Remodeling of HDL remnants generated by scavenger receptor class B type I

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Abstract **Scavenger receptor class B type I (SR-BI) mediates the selective transfer of cholesteryl ester from HDL to cells. We previously established that SR-BI overexpressed in livers of apolipoprotein A-I-deficient mice processes exogenous human HDL_2 to incrementally smaller HDL particles. When mixed with normal mouse plasma either in vivo or ex vivo, SR-BI-generated HDL "remnants" rapidly remodel to form HDL-sized lipoproteins. In this study, we analyzed HDLs throughout the process of HDL remnant formation and investigated the mechanism of conversion to larger particles. Upon interacting with SR-BI, α-migrating HDL_2 is initially converted to a preα-migrating particle that is ultimately processed to a smaller α-migrating HDL remnant. SR-BI does not appear to generate preβ-1 HDL particles. When incubated with isolated lipoprotein fractions, HDL remnants are converted to lipoprotein particles corresponding in size to the particle incubated with the HDL remnant. HDL remnant conversion is not altered in phospholipid transfer protein (PLTP)-deficient mouse plasma or by the addition of purified PLTP. Although LCAT-deficient plasma promoted only partial conversion, this deficiency was attributable to the nature of HDL particles in $LCAT^{-/-}$ mice rather than to a requirement for LCAT in the remodeling process. We conclude that HDL remnants, generated by SR-BI, are converted to larger particles by rapidly reassociating with existing HDL particles in an enzyme-independent manner.**—Webb, N. R., M. C. de Beer, B. F. Asztalos, N. Whitaker, D. R. van der Westhuyzen, and F. C. de Beer. **Remodeling of HDL remnants generated by scavenger receptor class B type I.** ***J. Lipid Res.*** **2004.** 45: **1666–1673.**

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Numerous population studies have established an inverse correlation between the risk for atherosclerosis and plasma HDL levels (1). The ability of HDL to protect against atherosclerosis is widely attributed to its role in reverse cholesterol transport, a process by which HDL promotes the flux of cholesterol from peripheral tissues to the liver for excretion. In the first step of this pathway, ABCA1 expressed on the surface of cells mediates the efflux of cellular cholesterol to lipid-poor apolipoprotein A-I (apoA-I). Free cholesterol in this nascent HDL is then esterified by LCAT. The cholesteryl oleoyl esters (CEs) that are formed move into the core of HDL particles by virtue of their hydrophobicity to produce α-migrating spherical HDL particles. HDL-CE is delivered primarily to the liver (2–4), either subsequent to transfer to apoB-containing lipoproteins or directly through a process involving scavenger receptor class B type I (SR-BI). The direct delivery of CE from HDL to cells is via a "selective uptake" mechanism whereby CE is selectively transferred from the core of the particles to cells without the intracellular accumulation and/or degradation of HDL apolipoproteins. Thus, the transport of cholesterol from extrahepatic tissue to the liver is envisioned to involve the lipidation (mediated by ABCA1) and delipidation (mediated by SR-BI) of HDL. Although the metabolic fate of HDL after SR-BI-mediated processing has not been completely delineated, studies in mice have shown that increased hepatic SR-BI expression leads to increased apoA-I catabolism, and at least some of this catabolism occurs in the kidney (5, 6).

We recently established a model system to study HDL particles after SR-BI selective lipid uptake (7). Our approach was to inject a bolus (750 μg) of radiolabeled human HDL_2 into apoA-$I^{-/-}$ mice overexpressing SR-BI by adenoviral vector and then reisolating these particles for analysis. As reported previously, SR-BI activity leads to the incremental formation of increasingly smaller HDL "remnants." When injected into C57BL/6 mice with normal SR-BI expression, these HDL remnants do not undergo

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Abbreviations: apoA-I, apolipoprotein A-I; CE, cholesteryl oleoyl ester; CETP, cholesteryl ester transfer protein; PLTP, phospholipid transfer protein; SR-BI, scavenger receptor class B type I.

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accelerated catabolism but rather are converted back to normal-sized HDL particles. This remodeling does not appear to involve the activity of ABCA1 or other cellular components, because conversion to larger particles occurred in a similar manner when HDL remnants were incubated with mouse plasma ex vivo. Cholesteryl ester transfer protein (CETP) is also not required for this conversion, because mice naturally lack this transfer protein. We have hypothesized that this remodeling pathway plays an important role in reverse cholesterol transport by protecting lipid-depleted apoA-I from rapid catabolism. The objective of the current study is to investigate the mechanism of HDL remnant remodeling.

EXPERIMENTAL PROCEDURES

Animals

C57BL/6 and apoA-I$^{-/-}$ (C57BL/6 background) mice were obtained from Jackson Laboratories. The animals were housed in a pathogen-free facility with an equal light/dark cycle and free access to water and regular rodent chow. All procedures were approved by the Veterans Administration Medical Center Institutional Animal Care and Use Committee.

Lipoprotein isolation and radiolabeling

VLDL (d = 1.006–1.019 g/ml), LDL (d = 1.019–1.063 g/ml), and HDL (d = 1.063–1.21 g/ml) were isolated from fresh mouse or human plasma by density gradient ultracentrifugation as described previously (8). Human HDL subfractions were defined as follows: HDL_2 (d = 1.08–1.13 g/ml), HDL_{2A} (d = 1.08–1.09 g/ml), HDL_{2B} (d = 1.09–1.11 g/ml), and HDL_3 (d = 1.13–1.18 g/ml). All isolated fractions were dialyzed against 150 mM NaCl and 0.01% EDTA, sterile filtered, and stored under nitrogen gas at 4°C. Protein concentrations were determined by the method of Lowry et al. (9). HDL was radioiodinated by the iodine monochloride method (10).

Generation of HDL remnants in apoA-I$^{-/-}$ mice

The production of SR-BI-generated HDL remnants in apoA-I$^{-/-}$ mice was described previously (7). ApoA-I$^{-/-}$ mice weighing at least 25 g were injected in the tail vein with 1.5×10^{11} particles of AdSR-BI, a replication-defective adenoviral vector expressing mouse SR-BI (11, 12). Three days after adenovirus infusion, the mice were injected via the jugular vein with 750 μg of human ^{125}I-HDL_2 in 100 μl of saline (specific activity, 20–30 cpm/ng). The samples described were obtained from three separate remnant preparations, each of which involved collecting plasma from two mice at 3 h after ^{125}I-HDL bolus injection. For each of the mice, two retro-orbital bleeds were done at earlier time points. By analyzing aliquots of each of the three time points by native gel electrophoresis, we were able to assess the kinetics of remnant generation and thus verify that each of the 3 h remnant preparations were processed to similar extents. Plasma collected from the mice was used for experiments without further manipulation. As we have reported previously, HDL remnants that have accumulated in apoA-I$^{-/-}$ mice at 3 h after bolus injection are too dense to refloat from plasma by density ultracentrifugation (7). Because SR-BI overexpression leads to an almost complete depletion of lipoprotein cholesterol and apolipoproteins in apoA-I$^{-/-}$ mice, plasma recovered from these mice contains virtually no endogenous lipoproteins (7). The concentration of HDL remnants in plasma at 3 h after bolus injection, determined from the counts recovered in the mouse plasma and the known specific activity of the injected ^{125}I-HDL, was ~0.15 mg/ml.

In vitro remodeling reactions

Plasma collected from mice at 3 h after ^{125}I-HDL injection (hereafter referred to as the 3 h HDL remnant) was mixed 1:10 (v/v) with mouse or human plasma, mouse plasma diluted in PBS, lipoprotein-deficient human serum, or lipoprotein fractions isolated by density gradient ultracentrifugation. Lipoprotein-deficient serum was prepared by clotting with thrombin (1 U/ml) the lipoprotein-deficient infranatant obtained after sequential ultracentrifugations of human plasma. The clot was removed by centrifugation, and the lipoprotein-deficient serum was passed through a 0.2 μm filter and stored frozen. Plasma from phospholipid transfer protein (PLTP)-deficient and LCAT-deficient mice was generously provided by X-c. Jiang (Columbia University) and S. Santamarina-Fojo (National Institutes of Health/National Heart, Lung, and Blood Institute), respectively. Partially purified human PLTP (Cardiovascular Targets, New York, NY), purified human recombinant LCAT (provided by J. S. Parks, Wake Forest University), or DTNB (5 mM final concentration) was added to some reactions, as indicated. After incubations at 37°C, the remodeling reactions were transferred to ice, immediately applied to a nondenaturing gradient gel (4–18% acrylamide) (13, 14), and then visualized by autoradiography.

Two-dimensional gel electrophoresis

^{125}I-HDL remnants and normal human plasma were separated by native two-dimensional gel electrophoresis, by charge in the first dimension and by size in the second dimension, as previously described (15, 16). Samples were applied on a low endosmosity 0.7% vertical slab agarose gel (SeaKem LE; FMC Bioproducts, Rockford, ME) and electrophoresed in a Pharmacia GE 2/4 recirculating apparatus (Uppsala, Sweden) at constant voltage (250 V) and temperature (10°C) in Tris-Tricine buffer (25 mM, pH 8.6) until the endogenous albumin, stained with bromphenol blue, was 3.5 cm from the origin. Lanes excised from the gel were then applied to a 3–35% concave gradient nondenaturing polyacrylamide gel and electrophoresed (24 h at 250 V) to completion. Samples were subsequently transferred to nitrocellulose membranes. The two-dimensional pattern of ^{125}I-HDL remnants was determined using a fluoroimager in X-ray mode, followed by visualizing the apoA-I-containing HDL subpopulations of human plasma, used as a control, by monospecific human apoA-I primary and ^{125}I-labeled secondary antibodies.

RESULTS

Two-dimensional gel analysis of HDL remnants

We recently described a method for generating HDL particles that have undergone extensive processing by SR-BI in vivo (7). For these studies, a bolus of radioiodinated human HDL_2 was injected into apoA-I$^{-/-}$ mice overexpressing SR-BI by adenoviral vector. By 3 h after bolus injection, essentially all of the exogenous HDL converted to small, dense HDL "remnants" (7) (**Fig. 1A**). Interestingly, the formation of small HDL particles appeared to occur in incremental steps, such that discrete populations of progressively smaller particles accumulated during the 3 h period after bolus injection (7).

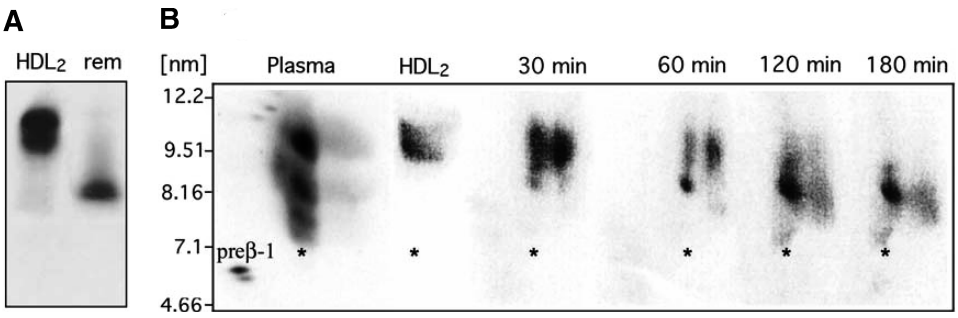

Fig. 1. Analysis of scavenger receptor class B type I (SR-BI)-generated HDL remnants by nondenaturing gel electrophoresis. Three days after infusion with 1.5×10^{11} particles of AdSR-BI, apolipoprotein A-I-deficient (apoA-I$^{-/-}$) mice were injected with human HDL_2 traced with ^{125}I. Plasma was collected at selected intervals 30–180 min after HDL injection. A: An aliquot of plasma collected 180 min after ^{125}I-HDL_2 injection (rem) was separated on a nondenaturing gradient gel (4–18% acrylamide) and visualized by autoradiography. An aliquot of the injected ^{125}I-HDL_2 was analyzed for comparison. B: ^{125}I-HDL_2 or plasma collected at the indicated time after ^{125}I-HDL_2 injection was electrophoresed on 0.7% low endosmotic agarose (first dimension) and nondenaturing 3–35% acrylamide (second dimension) gels and then visualized with a fluoroimager. For comparison, an aliquot of normal human plasma was separated under identical conditions and immunoblotted using monospecific anti-human apoA-I. The mobilities of α- (*) and preβ-1-migrating particles (first dimension) and standards with known diameters (second dimension) are indicated.

To further characterize HDLs during SR-BI processing, we analyzed HDL remnants at selected intervals after ^{125}I-HDL injection by two-dimensional gel electrophoresis (Fig. 1B). The injected ^{125}I-HDL_2 particles had a size range of 8.7–11.7 nm, with an average diameter of 9.2 nm. At 30 min after injection, the size range of the particles was 8.0–11.7 nm, with an average diameter of 8.8 nm. In addition, ~50% of the particles at this time point migrated with preα mobility. This contrasts to the injected ^{125}I-HDL_2, in which the majority of the particles migrated with α mobility. At 60 min after injection, the ratio of particles with α and preα mobility remained ~1:1, but the α-migrating particles were further decreased in size, to a mean diameter of 8.0 nm. At 120 and 180 min after injection, SR-BI processing resulted in the generation of small (7.7 nm diameter), mostly α-migrating particles. No SR-BI-generated preβ-1-migrating particles were evident in any of the HDL remnant samples analyzed.

HDL remnant remodeling in mouse plasma

We reported previously that small, dense HDL remnants rapidly converted to larger HDLs when incubated at 37°C with normal mouse plasma (7). This conversion appeared to involve more than a spontaneous fusion of particles, because remodeling did not occur when HDL remnants were incubated in PBS (7). To investigate the possibility that HDL remnant remodeling requires the contribution of plasma factor(s), we incubated HDL remnants at 37°C with serially diluted mouse plasma for 30 min and assessed the extent of conversion to larger HDLs. Whereas virtually all of the HDL remnants remodeled to HDL_2-sized particles when incubated with undiluted plasma, ~50% of the particles remained unaltered in size when incubated with plasma diluted 1:32 (**Fig. 2**). This result indicates that there is a component in mouse plasma required for the remodeling process that becomes limiting upon plasma dilution.

PLTP and HDL remnant remodeling

To investigate whether PLTP contributes to the remodeling process, HDL remnants were incubated with mouse plasma that was diluted 1:32 in PBS and supplemented with partially purified human PLTP. The final concentration of PLTP in the reaction mixture corresponded to ~4-fold the amount of PLTP present in diluted mouse plasma (Cardiovascular Targets, R. Brocia, personal communication). As shown in **Fig. 3A**, the addition of PLTP did not increase the extent of remodeling compared with diluted mouse plasma alone, suggesting that the deficiency in remodeling was not attributable only to insufficient PLTP activity. Additional experiments with twice the amount of PLTP produced the same result (data not shown). To further assess the role of PLTP in the remodeling process, HDL remnants were incubated with PLTP$^{-/-}$ mouse plasma. As depicted in Fig. 3B, HDL remnant remodeling in the presence of PLTP$^{-/-}$ and wild-type mouse plasma

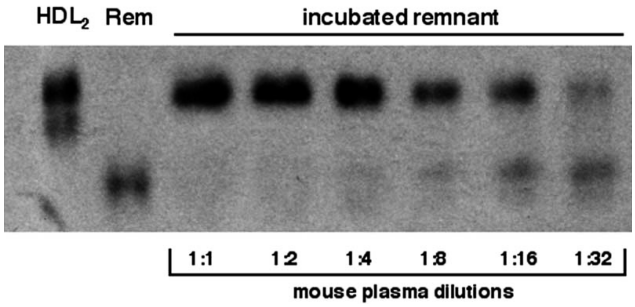

Fig. 2. Remodeling of HDL remnants in mouse plasma. Three hour ^{125}I-HDL remnant was mixed 1:10 (v/v) with C57BL/6 mouse plasma serially diluted in PBS. After incubating for 30 min at 37°C, the samples were separated on a nondenaturing 4–18% gradient polyacrylamide gel and visualized by autoradiography. Unmodified ^{125}I-HDL_2 and 3 h ^{125}I-HDL remnant (Rem) were analyzed for comparison.

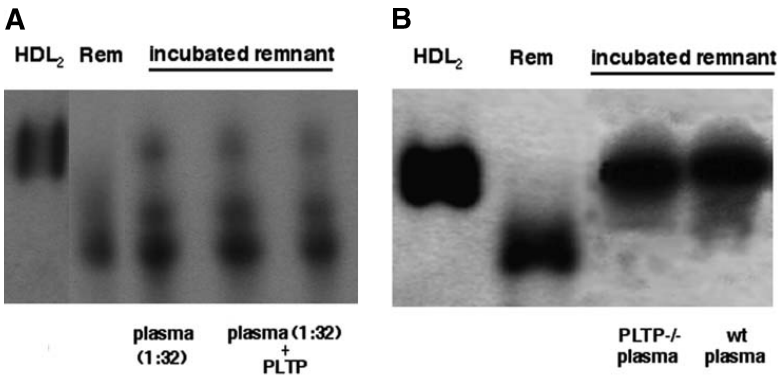

Fig. 3. Role of phospholipid transfer protein (PLTP) in HDL remnant remodeling. A: Three hour ^{125}I-HDL remnant was mixed with C57BL/6 mouse plasma diluted 1:32 in PBS with or without the addition of 0.7 μg of partially purified PLTP. After incubating for 30 min at 37°C, the samples were separated on a nondenaturing 4–18% gradient polyacrylamide gel and visualized by autoradiography. Unmodified ^{125}I-HDL$_2$ and 3 h ^{125}I-HDL remnant (Rem) were analyzed for comparison. B: Three hour ^{125}I-HDL remnant was mixed with plasma from wild-type (wt) or PLTP-deficient mice and incubated and analyzed as in A.

was similar. We conclude from these experiments that PLTP does not play a major role in the conversion of HDL remnants to larger HDLs.

LCAT and HDL remnant remodeling

To determine whether remodeling requires LCAT, HDL remnants were incubated with plasma obtained from LCAT$^{-/-}$ mice. Interestingly, the product of the remodeling process after incubation with LCAT$^{-/-}$ mouse plasma was different compared with normal mouse plasma (**Fig. 4**, lanes 3 and 4). Although most of the HDL remnants appeared to have converted to somewhat larger particles after incubation with LCAT$^{-/-}$ plasma, only a fraction migrated as HDL$_2$-sized particles. The addition of purified LCAT to LCAT$^{-/-}$ plasma during the incubation resulted in HDL remnant remodeling that was similar to what occurred with normal mouse plasma (Fig. 4, lanes 3 and 5). The ability of purified LCAT to enhance remodeling was reduced when it was heat inactivated (Fig. 4, lane 6) and totally abolished when the LCAT inhibitor DTNB was added to the remodeling reaction. Although these results suggested that LCAT may play a direct role in the conversion of HDL remnants to larger HDL particles, we considered an alternative possibility that the defect in remodeling in LCAT$^{-/-}$ plasma was attributable to the presence of aberrant HDL particles in this plasma. LCAT$^{-/-}$ mice have markedly reduced plasma concentrations of apoA-I-containing HDL particles that are heterogeneous in size and migrate primarily with preβ-1 or α-4 mobility (17). Thus, the addition of LCAT to LCAT$^{-/-}$ plasma could promote HDL remnant remodeling by altering the endogenous HDL present in this plasma. To investigate this possibility, LCAT$^{-/-}$ plasma was preincubated with purified LCAT for 120 min, and then enzymatic activity was abolished by either heat inactivation or by adding DTNB. Interestingly, LCAT$^{-/-}$ plasma that was pretreated with LCAT was capable of converting HDL remnants to larger HDLs similarly to normal mouse plasma even when LCAT was inactive during the remodeling process (Fig. 4, lanes 8 and 9). This suggests that the addition of LCAT to LCAT$^{-/-}$ plasma alters the product of HDL remnant remodeling by supplying CE to the HDL particles present in LCAT$^{-/-}$ plasma, rather than by actively participating in the remodeling process itself.

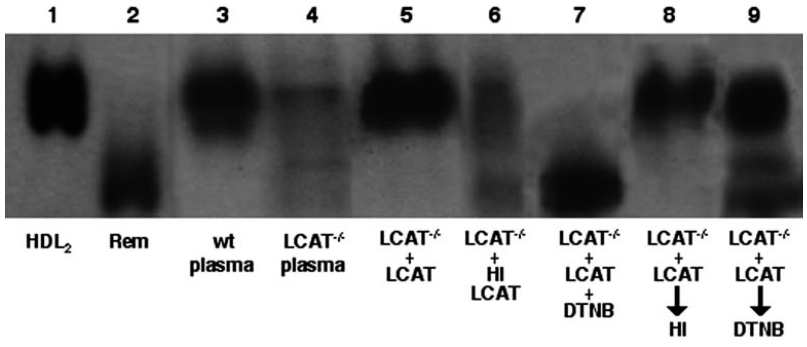

Fig. 4. Role of LCAT in HDL remnant remodeling. Three hour ^{125}I-HDL remnant was mixed with wild-type (wt) mouse plasma (lane 3), LCAT$^{-/-}$ mouse plasma (lane 4), LCAT$^{-/-}$ mouse plasma supplemented with 0.5 μg of purified human LCAT (lane 5), LCAT$^{-/-}$ mouse plasma supplemented with 0.5 μg of heat-inactivated (HI) LCAT (lane 6), LCAT$^{-/-}$ mouse plasma supplemented with 0.5 μg of purified LCAT and the LCAT inhibitor DTNB (lane 7), LCAT$^{-/-}$ mouse plasma preincubated with LCAT and then heat-inactivated before ^{125}I-HDL remnant addition (lane 8), or LCAT$^{-/-}$ mouse plasma preincubated with LCAT before the addition of ^{125}I-HDL remnant and DTNB (lane 9). After 120 min incubations at 37°C, the mixtures were separated by nondenaturing gradient gel electrophoresis and visualized by autoradiography. Unmodified ^{125}I-HDL$_2$ (lane 1) and 3 h ^{125}I-HDL remnant (Rem; lane 2) were analyzed for comparison.

HDL remnant remodeling and lipoprotein fractions

The results shown in Fig. 4 suggested that the product of the remodeling process was influenced by the nature of the lipoprotein particles that were incubated with the HDL remnants. To investigate this possibility further, HDL remnants were incubated with either human or mouse plasma, which contain characteristically distinct lipoprotein fractions. When incubated with human plasma, HDL remnants converted to larger particles of heterogeneous size that were reminiscent of the polydisperse nature of human HDL (**Fig. 5**). In contrast, HDL remnants incubated with mouse plasma converted to discretely sized particles similar to homogeneous mouse HDL. This finding raised the possibility that HDL remnant remodeling involved the association of the lipid-depleted particle with preexisting lipoproteins in plasma. This conclusion is consistent with the results shown in **Fig. 6**, which demonstrated that HDL remnants undergo virtually no conversion to larger particles when incubated with lipoprotein-deficient serum.

Interestingly, when HDL remnants were incubated with human plasma, there was no evidence of conversion to VLDL or intermediate density lipoprotein/LDL-sized particles, suggesting that the lipid-depleted particles interacted preferentially with HDL (Fig. 5). To directly assess the interaction of HDL remnants with various lipoprotein fractions, the particles were incubated with human VLDL, LDL, or HDL subfractions that were isolated by density ultracentrifugation. Analysis of the reaction mixtures after 30 min at 37°C showed that the size of the remodeled product corresponded to the size of the lipoprotein particles incubated with the HDL remnant (**Fig. 7A**). It was notable that the extent of HDL remnant remodeling varied for the different lipoprotein fractions. Whereas ~50% of the HDL remnants converted to larger particles when incubated with HDL subfractions, under the same incubation conditions only a small portion of the remnant particles converted to LDL- and VLDL-sized particles. Longer incubations with LDL (data not shown) or VLDL (Fig. 7B) did not allow for more complete remodeling of HDL remnants. The relative ability of VLDL and HDL to promote HDL remnant conversion was directly assessed by mixing these lipoprotein fractions at known ratios based on protein concentration and then incubating the mixture with HDL remnant particles (Fig. 7B). This analysis showed that the majority of remnants converted to HDL-sized particles, confirming the conclusion that lipid-depleted remnant particles preferentially associate with HDL.

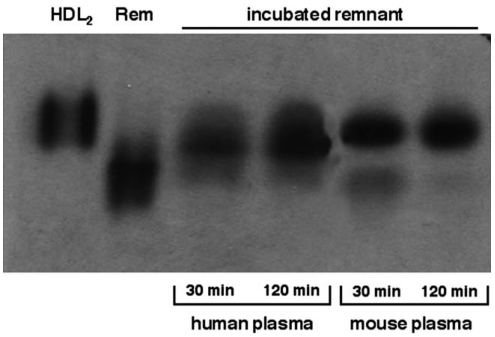

Fig. 5. HDL remnant remodeling with human and mouse plasma. Three hour ^{125}I-HDL remnant was mixed with mouse or human plasma (1:10, v/v). After incubating at 37°C for the indicated amount of time, the mixtures were separated on a nondenaturing 4–18% gradient polyacrylamide gel and visualized by autoradiography. Unmodified ^{125}I-HDL$_2$ and 3 h ^{125}I-HDL remnant (Rem) were analyzed for comparison.

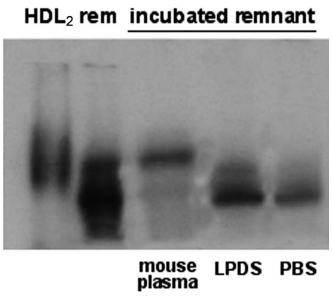

Fig. 6. HDL remnant remodeling in the presence of lipoprotein-deficient serum. Three hour ^{125}I-HDL remnant was mixed with C57BL/6 mouse plasma, human lipoprotein-deficient serum (LPDS), or PBS (1:10, v/v). After incubating at 37°C for 30 min, the mixtures were separated on a nondenaturing 4–18% gradient polyacrylamide gel and visualized by autoradiography. Unmodified ^{125}I-HDL$_2$ and 3 h ^{125}I-HDL remnant (rem) were analyzed for comparison.

DISCUSSION

In a previous study, we demonstrated that SR-BI has the capacity to generate small, dense HDL particles from spherical HDL$_2$ (7). These particles accumulate in mice with increased hepatic SR-BI expression brought about by adenoviral vector gene transfer (7). The HDL remnant particles appear to be protected from rapid catabolism in vivo by virtue of their ability to remodel in plasma to form larger HDLs. The mechanism of this remodeling process is of interest, because it may represent an important pathway in determining plasma HDL concentrations and the rate of reverse cholesterol transport. In the current study, we provide evidence that small, dense HDL remnants generated by SR-BI rapidly incorporate into preexisting lipoprotein particles, preferentially HDL, apparently in an enzyme-independent manner.

Two-dimensional gel analyses showed that during the generation of HDL remnants, α-migrating HDL$_2$ is initially processed to a preα-migrating particle that is not markedly altered in size compared with the starting HDL$_2$. Subsequent processing by SR-BI produces incrementally smaller HDLs with α-mobility. The reason for the initial increase in negative charge is not known. Previous analyses have shown that the surface charge of HDL can be influenced either by alterations in the content of negatively charged lipids in the shell of the particle (18, 19) or through conformational changes of resident apolipoproteins (18, 20). Although this has not been directly shown,

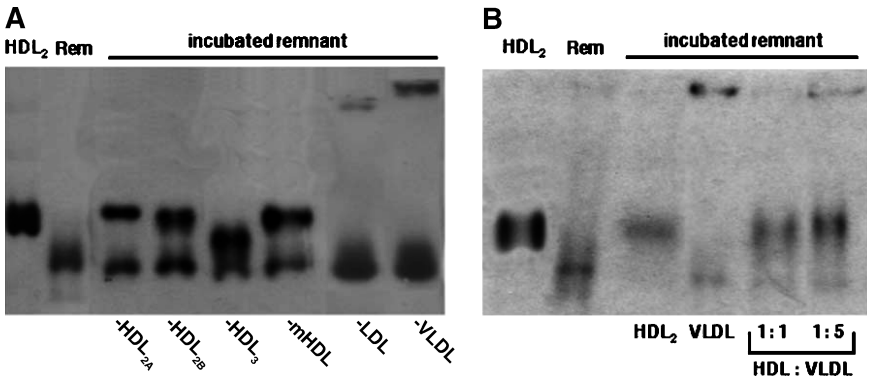

Fig. 7. HDL remnant remodeling with isolated lipoprotein fractions. A: Three hour ^{125}I-HDL remnant was mixed with the indicated mouse (mHDL) or human lipoprotein fraction (1 mg/ml) and incubated for 2 h at 37°C. Incubated samples were separated on a nondenaturing 4–18% gradient polyacrylamide gel and visualized by autoradiography. B: ^{125}I-HDL remnant was incubated at 37°C for 2 h with human HDL_2 (0.33 mg/ml), human VLDL (0.33 mg/ml), a mixture containing both HDL_2 and VLDL (0.3 mg/ml each), or a mixture of HDL_2 (0.33 mg/ml) and VLDL (1.65 mg/ml). Incubated samples were analyzed by nondenaturing gel electrophoresis and visualized by autoradiography. An aliquot of the injected ^{125}I-HDL_2 and unmodified 3 h ^{125}I-HDL remnant (Rem) was analyzed for comparison.

we envision that SR-BI-mediated depletion of neutral lipid from the core of HDL may initially be accommodated by a change in apoA-I conformation, which brings about a change in the surface charge of the HDL particle without a significant decrease in particle size. Further depletion of the core lipid may result in the progressive loss of apolipoprotein molecules from the HDL particle, leading to an incremental decrease in particle size. One of the most important findings in the current study is that there is no accumulation of preβ-migrating HDL particles, even in preparations extensively processed by SR-BI.

Our studies indicate that when mixed with mouse plasma, HDL remnants convert to larger particles by incorporating into preexisting lipoproteins. This conclusion is supported by the observation that the product of the remodeling process is dependent on the lipoprotein fraction incubated with the HDL remnant. Although the incorporation event appears to be rapid and spontaneous, the process is not indiscriminate. We have found no evidence for spontaneous fusion of HDL remnant particles in any of our experiments, suggesting that the association of HDL remnants with lipoprotein particles is under structural constraints, because HDL remnants apparently do not associate with other HDL remnants. Although both LDL and VLDL can induce some conversion of HDL remnants to larger particles, our data indicate that when incubated with mouse or human plasma, HDL remnants associate almost exclusively with HDL.

We investigated the role of PLTP in conversion to larger particles. Substantial evidence suggests that PLTP is an important factor in determining the size and composition of HDLs [reviewed in ref. (21)]. In studies using reconstituted HDL, the PLTP-mediated enlargement of HDL has been shown to involve a fusion event that is followed by the dissociation of apoA-I (22, 23). Our results show that the addition of partially purified human PLTP to HDL remnants does not promote the conversion to larger HDLs, indicating that these particles are not competent for PLTP-induced fusion. PLTP also mediates the transfer of phospholipids and cholesterol from triglyceride-rich lipoproteins into HDL particles to form larger HDLs (24). Studies in $PLTP^{-/-}$ mice indicate that this activity may play an important role in maintaining HDL cholesterol concentrations, possibly by promoting the conversion of nascent HDL to mature, spherical HDL particles (25, 26). To assess whether PLTP-mediated lipid transfer is involved in HDL remnant enlargement, remnants were incubated with $PLTP^{-/-}$ mouse plasma. The finding that $PLTP^{-/-}$ mouse plasma promotes the remodeling of HDL remnants in a manner similar to $PLTP^{+/+}$ mouse plasma demonstrates that this conversion process does not require the activity of PLTP.

We also assessed the role of LCAT in HDL remnant remodeling. LCAT plays a major role in HDL metabolism by esterifying free cholesterol present in nascent HDL to convert the preβ-migrating disc into an α-migrating, spherical HDL. In the current study, we showed that compared with normal mouse plasma, $LCAT^{-/-}$ plasma promotes only partial remodeling of HDL remnants. However, this deficiency appears to be attributable to the nature of HDL particles in $LCAT^{-/-}$ mice rather than to a requirement for LCAT in the remodeling process itself. Previous studies have shown that when LCAT increases the CE content of HDL, there is an associated increase in HDL particle size and an increase in the number of apoA-I molecules per particle. This increase in particle apoA-I content may be brought about either by particle fusion or by the incorporation of lipid-poor apoA-I into the HDL (27). It is tempting to speculate that SR-BI-generated HDL remnants may be one source of lipid-poor apoA-I that can participate in this LCAT-mediated HDL processing.

Our results with lipid-depleted HDL particles generated by SR-BI-mediated selective lipid uptake are similar in several respects to the recent findings of Parks and cowork-

ers, who investigated the metabolism of small (7.2–7.4 nm) HDL particles that represent a minor HDL fraction in human apoA-I transgenic mouse plasma (28). These HDLs, which were isolated by affinity chromatography and size exclusion chromatography, exhibited both α and preα mobility on agarose gels. When injected into human apoA-I transgenic mice or mixed with human apoA-I transgenic mouse plasma ex vivo, the majority of these particles rapidly remodeled to form medium or large HDLs through a process that did not require LCAT.

Nondenaturing gel electrophoresis analysis indicated that isolated HDL fractions do not increase substantially in size when incubated with HDL remnants, as might be expected if the HDL particles had incorporated additional apolipoprotein(s) as a result of their interaction with HDL remnants. In the experiments shown, HDL remnants were mixed with HDL fractions at an apoA-I ratio of ~1:50, respectively. Thus, it is possible that we did not detect a substantial increase in the overall size of the population of the added HDLs because only a small fraction of the particles could acquire apoA-I from HDL remnants. An increase in size may be difficult to detect if remnants preferentially associate with smaller HDL particles or if the interaction of remnants with spherical HDL leads to a rapid redistribution of apolipoproteins among all of the HDL particles.

Abundant data from in vitro studies have shown that apoA-I cycles between lipid-poor and lipid-associated forms as a result of the actions of LCAT, CETP, and PLTP (29). The HDL receptor SR-BI has the potential to contribute to the pool of lipid-poor apoA-I by virtue of its ability to selectively transfer lipid from the core of the particle to cells. Our studies establish that SR-BI modification of HDL_2 does not yield preβ-migrating HDL particles. Interestingly, apolipoproteins on these HDL remnants (or the remnant particles themselves) are capable of reincorporating into preexisting spherical HDL through a process that does not appear to require known HDL-remodeling enzymes. The ability of SR-BI-generated HDL remnants to directly incorporate into HDL may provide a mechanism for retaining HDL apolipoproteins in the circulation after selective lipid uptake. These findings provide new insights into the relationship of SR-BI with other HDL metabolic pathways.

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