SR-BI-mediated selective lipid uptake segregates apoA-I and apoA-II catabolism

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Abstract The HDL receptor scavenger receptor class B type I (SR-BI) binds HDL and mediates the selective uptake of cholesteryl ester. We previously showed that remnants, produced when human HDL_2 is catabolized in mice overexpressing SR-BI, become incrementally smaller, ultimately consisting of small α-migrating particles, distinct from preβ HDL. When mixed with mouse plasma, some remnant particles rapidly increase in size by associating with HDL without the mediation of cholesteryl ester transfer protein, LCAT, or phospholipid transfer protein. Here, we show that processing of HDL_2 by SR-BI-overexpressing mice resulted in the preferential loss of apolipoprotein A-II (apoA-II). Short-term processing generated two distinct, small α-migrating particles. One particle (8.0 nm diameter) contained apoA-I and apoA-II; the other particle (7.7 nm diameter) contained only apoA-I. With extensive SR-BI processing, only the 7.7 nm particle remained. Only the 8.0 nm remnants were able to associate with HDL. Compared with HDL_2, this remnant was more readily taken up by the liver than by the kidney. We conclude that SR-BI-generated HDL remnants consist of particles with or without apoA-II and that only those containing apoA-II associate with HDL in an enzyme-independent manner. Extensive SR-BI processing generates small apoA-II-depleted particles unable to reassociate with HDL and readily taken up by the liver. This represents a pathway by which apoA-I and apoA-II catabolism are segregated.— de Beer, M. C., D. R. van der Westhuyzen, N. L. Whitaker, N. R. Webb, and F. C. de Beer. **SR-BI-mediated selective lipid uptake segregates apoA-I and apoA-II catabolism. *J. Lipid Res.* 2005.** 46: **2143–2150.**

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Scavenger receptor class B type I (SR-BI) delivers cholesteryl ester (CE) from HDL to cells via a selective uptake mechanism whereby lipid is transferred from the core of the particle without concomitant direct degradation of the protein moiety (1). SR-BI-mediated selective lipid uptake generates incrementally smaller and denser HDL particles (HDL remnants) depleted in CE (2). These HDL remnants generated by SR-BI are mostly converted to larger particles by rapidly reassociating with existing HDL particles in the plasma in an enzyme-independent manner (2, 3). A portion of the HDL remnant apolipoprotein, however, is directed toward catabolism (2). This catabolic pathway may be significant because hepatic SR-BI overexpression can practically deplete mouse plasma of HDL apolipoproteins (4, 5). Thus, HDL remnant remodeling represents a process that could be important in determining the rate of apolipoprotein A-I (apoA-I) catabolism and HDL-mediated reverse cholesterol transport. This is relevant because variations in apoA-I and HDL cholesterol concentrations are to a significant extent determined by the rate of apoA-I catabolism rather than apoA-I synthesis (6, 7). In addition to SR-BI-mediated selective lipid uptake, many of the factors known to influence apoA-I catabolic rate also influence the CE content of the HDL particle. For example, LCAT deficiency, which results in the depletion of HDL CE, is associated with accelerated apoA-I catabolism (8). Conversely, the accumulation of CE in HDL, when cholesteryl ester transfer protein (CETP) is deficient, is associated with delayed apoA-I catabolism (9). In addition to HDL-CE content, studies in both humans and mice have indicated that the levels of HDL cholesterol and apoA-I are influenced by the rate of synthesis of apoA-II (10, 11). ApoA-II-deficient mice have markedly reduced HDL levels and increased HDL cholesterol and apoA-I fractional catabolic rates (12). We and others (13, 14) have

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Abbreviations: apoA-I, apolipoprotein A-I; apoA-I$^{-/-}$, apolipoprotein A-I-deficient; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; DLT, dilactitol tyramine; rHDL, reconstituted high density lipoprotein; SR-BI, scavenger receptor class B type I.

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previously shown that apoA-II exerts a marked effect on the interaction of native HDL with SR-BI, the apoA-II content of HDL being inversely correlated with HDL binding and selective cholesteryl ester uptake. In contrast, the selective uptake from apoA-I/apoA-II-reconstituted high density lipoprotein (rHDL) was greater than from apoA-I-rHDL, even though the binding of apoA-I/apoA-II-rHDL was less than that of apoA-I-rHDL (15).

Given the influence of apoA-II on HDL metabolism and HDL/SR-BI interaction, we investigated the role of apoA-II on the generation and subsequent remodeling of HDL remnants. ApoA-II might be predicted to affect HDL remodeling because it contains three amphipathic α helices that are likely to remain lipid-associated during SR-BI processing (16, 17). It has further been shown that apoA-II inhibits HDL remodeling mediated by CETP (18). To study the fate of apoA-II during HDL remnant metabolism, we used an established model that involves injecting a bolus (750 μg) of radiolabeled human HDL_2 into apolipoprotein A-I-deficient (apoA-$I^{-/-}$) mice with adenovirally mediated overexpression of SR-BI and then analyzing the remnant HDL particles that accumulate in the plasma of these mice (2, 3). The apolipoprotein composition of HDL remnants processed to different extents by SR-BI was determined. Data indicated a segregation of apoA-I and apoA-II into distinct remnant particles, such that extensive processing by SR-BI yielded a small α-migrating HDL remnant containing apoA-I only that was resistant to remodeling by failing to associate with larger HDLs. When injected into normal C57BL/6 mice, these apoA-I-only remnant particles exhibited enhanced uptake into the liver compared with unmodified HDL_2.

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. The Veterans Administration Medical Center Institutional Animal Care and Use Committee approved all procedures.

Lipoprotein isolation and radiolabeling

HDLs (d = 1.063–1.21 g/ml) were isolated from fresh mouse or human plasma by density gradient ultracentrifugation as described previously (19). Human HDL was subfractionated on an Isco fraction collector to obtain HDL_2 (d = 1.09–1.11 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. (20). To generate remnant particles, HDL_2 was radiolabeled by the iodine monochloride method (21). For tissue distribution of injected remnants, HDL_2 was traced with the nondegradable, intracellularly trapped radiolabel ^{125}I-dilactitol tyramine (DLT) (22).

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

The production of SR-BI-generated HDL remnants in apoA-$I^{-/-}$ mice was described previously (2, 3). 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. Three days after adenovirus infusion, the mice were injected via the jugular vein with a mixture of 700 μg of human HDL_2 and 50 μg of ^{125}I-HDL_2 in 100 μl of saline (specific activity of HDL mix, 20–30 cpm/ng). The experiments described were obtained from three separate remnant preparations, each of which involved collecting plasma from two mice at 0.5, 1, 2, and 3 h after ^{125}I-HDL bolus injection. By analyzing aliquots from each of the 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 was processed to a similar extent. 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 3 h after bolus injection are too dense to refloat from plasma by density ultracentrifugation (2). 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 (2). 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.

SDS-PAGE analysis of lipoprotein particles separated by gradient gel electrophoresis

HDL or remnant particles, identified and delineated by autoradiography of nondenaturing gels, were excised from the dried gels. The dried gel slices were hydrated for 30 min in electrophoresis buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine, and 0.1% SDS) to allow for dissociation of the paper backing from the gel slices. The hydrated gel slices were inserted into the wells of double-thick SDS gels and subjected to SDS-PAGE using 5–20% acrylamide gels for separation of the apoprotein components of the lipoprotein particles. Visualization was by autoradiography of dried gels.

In vitro remodeling reactions

Plasma collected from mice up to 3 h after ^{125}I-HDL injection (HDL remnant) was mixed 1:10 (v/v) with mouse HDL (1.7 mg/ml). After incubations at 37°C, the remodeling reactions were transferred to ice, mixed with an equal volume of saturated sucrose, and immediately applied to a nondenaturing gradient gel (4–18% acrylamide) (3) and visualized by autoradiography.

Tissue uptake and clearance of HDL_2 and HDL remnants in C57BL/6 mice

HDL remnants were generated in apoA-$I^{-/-}$ mice by injection of HDL_2 labeled with the nondegradable, intracellularly trapped ^{125}I-DLT as described above. To study tissue uptake and plasma clearance of HDLs, an aliquot (~100 μl) of apoA-$I^{-/-}$ mouse plasma containing ~15 μg of SR-BI-modified HDL remnants (~4×10^5 cpm) was injected via the jugular vein into C57BL/6 mice. An equivalent amount of unmodified HDL_2, mixed with ~100 μl of apoA-$I^{-/-}$ plasma obtained 3 days after administration of an adenovirus overexpressing SR-BI, was injected for comparison. Blood samples were collected at selected intervals after injection of labeled ligands and radioassayed to determine ^{125}I content. After 24 h, the animals were humanely killed, perfused with 50 ml of saline, and liver, kidney, heart, spleen, and sections of the small and large intestine were collected for radioassay.

RESULTS

To study HDL processing by SR-BI, we used an established model in which SR-BI was overexpressed in apoA-

$I^{-/-}$ mice by means of an adenoviral vector (2, 3). After 3 days, a period corresponding to maximal expression of SR-BI, HDL cholesterol was almost completely depleted (2), thus minimizing the remodeling of exogenously administered HDL by endogenous lipoproteins. A bolus of 750 μg of HDL_2 traced with ^{125}I was injected into the mice, and plasma samples were collected for analysis at selected intervals after bolus injection. The apoprotein composition of HDL processed by SR-BI was determined by direct SDS-PAGE analysis of plasma samples collected at the indicated times after bolus HDL_2 injection (**Fig. 1**). Visualization was by autoradiography. This analysis indicated a preferential loss from HDL_2 of apoA-II compared with apoA-I in plasma of mice overexpressing SR-BI (Fig. 1).

The size of HDL particles after SR-BI processing was determined by direct analysis of plasma samples by nondenaturing gel electrophoresis and autoradiography. As reported previously (2, 3), in this model system exogenously administered HDL_2 became progressively smaller, with the formation of HDL remnants occurring in incremental steps (**Fig. 2A**). As reported previously, no lipid-free apoA-I or lipid-poor particles were observed (2, 3). HDL remnant formation was the result of SR-BI overexpression, because this process did not occur in mice treated with an equal amount of adenovirus that does not express protein (2). Short-term SR-BI processing (~30 min) initially reduced the size of exogenous HDL_2 (average diameter, 9.2 nm) to a remnant particle (R1) with a diameter of ~8.8 nm. Intermediate processing (1–2 h) resulted in the formation of two distinct remnant particles, R2 and R3, both smaller than the injected HDL, with diameters of ~8.0 and ~7.7 nm, respectively. Prolonged/extensive processing (≥3 h) led to the formation of a single population of R3 remnant particles (~7.7 nm diameter). We have previously reported that although these small, dense R3 particles cannot be refloated by density gradient centrifugation, they do contain lipid and are larger than free apoA-I, as determined by Sudan Black staining and size exclusion chromatography (2). These HDL remnant particles seem to be the final stage of SR-BI processing, but they can already be detected during short-term (0.5 h) processing by SR-BI (Fig. 2A). We reported previously that two-dimensional analysis of HDL_2 processed for up to 3 h by SR-BI did not indicate the generation of any preβ HDL (3). Even with very extensive SR-BI processing of HDL_2 (~9 h) when the plasma contains only a small fraction of the injected ^{125}I, we found no evidence for the formation of preβ HDL.

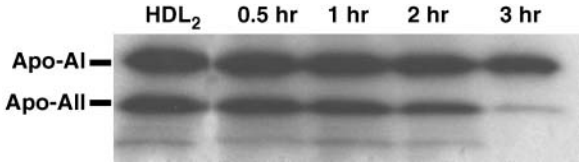

Fig. 1. SDS-PAGE analysis of ^{125}I-HDL_2 after scavenger receptor class B type I (SR-BI) processing. Apolipoprotein A-I-deficient ($apoA\text{-}I^{-/-}$) mice were injected with 1.5×10^{11} particles of adenoviral vector AdSR-BI. After 3 days, the mice were injected with a bolus of 750 μg of HDL_2 traced with ^{125}I. Plasma samples from individual mice were collected at the indicated times after bolus injection, and 5 μl was analyzed by SDS-PAGE (5–20% acrylamide gradient). An aliquot of the injected ^{125}I-HDL_2 is shown for comparison. Samples were visualized by autoradiography.

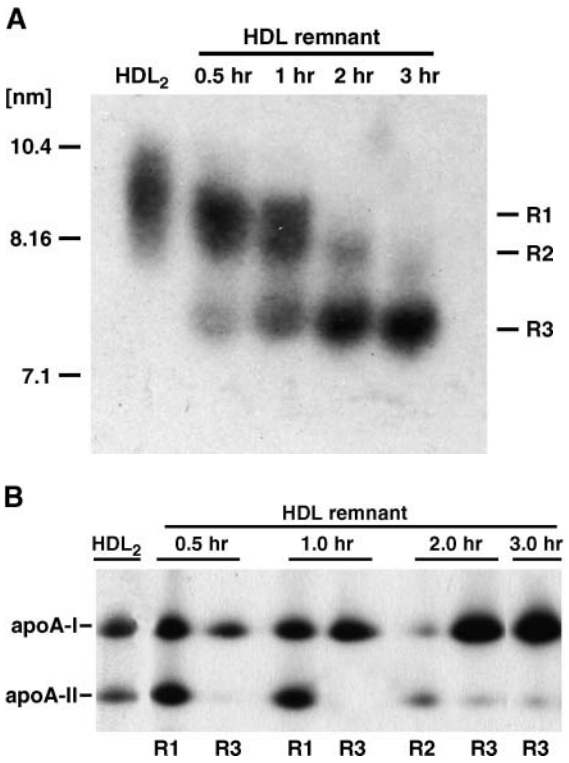

Fig. 2. A: Nondenaturing gel analysis of ^{125}I-HDL_2 after SR-BI processing. Plasma samples (7 μl), collected from a SR-BI-overexpressing mouse at the indicated times after injection of a bolus of ^{125}I-HDL_2, were separated on a 4–18% acrylamide nondenaturing gel. ^{125}I-HDL_2 injected into the mouse is shown for comparison. Samples were visualized by autoradiography. The mobilities of standards of known diameter are indicated. R1, R2, and R3 are remnant HDL particles of varying sizes. B: SDS-PAGE analysis of HDL remnant particles. HDL remnant particles R1, R2, and R3, generated by 0.5–3 h of SR-BI processing of injected ^{125}I-HDL_2, were excised from the nondenaturing gel depicted in A and subjected to SDS-PAGE (5–20% acrylamide gradient) and autoradiography to determine their apoprotein composition. ^{125}I-HDL_2 excised from the same nondenaturing gel is shown for comparison.

To further study the preferential loss of apoA-II compared with apoA-I during SR-BI processing (Fig. 1), the apoprotein composition of discretely sized HDL remnants was assessed. Bands corresponding to R1, R2, and R3 from the nondenaturing gel depicted in Fig. 2A were excised. Analysis of the bands by SDS-PAGE indicated that the injected ^{125}I-HDL_2 contained ^{125}I-apoA-I and ^{125}I-apoA-II at levels of ~60% and ~40%, respectively (Fig. 2B). Compared with HDL_2, the 0.5 h HDL remnant (R1) was relatively enriched in ^{125}I-apoA-II (Fig. 2B). In contrast, the very small amount of R3 generated by SR-BI processing at this time contained only ^{125}I-apoA-I (Fig. 2B). The same results were obtained from SDS-PAGE analysis of the 1.0 h remnant particles (Fig. 2B). The R2 particle generated after 2 h of SR-BI processing was enriched in ^{125}I-apoA-II compared with HDL_2, whereas the R3 particle consisted

of mainly ^{125}I-apoA-I (Fig. 2B). By 3 h of processing by SR-BI, R3 was the predominant remnant particle in plasma, with little evidence of R2 particles. These α-migrating R3 particles (3) contained practically only ^{125}I-apoA-I (Fig. 2B). These data indicate that as HDL_2 is processed by SR-BI, the particle becomes incrementally smaller, but early on a small, dense lipid-containing particle (R3) is formed that contains mainly apoA-I. This apoA-I-containing particle appears to accumulate with more extensive SR-BI processing, becoming the only remnant particle detected in the plasma 3 h after ^{125}I-HDL_2 injection.

We previously reported that HDL remnants, when injected into C57BL/6 mice, rapidly (within 3 min) increased in size to HDL-like particles (2). This conversion to larger particles also occurred ex vivo when HDL remnants were incubated with mouse plasma. We subsequently showed that this conversion to larger particles is attributable to the association of HDL remnants with lipoprotein particles, preferentially HDL, in a process that does not require CETP, LCAT, or phospholipid transfer protein (3). Thus, it was of interest to determine whether HDL remnants containing different amounts of apoA-I and apoA-II varied in their ability to associate with HDL. For these studies, plasma samples collected at selected intervals after bolus injection (and thus containing HDL remnants processed to different extents) were incubated at 37°C with C57BL/6 mouse HDL, analyzed by nondenaturing gel electrophoresis, and visualized by autoradiography (**Fig. 3A**). HDL remnants obtained after minimal (0.5 h) SR-BI processing readily associated with C57BL/6 mouse HDL, as indicated by an increase in particle size for the majority of this ^{125}I-HDL remnant sample (band T). The association of remnant particles with mouse HDL was verified by Western blotting of nondenaturing gels, which indicated that the radioactive band migrated with the bulk of mouse apoA-I (data not shown). In contrast, intermediate SR-BI processing (1 h) generated a subpopulation of HDL remnants that appeared to be completely resistant to HDL association, remaining unchanged in size when incubated with C57BL/6 mouse HDL (band B), as well as HDL remnants that associated with HDL particles to form either large (band T) or intermediate (band M) HDLs. Longer SR-BI processing (2 and 3 h) resulted in the generation of remnants that were mostly resistant to association with C57BL/6 mouse HDL (Fig. 3A). It is also possible that the small remnant did associate with HDL, but this association was sufficiently weak so that under the conditions of dilution and electrophoresis used, dissociation of remnant and HDL occurred.

We speculated that differences in the capacity of remnants to associate with mouse HDL could be correlated with variations in apoprotein composition. To investigate this possibility, bands corresponding to T, M, and B were excised from the nondenaturing gel (Fig. 3A) and subjected to SDS-PAGE (Fig. 3B). Surprisingly, the HDL remnant that was resistant to HDL association (band B) contained only apoA-I. The larger remodeled remnant (T) was enriched in apoA-II relative to apoA-I compared with the injected HDL_2 or the smaller remodeled remnant

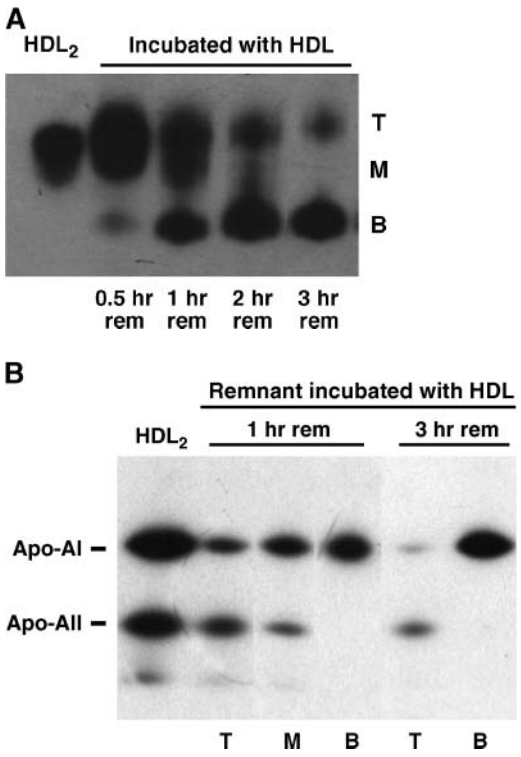

Fig. 3. A: Nondenaturing gel analysis of remodeled HDL remnants. Plasma samples containing remnants were collected from a SR-BI-overexpressing mouse at 0.5–3 h after bolus ^{125}I-HDL_2 injection. HDL remnants were mixed 1:10 (v/v) with C57BL/6 mouse HDL (1.7 mg/ml) for 2 h at 37°C to allow for remnant association with HDL. The resultant products were subjected to nondenaturing gel electrophoresis using a 4–18% acrylamide gradient and visualized by autoradiography. Human HDL_2 is shown for comparison. T, M, and B are remodeled and remodeling-resistant particles of varying sizes. B: SDS-PAGE analysis of remodeled HDL remnant particles. ^{125}I-HDL remnant particles (1 and 3 h) that remodeled by association with C57BL/6 mouse HDL (T and M) as well as remodeling-resistant remnant particles (B) were excised from the nondenaturing gel depicted in A and subjected to SDS-PAGE (5–20% acrylamide gradient) followed by autoradiography. HDL_2 excised from the same nondenaturing gel is shown for comparison.

(M). These results indicate that as HDL_2 is processed by SR-BI, progressively more remnant particles are generated that are resistant to remodeling through their association with HDL. These remodeling-resistant remnants contain mainly apoA-I, whereas remnants capable of remodeling by association with HDL contain both apoA-I and apoA-II.

We next investigated the in vivo metabolism of extensively processed HDL remnants that appear to be resistant to remodeling ex vivo. For this purpose, HDL remnants were generated by injecting mice overexpressing SR-BI with a bolus of HDL_2 radiolabeled with the nondegradable protein tracer ^{125}I-DLT. HDL_2 labeled with ^{125}I-DLT is processed in mice overexpressing SR-BI to remnant particles similar in size and apoprotein composition as seen for ^{125}I-HDL_2 (data not shown). Extensively processed remnants (R3) consisting mostly of apoA-I-only particles were obtained from these mice and then injected into normal C57BL/6 mice. An equal amount of unmodified ^{125}I-DLT-labeled HDL_2 was injected into additional C57BL/6 mice for com-

parison. Blood samples collected at selected intervals after injection of labeled ligands indicated no significant difference in the plasma clearance of ^{125}I-DLT-labeled HDL_2 and ^{125}I-DLT remnants (**Fig. 4A**). After 24 h, 11.2% of HDL_2 and 13.2% of the remnant particles remained in the plasma. Twenty-four hours after tracer injection, livers, kidneys, hearts, spleens, and intestine sections were collected from the mice and the uptake of ^{125}I-labeled DLT was determined. As expected, the liver and kidney were major sites of uptake of the ^{125}I-DLT-labeled HDL tracers (23), accounting for the uptake of 17% and 21% of the injected ^{125}I-DLT-labeled HDL_2 and ^{125}I-DLT remnant HDL, respectively (data not shown). However, there was a striking difference in the extent to which the liver and kidney took up HDL_2 and the extensively processed remnant (R3). In mice injected with ^{125}I-DLT-labeled HDL_2, significantly more of the tracer accumulated in the kidney on a per milligram tissue basis compared with the liver (Fig. 4B). In contrast, more ^{125}I-DLT accumulated in the livers of mice injected with the extensively processed remnant R3. Together, our findings show that SR-BI processing of HDL_2 can give rise to a small remnant particle that contains apoA-I only and is especially susceptible to uptake by the liver.

DISCUSSION

We have shown in an in vivo model system that SR-BI has the capacity to generate, in a progressive manner, small and dense HDL remnant particles (2, 3). Such particles are converted to larger particles by reassociating with existing HDL particles in an enzyme-independent manner (2, 3). This conversion can be viewed as a "salvage" pathway because in the absence of HDL, these remnants are directed to catabolism (2). This remodeling pathway may thus represent a significant determinant of the rate of apoA-I catabolism. Here, we focused on the role of apoA-II in the generation and remodeling of HDL remnants. Data presented in this article support the following contentions. *i*) SR-BI processing of HDL_2 results in the more rapid clearance of apoA-II compared with apoA-I. *ii*) SR-BI processing results in the segregation of apoA-I and apoA-II on HDL remnants, with apoA-II present on the larger remnants and the smallest remnant containing only apoA-I. *iii*) The larger apoA-II-containing HDL remnants that result from SR-BI processing rapidly reassociate with HDL, whereas the small apoA-I-only remnant is resistant to association with HDL. *iv*) Compared with HDL_2, small apoA-I-only remnants accumulate more in the liver than in the kidney, reflecting either a greater association of remnants with the liver or a more rapid conversion of remnants to a form that is quickly removed by the kidney and thus not taken up by this organ.

Data from these studies and previously published reports (2, 3) show that with SR-BI processing, HDL gets progressively smaller, with three discrete remnants emerging (R1, R2, and R3) (Fig. 2A). It is notable that the small apoA-I-only-containing remnant (R3) is rapidly generated, although in small amounts, and can already be detected by 30 min of SR-BI processing. At the same time, it is clear that SR-BI processing results in the more rapid clearance of apoA-II than apoA-I (Fig. 1). There is a remarkable segregation of apoA-I and apoA-II with respect to the remnants formed. ApoA-II is present only in the larger remnants (R1 and R2), in which it appears to be enriched compared with HDL_2 (Fig. 2B). The smallest remnant (R3) is apoA-II free and contains only apoA-I. R3 may in fact be the last particle remaining in a series of SR-BI-mediated modifications of HDL, because more extensive processing did not lead to the generation of smaller entities.

Human HDL_2 is polydisperse and generally consists of two subpopulations, one that contains apoA-I without apoA-II (LpA-I) and another that contains both apoA-I and apoA-II (LpA-I/A-II) (24). The question arises whether

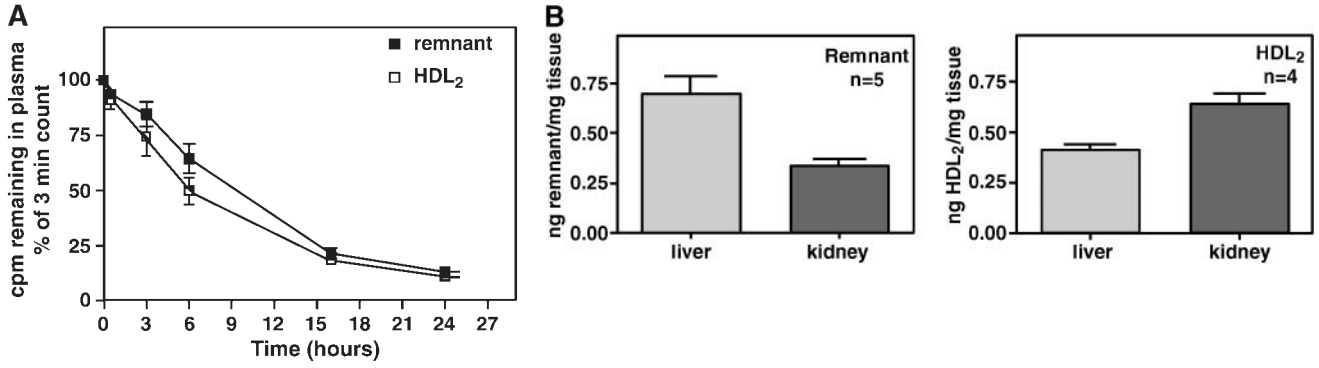

Fig. 4. A: Plasma clearance of ^{125}I-dilactitol tyramine (DLT)-labeled HDL_2 and remnant particles. Plasma samples were collected from C57BL/6 mice and assayed for radioactivity at the indicated times after injection of HDL remnants traced with the nondegradable, intracellularly trapped radiolabel ^{125}I-DLT or an equivalent amount of ^{125}I-DLT-labeled HDL_2. Each data point represents the mean ± SEM. For ^{125}I-DLT-labeled HDL_2, n = 4; for ^{125}I-DLT-labeled remnant particles, n = 5. Similar results were obtained in additional experiments. B: Liver and kidney uptake of unmodified HDL_2 and SR-BI-modified HDL_2 remnants. HDL remnants traced with the nondegradable, intracellularly trapped radiolabel ^{125}I-DLT were generated in apoA-I$^{-/-}$ mice as described in Experimental Procedures. Liver and kidney samples were collected for analysis 24 h after injection into C57BL/6 mice of equivalent amounts of ^{125}I-DLT-labeled HDL_2 or ^{125}I-DLT-labeled HDL remnants as described in Experimental Procedures. Values represent means ± SEM of the indicated number of animals. Similar results were obtained in a second experiment.

the small HDL remnants containing apoA-I only are derived solely from LpA-I or are a product of both LpA-I and LpA-I/A-II. Because apoA-I in LpA-I is catabolized faster than apoA-I in LpA-I/A-II (8, 25), it seems that the apoA-I-only remnant would be derived from both. This is supported by our finding that injection of normal mouse HDL into SR-BI-overexpressing apoA-I$^{-/-}$ mice gives rise to an apoA-I-only remnant particle and selective depletion of apoA-II, similar to what occurs with human HDL_2 (unpublished data). Given that mouse HDL is monodisperse and consists only of particles that contain both apoA-I and apoA-II (reviewed in 26), this supports the concept that the small apoA-I-only remnant (R3) is derived from both human LpA-I and LpA-I/A-II.

ApoA-II kinetics are complex, as is evident from the plasma disappearance curves of exogenous labeled tracer. It is likely that apoA-II catabolism occurs with different rates from different pools (27). An analogy is that apoA-I associated with apoA-II is catabolized more slowly than apoA-I not associated with apoA-II (25). The plasma clearance data in our model presented in Fig. 1 are thus likely integrals of various catabolic processes.

Our data are consistent with a model in which SR-BI processing results in the shedding of a small, dense HDL remnant that contains apoA-I only. ApoA-II is resistant to shedding, because of its higher affinity for lipid, and thus remains associated with a larger remnant. These apoA-II-containing remnants sustain a structural organization that allows reassociation with existing HDL. The small apoA-I-only remnant is destined to catabolism through interaction with a putative hepatic receptor.

Notwithstanding the fact that most of the HDL remnants would likely be salvaged by reassociation with HDL, a percentage is evidently directed toward catabolism (1, 2). The role of SR-BI in apoA-I catabolism is important because plasma levels of this apolipoprotein (and HDL) are to a significant extent determined by catabolism. The process whereby the selective uptake of lipid by SR-BI destines apoA-I to catabolism has not been well studied. The significance of this process is evident from the fact that alterations in HDL structure that influence the rate of selective uptake, such as modifications by phospholipases, have a major effect on apoA-I and plasma HDL concentrations (28). Additionally, overexpression of SR-BI by transgene or adenoviral vector can practically deplete plasma of apoA-I and HDL (4, 5, 29). Conversely, when selective lipid uptake is reduced, as in the ob/ob mouse or the SR-BI-deficient mouse, plasma levels of HDL are greatly increased (30, 31).

In exploring the relationship between the selective uptake and subsequent catabolism of the apolipoprotein component of HDL, it is important to evaluate the respective roles of the liver and kidney. When selective uptake from HDL is enhanced either by modification of HDL structure (secreted phospholipases) or by enhanced SR-BI expression, the uptake of apolipoprotein in the liver and kidney is increased, as detected by a nondegradable radiolabeled protein tracer. The bulk of the radiolabel, however, accumulates in the liver, with less in the kidney (28). A notable difference between kidney and liver is that nondegradable radiolabeled lipid, [^{3}H]CE, accumulates much more in the liver than in the kidney as a result of selective uptake (28). In the kidney, on the other hand, significantly more protein accumulates than lipid (28). Together, these data likely indicate that a very lipid-depleted protein entity results from SR-BI processing that is cleared in the kidney, whereas an alternative process exists whereby the majority of apoprotein is taken up into the liver. In this context, our data that SR-BI processing results in a small apoA-I-only-containing remnant (R3) with a predilection to hepatic clearance is important. It is remarkable that this α-migrating particle does not reassociate with normal HDL. This clearly distinguishes it from preβ HDL or free apoA-I.

It has been hypothesized that HDL contains two pools of apoA-I, one loosely associated exchangeable pool and a much more tightly bound pool that provides structural integrity (32). It is conceivable that our small remnant particle contains only the latter type of apoA-I. Current evidence suggests that amphipathic α helices of apoA-I are the recognition motifs for receptors and lipids (33, 34). With respect to SR-BI, marked differences have been observed in the affinity of rHDL particles of different sizes for this receptor, even when these rHDLs contain the same number of apoA-I molecules (35). Similarly, SR-BI exhibited a higher affinity for larger HDL_2 compared with the smaller HDL_3 (35, 36). The importance of apoA-I conformation affecting the display of the amphipathic α helices is further evident in the fact that preβ1 HDL exhibited a low affinity for SR-BI, whereas lipid-free apoA-I showed a high affinity for binding (8, 36). Cross-linking studies defined clear differences between SR-BI binding of apoA-I on HDL and lipid-free apoA-I (34). The conformation of apoA-I on the small α-migrating remnant (R3) that we describe is remarkable in that it not only fails to interact efficiently with SR-BI and ABCA1 (2) but it also is resistant to association with HDL. This rather unique conformation makes it possible that it acts as a ligand for another as yet undefined receptor that most likely resides in the liver.

Our small, dense remnant particle (R3) seems to be similar to the small LpA-I-only HDL particles isolated by anti-human apoA-I immunoaffinity chromatography (37). These particles also had α mobility and showed a significantly higher degradation by the liver compared with the kidney (37). In contrast, preβ-migrating HDL and free apoA-I were preferentially degraded by the kidney (37). We envisage SR-BI processing under physiological conditions to generate a small remnant destined for hepatic catabolism through an as yet unknown receptor. Whether this is merely the end of a spectrum of selective uptake activity of SR-BI from HDL or certain SR-BI molecules are in a configuration that allows them to be more active on HDL is unknown.

We have failed to show that even extensive SR-BI processing results in the generation of preβ-migrating HDL or free apoA-I (3). Our data are compatible with studies in nonhuman primates and mice that provided no evidence for the generation of preβ HDL during the catabolism of small HDLs containing LpA-I only (37, 38).

It is generally accepted that any disassociated lipid-poor apoA-I would not remain in this form for long. It would be relipidated by ABCA1, incorporated into preexisting HDL, or filtered by the glomerulus. Our data identify a fourth possibility: namely, that this lipid-poor particle (R3), with apoA-I likely in a unique conformation, could be a ligand for a hepatic receptor that results in apoA-I uptake and degradation.

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