In vivo modulation of HDL phospholipid has opposing effects on SR-BI- and ABCA1-mediated cholesterol efflux

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Abstract The effects of in vivo modulation of HDL phospholipid (PL) on scavenger receptor class BI (SR-BI) and ATP binding cassette transporter 1 (ABCA1)-mediated efflux were examined by overexpressing either endothelial lipase (EL) or phosphatidylserine phospholipase (PS-PLA2) in human apolipoprotein A-I (apoA-I) transgenic mice. Overexpression of EL led to large reductions in the serum PL/apoA-I ratio (~60%), total cholesterol (TC; ~89%), and HDL cholesterol (~91%). Relative to the serum before overexpression of EL, the efflux potential of the serum via SR-BI decreased by 90% and ABCA1-mediated efflux increased by 63%. In contrast to overexpression of EL, overexpression of PS-PLA2 led to increases in the PL/apoA-I ratio (88%), TC (78%), HDL cholesterol (57%), and HDL size. The efflux potential of the serum increased by 60% via SR-BI and decreased by 57% via ABCA1. There were significant positive correlations between SR-BI-mediated efflux and a number of serum parameters, including PL/apoA-I ratio, PL, TC, free cholesterol (FC), and HDL cholesterol. In striking contrast, the same correlations were seen with ABCA1-mediated efflux, but the relationships were inverse. In summary, in vivo modulation of HDL PL content affects ABCA1 and SR-BI-mediated efflux in a reciprocal manner. These findings indicate that the type of lipase acting on HDL in vivo will determine which FC efflux pathway the HDL serves. Additionally, the extent of lipolysis will determine the efficiency of FC removal via this pathway.—Yancey, P. G., M-a. Kawashiri, R. Moore, J. M. Glick, D. L. Williams, M. A. Connelly, D. J. Rader, G. H. Rothblat. In vivo modulation of HDL phospholipid has opposing effects on SR-BI- and ABCA1-mediated cholesterol efflux. J. Lipid Res. 2004. 45: 337–346.

HDL cholesterol levels are inversely correlated with the incidence of coronary artery disease (1–4). One mechanism by which HDL is thought to protect against atherosclerosis is by the removal of excess free cholesterol (FC) from peripheral cells and subsequent delivery to the liver for excretion (5–7). There are three known mechanisms by which HDL and/or its apolipoproteins can remove FC from cells. Aqueous diffusion is a relatively inefficient efflux mechanism that occurs with all cell types (8). In recent years, two proteins have been discovered that mediate efficient cholesterol efflux. The scavenger receptor class BI (SR-BI) facilitates the bidirectional flux of FC between cells and HDL (9, 10), and the ATP binding cassette transporter 1 (ABCA1) (11–13) mediates the unidirectional efflux of cellular FC and phospholipid (PL) to lipid-poor apolipoprotein A-I (apoA-I) and other exchangeable apolipoproteins. A number of studies suggest that both mechanisms of efflux may operate in atherosclerotic lesions (14–16).

The goal of the present study was to determine the effects of in vivo modulation of HDL PL on both ABCA1- and SR-BI-mediated FC efflux. The importance of studying the role of HDL PL in the flux of FC between cells and HDL is supported by a number of previous observations. The efflux of cholesterol from cells to serum is correlated with HDL PL content (17, 18). Importantly, it has been demonstrated that patients with coronary artery disease have low HDL PL levels (19). Past studies have used in vitro manipulation of HDL composition to probe the relationships between HDL PL and cell cholesterol flux. In the present study, we have taken advantage of the ability of two lipases that, when overexpressed in mice, have opposite effects on HDL levels and composition to establish how in vivo modification of HDL PL content affects ABCA1- and SR-BI-mediated cholesterol efflux.

Supplementary key words high density lipoprotein • scavenger receptor BI • ATP binding cassette transporter 1

Abbreviations: ABCA1, ATP binding cassette transporter 1; EL, endothelial lipase; PS-PLA2, phosphatidylserine phospholipase; SR-BI, scavenger receptor class BI.

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Recent studies have shown that two new members of the lipoprotein lipase gene family, endothelial lipase (EL) and phosphatidilserine phospholipase (PS-PLA1), modulate HDL metabolism in vivo (20). EL is capable of effectively hydrolyzing HDL PL, and HDL PL levels are markedly decreased when EL is overexpressed in both C57BL/6 and human apoA-I transgenic C57BL/6 mice (21). In contrast, PS-PLA1 is specific for PS, and overexpression of PS-PLA1 causes increases in HDL cholesterol and PL levels in both C57BL/6 and human apoA-I transgenic C57BL/6 mice (M-a. Kawashiri et al., submitted). Neither the mechanism by which PS-PLA1 influences HDL levels and composition nor its physiological role has been established.

The current studies address the role of HDL PL in both SR-BI- and ABCA1-mediated efflux by overexpressing either EL or PS-PLA1 in human apoA-I transgenic mice. Human apoA-I transgenic mice were chosen because their HDL subpopulation distribution more closely resembles that observed in humans, and in addition, the majority (80% to 90%) of the serum cholesterol is within the HDL fraction (22). These studies show that factors that modulate HDL PL in vivo will affect both SR-BI- and ABCA1-mediated efflux, but in an opposing manner.

MATERIALS AND METHODS

Materials

Calf serum (CS), FBS, BSA, penicillin, and streptomycin were purchased from Sigma Chemical Co. (St. Louis, MO). Tissue culture plasticware was obtained through Falcon (Lincoln, NJ). [1,2-3H]Cholesterol was purchased from NEN Life Sciences, Inc. (Boston, MA). All other reagents and organic solvents were purchased from Fisher (Pittsburgh, PA). The acyl-CoA:cholesterol acyltransferase inhibitor, compound CP113,818 (2), was a generous gift from Pfizer.

Recombinant adeno virus construction and animal studies

Recombinant adeno viral vectors encoding either EL or PS-PLA1 were constructed as previously reported (23, 24). Control adeno viruses (Adnull), replication-defective adeno viral vectors that contain no transgene, were made using the same procedures.

Female human apoA-I transgenic C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) were injected intravenously via the tail vein with 1.0 × 10^{11} particles of adeno viral vector encoding either human PS-PLA1 (AdhPS-PLA1) or human EL (AdhEL). As a control, a third group of mice was injected with 1.0 × 10^{11} Adnull particles. Blood was obtained from the retro-orbital plexus 1 day before injection and at several time points (as indicated in Results) after injection. Aliquots of the serum were stored at −80°C for subsequent lipid analyses and efflux experiments.

Analytical methods

HDL cholesterol and serum total cholesterol (TC), FC, PL, and triglyceride were measured on a Cobas Fara (Roche Diagnostic Systems, Inc., Montclair, NJ) using Sigma reagents. Human apoA-I was measured using a turbidometric assay (Sigma) on a Cobas Fara. Pooled serum samples from the time points indicated in Results were subjected to fast-protein liquid chromatography (FPLC) on two Superose 6 columns as described (25). The composition of HDL was measured using FPLC fractions 24–38, which constitute the HDL fraction. The protein, triglyceride, PL, and cholesterol of the HDL fraction were measured using enzymatic assay kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Cell culture and transient transfections

J774 macrophages were maintained on RPMI supplemented with 10% FBS and antibiotics. COS-7 cells were maintained on DMEM containing 10% CS and antibiotics. For transfection, COS-7 cells were seeded on 100 mm plates and incubated for 18 h at 37°C in 10% CS in DMEM. Cells were transfected with 10 μg of the indicated plasmid and diluted in serum-free DMEM and Fugene 6 (Roche Molecular Biochemicals) as described previously (26). The pSGS vector (Stratagene, Inc., La Jolla, CA) with or without murine SR-BI was prepared using endotoxin-free Qiagen (Chatsworth, CA) Maxiprep kits.

Measurement of SR-BI- and ABCA1-mediated cholesterol efflux

SR-BI-mediated cholesterol efflux was measured using control and SR-BI-transfected COS-7 cells as previously described (27). Briefly, after transfection, the cells were removed from the 100 mm plates by trypsinization. Then, the transfected cells were suspended in 10% DMEM containing the acyl-CoA:cholesterol acyltransferase inhibitor CP113,818 (2 μg/ml) and plated on 24-well plates. One 100 mm plate yielded one 24-well plate. For labeling, the cells were incubated for 24 h in 0.5 ml of 10% CS DMEM containing 12 μCi of [3H]cholesterol and 2 μg/ml CP113,818. For cholesterol efflux, the cells were washed once with 0.5 ml of 1% BSA in MEM and once with MEM. Medium containing 2% mouse serum was then added to the wells. After 1 h, 150 μl aliquots of the medium were removed and filtered through 0.45 μm multiscreen filtration plates to remove any floating cells. The [3H]cholesterol in 100 μl of the filtrate was then measured by liquid scintillation counting. The percentage efflux is based on the total [3H]cholesterol present in the cells before the efflux incubation. To measure the [3H]cholesterol present in the cells, the cell lipids were extracted by incubating the cell monolayers overnight in isopropanol. After lipid extraction, the total [3H]cholesterol present in the lipid extract was measured by liquid scintillation counting.

ABCA1-mediated FC efflux was measured using control J774 macrophages and J774 macrophages treated with cpt-cAMP to upregulate ABCA1 (28). J774 macrophages were plated on 24-well plates at a density of 350,000 cells per well. After 24 h, the cells were labeled by incubation for 24 h in 1% FBS RPMI containing 12 μCi/ml [3H]cholesterol and 2 μg/ml CP113,818. The cells were then incubated for 15 h in 0.2% BSA in RPMI containing 2 μg/ml CP113,818 alone or plus 0.3 mM cpt-cAMP. The cells were then washed once with 0.5 ml of 1% BSA in MEM and once with MEM. Medium containing the mouse serum to be tested, diluted to 1%, was then added to the cells and incubated at 37°C. After 4 h, the [3H]cholesterol content of the medium was measured as described above.

Calculation and presentation of cholesterol efflux data

In all SR-BI and ABCA1 FC efflux measurements, the percentage FC efflux was corrected for the small amount of [3H]cholesterol released to medium without acceptors present. The percentage SR-BI-mediated FC efflux was calculated as the percentage FC efflux from SR-BI-expressing cells minus the percentage FC efflux from control COS cells. The percentage efflux values for a representative experiment (n = 9) with the day 0 serum were 2.03 ± 0.24, 7.67 ± 0.60, and 5.64 ± 0.45 for control
cells, SR-BI-expressing cells, and SR-BI-specific FC efflux, respectively. The percentage ABCA1-mediated FC efflux was calculated as the percentage FC efflux from cells upregulated with cAMP minus the percentage FC efflux from control J774 cells. The percentage efflux values for a representative experiment (n = 9) with the day 0 serum were 7.87 ± 0.82, 12.59 ± 0.86, and 4.72 ± 0.31 for control cells, ABCA1-expressing cells, and ABCA1-specific FC efflux, respectively. These calculations control for the contribution of FC efflux from aqueous diffusion mechanisms and yield data that are specific for the contribution of ABCA1 or SR-BI. It should be noted that in both cases, the control cells lack significant levels of either ABCA1 (no cAMP treatment) or SR-BI (transfected with empty vector) (9, 10, 28). In addition, incubation times were short (1 or 4 h for SR-BI- or ABCA1-mediated efflux, respectively) to ensure the maintenance of initial receptor levels. As a control, a standard human serum pool was run in parallel with all SR-BI and ABCA1 FC flux assays. Lipid-free apoA-I (20 μg/ml of medium) was also tested for efflux in parallel with all ABCA1 efflux assays as a positive control for the up-regulation of ABCA1.

As already stated, a third group of mice was injected with control Adnull particles. Injection of the control Adnull particles had small effects on both serum parameters and efflux (Fig. 1A). To control for these small changes with the control particles, some data (as indicated in Results) obtained with EL or PS-PLA₁ serum were normalized to the mean data obtained from control Adnull serum collected at the same time after injection of the adenovirus particles. Thus, for example, the relative efflux value for SR-BI-mediated efflux to an individual day 4 EL serum was calculated from this equation: SR-BI-mediated efflux relative to control serum = (SR-BI-mediated efflux obtained with an individual day 4 EL serum / mean SR-BI-mediated efflux value obtained with the day 4 serum from control Adnull animals). The mean values before normalization for both SR-BI- and ABCA1-mediated efflux are included in the figure legends.

Statistical differences between groups were estimated by Student’s t-test. Linear correlation coefficients were used to describe relations between cholesterol efflux and various serum parameters. The slopes of regression lines for individual data sets were tested for significant differences from slope = 0 using the null hypothesis to calculate the P value from a Fisher ratio (F test) using the GraphPad Prism software program. Significance was considered at P < 0.05.

RESULTS

Effects of EL overexpression on the serum PL/apoA-I ratio and cholesterol efflux

Injection of the control vector had minimal effects on plasma lipids, including the serum PL/apoA-I ratio and apoA-I levels (Fig. 1A). No significant changes were observed with both SR-BI- and ABCA1-mediated cholesterol efflux.

As expected from previous studies (20), the peak day (4 days) of overexpression of EL led to large reductions in TC (89 ± 18%), HDL cholesterol (91 ± 10%), apoA-I (Fig. 1B), and serum PL/apoA-I ratio (Fig. 1B). The efflux potential of the serum via SR-BI decreased by 90% (Fig. 1B). In contrast, the ABCA1-mediated efflux increased by 63% despite the fact that substantially less apoA-I (77%) was present in the serum.

Shown in Fig. 2 are scattergrams of the relationships between either SR-BI- or ABCA1-mediated efflux and the PL/apoA-I ratio of serum collected at 4 and 7 days of overexpression of EL (n = 9 for each time point). Also shown are the P values resulting from comparing the slopes of the lines to 0. There was a positive relationship between SR-BI-mediated efflux and the serum PL/apoA-I ratio (r = 0.763; Fig. 2A). In contrast, there was a negative correlation between ABCA1-mediated efflux and the serum PL/apoA-I ratio (r = −0.869). The slopes of the lines for the relationships between the serum PL/apoA-I ratio and both SR-BI- and ABCA1-mediated efflux were significantly different from 0, indicating a strong effect of modulating the serum PL/apoA-I ratio on both mechanisms of efflux. As indicated by the increased PL/apoA-I ratio at day 7 compared with day 4 (Fig. 2), the effect of EL overexpression had begun to decrease by 7 days after adenovirus injection.

In addition to the positive correlation with the serum PL/apoA-I ratio, SR-BI-mediated efflux was also positively

**Fig. 1.** Changes in serum phospholipid (PL)/apolipoprotein A-I (apoA-I) ratio, apoA-I levels, and efflux potential via scavenger receptor class B1 (SR-BI) or ATP binding cassette transporter 1 (ABCA1) from mice injected with Adnull or AdhEL particles. Serum was obtained 4 days after injection of recombinant adenovirus that contained no transgene (A) or Adnull expressing human endothelial lipase (EL) (B). Serum parameters, SR-BI-, and ABCA1-mediated efflux were measured as described in Materials and Methods. SR-BI- and ABCA1-mediated efflux were calculated as the percentage cholesterol efflux that occurred with either SR-BI- or ABCA1-expressing cells minus the percentage cholesterol efflux that occurred with the respective control cells. The data are expressed as the percentage change relative to the measurements with day 0 serum. The values are means ± SD (n = 9). The asterisks indicate statistically significant differences when comparing the values obtained with day 4 serum and those obtained with day 0 serum. All P values were <0.05. For day 0 serum (A), the serum PL/apoA-I ratio, serum apoA-I content, SR-BI-mediated efflux, and ABCA1-mediated efflux were 1.2 ± 0.09 (w/w), 282 ± 70 mg/dl, 6.0 ± 0.7%, and 4.3 ± 1.5%, respectively. For day 0 serum (B), the serum PL/apoA-I ratio, serum apoA-I content, SR-BI-mediated efflux, and ABCA1-mediated efflux were 1.2 ± 0.08 (w/w), 315 ± 31 mg/dl, 5.6 ± 1.4%, and 4.7 ± 0.9%, respectively.
correlated with both serum PL ($r = 0.869$) and HDL cholesterol ($r = 0.772$) (Fig. 3A). However, there was not a significant correlation between SR-BI-mediated efflux and serum apoA-I levels (Fig. 3A). In contrast to SR-BI-mediated efflux, there were strong negative correlations between ABCA1-mediated efflux and both serum PL ($r = 0.0762$) and HDL cholesterol ($r = 0.0687$) (Fig. 3B). A strong positive correlation ($r = 0.696$) between ABCA1-mediated efflux and serum apoA-I levels (Fig. 3B) was also observed despite the reduction in serum apoA-I concentration upon EL overexpression. This result suggests that EL activity shifts serum apoA-I to a pool that is highly active in promoting ABCA1 efflux.

**Effects of PS-PLA$_1$ overexpression on serum PL/apoA-I ratio and cholesterol efflux**

Maximum changes in serum composition were obtained at 7 days after injection of an adenovirus encoding PS-PLA$_1$. Substantial increases in TC (78 ± 9%), FC (103 ± 9%), and HDL cholesterol (57 ± 8%) levels were observed (data not shown). The size of the lipoprotein particles remained constant throughout day 4 (Fig. 4A). By day 7, FPLC profiles revealed two peaks, both larger than that of control HDL (Fig. 4A). PL/apoA-I ratios increase with time, and by day 7, there was an 88% increase in the serum PL/apoA-I ratio (Fig. 4B) and a 16 ± 3% reduction ($P = 0.02$) in apoA-I levels. Efflux via SR-BI increased by 60%; in contrast, ABCA1-mediated efflux decreased by 57% (Fig. 4C).

Shown in Fig. 5 are scattergrams of the relationships between either SR-BI- or ABCA1-mediated efflux and the PL/apoA-I ratio of serum collected at multiple time points during the overexpression of PS-PLA$_1$. The relationship between SR-BI-mediated cholesterol efflux and serum PL/apoA-I ratio was curvilinear (Fig. 5A). At serum PL/apoA-I ratios <1.8, there was a strong positive relationship ($r = 0.773$; Fig. 6A) with SR-BI-mediated efflux. At serum PL/apoA-I ratios >1.8, there was no additional increase in the efficiency with which the serum stimulated SR-BI-mediated efflux. Coincident with this decrease in efficiency of the serum in stimulating SR-BI-mediated efflux, there was a shift in HDL size to larger particles at day 7 (Fig. 4A). In addition, compared with the HDL from the day 0 serum, the cholesteryl ester (CE) and TG content of the HDL from

**Fig. 2.** Scattergrams of the linear relationships between either SR-BI-mediated (A) or ABCA1-mediated (B) efflux and the PL/apoA-I ratio of serum from mice overexpressing EL. Serum was obtained at 4 and 7 days after injection of mice ($n = 9$) with a recombinant adenovirus expressing human EL. Serum parameters, SR-BI-mediated efflux, and ABCA1-mediated efflux were measured as described in Materials and Methods. The serum PL/apoA-I ratio and efflux values are presented relative to the mean values obtained with serum collected on the same day from control mice ($n = 9$) injected with Adnull particles that contained no transgene. Relative efflux was calculated according to the following equation: efflux relative to control serum = (efflux value obtained with each serum from day 4 or 7 ÷ the mean efflux value obtained with either the day 4 or 7 serum from control Adnull animals). The PL/apoA-I ratio relative to control serum was calculated similarly. Before normalization, the mean PL/apoA-I ratios (w/w) were 0.50 ± 0.14 and 0.88 ± 0.13 for the day 4 and 7 sera, respectively. Before normalization, the mean percentage SR-BI-mediated cholesterol efflux values were 0.62 ± 0.20% and 1.16 ± 0.22% for the day 4 and 7 sera, respectively. The mean percentage ABCA1-mediated cholesterol efflux values were 7.08 ± 0.47% and 2.65 ± 0.29% for the day 4 and 7 sera, respectively. The PL/apoA-I ratio, SR-BI efflux, and ABCA1 efflux values obtained with day 0 serum were 1.24 ± 0.07 (w/w), 6.03 ± 0.71%, and 4.34 ± 0.49%, respectively.

**Fig. 3.** Correlative data obtained with serum from mice at 7 days after injection with AdhEL particles. Shown are the correlation coefficients obtained from linear regression analysis for the relationships between SR-BI-mediated (A) or ABCA1-mediated efflux (B) efflux and serum HDL cholesterol, PL/apoA-I ratio, PL, and apoA-I. The relative cholesterol efflux values from Fig. 2 were used for the linear regression analyses. The asterisks indicate statistically significant differences when comparing the slopes of the regression lines with slope 0. All $P$ values were <0.001.
the day 7 serum was increased by 3- and 2-fold, respectively (data not shown). However, by day 14, the effects of PS-PLA1 overexpression on SR-BI-mediated efflux and the serum PL/apoA-I ratio had begun to dissipate (Fig. 5A). The relationship between efflux and the PL/apoA-I ratio were curvilinear, so the data were fit to either a one-phase exponential association equation or a one-phase exponential decay equation for SR-BI-mediated (A) and ABCA1-mediated (B) efflux, respectively. The serum PL/apoA-I ratio and efflux values are presented relative to the mean values obtained with serum collected on the same day from control mice (n = 10) injected with Adnull particles that contained no transgene. Relative efflux was calculated according to the following equation: efflux relative to control serum = (efflux value obtained with each serum from day 0, 3, 5, 7, or 14 + the mean efflux value obtained with day 0, 3, 5, 7, or 14 serum from control Adnull animals). The PL/apoA-I ratio relative to control serum was calculated similarly. Before normalization, the mean PL/apoA-I ratios (w/w) were 1.02 ± 0.08, 1.11 ± 0.05, 1.47 ± 0.13, 2.34 ± 0.44, and 1.59 ± 0.06 for the day 0, 3, 5, 7, and 14 sera, respectively. Before normalization, the mean percentage SR-BI-mediated cholesterol efflux values were 3.85 ± 0.18%, 4.09 ± 0.32%, 4.99 ± 0.38%, 5.37 ± 0.65%, and 5.03 ± 0.48% for the day 0, 3, 5, 7, and 14 sera, respectively. The mean percentage ABCA1-mediated cholesterol efflux values were 2.61 ± 0.36%, 2.05 ± 0.75%, 1.76 ± 0.79%, 1.08 ± 0.56%, and 1.43 ± 0.42% for the day 0, 3, 5, 7, and 14 sera, respectively.

Fig. 5. Scattergrams of the relationships between either SR-BI-mediated (A) or ABCA1-mediated (B) efflux and the PL/apoA-I ratio of serum from mice overexpressing PS-PLA1. Serum was obtained before (day 0) and at 3, 5, 7, and 14 days after injection of mice (n = 10) with a recombinant adenovirus expressing human AdhPS-PLA1. Serum parameters, SR-BI-mediated efflux, and ABCA1-mediated efflux were measured as described in Materials and Methods. The relationships between efflux and the PL/apoA-I ratio were curvilinear, so the data were fit to either a one-phase exponential association equation or a one-phase exponential decay equation for SR-BI-mediated (A) and ABCA1-mediated (B) efflux, respectively. The serum PL/apoA-I ratio and efflux values are presented relative to the mean values obtained with serum collected on the same day from control mice (n = 10) injected with Adnull particles that contained no transgene. Relative efflux was calculated according to the following equation: efflux relative to control serum = (efflux value obtained with each serum from day 0, 3, 5, 7, or 14 + the mean efflux value obtained with day 0, 3, 5, 7, or 14 serum from control Adnull animals). The PL/apoA-I ratio relative to control serum was calculated similarly. Before normalization, the mean PL/apoA-I ratios (w/w) were 1.02 ± 0.08, 1.11 ± 0.05, 1.47 ± 0.13, 2.34 ± 0.44, and 1.59 ± 0.06 for the day 0, 3, 5, 7, and 14 sera, respectively. Before normalization, the mean percentage SR-BI-mediated cholesterol efflux values were 3.85 ± 0.18%, 4.09 ± 0.32%, 4.99 ± 0.38%, 5.37 ± 0.65%, and 5.03 ± 0.48% for the day 0, 3, 5, 7, and 14 sera, respectively. Before normalization, the mean percentage ABCA1-mediated cholesterol efflux values were 2.61 ± 0.36%, 2.05 ± 0.75%, 1.76 ± 0.79%, 1.08 ± 0.56%, and 1.43 ± 0.42% for the day 0, 3, 5, 7, and 14 sera, respectively.
apoA-I ratio and ABCA1-mediated efflux. At PL/apoA-I ratios >1.8, there was no further decrease in the efficiency of ABCA1-mediated efflux (Fig. 5B).

There were significant positive relationships between SR-BI-mediated cholesterol efflux and a number of other serum parameters besides the PL/apoA-I ratio, including HDL cholesterol, PL/apoA-I ratio, PL, apoA-I, total cholesterol (TC), and free cholesterol (FC). The relative cholesterol efflux values from Fig. 5 were used for the linear regression analyses. As the relationships between efflux and the serum PL/apoA-I ratios were curvilinear (see Fig. 5A, B), the data corresponding to PL/apoA-I ratios >1.8 were not included in the linear regression analyses of efflux and the serum PL/apoA-I ratios shown in A and B. The asterisks indicate statistically significant differences when comparing the slopes of the regressions lines to slope 0. All \( P \) values were <0.001.

**Relationship between SR-BI- and ABCA1-mediated cholesterol efflux**

The opposing correlative data obtained when comparing the two mechanisms of efflux with the various serum parameters (Figs. 3, 6) suggest that a relationship exists between the two mechanisms of efflux. Strong inverse relationships were observed between SR-BI- and ABCA1-mediated efflux with serum from mice after either EL (\( r = -0.847, P < 0.0001 \)) or PS-PLA1 (\( r = -0.714, P < 0.0001 \)) overexpression (Fig. 7A, B).

**DISCUSSION**

Cellular cholesterol efflux is the first step in RCT and is thought to occur by a number of different mechanisms. In addition to aqueous diffusion that is thought to occur with all cell systems, there is protein-mediated efflux linked to the presence of either ABCA1 or SR-BI (8–13). The generally accepted model for efflux mediated by these two proteins is that the ligands for SR-BI-mediated efflux are mature, fully lipidated lipoproteins rich in PL (10, 27). In contrast to SR-BI, the ligands for ABCA1-mediated efflux appear to be poorly lipidated, exchangeable apolipoproteins such as apoA-I, apoA-II, apoA-IV, and apoE (28–31). This model of cellular cholesterol efflux is derived primarily from studies with cultured cells exposed to purified preparations of lipoproteins or apolipoproteins.

Because of the complex mixture of lipoproteins, apolipoproteins, enzymes, and lipid transfer proteins in whole serum, relatively few investigations have been done using serum as the cholesterol acceptor or using in vivo modulation of HDL properties by specific lipases. In the present investigation, we have used two enzymes as tools to manipulate in vivo the PL composition of HDL. This was accomplished by overexpressing either EL or PS-PLA1 in mice transgenic for human apoA-I. The use of animals overexpressing human apoA-I provided a mouse model in which there were high levels of both mature HDL particles and
unassociated human apoA-I (17, 22), thus magnifying the effects of shifting the distribution of apoA-I between these two pools. As in previous studies, overexpression of EL decreased serum PL content (20), whereas overexpression of PS-PLA_1 increased serum PL (Fig. 4B). Both of these treatments resulted in changes in the distribution of apoA-I between lipid-poor apoA-I and mature HDL from that present in the control serum (M-a. Kawasaki et al., submitted), and both treatments affected both mechanisms of efflux. Consistent with studies using purified apolipoproteins and lipoproteins, SR-BI-mediated efflux was positively correlated with the serum PL/apoA-I ratio, whereas ABCA1-mediated efflux was negatively correlated with the serum PL/apoA-I ratio. These findings indicate that the type of lipase acting on HDL in vivo will determine which FC efflux pathway the HDL serves. Additionally, the extent of lipolysis will determine the efficiency of FC removal via this pathway.

Effects of overexpression of either EL or PS-PLA_1 on SRBI-mediated cholesterol efflux

Earlier investigations of the efflux of cholesterol to either human or rat serum from Fu5AH hepatoma cells that express very high levels of SR-BI showed a good correlation between cholesterol efflux and HDL PL (18, 32, 33). Previous studies have shown that EL is effective at hydrolyzing HDL PL ex vivo (21) and at reducing HDL cholesterol, PL, and apoA-I when EL is overexpressed in vivo (20). In the present studies, EL-induced reductions in HDL cholesterol levels and the serum PL/apoA-I ratio resulted in a decreased efflux potential of the serum via SR-BI.

In contrast to the overexpression of EL, overexpression of PS-PLA_1 caused marked increases in HDL cholesterol, HDL PL, HDL size, and serum PL/apoA-I ratio (Fig. 4A–C). The relationship between SR-BI-mediated efflux and the serum PL/apoA-I ratio was curvilinear, demonstrating a saturation of the efflux response at the higher PL/apoA-I ratios (Fig. 5A). Coincident with this saturation in efflux efficiency, there was a shift in HDL size to larger particles (Fig. 4A). This is consistent with previous studies from our laboratory showing that large particles are less efficient than small particles at accepting cholesterol from Fu5AH cells, a cell type that naturally expresses high levels of SR-BI (34).

The in vivo modulation of HDL PLs by EL or PS-PLA_1 expression clearly shows that the SR-BI-mediated FC efflux pathway is strongly and positively affected by the PL/apoA-I ratio. The mechanistic basis for this effect is provided by a previous study (10) in which the enrichment of HDL PL, HDL size, and serum PL/apoA-I ratio was curvilinear, demonstrating a saturation of the efflux response at the higher PL/apoA-I ratios (Fig. 5A). Coincident with this saturation in efflux efficiency, there was a shift in HDL size to larger particles (Fig. 4A). This is consistent with previous studies from our laboratory showing that large particles are less efficient than small particles at accepting cholesterol from Fu5AH cells, a cell type that naturally expresses high levels of SR-BI (34).

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In contrast to the overexpression of EL, overexpression of PS-PLA_1 caused marked increases in HDL cholesterol, HDL PL, HDL size, and serum PL/apoA-I ratio (Fig. 4A–C). The relationship between SR-BI-mediated efflux and the serum PL/apoA-I ratio was curvilinear, demonstrating a saturation of the efflux response at the higher PL/apoA-I ratios (Fig. 5A). Coincident with this saturation in efflux efficiency, there was a shift in HDL size to larger particles (Fig. 4A). This is consistent with previous studies from our laboratory showing that large particles are less efficient than small particles at accepting cholesterol from Fu5AH cells, a cell type that naturally expresses high levels of SR-BI (34).

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Effects of overexpression of EL on ABCA1-mediated cholesterol efflux

Previous studies have shown that overexpression of EL induces the formation of smaller, PL-depleted HDL particles (20). Similarly, in the present study, overexpression of EL induced reductions in serum HDL cholesterol, PL, apoA-I, and PL/apoA-I ratio. Consistent with a shift in the distribution of apoA-I to a lipid-poor fraction, overexpression of EL caused a marked increase in the efflux potential of the serum via ABCA1 (Fig. 1B), and ABCA1-mediated efflux was inversely correlated with the serum PL/apoA-I ratio (Fig. 2B). The increase in the efflux potential of the serum via ABCA1 occurred even though the total apoA-I pool decreased by 77%. This indicates that there was a marked increase in the efficiency of serum apoA-I in stimulating ABCA1 efflux. The observation that ABCA1-mediated efflux was positively correlated with serum apoA-I also suggests that the total apoA-I pool was sufficiently delipidated to interact with ABCA1. In vitro modification studies have shown that PL depletion of HDL alone is not sufficient to promote the dissociation of lipid-poor apoA-I (35); rather, dissociation requires the net depletion of core lipids (35). However, studies have shown that PL depletion of HDL enhances the removal of core lipids by CE transfer protein (CETP) and that this remod-
The overexpression of PS-PLA1 are currently under way. To further characterize the lipoproteins produced by the expression of human LCAT in mice (38). Studies designed to follow in vivo changes in the serum PL of mice overexpressing human apoA-I. This system amplified the relationship between lipoprotein-bound and unassociated apoA-I. Consistent with this model, studies have shown that the selective uptake of HDL CE is accelerated when HDL PL is decreased by pretreatment with hepatic lipase (36, 37). In contrast to SR-BI-mediated efflux, PS-PLA1 overexpression caused a large decrease in the efflux potential of the serum for ABCA1-mediated efflux (Fig. 4C). At low serum PL/apoA-I ratios, there was a strong negative correlation with ABCA1-mediated efflux (Fig. 5B). At higher serum PL/apoA-I ratios (Fig. 5B), at which there was an increase in HDL size (Fig. 4A), a minimal level of ABCA1 efflux was observed (Fig. 5B). Taken together, these data suggest that the pool of lipid-poor apoA-I that is normally present in human apoA-I transgenic mice is incorporated into PL-enriched, larger HDL particles. In this regard, the effects of overexpression of PS-PLA1 may be similar to the effects caused by overexpression of human LCAT in mice (38). Studies designed to further characterize the lipoproteins produced by the overexpression of PS-PLA1 are currently under way.

Physiological significance

In the present study, we have not attempted to establish the physiological relevance of either EL or PS-PLA1. Our understanding of the role played by EL in HDL metabolism is expanding rapidly (39–42), whereas the mechanism(s) by which expression of PS-PLA1 modifies lipoprotein composition remains unknown. We have used these two enzymes, which have opposite effects on HDL composition, to demonstrate how the in vivo modification of HDL composition can influence cholesterol efflux by ABCA1 and SR-BI. The current concepts regarding the ligands that effectively promote cholesterol efflux from cells expressing ABCA1 or SR-BI are formulated largely from studies using isolated, purified acceptor particles such as HDL3 or lipid-free apolipoproteins. The importance of HDL PL in mediating cholesterol efflux has been directly demonstrated in studies in which HDL PL was modified by in vitro manipulations (27, 43) and from studies using whole serum (18, 33). The role of HDL PL in ABCA1-mediated efflux has not been carefully examined. The experimental systems used in this investigation specifically measured both ABCA1 and SR-BI efflux and followed in vivo changes in the serum PL of mice overexpressing human apoA-I. This system amplified the relationships between lipoprotein-bound and unassociated apoA-I. The present studies have shown that in vivo modulation of HDL PL affects both SR-BI- and ABCA1-mediated cholesterol efflux, but conditions that change HDL PL have opposing effects on the two mechanisms of efflux (Fig. 8). It follows that in vivo both mechanisms of efflux will be regulated by factors that modulate HDL PL content as well as the expression level of ABCA1 or SR-BI cholesterol donor cells. Together, the action of these factors will determine which mechanism of FC efflux is operating in the interstitial fluid, where the process of RCT is initiated, and in the liver, where cholesterol is delivered for excretion. The lipid composition of HDL results from a number of factors, such as LCAT, CETP, PL transfer protein, lipoprotein lipase, hepatic lipase, and EL. The model (Fig. 8) we are proposing to explain the results from the current study links cholesterol efflux to HDL PL by suggesting that increases in PL reduce the pool of unassociated apoA-I that mediate ABCA1 efflux and produce particles that are more efficient for SR-BI-mediated efflux. In contrast, treatments that reduce HDL PL would increase the pool of unassociated apoA-I, thus enhancing ABCA1-mediated efflux while reducing the efficiency of the particles for SR-BI-mediated efflux (Fig. 8). These changes in composition would also produce shifts in the size of the cholesterol acceptors that would influence the relative concentrations of particles in interstitial fluids, whereas the first steps in RCT are initiated. Thus, small HDL and unassociated apoproteins would be enriched in interstitial fluids, whereas the relative concentration of larger, PL-rich particles would be diminished. Perhaps equally important to atherosclerosis are the factors that are present in the lesion. These factors include lipoprotein lipase, hepatic TG lipase, and sphingomyelinase, as all are expressed by macrophages (44–48). In addition, secretory phospholipase A2, which is synthesized by smooth muscle cells (49), and EL, which is expressed in endothelial cells (20, 21), also may be present in the lesion.

In summary, the current studies have shown that both SR-BI- and ABCA1-mediated efflux are functions of the PL content of HDL and that modulation of HDL PL content affects the two mechanisms of efflux in a reciprocal manner. It follows that factors that modulate HDL PL content in vivo will have substantial effects on both mechanisms of efflux.

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