High density lipoprotein (HDL) mediates reverse transport of cholesterol from atheroma foam cells to the liver, but the mechanisms of hepatic uptake and trafficking of HDL particles are poorly understood. In contrast to its accepted role as a cell surface receptor, scavenger receptor class B type 1 (SR-BI) is shown to be an endocytic receptor that mediates HDL particle uptake and recycling, but not degradation, in both transfected Chinese hamster ovary cells and hepatocytes. Confocal microscopy of polarized primary hepatocytes shows that HDL particles enter both the endocytic recycling compartment and the apical canalicular region paralleling the movement of SR-BI. In polarized epithelial cells (Madin-Darby canine kidney) expressing SR-BI, HDL protein and cholesterol undergo selective sorting with recycling of HDL protein from the basolateral membrane and secretion of HDL-derived cholesterol through the apical membrane. Thus, HDL particles, internalized via SR-BI, undergo a novel process of selective transcytosis, leading to polarized cholesterol transport. A distinct process not mediated by SR-BI is involved in uptake and degradation of apoE-free HDL in hepatocytes.

High density lipoprotein (HDL) mediates the transport of cholesterol from peripheral tissues to the liver for excretion in bile in a process called reverse cholesterol transport (1–3). However, the mechanisms mediating the uptake of HDL by hepatocytes and other cells are poorly understood. Although a number of HDL binding proteins have been identified, none so far has been shown to mediate the uptake or degradation of HDL protein (4). Cholesterol enters the hepatocyte from the basolateral membrane in either LDL, remnants of triglyceride-rich lipoproteins, or HDL. HDL, and not LDL, appears to be the major donor of free cholesterol for bile acid production (5–7). HDL cholesterol must somehow traverse the hepatocyte from the basolateral membrane (sinusoidal membrane) to reach the apical membrane (bile canalicular membrane) followed by secretion into bile.

Scavenger receptor class B type 1 (SR-BI) is an authentic HDL receptor that is highly expressed in the liver and steroidogenic tissues and mediates the selective uptake of HDL cholesterol, i.e. the uptake of HDL free cholesterol and cholesteryl ester without the concomitant uptake or degradation of HDL protein (8). Based on studies in non-polarized cells, it is widely believed that SR-BI is a non-endocytic receptor that functions at the plasma membrane, perhaps forming a hydrophobic channel to facilitate entry of cholesteryl ester into the membrane (9–11). This view implies that there is a separate process mediating holo-HDL particle uptake. Intriguingly, SR-BI has been localized to the bile canaliculus (the apical membrane) in mice and rats (12, 13). Because hepatocytes primarily use transcytosis to localize membrane proteins to the apical surface (14–16), it is tempting to speculate that SR-BI is endocytosed from the basolateral membrane and traffics to the apical membrane. SR-BI appears to mediate rapid transport of HDL free cholesterol and cholesteryl ester into bile (17), suggesting that SR-BI plays an important role in HDL cholesterol transport across the cell.

Recently, we have shown that HDL particles are internalized by non-polarized hepatocytes and traffic to the endocytic recycling compartment (ERC, a transferrin-positive compartment) and that the majority of HDL particles that undergo endocytosis are resecreted in an intact form (18), in a process previously termed retroendocytosis (19–21). In addition, we found that selective HDL lipid uptake occurs during HDL recycling (18). The goals of the present study were to elucidate the route of HDL protein and cholesterol trafficking in polarized hepatocytes and to evaluate the role of SR-BI in HDL particle uptake and trafficking.

EXPERIMENTAL PROCEDURES

Hepatocyte Isolation—Hepatocytes were isolated from 8-week-old female C57BL/6J mice (purchased from The Jackson Laboratory, Bar Harbor, ME) as previously described (18). Hepatocyte couplets were isolated by following the above protocol except that the time of digestion of the liver was reduced from 18 to 12 min. The identity of the bile canalicus vacuole was verified by staining with filipin as previously described (18).

Lipoprotein Preparations—Human apoE-free HDL and LDL was isolated by buoyant density ultracentrifugation and radiolabeled with protein and lipid tracers as previously described (18). SR-BI-neutralizing Antibody Production—Preimmune and immune serum from rabbits was obtained by immunizing rabbits with a recombinant extracellular domain of mouse SR-BI (Cardiovascular Targets, New York, NY). IgG fraction was purified from preimmune and immune serum by protein A chromatography. A titration experiment was per-
formed to determine the optimal antibody concentration to inhibit SR-BI activity using CHO cells expressing SR-BI showed that a 1:1000 dilution of immune serum was sufficient to completely reduce HDL association to levels measured in CHO control cells. This concentration equaled ~10–20 μg/ml protein A-purified IgG. Preimmune serum was without effect at 1:1000.

**Pulse-chase Assays**—All cells types (freshly isolated hepatocytes, CHO, and MDCK cells) were treated as previously described (18). Trichloroacetic acid-soluble and -precipitable counts were determined as a measurement of degradation and secretion, respectively. Pulse-chase assays were also performed in the presence of protein A-purified neutralizing anti-SR-BI antibody or protein A-purified preimmune IgG (from the same rabbit used to produce the immune serum) by preincubating of cells at 4 °C for 1 h with 20 μg/ml antibodies followed by the pulse-chase assay as previously described (18). The values reported for the three components in all pulse-chase studies (i.e. association, secretion, and degradation) are considered specific, because they were calculated by subtracting nonspecific background levels as measured in the presence of 100-fold excess unlabeled HDL from total. Selective uptake was measured by subtracting nonspecific background levels as measured in the presence of 100-fold excess unlabeled HDL from total. Selective uptake was measured as previously described (18). For pulse-chase assays using MDCK cells, 150 μg of HDL/ml was used as the lipid acceptor in the apical chamber of Transwells (Costar).

**Adenovirus Infection**—Primary hepatocytes and CHO cells were infected with either an adenovirus harboring the DynK44A gene or a control adenovirus expressing green fluorescence protein (kind gifts of J. E. Pessin) according to a previous study (22). Cells were then incubated with either 125I-labeled HDL or LDL having specific activities of ~500 and 2300 cpm/ng, respectively.

**Immunolocalization of SR-BI**—Hepatocytes were incubated for 1 h with Alexa 568-labeled human holotransferrin (Sigma Chemical Co.). Cells were then washed with phosphate-buffered saline and fixed in 3.7% paraformaldehyde. Immunodetection was then performed using an anti-SR-BI antibody recognizing the carbohydrate terminus of SR-BI (Novus Biologicals). Alexa 488-labeled goat anti-rabbit IgG (Molecular Probes) was used as the secondary antibody.

**Fluorescence Confocal Microscopy**—10 mg of doubly labeled HDL (DiI and Alexa 488) was incubated for 1 h with primary hepatocyte couplets at 37 °C, and examined by confocal microscopy as previously described (18). A separate sample of HDL was double-labeled with the fluorescence cholesterol ester analog BODIPY-CE (Molecular Probes) as previously described (18). The protein component of BODIPY-CE-labeled HDL was then labeled with Alexa 568. This double-labeled HDL was incubated with primary hepatocyte couplets as described above and examined using a confocal microscopy.

**MDCK Cell Manipulations**—Stable cells expressing murine SR-BI in pCDNA3.1 (Invitrogen, Carlsbad, CA) or empty vector as control were made using Transfectamine (Life Technologies, Inc.). A pool of more than 300 individual clones were used for experiments. Polarization was carried out by growing cells for 5 days in 12-mm Transwell cell culture inserts (Corning). MDCK cell monolayer integrity was verified by determining the basal-to-apical diffusion of [3H]inulin (1 Ci/ml). Cells used for all experiments had <0.1% diffusion.

**Endocytosis Assays**—Measurements of endocytosis of SR-BI in all cells types (freshly isolated hepatocytes, CHO) using a reducible biotin cross-linker was performed according to the original method by Bretscher et al. (23) with modifications by McGwire et al. (24).

**Immunoprecipitation**—All cell types used (freshly isolated hepatocytes and CHO) were first lysed in radioimmune precipitation buffer. SR-BI was immunoprecipitated overnight using the anti-SR-BI antibody specific for the carboxyl terminus of SR-BI.

**RESULTS**

**Anti-SR-BI Antibody Inhibits HDL Particle Uptake in CHO Cells**—We previously demonstrated that HDL particles are actively taken up by primary hepatocytes and rapidly resecreted or degraded (18). The resecreted HDL was selectively depleted of free cholesterol and cholesteryl ester. To explore the possibility that SR-BI mediates HDL particle uptake and recycling, we used an antibody to the extracellular domain of SR-BI that neutralizes SR-BI selective uptake activity in vitro. Western blot analysis using the neutralizing SR-BI antibody in both primary hepatocytes and SR-BI-transfected CHO cells (25) yields a single band at the expected molecular mass of SR-BI (~82 kDa) (Fig. 1D). Lower M, bands seen in hepatocytes were also detected in pre-immune sera (Fig. 1D, PI), indicating specific recognition of SR-BI by immune sera (IM). Small amounts of this antibody were shown to markedly inhibit selective uptake of HDL cholesteryl ester (CE) by SR-BI-transfected CHO cells (Fig. 1A). Although not previously appreciated (26), we also found extensive uptake and recycling of HDL in CHO cells stably expressing SR-BI (Fig. 1A); the resecreted HDL was largely depleted in CE tracer (by ~75%, relative to protein), as found previously in hepatocytes (17). The anti-SR-BI antibody reduced HDL particle uptake, resecretion, and degradation in SR-BI-transfected CHO cells to the level found in vector control-transfected CHO cells (Fig. 1A). In a further characterization of the neutralizing antibody, CHO cells expressing SR-BI were incubated with HDL labeled with both diI as a lipid marker and Alexa-488 as a protein marker and incubated with cells pretreated with either preimmune antibody or the SR-BI-neutralizing antibody. Fig. 2A shows that the neutralizing SR-BI antibody (immune) blocked the uptake of both HDL lipid and protein fluorescent markers, thus confirming our findings with radiolabeled HDL. To further substantiate this finding in CHO cells and to test if HDL traffics to the ERC as in hepatocytes.
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SR-BI Mediates HDL Uptake and Recycling in Hepatocytes—but Is Not the Principal Receptor Mediating HDL Protein Degradation—The SR-BI-neutralizing antibody substantially reduced (63%, p < 0.001) the resecretion of HDL protein (Fig. 2B). Thus, SR-BI plays a major role in selective uptake and resecretion of HDL taken up by hepatocytes. However, in contrast to the CHO cells, the antibody was found to only partially inhibit (18%, p < 0.05) HDL protein uptake by hepatocytes and not to significantly affect HDL protein degradation (Fig. 1B). Thus, a distinct process is responsible for the major part of the uptake of the apoE-free HDL, in a pathway that leads to protein degradation. To further characterize this pathway, and to evaluate a possible role of other lipoprotein uptake pathways, such as the LDL receptor-related protein, proteoglycans, or hepatic lipase, cells were treated with heparinase or RAP. However, these treatments did not inhibit HDL protein uptake (Fig. 1C), indicating that neither proteoglycan nor LDL receptor-related protein is involved in HDL particle uptake. Treatments with heparin, as well as heparitinase and chondroitinase treatments were also without effect on HDL protein uptake (data not shown).

HDL Protein and Lipid Traffic to the ERC and Sub-apical Compartments—Because hepatocytes in vivo are polarized, we used fluorescence confocal microscopy to study HDL uptake in isolated primary hepatocyte couplets. Primary hepatocyte couplets have an intact bile canaliculus membrane cordonned off by tight junctions (27). In vivo, the canaliculus space shared by two hepatocytes is free-flowing to the bile. However, after couplet isolation the bile canaliculus is sealed and is thus described as a bile canaliculus vacuole (27). HDL was fluorescently labeled both on its protein component and with either a fluorescence non-degradable cholesteryl ester analog (BODIPY-CE) or a fluorescence marker for phospholipid (DiI). These double-labeled HDL particles were incubated with primary hepatocyte couplets for 1 h at 37 °C, then examined by confocal microscopy. Based on the current model of selective uptake, HDL-derived lipid should separate from HDL protein at the plasma membrane (9–11). On the contrary, Fig. 3, A and C, shows that both HDL protein and BODIPY-CE colocalized at the plasma membrane, in juxtanuclear regions previously identified as the ERC (18), and in a region near the bile canaliculus membrane, suggesting that HDL particles (protein and lipid) traverse the hepatocyte from the basolateral membrane to the apical canaliculus region via the ERC. To further substantiate this idea, we also examined the fate of HDL-derived phospholipid, a major component of HDL, using the fluorescence phospholipid analog, DiI. A similar localization pattern was found for HDL labeled with DiI (Fig. 3, B and C). Again upon closer examination at increased magnification (Fig. 3B, lower panels), the HDL protein and DiI were concentrated in sub-apical compartments beneath the canaliculus vacuole. Together the data indicate that intact HDL particles are moving from the basolateral plasma membrane to the bile canaliculus region.

SR-BI Is Localized to the ERC and Bile Canaliculus Membrane—The data in Fig. 1 suggest that SR-BI may mediate the trafficking of holo-HDL particles through the endosome system. If this is the case, then SR-BI should be localized to the ERC as previously shown for HDL (18). Freshly isolated hepatocyte couplets were pulsed with fluorescently labeled transferrin, fixed, permeabilized, and used to immunolocalize SR-BI. Fig. 4A shows that SR-BI is found in three locations: the basolateral plasma membrane, juxtanuclear compartments, and the bile canaliculus membrane. SR-BI protein is co-localized with transferrin in juxtanuclear compartments (Fig. 4A) indicating that SR-BI is found in the ERC, the same endosomal compartment that HDL enters (18). Transferrin is found only at low levels near the canaliculus membrane (28, 29). The localization of SR-BI is thus consistent with its proposed role in...
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**FIG. 3. Localization of HDL protein and lipid tracers in primary hepatocyte couplets.** A, colocalization of both HDL protein (Alexa-apoprotein) and the cholesteryl ester analog, BODIPY-CE, carried by HDL. Both protein and BODIPY-CE colocalize to the ERC and bile canicular vacuole (denoted by the arrow) as seen in the merged image. N denotes the nuclei. Similar patterns were observed in two independent experiments. B, colocalization of both HDL protein (Alexa-apoprotein) and the phospholipid tracer, Dil. The merged image shows colocalization around the nuclei (N) and the bile canicular vacuole (cv). The bottom panels show a 3× magnification of the cv. C, additional images showing the localization of HDL protein (Alexa-apoprotein) and BODIPY-CE or Dil around the canicular vacuole (denoted by the arrow) in hepatocyte couplets.

trafficking HDL particles to the ERC and bile canicular region.

**Anti-SR-BI Antibody Traffics to the ERC and Bile Canicular Region**—To test whether the localization to the ERC and bile canicular membrane was the result of SR-BI endocytosis from the basolateral plasma membrane, the neutralizing SR-BI antibody was fluorescently labeled and used as a tracer in living non-permeabilized hepatocytes. Fig. 4B shows that the anti-SR-BI antibody is internalized and localized in a punctate fashion both to a juxtanuclear compartment and to the bile canicular membrane. At higher magnification, the antibody appears to be localized to sub-apical compartments, and to the canicular membrane (Fig. 4B, center panel). The fluorescently labeled preimmune IgG showed little uptake in hepatocyte couplets. Taken together, the data indicate that SR-BI undergoes endocytosis and traffics to sub-apical vesicles and the canicular region in polarized hepatocytes.

**SR-BI Undergoes Endocytosis in Primary Hepatocytes**—The results above suggest that SR-BI is an endocytic receptor, mediating cellular uptake of HDL particles. Because SR-BI is widely considered to be a non-endocytic receptor (9–11), we wanted to provide further direct evidence that SR-BI undergoes endocytosis. Primary hepatocytes were surface biotinylated with a reducible cross-linker, warmed to 37 °C for the times indicated in Fig. 5A, then cooled to inhibit further uptake of biotinylated cell surface proteins. Biotin groups that remained on the cell surface were subsequently removed by reducing the disulfide bond of the cross-linker. Therefore, any SR-BI that is internalized remains biotinylated. Fig. 5A shows a time-dependent increase in protected, internalized SR-BI, indicating that SR-BI does undergo rapid endocytosis from the cell surface (~3-fold accumulation after 15 min). Fig. 5B shows that SR-BI also rapidly undergoes endocytosis from the plasma membrane of CHO cells. Maximum internalization of SR-BI occurred at 30 min (62% of total biotinylated SR-BI). After 30 min, further endocytosis (~glutathione) and total biotinylated SR-BI levels (~glutathione) were seen to decrease. The latter indicates slow degradation of SR-BI in CHO cells (21% of total biotinylated SR-BI degraded after 60 min).

**Mechanism of HDL Endocytosis**—To determine whether the uptake of HDL protein occurs via clathrin pits, primary hepatocytes and CHO-SR-BI cells were infected with an adenovirus expressing the dominant negative dynamin-1 mutant DynK44A, which inhibits the formation of clathrin-coated vesicles (30). Control cells were infected with an adenovirus expressing green fluorescence protein (GFP). Cells were then incubated with protein-labeled LDL as a positive control for DynK44A activity. It was found that DynK44A was unable to inhibit HDL protein uptake but did inhibit LDL uptake (~40% in both hepatocytes and CHO-SR-BI cells (Table 1). This level of inhibition of LDL uptake is similar to what was reported for uptake of transferrin, another protein that is internalized through clathrin-coated pits (22, 30). Furthermore, we tested whether a dominant negative mutant of caveolin-1, CavKSF, could inhibit HDL protein uptake, because it has been shown to inhibit
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This study begins to elucidate the pathways for HDL protein and cholesterol trafficking in hepatocytes. ApoE-free HDL particles are taken up by two distinct processes. One process is mediated by SR-BI and leads to trafficking of HDL through the ERC and sub-apical region, resorption of a FC and CE-depleted HDL particle from the basal cell surface, and polarized secretion of HDL-derived cholesterol through the apical membrane. We term this process selective transcytosis of lipoprotein cholesterol. The other hepatocyte-specific uptake pathway leads to degradation of HDL protein and appears to involve a novel, hepatocyte-specific process not mediated by known lipoprotein receptors. SR-BI mediates the selective uptake of HDL cholesterol at the plasma membrane and does not mediate the uptake of holo-HDL particles (9–11). This hypothesis has been based primarily on studies using pharmacological inhibitors of clathrin-coated pit-mediated endocytosis, which do not inhibit HDL-selective uptake (35). Our experiments employed several different approaches, such as transfection of SR-BI cDNA, specific SR-BI-neutralizing antibodies, confocal microscopy, and cell surface biotinylation of SR-BI, to show that SR-BI is an endocytic receptor that mediates uptake and recycling of HDL particles in transfected CHO cells, MDCK cells, and hepatocytes. A dominant negative dynamin-1 mutant K44A did not inhibit HDL protein uptake or HDL-selective uptake, indicating that HDL enters the cell through a non-clathrin pathway, possibly explaining the previous results (9–11). In agreement with this finding, the amino acid sequence of SR-BI does not contain obvious signals for selective inclusion in clathrin-coated vesicles, such as NPXY, di-leucine-, or tyrosine-based motifs (33). In addition, HDL uptake may not be through caveolae, because it was not inhibited by a dominant negative caveolin-1 mutant, even though SR-BI has been reported to partially colocalize with caveolin-1 in CHO cells (36). Examples of clathrin- and caveolae-independent endocytosis have been reported (37–39), although a mechanism for these pathways has yet to be elucidated.

Our data show that resecreted HDL after uptake via SR-BI is cholesterol-depleted, indicating that selective uptake occurs during the process of recycling, either at the plasma membrane or at intracellular sites. The physiological significance of the SR-BI pathway for HDL uptake is indicated by the finding of elevated HDL cholesterol and increased atherosclerosis in mouse models of SR-BI deficiency (40, 41). Furthermore, HDL recycling and selective uptake are dramatically reduced in hepatocytes of ob/ob mice, in association with depletion of sterol in the ERC and elevated plasma HDL cholesterol levels. Together the studies suggest that SR-BI-mediated HDL recycling plays a role in delivering cholesterol to the ERC and regulates plasma HDL cholesterol levels (18). SR-BI protein and mRNA levels are not altered in ob/ob mice, suggesting that SR-BI is dysfunctional.

The endocytic recycling pathway for uptake of HDL protein and cholesterol by hepatocytes contrasts with the well-defined uptake of SV40 (31, 32). Overexpression of CavKSF did not inhibit HDL nor LDL protein uptake by CHO-SR-BI cells (data not shown). Together, these data indicate that HDL is endocytosed through a non-clathrin-coated pit pathway, not requiring caveolin-1.

**SR-BI Facilitates Transcytosis of HDL Cholesterol in Polarized MDCK Cells**—Hepatocytes represent a polarized epithelium but do not readily form tight junctions when grown on filters. Thus, we next examined transport of HDL cholesterol and protein in polarized MDCK cells, an established model of polarized epithelium (33). To determine the membrane distribution (i.e. apical versus basal) of SR-BI, MDCK cells expressing SR-BI (MDCK-SR-BI) were polarized on filters and either basolateral or apical membranes were selectively biotinylated, followed by immunoprecipitation of SR-BI. Fig. 6A shows that SR-BI is localized to both the basolateral and apical membranes with a ratio of ~4:1. Polared MDCK cells expressing SR-BI or vector control cells, which express extremely low levels of SR-BI (data not shown; SR-BI expression is ~5-fold above vector control cells) were pulse-chased with protein and lipid-labeled HDL in the basolateral compartment and the amount of lipid and protein tracer that enters the cells and is secreted into the apical compartment was measured. The apical compartment contained unlabeled HDL, which acts as an acceptor for cellular cholesterol (34). Fig. 6B shows that MDCK cells expressing SR-BI have greatly enhanced uptake of HDL protein (4-fold), increased resecretion of intact HDL protein from basolateral membranes (6-fold), but very little apical secretion of HDL protein. In contrast, there was substantial apical secretion of HDL FC and CE (Fig. 6C). The HDL that is resecreted from the basal membrane is depleted in CE and FC tracer (~22% less CE and FC compared with starting material). SR-BI expression results in increased selective uptake of HDL CE and FC, as expected (Fig. 6D). Our results show that “selective uptake” comprises both selective cell association of HDL CE and FC, as well as selective apical secretion of CE and FC compared with HDL protein. To determine if efflux of cholesterol from the apical membrane was SR-BI-dependent, MDCK cells were labeled with [3H]cholesterol tracer, and the percentage of tracer efflux to HDL was compared between SR-BI-expressing cells and control. We found that, after a 6-h chase period, SR-BI-expressing cells effluxed ~2-fold more tracer (15.0 ± 0.92 versus 7.0 ± 0.22%; p < 0.001). Therefore, efflux from the apical membrane was dependent on SR-BI.

**DISCUSSION**

This study begins to elucidate the pathways for HDL protein and cholesterol trafficking in hepatocytes. ApoE-free HDL particles are taken up by two distinct processes. One process is mediated by SR-BI and leads to trafficking of HDL through the ERC and sub-apical region, resorption of a FC and CE-depleted HDL particle from the basal cell surface, and polarized secretion of HDL-derived cholesterol through the apical membrane. This process is selective transcytosis of lipoprotein cholesterol. The other hepatocyte-specific uptake pathway leads to degradation of HDL protein and appears to involve a novel, hepatocyte-specific process not mediated by known lipoprotein receptors.

**TABLE I**

| Adenovirus | DynK44A | GFP | DynK44A | GFP |
|------------|--------|----|--------|----|
| ng HDL/mg cell protein | ng LDL/mg cell protein |
| Hepatocyte | 101.6 ± 10.5 | 90.0 ± 12.3 | 263.3 ± 21.9 | 42.0 ± 1.9 |
| SR-BI CHO | 263.7 ± 16.6 | 231.2 ± 23.6 | 167.8 ± 13.8 | 268.7 ± 11.9 |

*p < 0.001 for dynK44A versus GFP, respectively.

**Fig. 5.** Endocytosis of endogenous SR-BI in primary hepatocytes, A. Endocytosis of biotinylated SR-BI from the cell surface of primary hepatocytes. At the times indicated, cells were treated with glutathione to remove cell surface biotin, and internalized SR-BI (protected from glutathione treatment) was detected by immunoprecipitation and immunodetection against biotin using streptavidin-HRP. B, endocytosis of SR-BI in CHO cells having stable expression of SR-BI (CHO-SR-BI). The experiment was performed as in A. Cells that were not treated with glutathione (−) represent the total level of biotinylated SR-BI throughout the experiment. Experiments shown in A and B are representative of two independent experiments.

**Fig. 6.** Transcytosis of HDL cholesterol through hepatocytes. A, hepatocytes were polarized on filters and either basolateral or apical membranes were selectively biotinylated, followed by immunoprecipitation of SR-BI. The HDL that is rescued from the apical membrane is depleted in CE and FC, as expected (Fig. 6D). Our results show that “selective uptake” comprises both selective cell association of HDL CE and FC, as well as selective apical secretion of CE and FC compared with HDL protein. To determine if efflux of cholesterol from the apical membrane was SR-BI-dependent, MDCK cells were labeled with [3H]cholesterol tracer, and the percentage of tracer efflux to HDL was compared between SR-BI-expressing cells and control. We found that, after a 6-h chase period, SR-BI-expressing cells effluxed ~2-fold more tracer (15.0 ± 0.92 versus 7.0 ± 0.22%; p < 0.001). Therefore, efflux from the apical membrane was dependent on SR-BI.
process of LDL particle uptake, which terminates in the degradation of LDL cholesteryl ester and protein in lysosomes (42). In contrast, HDL uptake and trafficking is more analogous to the process of iron delivery via transferrin. Iron-saturated transferrin undergoes receptor-mediated endocytosis and enters the early endosome system where transferrin unloads its iron. Iron-depleted transferrin remains bound to its receptor and recycles back to the plasma membrane through the ERC (43). In a similar fashion, we found that HDL particles undergo SR-BI-mediated endocytosis into the ERC and sub-apical compartments followed by the recycling of HDL protein back to the basolateral plasma membrane. However, unlike the trafficking of transferrin and its receptor, the removal of HDL cholesterol involves a further sorting event leading to the polarized secretion of HDL cholesterol from the apical membrane.

The transcytosis of the well characterized polymeric IgA receptor (44) involves its movement through sorting endosomes and apical recycling compartments followed by vesicular transport to the apical membrane where secretory component is cleaved and released into bile. The transcytosis of SR-BI may be partly analogous to the polymeric IgA receptor. However, in contrast to IgA, the trafficking of HDL likely involves the selective sorting of cholesterol away from the apoprotein component. Our analysis of the movement of fluorescent lipid and HDL protein tracers in primary mouse hepatocytes showed that both HDL protein and cholesterol traffic together to the ERC and sub-apical compartments (Fig. 4, A and C). It is likely that SR-BI brings HDL particles into the ERC and sub-apical regions, where a sorting event occurs. This could involve release of a lipid-depleted HDL remnant from SR-BI, while cholesterol bound to SR-BI traffics in a vesicle to the apical membrane, where the HDL-derived cholesterol is taken up by biliary micelles. Cholesterol secretion from the apical surface of the hepatocyte may be facilitated by SR-BI as demonstrated in polarized MDCK cells. The level of HDL apoproteins in bile is quite low, despite substantial levels of HDL-derived free cholesterol (45). Our model of SR-BI-mediated trafficking and selective sorting of HDL protein from cholesterol in the ERC and sub-apical region suggests a mechanism to explain the selective exclusion of HDL apoprotein from the bile despite the enrichment with HDL cholesterol.

These studies provide the first clear-cut evidence for a HDL protein catabolic process in hepatocytes that is not mediated by SR-BI. The finding that acute inhibition of SR-BI function using a SR-BI antibody in vitro does not substantially inhibit HDL protein uptake and degradation is consistent with the fact that SR-BI-deficient mice have similar HDL apoprotein levels (46) and hepatic apoprotein catabolism (47) as wild-type mice. However, the previous in vivo findings did not exclude a role of SR-BI in mediating HDL protein degradation, because there could be compensation by other pathways, such as receptors that could clear apoE-rich HDL particles in SR-BI-deficient mice (48, 49); furthermore, SR-BI overexpression is associated with a marked increase in HDL protein degradation in liver and kidney (50). Thus, our results provide the first direct evidence for the existence of a distinct, novel pathway of apoE-free HDL particle uptake leading to protein degradation in hepatocytes. Because there is a marked defect in clearance of HDL apoA-I and apoA-II in ob/ob mice (51), this pathway may be down-regulated. Further studies on the mechanisms of SR-BI-mediated selective transcytosis and the molecular characterization and regulation of the HDL protein degradation pathway may be rewarding.

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