Scavenger receptor class B type I affects cholesterol homeostasis by magnifying cholesterol flux between cells and HDL

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Abstract **Results from several laboratories clearly indicate that expression of scavenger receptor class B type I (SR-BI) enhances the bidirectional flux of cholesterol between cells and lipoproteins. Because the activity of HMG-CoA reductase, the key enzyme in cholesterol biosynthesis, is regulated by cell cholesterol content, we designed experiments to investigate the effect of SR-BI expression on the activity of this enzyme and on net cellular cholesterol mass. In addition, we compared the function of SR-BI with its human homolog, CD36 and LIMPII analogous 1. Our experiments demonstrate that both receptors enhance the flux of unesterified or free cholesterol bidirectionally, down a concentration gradient. Receptor-mediated cholesterol flux can effectively modulate multiple aspects of cellular cholesterol metabolism, including the pool that regulates the activity of HMG-CoA reductase. We also found that constitutive expression of SR-BI alters the steady state level of cellular cholesterol and phospholipid when SR-BI-expressing cells are maintained in medium containing serum lipoproteins. All of these effects are proportional to the level of receptor on the cell surface. These data indicate that the level of SR-BI expression determines both the rate of free cholesterol flux and the steady state level of cellular cholesterol.**—de la Llera-Moya, M., M. A. Connelly, D. Drazul, S. M. Klein, E. Favari, P. G. Yancey, D. L. Williams, and G. H. Rothblat. **Scavenger receptor class B type I affects cholesterol homeostasis by magnifying cholesterol flux between cells and HDL.** ***J. Lipid Res.*** **2001.** 42: **1969–1978.**

Supplementary key words CLA-1 • cholesterol flux • cholesterol efflux • high density lipoprotein • HMG-CoA reductase • SR-BI

The maintenance of optimum cellular cholesterol content is basic to proper cell physiology. Cells reciprocally regulate cholesterol biosynthesis and the exchange of cholesterol with the extracellular lipoproteins (1, 2). All cells can exchange cholesterol with extracellular acceptors and a variety of mechanisms have been proposed to explain this movement or flux of cellular cholesterol (3). Receptor-mediated cholesterol flux has been described in a number of different cell types (4, 5). More specifically, it has been repeatedly demonstrated that the expression of the scavenger receptor class B type I (SR-BI) can greatly enhance cholesterol movement between cells and phospholipid-containing acceptors such as lipoproteins (4, 6, 7). SR-BI interacts with both LDL and HDL to promote the bidirectional exchange of free or unesterified cholesterol (FC) between these lipoproteins and SR-BI-expressing cells (8, 9). In addition, SR-BI promotes the selective uptake of cholesteryl ester (CE) by interacting with HDL (10, 11). Although the mechanism whereby SR-BI promotes movement of cholesterol between cells and lipoproteins is not well understood, it is apparent that cholesterol flux mediated by SR-BI can effectively modulate cellular cholesterol content. Because the activity of HMG-CoA reductase (HMGR), a key enzyme in cholesterol biosynthesis, is tightly regulated by cell cholesterol content we designed experiments to investigate the effect of SR-BI expression on both cell cholesterol and the activity of this enzyme.

MATERIALS AND METHODS

Reagents

Human serum was obtained by the Lipoprotein Core Laboratory under an approved protocol and with approved consent from healthy, normolipemic volunteers. High density lipoprotein class 3 (HDL_3) and lipoprotein-deficient serum (LPDS)

Abbreviations: CE, cholesteryl ester; CLA-1, CD36 and LIMPII analogous 1; CS, calf serum; FC, free cholesterol; HMGR, HMG-CoA reductase; LPDS, lipoprotein-deficient serum; PL, phospholipid; SM, sphingomyelin; SR-BI, scavenger receptor class B type I.

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were isolated from human serum by sequential centrifugation in the Lipoprotein Core Laboratory (8). HDL_3 was modified as previously described by incubation with phospholipid micelles at the transition temperature (6); both native and modified HDL were characterized by measuring cholesterol and phospholipid content relative to total protein. Phospholipids were obtained from Avanti Polar Lipids (Alabaster, AL). Chemicals used in the HMGR assay were purchased from Sigma (St. Louis, MO) except for hydroxy-3-methylglutaryl-coenzyme A, DL-3-[glutaryl-3-^{12}C] and mevalonic acid, ammonium salt, (*R*)-[5-^{3}H], which were purchased from NEN Life Sciences Products (Boston, MA). [1,2-^{3}H]-cholesterol and ^{125}I-labeled iodine (^{125}I-iodine) were also purchased from NEN Life Sciences Products. Whatman LK6 silica gel 60A thin-layer chromatography plates were from Whatman (Clifton, NJ). All organic solvents were purchased from Fisher Scientific (Pittsburgh, PA). Sterols used as gas–liquid chromatography (GLC) standards such as cholestenone, cholesterol, and cholesterol methyl ether were from Sigma. Cholesterol oxidase from *Nocardia erythropolis* were purchased from Boehringer Mannheim (Indianapolis, IN). DMEM, Eagle's minimal essential medium (EMEM), PBS, and geneticin (G418) were obtained from Cellgro-Mediatech (Fisher Scientific). FBS, calf serum (CS), trypsin-EDTA, penicillin-streptomycin, and gentamicin were purchased from Sigma. Penicillin-streptomycin-glutamine antibiotic solution was purchased from GIBCO (Grand Island, NY). Tissue culture flasks and plates were obtained from Corning (Corning, NY). Other materials and reagents were obtained as noted below.

Routine cell maintenance and transfection

COS-7 cells were grown and transiently transfected as previously described (8). Briefly, 1.5×10^6 cells were seeded in 100-mm plates in growth medium (DMEM, 10% CS, 1 mM sodium pyruvate, antibiotics). Cells were transfected with a mixture of 10 μg of the desired plasmid diluted in 470 μl of serum-free medium and 30 μl of FuGENE 6 (Boehringer Mannheim, Indianapolis, IN) in a sterile polystyrene tube (Falcon 2058; Becton Dickinson Labware, Lincoln Park, NJ) after 18 h of incubation (37°C, humidified 95% air–5% CO_2). Two different plasmids were used to express SR-BI. Plasmid pSG5(SR-BI) has been described (8). Plasmid $pcDNA_3$(SR-BI) was used to express SR-BI in experiments comparing SR-BI with CD36 and LIMPII analogous 1 (CLA-1). This plasmid was constructed from pmSR-BI 77, a gift from M. Krieger (Massachusetts Institute of Technology, Cambridge, MA). The SR-BI-coding region was digested with *Hin*dIII and *Xba*I and inserted into a $pcDNA_3$ vector modified to contain a cytomegalovirus translational enhancer upstream of the multiple cloning site to boost protein expression. The plasmid used to express CLA-1, $pcDNA_3$(Cla-I), was a gift from O. Quehenberger (University of California, San Diego). All plasmids were prepared with endotoxin-free Qiagen (Valencia, CA) Maxi-prep kits and were sequenced throughout the coding region to confirm correct fragment insertion and that no point mutations had occurred. DNA sequencing was performed by the automated sequencing facility at the State University of New York (SUNY, Stony Brook, NY). Reactions were performed with a dye termination cycle sequencing kit and were analyzed on an Applied Biosystems model 373 DNA sequencer (PE Applied Biosystems, Foster City, CA). Transfections with empty vector were done to yield control cells. Experiments were typically carried out 48–72 h after transfection. SR-BI expression was confirmed by Western blot assays of whole cell lysates as described previously (12), using antibody directed against the C-terminal tail of SR-BI (a gift from S. Azhar, Veterans Affairs Palo Alto Health Care Systems, Palo Alto, CA).

Stably transfected SR-BI-expressing clones were obtained from COS-7 cells transfected as described above with $pcDNA_3$(SR-BI). Clones were maintained in growth medium supplemented with geneticin (400 μg/ml), which was used for selection. Stably transfected WI38-VA13 human fibroblastic cells were obtained after transfection with the same SR-BI-expressing plasmid, using geneticin selection (800 μg/ml). Cultures of WI38-VA13 human fibroblastic cells were maintained in DMEM supplemented with 10% FBS, antibiotics, and geneticin (800 μg/ml). SR-BI expression in stably transfected cell lines was confirmed by Western blots of whole cell lysates as described above. Cell surface SR-BI expression in COS-7 clones was measured using specific binding of HDL_3 (see below).

Assay of HMGR enzyme

Frozen cell pellets were thawed and resuspended in 75 μl of TEDK buffer (50 mM Tris-HCl, 1 mM EDTA, 10 mM DTT, 10 M KCl, pH 7.5). The cells were lysed by sonication (10 min at room temperature). Lysates were incubated for 10 min at 37°C and then centrifuged for 1 min at 12,000 rpm to obtain postnuclear supernatants (PNS). PNS protein was measured by modified Lowry assay (13). HMGR activity was assayed by a modification of the assay published by Harwood, Bridge, and Stacpoole (14). Typically, 0.1–0.2 mg of PNS protein was preincubated for 20 min at 37°C in a 75-μl reaction mixture containing 33 mM Tris (pH 7.5), 33.3 mM KCl, 3.3 mM DTT, and 68 mM EDTA (inhibits mevalonic acid kinase). After the preincubation, 30 mM glucose 6-phosphate, 3.4 mM $NADP^+$, and 0.2 U of glucose-6-phosphate dehydrogenase were added to regenerate NADPH. To start the reaction, [3-^{14}C]HMG-CoA (specific activity, ~15–30 cpm/pmol) was added, and the mixture was then incubated for an additional 90 min at 37°C. The reaction was stopped by adding 20 μl of 12 N HCl to inactivate the reductase, and convert the mevalonic acid to the lactone. The acidified mixture was placed on ice and [5-^{3}H]mevalonic acid was added as an internal standard (0.6–1.2 Ci/mmol, 15,000–20,000 cpm per reaction). The mixture was incubated for 30 min at 37°C to assure complete lactonization. Unlabeled mevalonic acid lactone (10 μl, 100 mg/ml) was added as a carrier, and TLC was used to separate the product [silica gel plates run in toluene–acetone 1:1 (v/v)]. The lactone band was visualized with iodine and scraped. Radioactivity was detected by double isotope liquid scintillation counting. The reaction product obtained was corrected for recovery and enzyme activity was expressed as units (picomoles of mevalonic acid per minute per milligram cell protein). A reaction blank was always measured and subtracted.

Assay of cell cholesterol content, cholesterol efflux, selective uptake, and the cholesterol oxidase-sensitive pool

Cell cholesterol content was measured in aliquots of 2-propanol extracts from monolayers, using GLC as previously described (15). Total cell phospholipids were measured by quantitating inorganic phosphorus in 2-propanol extracts as described previously (6). Cell lipids were normalized to total cell protein measured by modified Lowry assay (13). Cell cholesterol efflux to HDL_3 was measured as previously described (8). Briefly, cells plated in multiwell plates were prelabeled by incubation for 24–48 h in growth medium containing serum labeled with [1,2-^{3}H]-cholesterol. The radioactive cholesterol released to HDL_3 at a given time was obtained by counting an aliquot of medium by liquid scintillation. Total cell radioactivity was measured in 2-propanol extracts obtained from washed monolayers. Radioactive cholesterol released to the medium was expressed as a fraction of the total radioactivity in the well. Selective uptake was measured with doubly labeled HDL to establish CE uptake independent of internalization as previously described (16).

The cholesterol oxidase-sensitive pool was measured as previously described, using a method that assays cholesterol found in

caveolae (17). Briefly, cells were prelabeled with [1,2-^{3}H]cholesterol and exposed to cholesterol oxidase, without previous fixation, for 1–4 h at 37°C. Extracts were prepared from washed monolayers, using 2-propanol, and the radioactive cholestenone and cholesterol were separated by TLC and quantitated by liquid scintillation counting. The radioactive cholestenone produced was expressed as a percentage of the total radioactive cell cholesterol. Cholestenone production after oxidase treatment was always compared with that found in replicate cell cultures harvested at the start of the experiment (T_0 cells). Cholestenone formed in replicate cell cultures incubated at 37°C for up to 4 h without oxidase was no different than that found in T_0 cells. In some experiments the fraction of cholesterol oxidized to cholestenone was measured by GLC. Cholestenone standards were used to establish its retention time. Both cholesterol and cholestenone were quantitated in the same chromatogram and the ratio of the area under the cholesterol peak to that under the cholestenone peak was used to estimate the fraction of cell cholesterol oxidized. In preliminary experiments estimates of cholestenone production, using TLC, of labeled cell extracts were compared with estimates obtained by GLC in identical cell extracts and the two estimates were found to be similar.

Assay of HDL_3-specific binding

Stably transfected COS-7 clones were characterized for SR-BI expression by measuring the specific binding of HDL_3 labeled with ^{125}I-iodine. Binding was measured by incubating cells and radioactive HDL_3 (25 μg of HDL protein per ml) for 1.5 h at 4°C as described (18). Nonspecific binding was measured in duplicate incubations containing a 20× excess of nonradioactive ligand. Specific binding was calculated by subtraction of nonspecific binding from total binding. The mass of bound ligand was calculated from the specific activity of the radioactive HDL_3. The iodine monochloride method was used to label HDL_3 (19).

Statistical analysis

The figure legends specify whether the data represent means of multiple assays or of multiple experiments and whether the error bars represent the standard error or the standard deviation. Significant differences were established by unpaired *t*-tests. Rate constants were estimated by nonlinear regression after the model described by Johnson et al. (20). All statistical analyses were done using GraphPad (San Diego, CA) Prism.

RESULTS

Effect of SR-BI expression on the relationship between medium cholesterol levels and HMGR activity

Initial experiments were designed to validate our HMGR assay by demonstrating the well-documented dependence of HMGR activity on medium cholesterol content (1). Transiently transfected COS-7 cells were prepared as described in Materials and Methods and then incubated with medium containing either 5% CS, 2.5% CS, or LPDS (5 mg/ml). The control cells used in these experiments were transfected with empty vector and have no detectable endogenous SR-BI as measured by Western blots of whole cell lysates (data not shown). As expected, when COS-7 cells were incubated for 24 h with medium containing increasingly lower serum concentrations, a reciprocal increase in HMGR activity was obtained in both control and SR-BI-expressing cells (**Fig. 1**), signifying a drop in cell cholesterol content. The increase in enzyme activity was most pronounced when COS-7 cells were exposed to LPDS, and HMGR activity measured in these cells was significantly greater than the activity measured in cells exposed to 5% CS in both control and SR-BI cells.

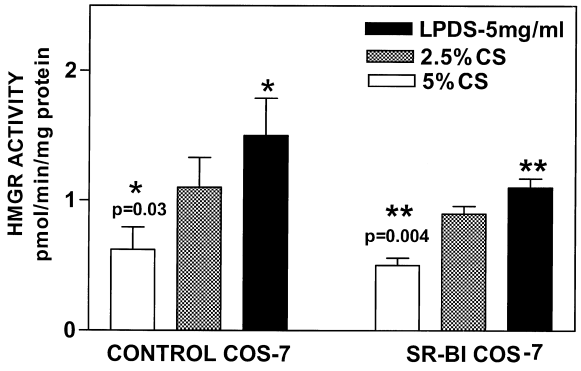

Fig. 1. Dependence of HMGR activity on cholesterol content of medium. COS-7 cells in 100-mm dishes were transfected with either empty vector (control) or vector containing the SR-BI gene (SR-BI) in DMEM supplemented with 10% CS as described in Materials and Methods. The transfected cells were incubated for 24 h with DMEM supplemented with either lower concentrations of calf serum (5% and 2.5%) or LPDS (5 mg/ml) before assay of HMGR activity as described in Materials and Methods. Results represent means and standard deviations of triplicate assays.

Effect of SR-BI expression on HMGR activity in cells incubated with isolated lipoproteins

The effect of incubation with HDL_3 on cell cholesterol content and HMGR activity was measured in transiently transfected COS-7 cells that had been maintained for 48 h in growth medium supplemented with 10% CS (T_0 cells). **Figure 2** shows that overnight incubation with HDL_3 (100 μg of protein per ml) had little effect on HMGR activity in control cells. On the other hand, the same treatment caused a significant increase in enzyme activity in cells expressing SR-BI (T_0 cells, 0.34 ± 0.10 units; vs. HDL_3-treated cells, 2.10 ± 0.5 units; $P = 0.002$).

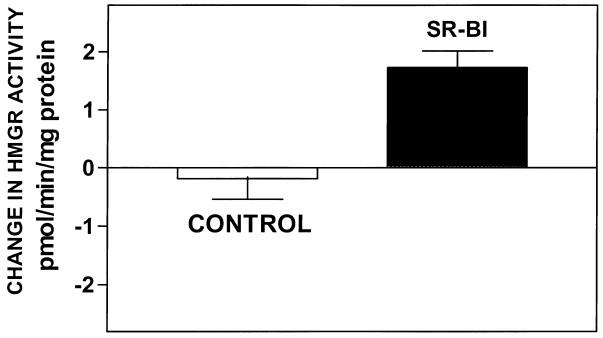

Fig. 2. Effect of incubation with HDL_3 on HMGR activity. Control and SR-BI-expressing COS-7 cells were transfected as described in Fig. 1. The transfected cells were maintained in medium supplemented with 10% CS for 48 h (T_0 cells). Some of the cell cultures were then incubated with isolated HDL_3 (100 μg/ml) for an additional 18 h. HMGR activity in T_0 and HDL_3-treated cells was assayed as described in Materials and Methods. Results represent means and standard deviations of triplicate assays.

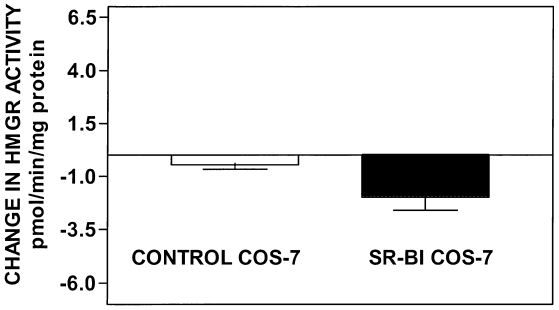

Fig. 3. Effect of incubation with HDL_3 on HMGR activity in LPDS-treated COS-7 cells. Control and SR-BI-expressing COS-7 cells were transfected as described in Fig. 1. The transfected cells were then treated and assayed as described in Fig. 2 except that the cells were incubated for 24 h with LPDS (5 mg/ml) before incubation with isolated HDL_3 (100 μg/ml) for an additional 18 h. Results represent the average of eight experiments and the data are plotted as the average difference in HMGR activity units between HDL_3-treated and T_0 cells. In every experiment HMGR activity measured in SR-BI-expressing cells after HDL_3 incubation was significantly different from that measured at T_0. Average values for HMGR activity at T_0 and the percent change in enzyme activity were as follows: Control cells, 1.7 ± 0.6, no significant change after HDL_3; SR-BI-expressing cells, 3.4 ± 2, 60% average decrease after HDL_3 incubation.

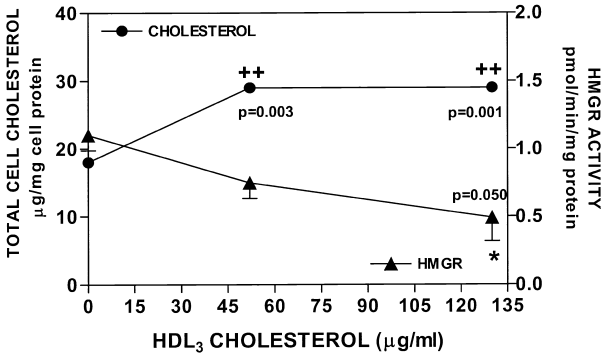

Fig. 4. Effect of incubation with increasing concentrations of HDL_3 on HMGR activity and cell cholesterol content in LPDS-treated SR-BI COS-7 cells. Control and SR-BI-expressing COS-7 cells were transfected as described in Fig. 1. All the cells were then exposed for 24 h to LPDS (5 mg/ml), which was considered the T_0 treatment. Some cultures were then incubated for an additional 18 h with increasing concentrations of HDL_3. HMGR activity and cell cholesterol content were measured both at T_0 and after HDL_3 treatment as described in Materials and Methods. The graph shows the average of triplicate assays of HMGR activity and cell cholesterol levels obtained with SR-BI-expressing cells in two experiments. Significant change ($P < 0.05$) in HMGR (*) and cell cholesterol ($^{++}$) obtained as compared with values measured in T_0 cells.

To test the effect of SR-BI expression on HMGR activity in cholesterol-depleted cells, either control or SR-BI COS-7 cells were exposed to LPDS (5 mg protein/ml) for 24 h to deplete cell cholesterol and increase HMGR activity (T_0 cells). Replicate cultures were then incubated for an additional 18 h with HDL_3. Enzyme activity was measured in both T_0 and HDL_3-treated cells; in some experiments cellular cholesterol content was measured in identically treated, replicate cell cultures. Because LPDS treatment was expected to cause a significant decrease in cell cholesterol, we first confirmed that, in LPDS-treated cells, SR-BI retained its ability to enhance cell cholesterol efflux to HDL_3 (data not shown). **Figure 3** summarizes results from four different experiments in which LPDS-treated control and SR-BI-expressing cells were incubated with HDL_3 (100 μg of protein per ml) for an additional 18 h. Compared with the activity measured at T_0 time, incubating control cells with HDL_3 did not significantly change HMGR activity. This result is in agreement with experiments published by Brown, Dana, and Goldstein (1) and Daerr et al. (21), who have shown that incubation of LPDS-treated skin fibroblasts with a broad range of HDL concentrations did not change HMGR activity. In contrast, when LPDS-treated COS-7 cells expressing SR-BI were incubated with HDL_3, a statistically significant decrease in HMGR activity was consistently obtained. Because changes in HMGR activity typically signal a change in cell cholesterol content, both cell cholesterol content and HMGR activity were measured in LPDS-treated COS-7 cells incubated with increasing concentrations of HDL_3 for 18 h. As shown in **Fig. 4**, SR-BI expression promoted net influx of cholesterol from HDL_3, resulting in significant increases in cell cholesterol content and a reciprocal decrease in HMGR activity. In these experiments identical treatments using control cells resulted in minimal changes in cell cholesterol content and no change in HMGR activity (data not shown, $P > 0.05$).

Direction of SR-BI-dependent net cholesterol flux between COS-7 cells and HDL depends on cellular cholesterol content

The results described above indicate that, although SR-BI enhances FC flux in or out of cells, the direction of this movement depends on the FC concentration gradient between the cell and HDL in the medium. To better characterize the effect of the cellular cholesterol status on the gradient for FC flux, experiments were done in which control and SR-BI-expressing cells having different initial cell cholesterol mass were incubated in parallel with the same HDL_3 preparation. In these experiments cell cholesterol content and HMGR activity were measured in transiently transfected COS-7 cells that had been either maintained in growth medium supplemented with 10% CS or exposed to LPDS for 24 h before incubation with HDL_3 for 18 h. The data in **Fig. 5** show that, compared with T_0 cells, when cholesterol-depleted, SR-BI-expressing cells were incubated with HDL_3 (100 μg of protein per ml) there was net cholesterol influx (Fig. 5B, hatched column), and a reciprocal decrease in HMGR activity (Fig. 5A, hatched column). On the other hand, incubation of cholesterol-rich, SR-BI-expressing cells with HDL_3 resulted in net efflux of cell cholesterol (Fig. 5B, solid column), and a reciprocal increase in HMGR activity (Fig. 5A, solid column). Overnight incubation with 100 μg of HDL_3 protein per ml did not change either cholesterol mass or HMGR activity in LPDS-treated control cells and caused minimal changes in these parameters in control cells that

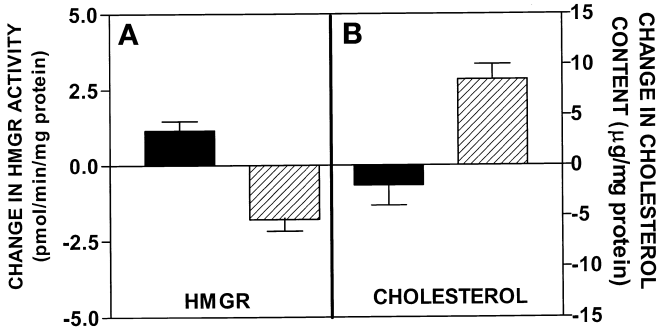

Fig. 5. Effect of initial cell cholesterol content on HDL_3-dependent changes in HMGR activity and cell cholesterol content in SR-BI-expressing COS-7 cells. Replicate cultures of control and SR-BI-expressing COS-7 cells, prepared as described in Fig. 1, were incubated with HDL_3 (100 μg/ml) for 18 h and both HMGR activity and cell cholesterol content were measured in T_0 cells and after HDL treatment as described in Materials and Methods. In these experiments one-half of the cultures were maintained in DMEM supplemented with 10% CS and the other half was incubated with LPDS (5 mg/ml) for 24 h before incubation with HDL_3. Results shown represent averages of triplicate assays from two experiments. Data, shown for SR-BI cells only, are expressed as the difference between HDL_3-treated and T_0 cells. Solid columns, cells maintained on 10% CS serum; hatched columns, cells treated with LPDS (5 mg/ml).

had been maintained in 10% CS (data not shown, $P > 0.05$). These results confirm our previous observations (Figs. 2–4). Because significant changes in cell cholesterol content could be measured only in cells expressing SR-BI, we can conclude that under these conditions SR-BI magnifies the effect of HDL_3 on cell cholesterol homeostasis. We can also conclude that, although SR-BI enhances both the bidirectional flux of FC and CE uptake, the gradient that determines net cholesterol movement depends not only on the concentration of extracellular lipoproteins that can interact with this receptor but also on factors that affect cellular cholesterol content.

CLA-1 behaves like SR-BI in transfected COS-7 cells

Our results demonstrate that SR-BI expression can profoundly affect cellular cholesterol homeostasis. Specifically, SR-BI expression markedly enhanced the exchange of cellular cholesterol with lipoproteins and changed the distribution of cellular cholesterol as evidenced by the increased sensitivity of intact cells to cholesterol oxidase (17). To test whether CLA-1, the human homolog of rodent SR-BI (22), has similar effects on cell cholesterol metabolism, we compared CLA-1 with SR-BI in transiently transfected COS-7 cells. The data in **Table 1** show that both receptors are equally efficient in promoting the selective uptake of HDL CE and can substantially increase the rate of cell cholesterol flux to HDL_3. Similar to SR-BI, CLA-1 changed the distribution of cellular cholesterol as evidenced by increased production of cholestenone when intact cells were exposed to cholesterol oxidase. In addition, both receptors caused a marked decrease in HMGR activity in LPDS-treated cells incubated overnight with 100 μg of HDL_3 protein per ml. These results demonstrate that CLA-1 has the same spectrum of effects on cholesterol metabolism as SR-BI.

Effect of HDL on HMGR activity in WI 38-VA 13 cells stably transfected with SR-BI depends on its ability to promote net cholesterol influx

We have previously shown that HDL phospholipid composition modulates the exchange of HDL cholesterol with SR-BI-expressing cells; more specifically, sphingomyelin (SM) enrichment inhibited influx of HDL FC without changing the K_d for HDL binding to SR-BI (6). To confirm that the decrease in HMGR activity observed in LPDS-treated, SR-BI-expressing COS-7 cells exposed to HDL_3 was due to SR-BI-dependent net FC influx, we incubated stably transfected WI38-VA13 fibroblasts with native HDL_3 and with the same HDL_3 after approximately 3× enrichment with SM. In this experiment the cells were treated with LPDS for 48 h (T_0 cells) and replicate cul-

TABLE 1. Comparison of effect of SR-BI and CLA-1 on cell cholesterol metabolism

Receptor Activity Assayed	Receptor Expressed		
	None	SR-BI	CLA-1
Selective uptake efficiency (ng CE/ng cell-associated HDL)	0	25 ± 0.5	20 ± 2
Rate of cell cholesterol efflux to HDL_3 (%/h)			
25 μg protein/ml	0.438 ± 0.034	3.36 ± 0.025	2.33 ± 0.028
250 μg protein/ml	1.38 ± 0.035	8.37 ± 0.041	7.12 ± 0.062
Rate of cholestenone formation (%/h)	2.67 ± 0.069	7.61 ± 0.039	5.99 ± 0.095
Change in HMGR activity: HDL treatment compared with LPDS-treated T_0 cells (pmol/min/mg cell protein)	−0.18 ± 0.11	−1.07 ± 0.72	−0.60 ± 0.10

COS-7 cells were transfected either with empty plasmid (Controls) or with plasmid containing receptor DNA (+SR-BI) and then used to measure the various activities as described in Materials and Methods. HMGR activity in LPDS-treated cells at T_0 was as follows: Control = 0.8 ± 0.2; +SR-BI = 3.4 ± 0.9; +CLA-1 = 1.2 ± 0.3. Data represent means ± SE of triplicate measurements. All values obtained with receptor-expressing cells are significantly different from control cells ($P < 0.0001$).

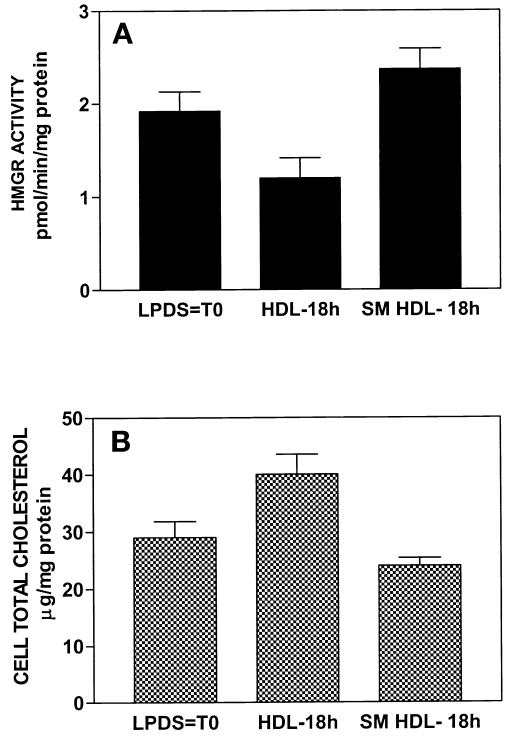

Fig. 6. Effect of incubation with native and sphingomyelin-enriched HDL_3 on HMGR activity and cell cholesterol content in LPDS-treated WI38-VA13 cells expressing SR-BI. Stably transfected control and SR-BI-expressing WI38-VA13 cells were grown in 100-mm culture plates, using DMEM supplemented with 10% FBS. When almost confluent, all the cells were incubated with LPDS (5 mg/ml) for 24 h to deplete cell cholesterol content. Some of the LPDS-treated cells were then incubated for an additional 18 h with HDL_3 protein (100 µg/ml), using either native HDL_3 or the same HDL_3 modified with sphingomyelin as described in Materials and Methods (mg phospholipid/mg protein: native HDL_3, 0.372; SM-HDL_3, 1.056 mg). HMGR activity (A) and cell total cholesterol content (B) were measured in triplicate in T_0 and HDL_3-treated cell cultures as described in Materials and Methods. Results are shown for SR-BI cells only and are the average of two experiments. Only the values measured after treatment with native HDL_3 were different from T_0 ($P < 0.05$).

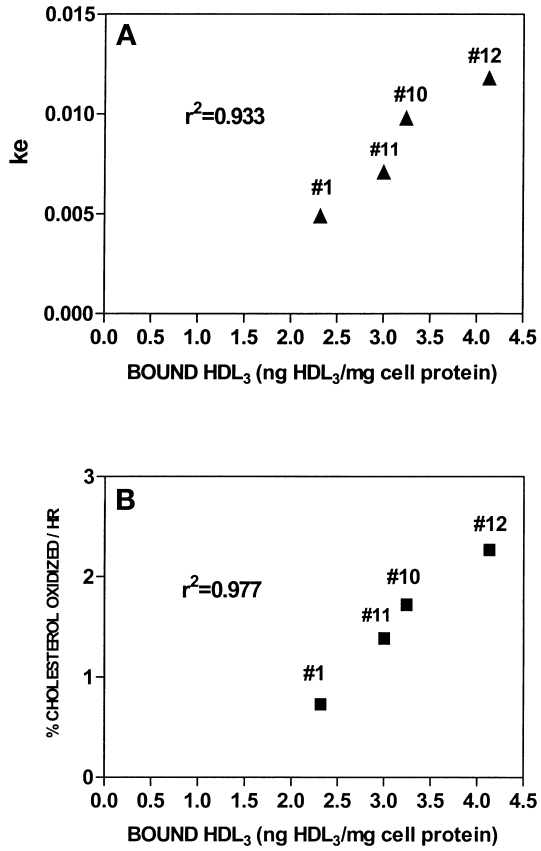

Fig. 7. Effect of SR-BI expression level on cell cholesterol metabolism. Stably transfected clones of COS-7 cells expressing varying amounts of SR-BI were obtained as described in Materials and Methods. GraphPad Prism was used to obtain regression lines and to estimate rate constants from the respective time courses for cholesterol efflux and oxidation as described in Materials and Methods. A: The rate of cholesterol efflux (ke) to HDL_3 (25 µg of protein per ml) plotted against the specific binding of HDL_3 at the same concentration. B: The rate of oxidation of membrane cholesterol is plotted against the specific binding of HDL_3 (25 µg of protein per ml). Triplicate assays for cholesterol efflux to HDL_3, cholesterol oxidation, and specific binding of HDL_3 were as described in Materials and Methods.

tures were then incubated with the HDL_3 preparations for an additional 24 h. As shown in **Fig. 6**, incubation with HDL_3 decreased HMGR activity (Fig. 6A) and increased total cellular cholesterol (Fig. 6B). However, incubation with SM-enriched HDL_3 did not significantly change cellular cholesterol content or HMGR activity (Fig. 6A and B). This result supports the conclusion that receptor-dependent cholesterol influx regulates HMGR activity in SR-BI-expressing cells.

Effect of SR-BI on cell cholesterol metabolism and HMGR activity depends on the level of SR-BI expression

To test the effect of varying SR-BI expression on cholesterol metabolism, clones of COS-7 cells stably expressing different levels of SR-BI were isolated. Control clones were obtained from COS-7 cells transfected with empty vector (clone 1). **Figure 7** shows that, in these cloned cells, both the rate of cellular cholesterol release to 25 µg of HDL_3 protein per ml (Fig. 7A), and the rate of cholestenone formation (Fig. 7B), were highly correlated to receptor expression as measured by the specific binding of radiolabeled HDL_3 (25 µg of protein per ml). We selected the control clone (1) and the two high expressing clones (10 and 12) and measured the change in HMGR activity resulting when LPDS-treated cells were incubated with HDL_3. The results shown in **Fig. 8** indicate that clone 1, which does not express SR-BI, behaved like control cells in that incubation with HDL_3 (50 µg of protein per ml, 10 h) caused a slight increase in HMGR when compared with the activity measured after LPDS treatment. On the other hand, incubation of both SR-BI-expressing clones (10 and 12) with HDL_3 after exposure to LPDS caused a decrease in HMGR that was proportional to the level of receptor expression as measured by specific binding of HDL_3 (Fig. 7). These results indicate that the effects of SR-BI expression on cholesterol metabolism are dependent on the level of receptor present on the cell surface.

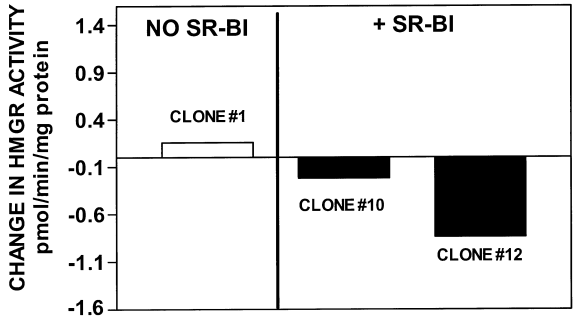

Fig. 8. Effect of SR-BI expression level on the regulation of HMGR activity in stably transfected COS-7 clones incubated with HDL_3. Stably transfected clones of COS-7 cells (clones 1, 10, and 12) described in Fig. 7 were used. The clones were grown in 100-mm plates until almost confluent and were then incubated with LPDS (5 mg/ml) for 12 h to deplete cell cholesterol (T_0 cells). Replicate cultures of each clone were then incubated with HDL_3 (50 μg of protein per ml) for 10 h. HMGR activity was measured in triplicate as described in Materials and Methods in T_0 and HDL_3-treated cultures. Data are expressed as the difference in HMGR activity between HDL_3-treated and T_0 cells. HMGR activity measured after HDL_3 treatment in clone 12 was significantly different from that measured at T_0 ($P < 0.05$).

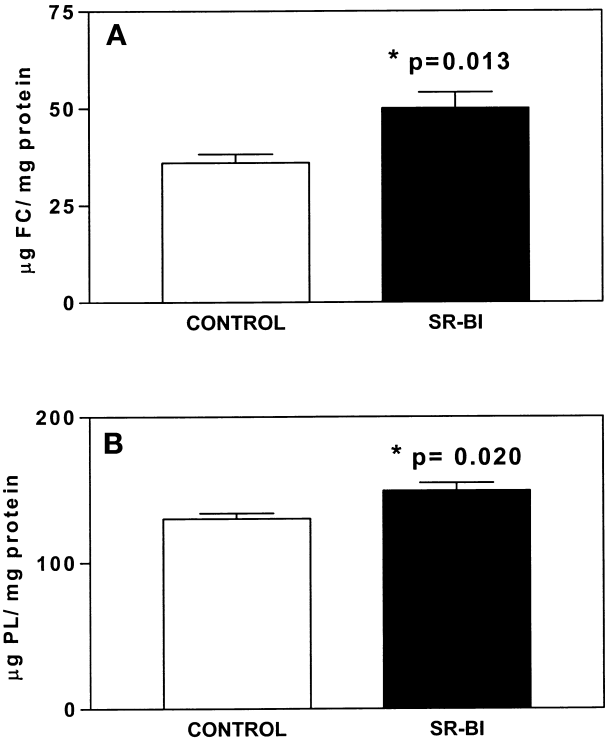

Fig. 9. Steady state contents of free cholesterol and total cell phospholipid (PL) in control and SR-BI-expressing WI38-VA13 cells grown in 10% FBS. Control and stably transfected SR-BI-expressing WI38-VA13 cells were grown in serum-containing medium for 6 months and assayed over a period of 8 weeks. Cell FC and PL contents normalized to cell protein were measured in triplicate assays as described in Materials and Methods. A: FC content in control and SR-BI cells. Results represent the average and standard deviation of seven experiments. B: PL content in control and SR-BI cells. Results represent the average and standard deviation of eight experiments. The average FC-to-PL ratio of SR-BI-expressing cells was higher but not significantly different from that of control cells (control cells, 0.312 ± 0.04; SR-BI-expressing cells, 0.353).

Constitutive expression of SR-BI increases the steady state level of cellular cholesterol and the sensitivity to cholesterol oxidase

WI38-VA13 cells stably transfected with SR-BI express this receptor constitutively. **Figure 9** shows that when these cells were grown for several generations in serum-containing medium, both cellular FC (Fig. 9A) and phospholipid (Fig. 9B) content were significantly higher when compared with control cells grown in the same medium. This result indicates that constitutive expression of SR-BI changes the steady state level of cholesterol. The increased phospholipid content is likely to be a cellular response to the increased cell FC content induced by SR-BI; however, we have not studied the mechanism of this response.

Because the increased sensitivity to cholesterol oxidase seen in cells that express SR-BI could simply reflect an increase in membrane FC content, control and SR-BI-expressing WI38-VA13 cells were pretreated to equalize cellular FC levels before exposure to cholesterol oxidase. For this purpose, stable WI38-SR-BI cells were grown for 3 days in medium containing LPDS whereas the WI38-VA13 control cells were grown in medium containing either 10% or 40% FBS. FC contents for the cells were 29.4 ± 4.1, 30.9 ± 3.7, and 34.4 ± 1.1 μg/mg cell protein for 10% FBS controls, 40% FBS controls, and LPDS SR-BI cells, respectively. These values were not significantly different ($P > 0.05$). Note that CE contents were low, 1–2 μg/mg cell protein, for all three cell types. Sensitivity to cholesterol oxidase (percent FC oxidized in 4 h) for the same cells were as follows: 2.4 ± 0.4, 1.4 ± 0.3, and 26.7 ± 0.8 for 10% FBS controls, 40% FBS controls, and LPDS SR-BI cells, respectively. Thus the increased sensitivity to cholesterol oxidase in the SR-BI-expressing cells was present even when the FC content was the same as in control cells. These results argue strongly that SR-BI-expressing cells have something fundamentally different about the organization of plasma FC rather that simply having increased content of FC. Taken together these results suggest that the presence of SR-BI in the plasma membrane provokes a reorganization of membrane FC that can somehow alter membrane properties.

DISCUSSION

It has been amply demonstrated that cells have a novel regulatory pathway that senses cellular cholesterol levels and inversely controls both the activity of HMGR, the rate-limiting enzyme in cholesterol biosynthesis, and the expression of the LDL receptor on the cell surface (1, 23). Until recently the interaction of LDL with the LDL receptor was considered the main mechanism whereby cells acquired lipoprotein cholesterol. However, another cell surface receptor, the SR-BI, has been shown to enhance the bidirectional movement of FC between lipoproteins and cells, promote selective uptake of CE from HDL, increase

cellular FC content, and alter the distribution of membrane cholesterol pools (10).

Although it is hypothesized that, similarly to the LDL receptor, SR-BI must affect cell cholesterol balance, little is known about the effect that SR-BI has on cellular cholesterol homeostasis. To date, the only data available showed that SR-BI expression in cholesterol-depleted CHO ldlA7 cells, which lack the LDL receptor, resulted in the loss of immunodetectable HMGR protein when these cells were exposed to HDL (400 μg/ml) and to high (100 μg/ml) but not low (10 μg/ml) levels of LDL (24). In addition, it was demonstrated that the presence of sterol regulatory element-binding protein 2 (SREBP-2) in nuclear extracts from these cells mirrored the pattern of expression of HMGR protein; that is, nuclear SREBP-2 levels were high when HMGR protein could be detected. These results suggest that SR-BI-dependent cholesterol influx can modulate HMGR expression via the same mechanism as the cholesterol brought in by the LDL receptor. However, HMGR activity has been shown to be regulated by mechanisms other than changes in genetic expression and, therefore, to document an effect on HMGR regulation it is important to measure enzymatic activity (25). In the present report we have extended the initial observations of Stangl, Hyatt, and Hobbs (24) by measuring both HMGR activity and cholesterol content in cells expressing SR-BI. In addition, we have tested the effect of altering the cholesterol gradient between cells and lipoproteins in both directions, by altering the gradient via changes in the cellular cholesterol status and by changing the concentrations of extracellular lipoprotein.

An important result documented by our experiments was that incubation with HDL_3 consistently affected HMGR activity in cells expressing SR-BI but had no effect in control cells (Figs. 2 and 3). In addition, the data in Table 1 clearly demonstrate that CLA-1 affected cellular cholesterol metabolism and HMGR activity identically to SR-BI. Although CLA-1 is generally considered to be the human homolog of SR-BI (22, 26), no direct comparisons of the function of these receptors have been published. Because we show that, similarly to SR-BI, CLA-1 plays an important role in cholesterol homeostasis, studies of SR-BI function are relevant to human physiology. Using transiently transfected COS-7 cells we also demonstrated that, although SR-BI enhances cholesterol flux between cells and HDL_3, net movement of cholesterol occurs down a cholesterol concentration gradient (Figs. 2 and 5 vs. Figs. 3, 4, and 5). The observation that cholesterol flux occurs down a concentration gradient is not new. What is significant from our results is the observation that this flux is significant only in SR-BI-expressing cells and that the magnitude of this flux is related to the level of SR-BI expression. The concentration gradient to which SR-BI responds can be set either by changes in the concentration of extracellular lipoproteins or by changes in cellular cholesterol contents. Expression of SR-BI also increased net cholesterol movement between HDL_3 and WI38-VA13 cells stably transfected with SR-BI (Fig. 6). However, when LPDS-treated WI38-VA13 cells expressing SR-BI were incubated with HDL_3 enriched in SM, which results in HDL_3 particles that bind to SR-BI but do not promote net FC influx (6), there was no significant effect on either HMGR activity or cellular cholesterol content (Fig. 6). This result supports the conclusion that HMGR activity is responsive to the SR-BI-mediated influx of cholesterol.

These data demonstrate that a consequence of either SR-BI or CLA-1 expression is to move cholesterol in or out of the cellular pool that regulates HMGR. Other than testing the effect of SM-enriched HDL_3 on total cell cholesterol accumulation, we did not attempt to assess the relative importance of SR-BI-mediated FC transport versus CE uptake on the acute regulation of HMGR. The question concerning the quantitative contribution of HDL FC and CE to SR-BI-mediated cholesterol flux is difficult to address for a number of reasons. For example, *1*) the relationships between HDL dose and influx are different for FC and CE, with FC influx never showing a clear saturation whereas CE uptake does show saturation, *2*) FC flux is bidirectional whereas CE efflux does not occur unless the CE is first hydrolyzed to FC, a reaction that complicates the estimates of CE flux, and *3*) the use of nonhydrolyzable tracers, such as cholesteryl oleolyl ether, may not provide an accurate estimate of CE influx. However, earlier studies (27, 28), the experiment shown in Fig. 6, and the present observation that SR-BI can enhance net cholesterol movement out of cells to HDL strongly indicate that the ability of SR-BI to facilitate the movement of FC in the direction of its concentration gradient is of particular importance in the modulation of cell cholesterol homeostasis. Taken together, these results support the concept that SR-BI functions by creating an environment in the plasma membrane that facilitates cholesterol movement (3, 29).

Previously Chen et al. (30) demonstrated a direct relationship between cholesterol efflux and the level of SR-BI expression in RAW cells. Comparison of COS-7 clones having different levels of SR-BI expression shows that the expected enhancement of cholesterol transport by SR-BI, as reflected by changes in HMGR activity, is proportional to the level of receptor expression (Figs. 7A and 8). In addition, the sensitivity of membrane cholesterol to exogenous cholesterol oxidase in COS-7 clones is also proportional to SR-BI expression (Fig. 7B). This effect of SR-BI is believed to reflect not just an increase in plasma membrane cholesterol but rather a difference in the organization of cholesterol within membrane domains. Thus different amounts of SR-BI proportionally change the amount of cholesterol in the cell as well as plasma membrane properties as if SR-BI has a stoichiometric effect on these parameters. Moreover, compared with control cells that lack SR-BI, WI38-VA13 cells stably transfected to express this receptor have significantly higher cell FC and phospholipid content after growing in 10% FBS for several generations (Fig. 9A and B). It has been shown that macrophages respond to cholesterol loading by upregulating the synthesis of phosphatidylcholine (31, 32). Thus it is likely that the increased phospholipid content of SR-BI-expressing WI38-VA13 cells occurs in response to the increase in FC

induced by SR-BI; however, we have not investigated this. These stably transfected cells showed all the changes in cholesterol metabolism characteristic of the transiently transfected COS-7 cells, including the increased sensitivity to cholesterol oxidase even when both SR-BI-negative and -positive cells have the same content of FC.

The observations that constitutive expression of SR-BI increases the steady state content of FC and expands the pool of FC that is sensitive to cholesterol oxidase indicate that SR-BI is not just a catalyst that simply increases cholesterol flux between cells and lipoproteins. Rather, our data provide further evidence that SR-BI can alter the distribution of cellular cholesterol pools. In addition, we show that cholesterol flux mediated by this receptor can effectively modulate multiple aspects of cholesterol metabolism, including regulation of HMGR activity. Because all the effects of SR-BI/CLA-1 are proportional to the level of receptor on the cell surface, future studies to elucidate how SR-BI functions and the mechanisms that control its expression will be basic to our understanding of the regulation of cellular cholesterol homeostasis.

This work was supported by an Atorvastatin Research Award to M.L.L.M., National Institutes of Health Grants HL63768 and HL58012, and Program Project Grant HL22633. The authors acknowledge the following individuals: R. E. Temel and R. B. Dematttos for construction of pcDNA3(SR-BI), R. Wang for help with the Western blots, S. Azhar for the antibody to the C terminal of SR-BI, and Sun Yi for excellent technical assistance.

Manuscript received 27 March 2001 and in revised form 24 July 2001.

REFERENCES

1. Brown, M. S., S. E. Dana, and J. L. Goldstein. 1973. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts by lipoproteins. *Proc. Natl. Acad. Sci. USA.* **70:** 2162–2166.
2. Daerr, W. H., S. H. Gianturco, J. R. Patsch, L. C. Smith, and A. M. Gotto, Jr. 1980. Stimulation and suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in normal fibroblasts by high density lipoprotein subclasses. *Biochim. Biophys. Acta.* **619:** 287–301.
3. Rothblat, G. H., M. de la Llera-Moya, V. Atger, G. Kellner-Weibel, D. L. Williams, and M. C. Phillips. 1999. Cell cholesterol efflux: integration of old and new observations provides new insights. *J. Lipid Res.* **40:** 781–796.
4. Jian, B., M. de la Llera-Moya, Y. Ji, N. Wang, M. C. Phillips, J. B. Swaney, A. R. Tall, and G. H. Rothblat. 1998. Scavenger receptor class B type I as a mediator of cellular cholesterol efflux to lipoproteins and phospholipid acceptors. *J. Biol. Chem.* **273:** 5599–5606.
5. Lawn, R. M., D. P. Wade, M. R. Garvin, X. Wang, K. Schwartz, J. G. Porter, J. J. Seilhamer, A. M. Vaughan, and J. F. Oram. 1999. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J. Clin. Invest.* **104:** R25–R31.
6. Yancey, P. G., M. de la Llera-Moya, S. Swarnakar, P. Monzo, S. M. Klein, M. A. Connelly, W. J. Johnson, D. L. Williams, and G. H. Rothblat. 2000. HDL phospholipid composition is a major determinant of the bi-directional flux and net movement of cellular free cholesterol mediated by scavenger receptor-BI (SR-BI). *J. Biol. Chem.* **275:** 36596–36604.
7. Jian, B., M. de la Llera-Moya, L. Royer, G. Rothblat, O. Francone, and J. B. Swaney. 1997. Modification of the cholesterol efflux properties of human serum by enrichment with phospholipid. *J. Lipid Res.* **38:** 734–744.
8. de la Llera-Moya, M., G. H. Rothblat, M. A. Connelly, G. Kellner-Weibel, S. W. Sakr, M. C. Phillips, and D. L. Williams. 1999. Scavenger receptor BI (SR-BI) mediates free cholesterol flux independently of HDL tethering to the cell surface. *J. Lipid Res.* **40:** 575–580.
9. Ji, Y., B. Jian, N. Wang, Y. Sun, M. de la Llera Moya, M. C. Phillips, G. H. Rothblat, J. B. Swaney, and A. R. Tall. 1997. Scavenger receptor B1 promotes high density lipoprotein-mediated cellular cholesterol efflux. *J. Biol. Chem.* **272:** 20982–20985.
10. Williams, D. L., M. A. Connelly, R. E. Temel, S. Swanakar, M. C. Phillips, M. de la Llera-Moya, and G. H. Rothblat. 1999. Scavenger receptor BI and cholesterol trafficking. *Curr. Opin. Lipidol.* **10:** 329–339.
11. Rigotti, A., B. Trigatti, J. Babitt, M. Penman, S. Xu, and M. Krieger. 1997. Scavenger receptor BI—a cell surface receptor for high density lipoprotein. *Curr. Opin. Lipidol.* **8:** 181–188.
12. Temel, R. E., B. Trigatti, R. B. DeMattos, S. Azhar, M. Krieger, and D. L. Williams. 1997. Scavenger receptor class B, type I (SR-BI) is the major route for the delivery of high density lipoprotein cholesterol to the steroidogenic pathway in cultured mouse adrenocortical cells. *Proc. Natl. Acad. Sci. USA.* **94:** 13600–13605.
13. Markwell, M. A. K., S. M. Haas, L. L. Bieber, and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87:** 206–210.
14. Harwood, H. J., D. M. Bridge, and P. W. Stacpoole. 1987. In vivo regulation of human mononuclear leukocyte 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Clin. Invest.* **79:** 1125–1132.
15. Klansek, J. J., P. G. Yancey, R. W. St. Clair, R. T. Fischer, W. J. Johnson, and J. M. Glick. 1995. Cholesterol quantitation by GLC: artifactual formation of short-chain esters. *J. Lipid Res.* **36:** 2261–2266.
16. Swarnakar, S., R. E. Temel, M. A. Connelly, S. Azhar, and D. L. Williams. 1999. Scavenger receptor class B, type II, mediates selective uptake of low density lipoprotein cholesteryl ester. *J. Biol. Chem.* **42:** 29733–29739.
17. Kellner-Weibel, G., M. de la Llera-Moya, M. A. Connelly, G. Stoudt, A. E. Christian, M. P. Haynes, D. L. Williams, and G. H. Rothblat. 2000. Expression of scavenger receptor BI in COS-7 cells alters cholesterol content and distribution. *Biochemistry.* **39:** 221–229.
18. Williams, D. L., M. de la Llera-Moya, S. T. Thuahnai, S. Lund-Katz, M. A. Connelly, S. Azhar, G. M. Anantharamaiah, and M. C. Phillips. 2000. Binding and cross-linking studies show that scavenger receptor BI (SR-BI) interacts with multiple sites in apolipoprotein A-I and identify the class A amphipathic α-helix in a recognition motif. *J. Biol. Chem.* **275:** 18897–18904.
19. Goldstein, J. L., S. K. Basu, and M. S. Brown. 1983. Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. *Methods Enzymol.* **98:** 241–260.
20. Johnson, W. J., M. J. Bamberger, M. J. Latta, R. A. Rapp, M. C. Phillips, and G. H. Rothblat. 1986. The bidirectional flux of cholesterol between cells and lipoproteins. *J. Biol. Chem.* **261:** 5766–5776.
21. Daerr, W. H., S. H. Gianturco, J. R. Patsch, L. C. Smith, and A. M. Gotto. 1980. Effects of HDL subclasses on 3-hydroxy-3-methylglutaryl coenzyme A reductase in human fibroblasts. *Nutr. Metab.* **24:** 26–33.
22. Calvo, D., and M. A. Vega. 1993. Identification, primary structure, and distribution of CLA-1, a novel member of the CD36/LIMPII gene family. *J. Biol. Chem.* **268:** 18929–18935.
23. Brown, M. S., and J. L. Goldstein. 1999. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc. Natl. Acad. Sci. USA.* **96:** 11041–11048.
24. Stangl, H., M. Hyatt, and H. H. Hobbs. 1999. Transport of lipids from high and low density lipoproteins via scavenger receptor-BI. *J. Biol. Chem.* **274:** 32692–32698.
25. Petras, S. E., S. Lindsey, and H. J. Harwood. 1999. HMG-CoA reductase regulation: use of structurally diverse first half-reaction squalene synthetase inhibitors to characterize the site of mevalonate-derived nonsterol regulation production in cultured IM-9 cells. *J. Lipid Res.* **40:** 24–38.
26. Calvo, D., D. Gomez-Coronado, M. A. Lasuncion, and M. A. Vega. 1997. CLA-1 is an 85-kD plasma membrane glycoprotein that acts as a high-affinity receptor for both native (HDL, LDL, and VLDL) and modified (OxLDL and AcLDL) lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2341–2349.
27. Nestler, J. E., M. Bamberger, G. H. Rothblat, and J. F. Strauss. 1985. Preferential utilization of the free cholesterol moiety of high density lipoproteins by cultured rat granulosa cells. *In* Lipoprotein

and Cholesterol Metabolism in Steroidogenic Tissues. J. F. Strauss and K. M. J. Menon, editors. G. F. Strickley, Philadelphia, PA. 135–139.
28. Bamberger, M., J. M. Glick, and G. H. Rothblat. 1983. Hepatic lipase stimulates the uptake of high density lipoprotein cholesterol by hepatoma cells. *J. Lipid Res.* **24:** 869–876.
29. Rodrigueza, W. V., S. T. Thuahnai, R. E. Temel, S. Lund-Katz, M. C. Phillips, and D. L. Williams. 1999. Mechanism of scavenger receptor class B type I (SR-BI) mediated selective uptake of cholesteryl ester from high density lipoprotein to adrenal cells. *J. Biol. Chem.* **274:** 20344–20350.
30. Chen, W., D. Silver, J. D. Smith, and A. R. Tall. 2000. Scavenger receptor-BI (SR-BI) inhibits ATP binding cassette transporter 1 (ABC1)-mediated cholesterol efflux in macrophages. *J. Biol. Chem.* **275:** 20822–20828.
31. Tabas, I., S. Marathe, G. A. Keesler, N. Beatini, and Y. Shiratori. 1996. Evidence that the initial up-regulation of phosphatidylcholine biosynthesis in free cholesterol-loaded macrophages is an adaptive response that prevents cholesterol-induced cellular necrosis—proposed role of an eventual failure of this response in foam cell necrosis in advanced atherosclerosis. *J. Biol. Chem.* **271:** 22773–22781.
32. Kellner-Weibel, G., Y. J. Geng, and G. H. Rothblat. 1999. Cytotoxic cholesterol is generated by the hydrolysis of cytoplasmic cholesteryl ester and transported to the plasma membrane. *Atherosclerosis.* **146:** 309–319.