cells at 37°C for 4 h with increasing concentrations of competitor, washed the cells, and then measured binding of $^{125}$I-labeled PLTP or apoA-I after subsequent 2-h incubations at 0°C. This sequential protocol is effective because apolipoproteins dissociate slowly from their high-affinity binding sites at 0°C, and thus most of these sites remain blocked at this temperature during the first several hours after removal of apolipoproteins (21). This approach has the advantage of minimizing interactions between ligand and competitor in solution, which can give false positive results. Preincubating cells with increasing concentrations of unlabeled apoA-I blocked 80% of the subsequent binding of $^{125}$I-PLTP (Fig. 6A), and preincubating cells with increasing concentrations of PLTP blocked 67% of the
subsequent binding of $^{125}$I-apoA-I (Fig. 6B). These results indicate that PLTP and apoA-I interact with common high-affinity binding sites on ABCA1-expressing cells.

To obtain evidence for the functional significance of these binding sites, we tested the effects of increasing concentrations of PLTP on apoA-I-mediated cholesterol efflux from BHK cells. Since PLTP has less ability than apoA-I to promote cholesterol efflux in the absence of HDL (Fig. 2), increasing ratios of PLTP to apoA-I should inhibit cholesterol efflux if saturable common binding sites are involved. Although PLTP had no effect on ABCA1-dependent lipid efflux at similar apoA-I to PLTP mass ratios (10 µg/ml each, Fig. 2), raising the concentration of PLTP progressively decreased apoA-I-mediated cholesterol efflux from ABCA1-expressing cells (Fig. 6C). A significant inhibition was observed at a PLTP to apoA-I mass ratio of 2.5 to 1. Thus, the binding sites shared by apoA-I and PLTP appear to play a role in ABCA1-dependent cholesterol transport.

Cross-linking studies have suggested that apoA-I binds directly to ABCA1 (26,31,32). We tested whether this is also the case for PLTP by incubating mock- and ABCA1-transfected BHK cells with $^{125}$I-PLTP, treating cells with the cross-linking agent DSS, isolating ABCA1 by immunoprecipitation, and detecting $^{125}$I-labeled ABCA1 by phosphorimaging of SDS PAGE gels. Results with ABCA1-transfected cells showed a single iodinated band with an apparent molecular mass greater than 300 kDa that was barely visible in mock-transfected cells and was markedly reduced in intensity when the binding incubations were performed in the presence of 100-fold excess unlabeled PLTP or apoA-I (Fig. 7A). No band was evident in absence of DSS (not shown). Excess unlabeled PLTP or apoA-I also blocked cross-linking of $^{125}$I-apoA-I to ABCA1 (Fig. 7B).
These findings suggest that PLTP and apoA-I bind to ABCA1 by the same or very similar mechanisms.

The interaction of apoA-I with cells stabilizes ABCA1 protein by interfering with its calpain-mediated degradation (33,34). We examined the possibility that PLTP also stabilizes ABCA1 by comparing the effects of apoA-I and PLTP on the level of ABCA1 protein in J774 macrophages, which were shown previously to have a rapid turnover rate of ABCA1 protein (35). When ABCA1 was induced by cholesterol loading and 8-Br-cAMP treatment, addition of either apoA-I or PLTP to the cAMP medium for the last 6 h of incubation led to a several fold increase in the membrane content of ABCA1 (Fig. 8A). When cAMP was removed from the medium during this 6 h, the level of ABCA1 was markedly reduced, consistent with degradation of the preformed protein after removal of inducer (26). Addition of either apoA-I or PLTP to this medium prevented this reduction in protein, suggesting that they both inhibited ABCA1 degradation.

To confirm that PLTP was affecting ABCA1 protein turnover rather than synthesis, we pulsed cells for 15 min with $^{35}$S-methionine after the 6-h incubations, isolated ABCA1 by immunoprecipitation and SDS-PAGE, and compared the relative levels of $^{35}$S-labeled ABCA1. Removal of 8-Br-cAMP from the medium decreased incorporation of $^{35}$S-methionine into ABCA1 (Fig. 8C), consistent with a reduced transcription of ABCA1 after cAMP removal (26). Addition of PLTP to medium containing or lacking 8-Br-cAMP had no effect on $^{35}$S-labeling of ABCA1. Thus, PLTP increased ABCA1 levels by stabilizing the protein rather than stimulating protein synthesis.
DISCUSSION

We showed previously that PLTP enhanced HDL-mediated cholesterol efflux from cholesterol-loaded human fibroblasts (15). This enhancement was absent with fibroblasts from a patient with Tangier disease, an HDL deficiency syndrome characterized by an inability of lipid-poor apolipoproteins to remove cellular lipids (36). The subsequent discovery of ABCA1 as the defective gene product in Tangier disease (16-19) implicated this cell transporter as being involved in PLTP-enhanced lipid efflux. Here we confirm that ABCA1 plays a role in this efflux and show that this likely involves a direct interaction of PLTP with ABCA1.

We used two cell models to show that induction of ABCA1 enhances the ability of PLTP to remove cellular cholesterol and phospholipids in the presence of HDL particles. In the basal state, J774 macrophages and BHK cells have a relatively inactive ABCA1 lipid transport pathway, but HDL phospholipids promote efflux of cholesterol, and to a lesser extent, phospholipids by ABCA1-independent diffusional mechanisms. This efflux was unaffected by the addition of PLTP. However, when we induced ABCA1 in macrophages or in ABCA1-transfected BHK cells, PLTP markedly enhanced cholesterol and phospholipid efflux in the presence of HDL particles. Thus, when ABCA1 was induced, PLTP gained the ability to remove cellular cholesterol and phospholipids when co-incubated with phospholipid-rich lipoproteins that promote cholesterol efflux largely by diffusion. In contrast, PLTP had no effect when ABCA1-mediated cholesterol and phospholipid efflux was maximally stimulated by apoA-I.
One difference between these two cell types was that PLTP alone stimulated cholesterol and phospholipid efflux from the ABCA1-transfected BHK cells but not from the cAMP-treated macrophages. Our previous studies also showed that PLTP alone did not promote lipid efflux from cholesterol-loaded fibroblasts (15). These differences may reflect the level of ABCA1 protein expressed in these cells. Our ABCA1-transfected BHK cells contain a 10-fold or higher level of ABCA1 protein compared to either cAMP-treated J774 macrophages or cholesterol-loaded fibroblasts (25). Perhaps PLTP cannot promote lipid efflux from cells in the absence of acceptors below a threshold of ABCA1 expression.

With ABCA1-transfected BHK cells, the cholesterol efflux-promoting activity of PLTP alone was partially saturable and was abolished by heat inactivation. Moreover, a mutant form of PLTP with markedly reduced phospholipid transfer activity (24) also had a reduced ability to remove cellular cholesterol. These results suggest that the lipid efflux activity of PLTP was not a simple mass effect and required some structural determinants related to its phospholipid transfer activity.

The most likely explanation for the above findings is that PLTP interacts with cells and removes lipids by the ABCA1 pathway. In support of the role of cellular interactions are results showing that PLTP had the same cholesterol efflux stimulatory pattern, although to a lesser extent, when ABCA1-transfected cells were first treated with PLTP alone and then incubated with PLTP-free medium containing different lipid acceptors. Because of its low lipid binding capacity compared to apolipoproteins, PLTP may require acceptor particles to act as sinks for the lipids it transports from cells. This would explain why PLTP had a greater ability to stimulate lipid efflux when co-incubated
with HDL particles. PLTP plus HDL or TrHDL removed cholesterol and phospholipids from ABCA1-expressing cells to the same or greater extent than apoA-I did. Although PLTP also enhanced cholesterol efflux from ABCA1-transfected cells when added to medium containing LDL or phospholipid vesicles, the increase was similar to that seen when PLTP alone was added to particle-free medium. This suggests that HDL particles are the preferred recipients of PLTP-transported cellular lipids.

Although the lipid transport requirements may differ, apoA-I and PLTP appear to share common mechanisms for interacting with ABCA1-expressing cells and with ABCA1 itself. ABCA1 induction in BHK cells dramatically increased cell-surface binding of both apoA-I and PLTP, and cross-competition experiments showed that most of this binding was to common sites. As shown previously for apoA-I (26,31,32), 125I-labeled PLTP covalently attached to ABCA1 when cells were treated with the cross-linking agent DSS, indicating that PLTP comes in close proximity (<1.2 nm) to ABCA1 on cells. Excess unlabeled PLTP and apoA-I blocked cross-linking of both 125I-PLTP and 125I-apoA-I to ABCA1, consistent with these molecules interacting with the same or closely associated sites on ABCA1. Lastly, we found that addition of both apoA-I and PLTP blocked the degradation of cAMP-induced ABCA1 that follows the removal of inducer from the medium. Thus, PLTP mimics apoA-I in its ability to stabilize ABCA1 (33,34), which is presumably due to direct binding to ABCA1.

It is unclear what common properties of apoA-I and PLTP mediate their interactions with ABCA1-expressing cells. Studies with synthetic peptides implicate amphipathic α-helices in apolipoproteins as promoting these interactions (27,37,38). PLTP is predicted to have α-helices with amphipathic properties (39), particularly
residues 247 to 263. It is possible that α-helices in PLTP mimic those in apolipoproteins in their ABCA1 binding activity. These findings also support the idea that membrane phospholipids play a role in facilitating the interaction of proteins with ABCA1 (40).

The current study shows that HDL apolipoproteins and PLTP each have the potential of transporting lipids from cells by the ABCA1 pathway. Whereas apolipoproteins become lipidated to form nascent particles that eventually mature into HDL, PLTP may function to transfer cellular lipids to other acceptor particles. This could occur by shuttling lipids from cells to lipoproteins or by forming a PLTP-lipoprotein complex that directly interacts with cell-surface lipid domains generated by ABCA1. Moreover, PLTP may remodel HDL particles so as to generate more lipid-poor apolipoprotein acceptors for ABCA1-dependent lipid transport (2,15). A role for PLTP in macrophage cholesterol efflux is further suggested by studies showing that the sterol-responsive liver X receptor induces macrophage PLTP in concert with other proteins involved in cholesterol efflux, including ABCA1 and apoE (41-43). PLTP accumulates in atherosclerotic lesions in association with macrophage foam cells (43,44), consistent with PLTP playing a role in atherogenesis.

Our findings suggest that PLTP could either enhance or suppress mobilization of excess cholesterol from cells, depending on the lipoprotein environment. In the presence of predominantly lipidated HDL particles, PLTP would be predicted to stimulate ABCA1-dependent cholesterol efflux. However, in the presence of lipid-poor apolipoproteins, PLTP could inhibit cholesterol efflux by interfering with apolipoprotein interactions with ABCA1. Indeed, our data shows that a high ratio of PLTP to apoA-I reduces apoA-I-mediated cholesterol efflux from ABCA1-expressing cells.
Studies of genetically modified mice have suggested that PLTP is atherogenic (8,9), which would be consistent with an inhibitory effect on cholesterol efflux from arterial macrophages. It is possible, however, that PLTP enhances HDL-mediated cholesterol efflux from macrophages in vivo, but that this can be overwhelmed by other atherogenic effects of PLTP, such as stimulating hepatic production of apoB-containing lipoproteins (8,9). It is difficult to assess from existing animal models the contribution of PLTP/ABCA1 interactions to reverse cholesterol transport, since PLTP can directly remodel HDL in the plasma. It is noteworthy, however, that mice deficient in either PLTP or ABCA1 have a severe HDL deficiency (7, 45).

In summary, this study shows that PLTP can mimic HDL apolipoproteins in removing cholesterol and phospholipids from cells by the ABCA1 pathway. Unlike apolipoproteins, however, PLTP requires the presence of HDL acceptors for its optimum activity. PLTP therefore appears to function as an intermediary in the transfer of excess cellular lipids to lipoproteins through its interaction with ABCA1. This transport mechanism may play an important role in helping rid cells of excess cholesterol, particularly when lipid-poor apolipoproteins are sparse. Additional studies with appropriate animal models are needed to define the metabolic roles of PLTP/ABCA1 interactions and their effects on atherogenesis.
REFERENCES


Footnotes:

* This work was supported by National Institutes of Health Grants HL30086, HL18645, HL55362, and DK02456.

The abbreviations used are: PLTP, phospholipid transfer protein; HDL, high density lipoprotein; ABCA1, ATP-binding cassette transporter A1; LDL, low density lipoprotein; ApoA-I, apolipoprotein A-I; BHK, baby hamster kidney; DMEM, Dulbecco’s modified Eagle’s medium; BSA, bovine serum albumin; 8-Br-cAMP, 8-bromo-cAMP; DSS, 2,2-dimethyl-2-silapentanesulfonic acid; TrHDL, trypsinized HDL.
FIG. 1. **PLTP enhances cholesterol and phospholipid efflux from ABCA1-expressing J774 macrophages in the presence of HDL but not apoA-I**  
J774 macrophages were incubated for 24 h with 50 µg/ml acetylated LDL followed by 24-h incubations without (Basal) or with (+cAMP) 0.5 mM 8-Br-cAMP, and [³H]cholesterol (A and B) and [³H]choline-labeled phospholipid (C and D) efflux was measured during 6h incubations with basal or cAMP media containing albumin without or with 10 µg/ml PLTP plus either no additions (Control), 20 µg/ml HDL protein, 20 µg/ml trypsinized HDL protein (TrHDL), or 10 µg/ml apoA-I. Each value is the mean ± S.D. of triplicate incubations expressed as percent total (medium plus cell) radiolabeled free cholesterol or phospholipids.

FIG. 2. **PLTP enhances cholesterol and phospholipid efflux from ABCA1-transfected BHK cells in the presence of HDL but not apoA-I**  
[³H]cholesterol (A and B) and [³H]choline-labeled phospholipid (C and D) efflux from mock- and ABCA1-transfected BHK cells was measured during 6h incubations with medium containing albumin without (-) or with (+) 10 µg/ml PLTP plus either no additions (Control), 20 µg/ml HDL protein, 20 µg/ml trypsinized HDL protein (TrHDL), or 10 µg/ml apoA-I. Each value is the mean ± S.D. of triplicate incubations expressed as percent total (medium plus cell) radiolabeled free cholesterol or phospholipids.
FIG. 3. **Active PLTP promotes cholesterol efflux from ABCA1-transfected BHK cells in the absence of lipid acceptors.** [3H]cholesterol efflux was measured as described in Fig. 2 except that the efflux media contained either no additions (Control) or the indicated concentrations of PLTP, mutant PLTP (mPLTP), or heat-inactivated PLTP (hiPLTP). Each value is the mean ± S.D. of triplicate incubations.

FIG. 4. **PLTP enhances cholesterol efflux from ABCA1-transfected BHK cells in the presence of LDL and phospholipid vesicles.** [3H]cholesterol efflux was measured as described in Fig. 2 except that the albumin-containing efflux media had either no additions (Control), 20 µg/ml LDL protein, or 20 µM phospholipid vesicles (PLV). Each value is the mean ± S.D. of triplicate incubations.

FIG. 5. **Pretreating cells with PLTP enhances cholesterol efflux from ABCA1-transfected BHK cells during subsequent incubations.** [3H]cholesterol efflux was measured as described in Fig. 2 except that cells were first incubated for 4 h without (-) or with (+) 10 µg/ml PLTP, and radiolabeled cholesterol efflux was measured after subsequent 2 h incubations with albumin alone (Control) or albumin plus the indicated acceptors added as described in Fig. 2 and 3. Each value is the mean ± S.D. of triplicate incubations.

FIG. 6. **PLTP and apoA-I block binding of each other to ABCA1-transfected BHK cells, and high levels of PLTP inhibit apoA-I-mediated cholesterol efflux.** A. Mock- and ABCA1-transfected BHK cells were first incubated for 4 h at 37°C with the indicated
concentrations of either apoA-I (A) or PLTP (B), and high-affinity binding of either $^{125}$I-PLTP (A) or $^{125}$I-apoA-I (B) (both added at 2 µg/ml ± 300 µg/ml unlabeled protein) was measured after subsequent 2-h incubations at 0°C. B. [3H]cholesterol efflux was measured as described in Fig. 2 except that cells were incubated for 2 h with 10 µg/ml apoA-I plus the indicated concentrations of PLTP. Each value is the mean ± S.D. of triplicate incubations representative of at least 3 experiments.

FIG. 7. PLTP cross-links to ABCA1 by an apoA-I-inhibited process and blocks apoA-I cross-linking to ABCA1. Mock- and ABCA1-transfected BHK cells were incubated for 2 h at 37°C with either 2 µg/ml $^{125}$I-PLTP or $^{125}$I-apoA-I alone or with 200 µg/ml either PLTP (+PLTP) or apoA-I (+ApoA-I) and treated with the cross-linker DSS. ABCA1 was isolated by immunoprecipitation and SDS-PAGE, and $^{125}$I-labeled ABCA1 was detected by PhosphorImaging.

FIG. 8. Both apoA-I and PLTP stabilize ABCA1 protein. J774 macrophages were incubated for 24 h with 50 µg/ml acetylated LDL followed by 20-h incubations with 0.5 mM 8-Br-cAMP and then incubated for 6 h with (+cAMP) or without (-cAMP) 8-Br-cAMP without (-) or with (+) either 10 µg/ml apoA-I or PLTP. A. Immunoblot analysis of membrane ABCA1 was performed for cells treated with apoA-I or PLTP in separate experiments. B. Cells were incubated for 15 min with $^{35}$S-methionine, ABCA1 was isolated by immunoprecipitation and SDS-PAGE, and $^{35}$S-labeled ABCA1 was detected by PhosphorImaging.
Fig. 1

- Control HDL
- TrHDL
- ApoA-I

% Free $^3$H-Cholesterol

Basal +cAMP

% $^3$H-Phospholipid

A
B
C
D

PLTP
- -
+ +

Control HDL TrHDL ApoA-I
Fig. 2

Mock

<table>
<thead>
<tr>
<th>% Free $^3$H-Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 10 20 30 40 50 60</td>
</tr>
</tbody>
</table>

ABCA1

<table>
<thead>
<tr>
<th>% $^3$H-Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 2 4 6 8 10 12</td>
</tr>
</tbody>
</table>

A

PLTP

- -

+ +

B

C

D

Control HDL TrHDL ApoA-I

Control HDL TrHDL ApoA-I

- -

+ +
Fig. 3

A Mock

B ABCA1

% Total Free $^3$H-Cholesterol

$\mu$g/ml

- PLTP
- mPLTP
- hiPLTP
Fig. 4

[Graph showing the effect of PLTP and ABCA1 on % Free $^3$H-Cholesterol in Control LDL PLV conditions.]

A Mock

B ABCA1

- PLTP
  - -
  - +

% Free $^3$H-Cholesterol

Control LDL PLV Control LDL PLV
Fig. 5

A  ABCA1

B  Mock
Fig. 6

**Binding**

**A** $^{125}\text{I}-\text{PLTP}$

**B** $^{125}\text{I}-\text{ApoA-I}$

- **ABCA1**
- **Mock**

**Efflux**

**C** ApoA-I

% Free $^3\text{H}$-Cholesterol

- $\mu\text{g/ml ApoA-I}$
- $\mu\text{g/ml PLTP}$

ng/mg Cell Protein

0 5 10 15 20

0 40 80 120

0 5 10 15 20

0 1 2

0 50 100 150 200

0 1 2 3
Fig. 7

Mock  ABCA1

240 kD-

$^{125}$I-PLTP

240 kD-

$^{125}$I-ApoA-I

+PLTP  +ApoA-I
Fig. 8

A

<table>
<thead>
<tr>
<th>ApoA-I</th>
<th>PLTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>+cAMP</th>
<th>+cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>-cAMP</td>
<td>-cAMP</td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th>PLTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
</tr>
<tr>
<td>+</td>
</tr>
</tbody>
</table>
Phospholipid transfer protein interacts with and stabilizes ABCA1 and enhances cholesterol efflux from cells
John F. Oram, Gertrud Wolfbauer, Ashley M. Vaughan, Chongren Tang and John J. Albers

*J. Biol. Chem.* published online October 14, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M310695200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/early/2003/10/14/jbc.M310695200.citation.full.html#ref-list-1