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Role of the hepatic ABCA1 transporter in modulating intrahepatic cholesterol and plasma HDL cholesterol concentrations

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Abstract The current model for reverse cholesterol transport proposes that HDL transports excess cholesterol derived primarily from peripheral cells to the liver for removal. However, recent studies in ABCA1 transgenic mice suggest that the liver itself may be a major source of HDL cholesterol (HDL-C). To directly investigate the hepatic contribution to plasma HDL-C levels, we generated an adenovirus (rABCA1-GFP-AdV) that targets expression of mouse ABCA1-GFP in vivo to the liver. Compared with mice injected with control AdV, infusion of rABCA1-GFP-AdV into C57Bl/6 mice resulted in increased expression of mouse ABCA1 mRNA and protein in the liver. ApoA-I-dependent cholesterol efflux was increased 2.6-fold in primary hepatocytes isolated 1 day after rABCA1-GFP-AdV infusion. Hepatic ABCA1 expression in C57Bl/6 mice (n = 15) raised baseline levels of TC, PL, FC, HDL-C, apoE, and apoA-I by 150–300% ($P < 0.05$ all). ABCA1 expression led to significant compensatory changes in expression of genes that increase hepatic cholesterol, including HMG-CoA reductase (3.5-fold), LDLr (2.1-fold), and LRP (5-fold) in the liver. These combined results demonstrate that ABCA1 plays a key role in hepatic cholesterol efflux, inducing pathways that modulate cholesterol homeostasis in the liver, and establish the liver as a major source of plasma HDL-C.—Basso, F., L. Freeman, C. L. Knapper, A. Remaley, J. Stonik, E. B. Neufeld, T. Tansey, M. J. A. Amar, J. Fruchart-Najib, N. Duverger, S. Santamarina-Fojo, and H. B. Brewer, Jr. **Role of the hepatic ABCA1 transporter in modulating intrahepatic cholesterol and plasma HDL cholesterol concentrations. *J. Lipid Res.* 2003.** 44: **296–302.**

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The role of HDL in the removal of excess cholesterol from peripheral cells was postulated nearly 30 years ago and designated reverse cholesterol transport (RCT) (1, 2). In the current working model of RCT, excess cholesterol is removed from peripheral cells and esterified by lecithin cholesterol acyltransferase. The cholesteryl ester is then transported back to the liver for removal from the body, either directly by HDL or following transfer by cholesterol ester transfer protein to the apoB-containing lipoproteins. This model proposes that cholesterol and lipids used for the formation and maturation of HDL are derived from nonhepatic, peripheral cells as well as from the metabolism and remodeling of the triglyceride-rich, apoB-containing lipoproteins. Previous reports indicate that the liver is an important source of apoA-I and thus contributes significantly to the plasma pool of nascent HDL (3, 4). However, although the liver is the most important modulator of cholesterol homeostasis in the body, it has not been implicated as a major in vivo source of cholesterol to lipidate HDL in the circulation.

A major advance in our understanding of the first step in reverse cholesterol transport was the identification of the ABCA1 transporter as the genetic defect in patients with Tangier disease (5–10). ABCA1 is the major transporter that facilitates the efflux of cholesterol and phospholipids to poorly lipidated apoA-I to form nascent or preβ HDL. In the absence of a functional ABCA1 transporter, patients with Tangier disease are unable to efflux cholesterol to apoA-I and accumulate cholesteryl esters in many tissues, including arterial macrophages. Attie et al. reported similar findings in the WHAM chicken, an animal model with ABCA1 transporter deficiency (11).

The importance of the ABCA1 transporter in RCT and cholesterol metabolism has been substantiated in studies with ABCA1-KO mice that are characterized by virtually undetectable plasma concentrations of HDL cholesterol

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(HDL-C) and significant accumulations of lipid-laden macrophages in various tissues (12–14). Characterization of ABCA1 transgenic mice has also provided important new insights into the role of ABCA1 in HDL metabolism and atherosclerosis (15). In studies performed in our laboratory, we overexpressed the ABCA1 transporter in the liver and macrophages of C57Bl/6 mice utilizing the apoE promoter and human ABCA1 cDNA (16, 17). On an atherogenic diet, ABCA1 transgenic mice had a marked increase in HDL, decreased LDL, and significantly reduced atherosclerosis when compared with control mice (17). However, the actual source of the increased plasma HDL-C, liver or macrophages, was not determined. Recently, bone marrow transplantation studies in ABCA1-KO mice (18, 19) have provided evidence that ABCA1-mediated cholesterol efflux by macrophages does not make a significant contribution to the plasma HDL-C level. These combined results suggest that the major source of the increase in plasma HDL-C observed in our transgenic mice that overexpress ABCA1 in both macrophages and liver may be the liver.

In the present report, we directly test the hypothesis that hepatic expression of the ABCA1 transporter results in increased plasma HDL-C in C57Bl/6 mice by facilitating hepatic cholesterol efflux. These results provide a major paradigm shift in the current concept of RCT in which we propose that the liver is a major source of plasma HDL-C and the level of expression of hepatic ABCA1 modulates intracellular cholesterol levels as well as plasma HDL-C concentrations.

MATERIALS AND METHODS

Animals

Male C57Bl/6 mice (Jackson Labs, Bar Harbor, ME) aged 2–4 months (20–30 g in weight) were maintained on a regular chow diet (NIH-31; Harlan Teklad, Madison WI). All experiments were performed according to a research protocol approved by the Animal Care and Use Committee of the NHLBI, NIH.

Adenovirus construct

Recombinant adenovirus was generated as previously described (20). Briefly, an ABCA1-GFP expression cassette containing mouse ABCA1-GFP under control of the TRE/minCMV promoter (Clontech, Palo Alto, CA) and the SV40 polyA signal was blunt-end ligated into vector pCA350 previously digested with *Swa*I/*Sal*I. The resulting shuttle plasmid, pJJABC1GFP, which contained the ABCA1-GFP expression cassette flanked by adenovirus sequences (5′ITR-psi and 3′pIX), was cotransfected with a truncated adenoviral backbone-containing plasmid, pOSE 17 (E4R), into JM83 for recombination. The resulting recombinant ABCA1 adenovirus, rABCA1-GFP-AdV, was linearized with *Pac*I and propagated in 293 cells, prepared, isolated, titered, and stored as previously described (20). The rTetOff recombinant adenovirus was created in a similar fashion by ligating the filled *Nco*I-*Hind*III fragment of pTet-OFF (Clontech) into the pCA350 plasmid, previously digested with *Nco*I and *Sal*I. The shuttle plasmid was then cotransfected with pOSE as above to generate the rTetOff-AdV. The Tet-OFF adenovirus system uses a pTet-OFF regulator plasmid (Clontech) that expresses a fusion protein (the Tetracycline-Controlled Activator) that activates transcription in the absence of tetracycline.

Adenovirus injection and blood sampling

A volume of 100–200 μl of the purified recombinant adenovirus (1–5 × 10^8 pfu) was injected via the saphenous vein of mice anesthetized with IP avertin (1.25%; 0.015–0.017 ml/ body weight; Sigma, St. Louis, MO) on day 0 of the study. For all blood sampling, mice were fasted for 4 h. Bleeding was performed from the retrorbital plexus, and the mouse plasma was kept on ice until it was centrifuged at 2,500 *g* for 20 min. Plasma was removed, aliquoted, immediately frozen in dry ice, and stored at −70°C.

RNA isolation and Northern blot analysis

Total RNA was isolated from the tissues of age- and sex-matched mice using TRIZOL (Invitrogen, Carlsbad, CA). RNA (20–30 μg) was subjected to Northern analysis using a ^{32}P-labeled full-length human 6.78 kb ABCA1 cDNA fragment (16), pEGFP-1 probe (Clontech), or nucleotides 639–1,388 of HMG-CoA reductase cDNA. The blots were rehybridized with a ^{32}P-labeled 693 bp cyclophilin cDNA probe (Ambion, Austin, TX). ABCA1 mRNA was quantified using a scanning densitometer (Molecular Dynamics, Sunnyvale, CA) and normalized to cyclophilin mRNA.

Cholesterol efflux from primary hepatocytes

Primary hepatocytes were isolated from mouse liver after perfusion with collagenase, as previously described (21). After catheterization of the common bile duct, the liver was perfused with 3 ml of Liver Perfusion Media (Invitrogen) followed by 10 ml of prewarmed (37°C) Liver Digest Medium (Invitrogen). The liver was resected, the gallbladder removed, and the manually dissected liver was placed in 25 ml of L-15 medium and mechanically agitated (rpm = 200) for 5 min at room temperature. After centrifugation, the cell pellet was resuspended in 30 ml Hepatocyte Wash Medium (Invitrogen), filtered through a sterile 100 μm nylon mesh, and plated at a density of 2 × 10^6 cells/ml in 24 well Biocoat Poly-D-Lysine plates (Becton Dickinson Labware, Bedford, MA) in 10% FBS DMEM/F-12 media. Unattached cells were removed 3 h after plating. The cells were then labeled with [^{3}H]cholesterol (10 μCi/ml) for 24 h, and cholesterol efflux was performed, as previously described (16) with α-MEM medium plus 1% BSA with or without HDL (50 μg/ml) or apoA-I (10 μg/ml) as the cholesterol acceptor.

Analyses of the plasma lipid profile

Plasma lipids were determined after a 4 h fast using enzymatic kits as previously described (16, 17). HDL-C was determined as the cholesterol remaining in the plasma after precipitation of apoB-containing lipoproteins with heparin sodium (Lyphomed, Deerfield, IL). Plasma levels of apoA-I and apoE were quantified by densitometric scanning following electrophoresis on 4–12% Bis-Tris acrylamide gels (Invitrogen) and transferred onto Invitrolon PDVF membranes (Invitrogen). The membranes were incubated with rabbit anti-mouse apoE and apoA-I polyclonal antibodies from Biodesign International (Saco, ME) followed by the secondary antibody (donkey anti-rabbit antibody) conjugated to horseradish peroxidase (Amersham, Piscataway, NJ). Proteins were detected after development with the Duolox chemiluminescence kit according to the manufacturer's instructions (Vectastain, Burlingame, CA) (17).

Lipoprotein analysis

Plasma lipoproteins from 50 μl of pooled mouse plasma (n = 8) were separated by gel filtration using two Superose 6 HR 10/30 columns (Pharmacia Biotech Inc., Piscataway, NJ) connected in series (16). Lipids in the recovered fractions were assayed as

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described above. The elution volumes of the plasma lipoproteins separated by FPLC were VLDL, 15.0–16.0 ml; IDL/LDL, 20.0–24.0 ml; and HDL, 30.0–31.0 ml. Native agarose gel electrophoresis of pooled mouse plasma (1–2 μl) was performed by using the Titan Gel Electrophoresis System (Helena Laboratories, Beaumont TX) under nondenaturing conditions according to the manufacturer's instructions. 2D gel electrophoresis was performed as described previously (16). ApoA-I-containing lipoproteins were detected by the use of rabbit antibodies against murine apoA-I (Biodesign, Saco, ME) followed by visualization with the Vectasin ABC kit (Vector Labs, CA).

Hepatic cholesterol content

For extraction of lipids, ~100 mg (wet weight) liver was initially homogenized in 1 ml H_2O. Seven microliter methanol and 14 ml chloroform were added, homogenized, and the mixture incubated at 37°C for 40 min. Following incubation, 4.4 ml H_2O was added and the solution was inverted three times and stored at 4°C overnight. The upper phase, as well as the interphase, was removed, and 0.83 vol of a 47:3:48 mixture of H_2O-chloroform-methanol was added to the remaining organic phase. The mixture was inverted three times and stored at 4°C (22). Aliquots of the organic phase were dried under nitrogen, followed by heating at 90°C for 10 min. The samples were dissolved in 50 μl isopropanol and assayed for total cholesterol using an enzymic procedure as previously described (23). A total of four livers were analyzed in each study group. Data were expressed as micrograms of cholesterol per grams of liver.

Western blot analysis

Mouse liver membrane proteins were purified as previously described (16), separated on Nu-PAGE 3–8% Tris Acetate gels (Novex, San Diego, CA) under reducing conditions, transferred onto an Immobilon membrane (Millipore, Bedford, MA), and probed with polyclonal-IgG-antibodies according to manufacturer's instructions against either the ABCA1 transporter (Novus Biologicals, Littleton, CO), LDLr (RDI, Flanders, NJ), SR-BI (Novus Biologicals), or LRP (a generous gift from Dr. Dudley Strickland).

Fluorescence microscopy

Living primary hepatocytes isolated from mouse liver were photographed 1 h after plating, on an Olympus IX-70 inverted fluorescence microscope using a 40× objective lens, the manufacturer's filter set for GFP fluorescence, and a SPOT RT camera (Diagnostic Instruments, Inc.). All images were acquired and processed under identical conditions.

RESULTS

Adenovirus expression of functional hepatic ABCA1 transporter

To determine the effects of hepatic ABCA1 expression, rABCA1-GFP-AdV as well as the control rTetOff AdV were infused into C57Bl/6 mice. Northern analysis (**Fig. 1A**) demonstrated that prior to infusion (Pre-Tx), endogenous mouse ABCA1 mRNA (>9.5 kb) (24) is transcribed in C57Bl/6 liver. Six hours after infusion of rABCA1-GFP-AdV, mouse ABCA1-GFP mRNA (7,319 kb) was increased 10-fold in the livers of C57Bl/6 mice compared with the mouse endogenous ABCA1 mRNA. Northern blot hybridization using a ^{32}P-labeled GFP probe failed to detect significant expression of the ABCA1-GFP RNA in heart, spleen, kidney, and intestine (data not shown). These findings are consistent with previous studies (20, 25, 26), which have established that systemic infusion of adenovirus vectors results in virtually specific transgene expression in the liver. Western blot analysis at 6 h and 24 h following rABCA1-GFP-AdV infusion revealed a 1.6-fold and 1.7-fold (in arbitrary units: 11.4 ± 1 and 12.4 ± 5, respectively, compared with 7.3 ± 0.1 at day 0; $P < 0.02$, all) increase in hepatic expression of ABCA1 protein (Fig. 1B). Fluorescence microscopic analysis of isolated hepatocytes at 6 h and 24 h following rABCA1-GFP adenovirus infusion demonstrated that the ABCA1-GFP protein was present on the cell membrane as well as in the late endocytic vesicles, consistent with our previously reported studies in CHO (27) and WIF-B cells (28) (Fig. 1C). Thus, the infusion of rABCA1-GFP-AdV successfully targeted hepatic expression of human ABCA1 mRNA and protein in C57Bl/6 mice.

To demonstrate that the ABCA1-GFP fusion protein expressed by the adenovirus is functional, primary hepatocytes isolated from C57Bl/6 mice 24 h after infusion with rABCA1-GFP-AdV were analyzed for cholesterol efflux to HDL and apoA-I (**Fig. 2**). ApoA-I-mediated cholesterol efflux was significantly increased 2.6-fold ($P <$ 0.001) in hepatocytes isolated from mice infused with rABCA1-GFP-AdV compared with mice infused with control rAdV. These studies established that the ABCA1-GFP fusion protein expressed by the rABCA1-GFP-AdV is functional.

Effect of selective hepatic ABCA1 expression on the plasma lipid and lipoprotein profile

The plasma lipid and HDL levels of C57Bl/6 mice before (day 0) and after days 2 and 4 of adenovirus infusion are illustrated in **Fig. 3A**. The lipid and lipoprotein changes following rABCA1-GFP-AdV infusion in C57Bl/6 mice ($n = 15$) reached a maximum on day 2 with a 2- to 3-fold increase in TC, PL, FC, CE, and HDL-C ($P <$ 0.05, all). Immunoblot analysis of plasma isolated from mice after infusion of rABCA1-GFP-AdV (Fig. 3A, inset) revealed no significant increase in apoA-I (83 ± 4 vs. 87.2 ± 4.2 mg/dl; $P > 0.05$) and a 1.5-fold increase in apoE (2 ± 0.02 vs. 3 ± 0.01 mg/dl; $P < 0.05$) in C57Bl/6 mice (day 4). Native agarose gel electrophoresis (Fig. 3B) of plasma from C57Bl/6 mice before (day 0) and after rABCA1-GFP-AdV infusion (days 2, 3, and 4) demonstrated a progressive increase in the α-HDL lipoproteins over the time course of the study (Fig. 3B, upper and lower panels).

Effect of selective hepatic ABCA1 expression on the plasma HDL profile

The plasma FPLC profiles of C57Bl/6 mice revealed that the content of cholesterol (Fig. 3C) as well as PL and FC (data not shown) in HDL_1 and HDL were significantly increased after rABCA1-GFP-AdV infusion (Post-Tx) when compared with Pre-Tx values. Analysis of the plasma lipoprotein before and following rABCA1-GFP-AdV infusion by 2D gel electrophoresis revealed a small increase in pre-β

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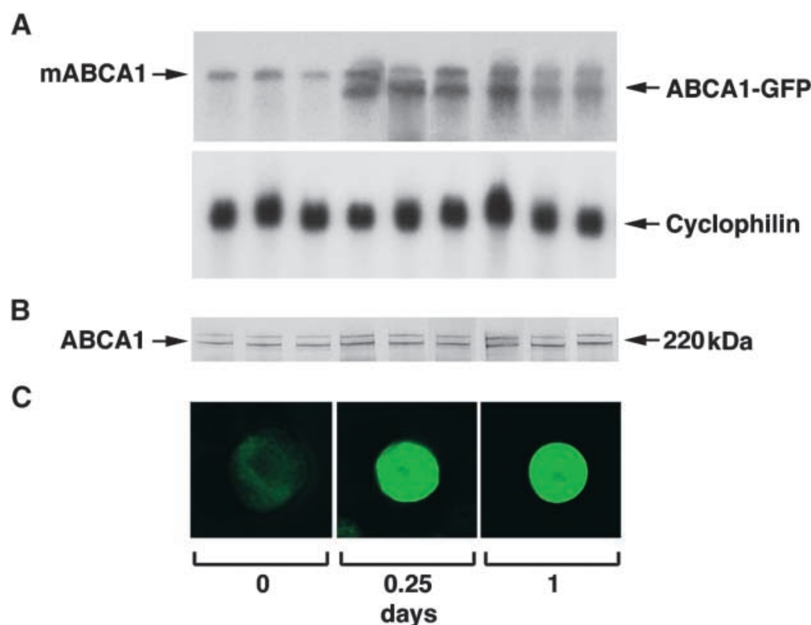

Fig. 1. Expression of hepatic ABCA1-GFP following rABCA1-GFP-AdV infusion into C57Bl/6 mice. A: Total RNA isolated from livers of three C57Bl/6 mice at various times (0 h, 6 h, and 24 h) after infusion with rABCA1-GFP-AdV was analyzed by Northern blotting using human ABCA1 cDNA (upper panel) or mouse cyclophilin (lower panel) as a probe. Left and right arrows (upper panel) indicate migration of endogenous mouse ABCA1 mRNA (over 9.5 kb) or mouse ABCA1-GFP (7319 bp), respectively. B: Western blot analysis of hepatic tissue from C57Bl/6 mice 6 h and 24 h following rABCA1-GFP-AdV infusion. C: Fluorescence microscopy of primary hepatocytes from control rTet-Adv and rABCA1-GFP-AdV mice isolated 6 h and 24 h following rABCA1-GFP-AdV infusion.

HDL and a significant increase in mature, α-migrating HDL particles (Fig. 3D). These combined data indicate that targeted expression of ABCA1 to the liver in C57Bl/6 mice significantly increases the plasma concentrations of HDL-C.

Effect of selective ABCA1 expression in liver on hepatic cholesterol content, receptor, and enzyme expression

Since selective expression of the ABCA1 transporter increases hepatic cholesterol efflux and plasma HDL-C levels, a decrease in cholesterol content in the liver would be anticipated following rABCA1-GFP-AdV infusion. To evaluate this possibility, hepatic cholesterol content was quantified after infusion of rABCA1-GFP-AdV into C57Bl/6 mice. In mice at day 0, hepatic cholesterol was 1.8 ± 0.1 mg/g and did not change significantly 6 h after infusion (2 ± 0.1 mg/g). However, at days 1 and 4 after infusion, significant increases of 1.4- and 1.6-fold (2.6 ± 0.4 mg/g; $P < 0.01$ and 3 ± 0.8 mg/g; $P < 0.02$, respectively) were observed compared with day 0. Thus, rather than decreasing, hepatic cholesterol remained stable or even increased during the time course of the study.

One possible explanation for the inability to detect a decrease in hepatic cholesterol content is that overexpression of the ABCA1 transporter initially decreases hepatic cholesterol levels and this depletion induces a compensatory response in the liver to return the cholesterol levels to normal levels. Northern and Western blot analysis of liver RNA and protein levels from C57BL/6 mice (**Fig. 4**) revealed increased HMG-CoA reductase mRNA (3.5-fold), LDLr (2.1-fold), and LRP (5-fold) protein levels as early as 6 h after infusion of ABCA1-GFP. SR-BI protein levels were not significantly changed. These findings indicate that ini-

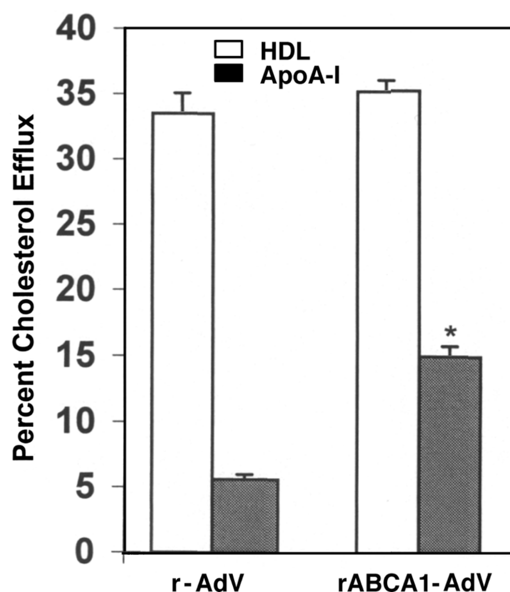

Fig. 2. HDL and apoA-I-mediated cholesterol efflux from primary hepatocytes isolated from control rTet-AdV (r-AdV) and rABCA1-GFP-AdV-infused mice 24 h after adenovirus injection ($P < 0.001$; rABCA1-GFP-AdV vs. rTet-AdV).

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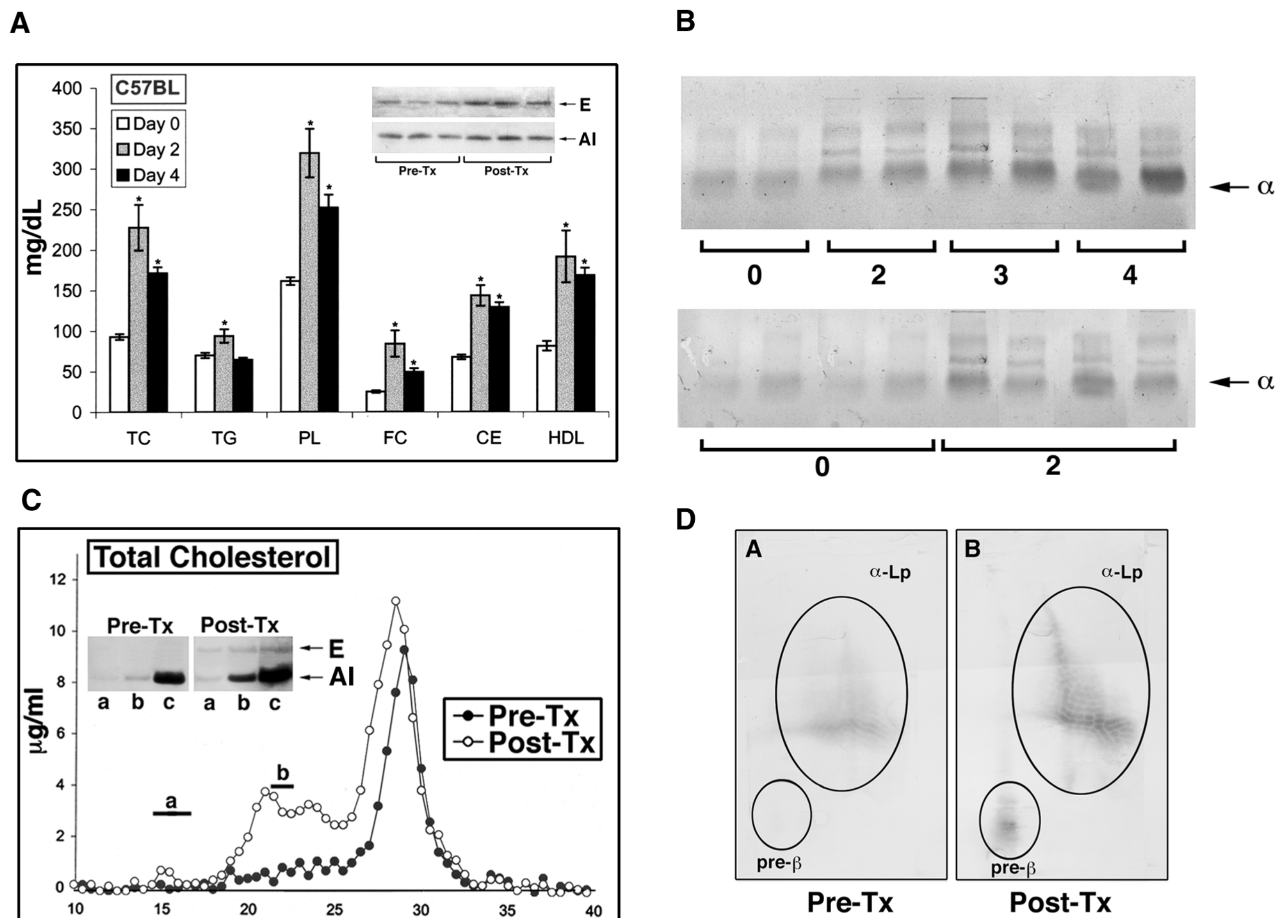

Fig. 3. Increased plasma HDL after rABCA1-GFP-AdV infusion. A: Plasma lipid levels in C57Bl/6 mice 0, 2, and 4 days after infusion with rABCA1-GFP-AdV. Inset: Western analysis of plasma (0.1 μl of plasma) to apoE (upper panel) or apoA-I (lower panel) before (Pre-Tx) or 4 days after (Post-Tx) infusion of rABCA1-GFP-AdV. B: Native agarose gel electrophoresis of plasma from two C57Bl/6 mice isolated 0, 2, 3, or 4 days after infusion with rABCA1-GFP-AdV (upper panel) and from four C57Bl/6 mice isolated 0 and 2 days after infusion with rABCA1-GFP-AdV (lower panel). Arrow (right) indicates migration of α-lipoproteins. C: FPLC elution profile of pooled plasma (n = 8) from C57Bl/6 mice before (Pre-Tx, closed circles) and 2 days after (Post-Tx, open circles) infusion with rABCA1-GFP-AdV. Bars (a, b, and c) designate the three pools of FPLC fractions used for Western analysis for apoE or apoA-I (inset). D: 2D gel electrophoresis of apoA-I-containing lipoproteins in plasma from C57Bl/6 before (A) and after (B) rABCA1-GFP-AdV infusion.

tial ABCA1-induced changes in hepatic cholesterol content are rapidly corrected by homeostatic mechanisms that include increases in HMG-CoA reductase, LDLr, and LRP levels. These results are consistent with an integrated compensatory response of the liver to maintain cholesterol homeostasis following ABCA1 overexpression and increased cholesterol efflux.

DISCUSSION

In the current classic concept of reverse cholesterol transport, peripheral cells and the metabolism of triglyceride-rich apoB-containing lipoproteins are considered the principal source of plasma HDL-C (1, 2). Cholesterol is then transported either directly by HDL or via the apoB-containing lipoproteins to the liver for removal from the body. Our initial studies with transgenic mice that selectively overexpress ABCA1 in the liver and macrophages demonstrated a significant increase in plasma HDL (16). Since recent bone marrow transplant studies with ABCA1-KO mice macrophages in control, ABCA1-KO, and LDLr-KO mice indicated that macrophage expression of ABCA1 does not significantly alter plasma levels of HDL-C (18, 19), hepatic ABCA1 expression appears to be the most probable source of the increased HDL-C observed in the hABCA1-Tg mice.

In the present study, we provide in vivo evidence to support the concept that the hepatic ABCA1 transporter modulates levels of hepatic cholesterol and plasma HDL-C. In vivo infusion of rABCA1-GFP-AdV resulted in selective hepatic expression of ABCA1 and a 2- to 3-fold increase in plasma HDL-C levels in C57Bl/6 mice. It is also of interest that despite marked increases in the plasma total and HDL-C levels mediated by AdV-mediated enhanced expression of ABCA1 in the liver, normal hepatic cholesterol content was maintained in the mouse model as early as 6 h after rABCA1-GFP-AdV infusion. At that

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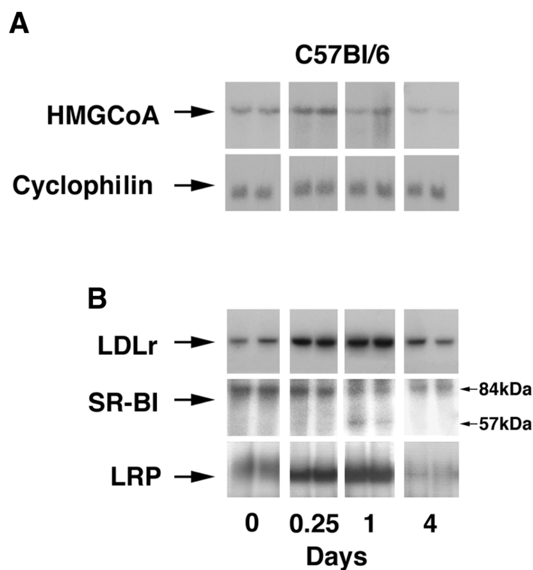

Fig. 4. Increased expression of genes involved in cholesterol homeostasis in liver after infusion with rABCA1-GFP-AdV. A: Total RNA isolated from livers of two C57Bl/6 mice 0, 0.25, 1, or 4 days after infusion with rABCA1-GFP-AdV was subjected to Northern analysis using probes for either HMG-CoA reductase (upper panel) or cyclophilin (lower panel) as a probe. B: Western blot analysis of liver membrane proteins from two C57Bl/6 mice 0, 0.25, 1, and 4 days after infusion with rABCA1-GFP-AdV. The individual blots were developed with antibodies to LDLr (upper panel), SR-BI (middle panel), or LRP (lower panel). Both gylcosylated (84 kDa) and nonglycosylated (57 kDa) SR-BI are present (middle panel).

time, significant upregulation of HMG-CoA reductase, SR-BI, LRP, and LDLr was already evident. Between days 1 and 3, increased expression of these genes maintained the intrahepatic cholesterol level and led to overcompensation with increased hepatic cholesterol content as the level of expression of the ABCA1 transporter decreased. These findings demonstrate significant intracellular regulatory compensation of hepatic cholesterol metabolism with hepatic ABCA1 overexpression, suggesting the cholesterol is effluxed from a regulatory pool in the liver.

Additional studies support the concept of the importance of the hepatic ABCA1 transporter in the synthesis of plasma HDL-C. The ABCA1 transporter has recently been localized to the basolateral surface of Wif-B cells, a polarized liver cell line, indicating that enhanced ABCA1 expression would result in increased efflux of cholesterol into the plasma rather than the bile, which would have occurred if the ABCA1 transporter were present on the apical surface (28). In addition, increased hepatocellular cholesterol has been observed in a patient with Tangier disease (29) and in the liver of the WHAM chicken (11), consistent with the role of the ABCA1 transporter in modulating intrahepatic cholesterol levels. This hypothesis is also consistent with the abundance of the ABCA1 mRNA in the liver and with hepatic in vitro cell culture studies (30, 31). These combined results establish that hepatic expression of ABCA1 increases plasma HDL-C levels and indicates that liver expression of the ABCA1 transporter is an important source of plasma HDL-C.

Our findings provide evidence to support two novel concepts in hepatic cholesterol metabolism and HDL-mediated reverse cholesterol transport. The first concept can be conceptualized as “reverse reverse cholesterol transport,” in which the liver is an important source of HDL-C and of HDL particles that can be remodeled by LCAT into mature HDL particles following cholesterol esterification, as well as the site of synthesis of nascent HDL, which can be transported to the periphery to function as an acceptor for ABCA1-mediated cholesterol efflux from nonhepatic peripheral cells. Thus, the liver may serve as a source of both cholesterol for plasma HDL acceptors as well as nascent HDL particles, which mediate cholesterol efflux from peripheral cells. The cholesterol carried in liver-derived mature HDL particles can be either transported to peripheral cells, including the ovary, adrenal, and testis, for cholesterol delivery to these tissues, or ultimately transported back to the liver for removal from the plasma via the SR-BI receptor. Thus, there is not a simple one-way movement of cholesterol from peripheral cells via reverse cholesterol transport back to the liver, as previously believed, but the liver may also function as a source of cholesterol for plasma apolipoprotein acceptors as well as nascent HDL-C particles.

The second concept suggested by the data presented in this manuscript is the role of the ABCA1 transporter in modulating the intracellular concentration of cholesterol in the hepatocyte. Thus, the liver, as well as the peripheral cells, modulates the intracellular level of cholesterol by the level of expression of the ABCA1 transporter. To date, the VLDL-apoB secretion has been proposed to be the major pathway for the secretion of cholesterol from the liver into the plasma. The present results indicate that the ABCA1 transporter pathway, in addition to the VLDL-apoB pathway, may play a pivotal role in modulating intrahepatic cholesterol levels and the transport of cholesterol into the plasma. The upregulation of the ABCA1 transporter would facilitate the efflux of excess hepatic cholesterol either to acceptor apolipoproteins secreted from the liver in conjunction with the increased expression of ABCA1 or to acceptors already present in plasma. It is interesting to note that in the liver, the VLDL-apoB secretion pathway is modulated by levels of triglyceride synthesis via SREPB1c (32, 33), whereas cholesterol/oxysterols regulate ABCA1 expression via LXR (34, 35). Thus, in the liver, excess levels of fatty acids and triglycerides may trigger export of triglyceride-rich, apoB-containing VLDL, whereas excess levels of cholesterol and its oxysterol derivatives would be expected to facilitate export of cholesterol via the ABCA1 transporter pathway. Additional studies will be required to more fully understand the coordinate regulation of cholesterol and triglyceride metabolism in the liver, and the factors which modulate the important role of the ABCA1 transporter in regulating hepatic cholesterol as well as plasma HDL levels.

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