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Analysis of Chimeric Receptors Shows That Multiple Distinct Functional Activities of Scavenger Receptor, Class B, Type I (SR-BI), Are Localized to the Extracellular Receptor Domain†

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ABSTRACT: Scavenger receptor BI (SR-BI) mediates the selective uptake of high-density lipoprotein (HDL) cholesteryl ester (CE), a process by which HDL CE is taken into the cell without degradation of the HDL particle. In addition, SR-BI stimulates the bi-directional flux of free cholesterol (FC) between cells and lipoproteins, an activity that may be responsible for net cholesterol efflux from peripheral cells as well as the rapid hepatic clearance of FC from plasma HDL. SR-BI also increases cellular cholesterol mass and alters cholesterol distribution in plasma membrane domains as judged by the enhanced sensitivity of membrane cholesterol to extracellular cholesterol oxidase. In contrast, CD36, a closely related class B scavenger receptor, has none of these activities despite binding HDL with high affinity. In the present study, analyses of chimeric SR-BI/CD36 receptors and domain-deleted SR-BI have been used to test the various domains of SR-BI for functional activities related to HDL CE selective uptake, bi-directional FC flux, and the alteration of membrane cholesterol mass and distribution. The results show that each of these activities localizes to the extracellular domain of SR-BI. The N-terminal cytoplasmic tail and transmembrane domains appear to play no role in these activities other than targeting the receptor to the plasma membrane. The C-terminal tail of SR-BI is dispensable for activity as well for targeting to the plasma membrane. Thus, multiple distinct functional activities are localized to the SR-BI extracellular domain.

The risk for developing atherosclerotic heart disease is inversely related to plasma concentrations of high-density lipoprotein (HDL)[1] (*1*). Although the mechanism of this protective effect remains uncertain, it has been known for some time that HDL plays a pivotal role in the transport of free cholesterol (FC) and cholesteryl ester (CE) through the plasma. HDL participates in reverse cholesterol transport (*2*), a process involving the uptake of cholesterol from vascular and other tissues and its delivery to steroidogenic cells for hormone synthesis and to the liver for bile acid synthesis. HDL provides CE to cells via a novel selective uptake pathway in which HDL CE is taken into the cell without the uptake and lysosomal degradation of the HDL particle (*3*−*8*). Recent studies indicate that scavenger receptor BI (SR-BI) is the cell surface receptor responsible for HDL CE selective uptake (*9*−*11*). SR-BI is expressed in rodents abundantly in the liver and in steroidogenic cells of the adrenal gland and ovary (*9, 12, 13*) where SR-BI levels are regulated by tropic hormones coordinately with the selective uptake of HDL CE and steroidogenesis (*3, 6, 12, 13*). In addition, antibody blocking experiments show that SR-BI is the receptor responsible for the uptake of HDL CE and its delivery to the steroidogenic pathway in adrenocortical cells (*14*). Inactivation of the SR-BI gene in mice alters plasma HDL metabolism and reduces adrenal gland CE accumulation, results consistent with a major role for SR-BI in cholesterol metabolism in vivo (*15, 16*). Taken together, these studies indicate that SR-BI is a physiologically relevant HDL receptor.

SR-BI was originally defined as a class B scavenger receptor (*17*−*19*) in a family that includes CD36, LIMPII, and SR-BII, a form of SR-BI with an alternate C-terminal cytoplasmic tail (*20*). SR-BI and CD36 are similar in size, show considerable amino acid sequence identity, and are very similar in predicted secondary structure. Structure predictions suggest that each protein contains two transmembrane and two cytoplasmic domains (the amino- and carboxy-terminal

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[1] Abbreviations: HDL, high-density lipoprotein; PBS, phosphate-buffered saline; CE, cholesteryl ester; FC, free cholesterol; SR-BI, scavenger receptor, class B, type I.

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domains) as well as a large extracellular domain containing a cysteine-rich region and nine putative sites for N-linked glycosylation. In addition, both proteins are fatty acylated and localized to caveolae (*21*−*23*).

CD36 and SR-BI have a number of common ligands, including HDL that binds with high affinity to both receptors (*9, 24*). In previous studies, analysis of SR-BI, CD36, and chimeric receptors derived by swapping various domains between these receptors showed that the extracellular domain of SR-BI, but not CD36, mediates the high-efficiency selective uptake of CE from receptor-bound HDL particles (*25, 26*). The transmembrane domains and cytoplasmic tails of CD36 could substitute for those in SR-BI and preserve full HDL CE selective uptake activity. In contrast, the transmembrane domains and cytoplasmic tails of SR-BI were unable to confer HDL CE selective uptake activity upon the extracellular domain of CD36. Thus, the transfer of HDL CE into the cell appears to be an inherent activity of the extracellular domain of SR-BI. Interestingly, SR-BII showed a reduced efficiency of HDL CE selective uptake similar to that of CD36, suggesting that the C-terminal tail of SR-BI may be important for function (*25*).

In addition to mediating HDL CE selective uptake, SR-BI has a variety of effects on free cholesterol flux and the organization of plasma membrane lipid domains (*11, 27*). For example, SR-BI stimulates the bi-directional flux of free cholesterol between cells and lipoproteins (*27*−*31*), an activity that may be responsible for the rapid hepatic clearance of FC from plasma HDL and its resultant secretion into bile (*32, 33*). In cultured cells, SR-BI also increases cellular cholesterol mass and alters the cholesterol distribution in plasma membrane domains. The altered distribution of membrane cholesterol is reflected by an SR-BI-dependent increase in the sensitivity of membrane cholesterol to extracellular cholesterol oxidase (*28, 34*). In addition, SR-BI expression enlarges the fast kinetic pool for cellular cholesterol efflux to cyclodextrin acceptors (*34*).

In the present study, analyses of chimeric SR-BI/CD36 receptors and domain-deleted SR-BI were performed to test the various domains of SR-BI for a spectrum of functional activities related to HDL CE selective uptake, bi-directional FC flux, alteration of membrane cholesterol distribution, and the accumulation of cellular free cholesterol mass. The results show that each of these activities localizes to the extracellular domain of SR-BI. The N-terminal cytoplasmic tail and transmembrane domains appear to play no role in these activities other than proper targeting of the receptor to the plasma membrane. The C-terminal tail of SR-BI is dispensable for activity as well for targeting to the plasma membrane. Thus, the extracellular domain of SR-BI provides multiple functions necessary for the flux of FC, the selective uptake of lipoprotein CE, and the alteration of plasma membrane properties.

EXPERIMENTAL PROCEDURES

Materials. The following antibodies were used: monoclonal anti-Flag IgG (M2; Stratagene) (1:1000 for immunofluorescence and 1:2000 for immunoblot); monoclonal anti-*myc* IgG (Invitrogen) (1:1000 for immunofluorescence and 1:5000 for immunoblot); monoclonal anti-Histone IgG (mAb052; Chemicon International) (1:1000 for immunofluorescence); Texas Red sulfonyl chloride (TRSC)-conjugated donkey anti-mouse or anti-rabbit secondary IgG (Jackson ImmunoResearch Laboratories) (1:1000 for immunofluorescence); peroxidase-conjugated goat anti-mouse or anti-rabbit secondary IgG (Jackson ImmunoResearch Laboratories) (1:10 000 for immunoblot); polyclonal anti-SR-BI extracellular antibody #356 (*14*) (1:250 for immunofluorescence); polyclonal anti-SR-BI C-terminal antibody (*35*) (2 μg/mL purified IgG for immunoblot); polyclonal anti-CD36 antibody 39815 (1:250 for immunofluorescence) (*36*).

Plasmids and Sequencing. Murine SR-BI and rat CD36 were analyzed in this study. PCR amplifications were performed using a Perkin-Elmer Cetus DNA Thermal Cycler 9700. Oligonucleotides were purchased from Integrated DNA Technologies. Expression plasmids for *pSG5(CD36)*, *pSG5(CD/SRT)*, *pSG5(SR/CD/SR)*, *pSG5(SR-BI)*, and *pSG5(SR/CDT)* were described previously (*25*). For construction of *pSG5(CD/SR/CD)*, primers 5′-AGGTTGCTCTTCAAGGAATGTCCGCATAGACCCG-3′ and 5′-AGCTTGCTCTTCACAGAACCTGGGGCATCAGCACC-3′ and primers 5′-AGCTTGCTCTTCACTGGGCCTGGTTGAGATGGTC-3′ and 5′-AGCGTGCTCTTCACCTCTTTATTGTCTTCTCAATGAGTAGGTC-3′ were employed to amplify pSG5(SR-BI) and pSG5(CD36), respectively. The resulting PCR products were digested with *Sap*I and ligated.

For construction of *pSG5(SR-BI/D1)*, primers 5′-GACCGAATTCCAATTGCCGTCTCCTTCAGGTCCTGAGC-3′ and 5′-ACTCAAGATCTCTACTGGCTCCGCAGTTGGCAGATGATGG-3′ were employed to amplify pSG5(SR-BI). For addition of the myc-epitope tag to the C-terminus of SR-BI, *pSG5(SR-BI)MC*, primers 5′-GACCGAATTCCAATTGCCGTCTCCTTCAGGTCCTGAGC-3′ and 5′-ACTCAAGATCTTTACAGATCCTCTTCGGAGATGAGTTTCTGCTCTAGCTTGGCTTCTTGCAGCACC-3′ were employed to amplify pSG5(SR-BI). For addition of the flag-epitope tag to the N-terminus of SR-BI, *pSG5(SR-BI)FN*, primers 5′-ACTCACAATTGGACATGGGCGATTACAAGGATGACGACGATAAGGGCAGCTCCAGGGCGCGC-3′ and 5′-GACCGGATCCAGATCTGCGGACAGGTGTGACATCTGG-3′ were employed to amplify pSG5(SR-BI). For addition of the myc-epitope tag to the C-terminus of CD36, *pSG5(CD36)-MC*, primers 5′-ACTCACAATTGAGGAGAATGGGCTGCGATCGG-3′ and 5′-ACTCAAGATCTCTACAGGTCCTCTTCGGAGATGAGTTTCTGCTCTTTTCCATTCTTGGATCTGCAAGCACAG-3′ were employed to amplify pSG5-(CD36). For addition of the flag-epitope tag to the N-terminus of CD36, *pSG5(CD36)FN*, primers 5′-ACTCACAATTGAAGAGAATGGGCGATTACAAGGATGATGATGATAAGTGCGATCGGAACTGTGGGC-3′ and 5′-ACTGAAGATCTCTATTTTCCATTCTTAGACCTGCAAGCACAG-3′ were employed to amplify pSG5(CD36). The resulting PCR products were digested with *Mfe*I and *Bgl*II and ligated into *Eco*RI and *Bgl*II digested pSG5 (Stratagene, Inc.).

All plasmids were prepared using Endotoxin-free Qiagen Maxi-prep kits and sequenced throughout the coding region to confirm that no point mutations had been generated. Reactions were prepared using a dye termination cycle sequencing kit and analyzed on an Applied Biosystems model 373 DNA Sequencer with an Excel Upgrade as recommended by the manufacturer (PE Applied Biosystems).

Transient Transfection of COS-7 Cells. COS-7 cells were maintained in Dulbecco's minimal essential medium, 10%

calf serum, 2 mM L-glutamine, 50 units/mL penicillin, 50 μg/mL streptomycin, and 1 mM sodium pyruvate, and transfected as described (*25*). The following day, two 10 cm dishes of transfected cells were trypsinized and resuspended in a total volume of 27 mL with fresh medium, and 0.5, 1, or 2 mL was added to each of 11 (24 well plate), 22 (12 well plate), or 35 (6 well plate) mm wells, respectively. The cells were assayed 48 h post-transfection unless otherwise indicated.

Immunoblot Analysis. Transiently transfected cells expressing wild-type or chimeric receptors (in 35 mm wells) were washed twice with PBS (pH 7.4) and lysed with 300 μL of NP-40 cell lysis buffer (*13, 14*) containing 1 μg/mL pepstatin, 0.2 mM phenylmethylsulfonyl fluoride, 1 μg/mL leupeptin, and 10 μg/mL aprotinin. Protein concentrations were determined by the method of Lowry (*37*). Immunoblots with antibodies directed to different regions of SR-BI and CD36 confirmed the presence of the respective region of either SR-BI or CD36 in each of the chimeric receptors (data not shown).

Preparation of [^{125}I, ^{3}H]-hHDL$_3$. Human (h) HDL$_3$ (1.125 g/mL < ρ < 1.210 g/mL) was isolated by sequential ultracentrifugation (*38*). The hHDL$_3$ was labeled with [^{3}H]-cholesteryl oleyl ether ([^{3}H]-CE) (Amersham Life Sciences) using recombinant cholesteryl ester transfer protein as described (*39*) with the following modifications. HDL and cholesteryl ester transfer protein were incubated with [^{3}H]-CE (dried down on the glass tube as an alternative to Celite) for 5 h at 37 °C. Labeled particles were reisolated by gel exclusion chromatography on a 25 mL Superose 6 (Pharmacia) column. The hHDL$_3$ was then labeled with [^{125}I]-dilactitol tyramine as previously described (*25*). Particles were dialyzed versus four changes of 150 mM NaCl, 10 mM potassium phosphate buffer, pH 7.4, 1 mM EDTA, and stored at 4 °C under argon. The specific activity of the [^{125}I, ^{3}H]-hHDL$_3$ was 790 dpm/ng of protein for ^{125}I and 67 dpm/ng of protein for ^{3}H.

HDL Cell Association, Selective CE Uptake, and Apolipoprotein Degradation. Transiently transfected COS-7 cells (in 35 mm wells) were washed once with serum-free medium/0.5% BSA. [^{125}I]-Dilactitol tyramine−[^{3}H]-cholesterol oleyl ether hHDL$_3$ particles were added at a concentration of 10 μg of protein/mL (unless otherwise indicated) in serum-free medium/0.5% BSA. After incubation for 1.5 h at 37 °C, the medium was removed, and the cells were washed 3 times with PBS/0.1% BSA (pH 7.4) and 1 time with PBS (pH 7.4). The cells were lysed with 1.1 mL of 0.1 N NaOH, and the lysate was processed to determine trichloroacetic acid soluble and insoluble ^{125}I radioactivity and organic solvent-extractable ^{3}H radioactivity (*25*). The values for cell-associated HDL apolipoprotein (expressed as HDL CE), endocytosed and degraded HDL apolipoprotein (expressed as HDL CE), total cell associated HDL CE, and the selective uptake of HDL CE were obtained as previously described (*25*).

Cholesterol Efflux and Influx Assays. Transiently transfected COS-7 cells were replated into 11 mm wells in growth medium. For cholesterol efflux, cells were labeled for 24 h with 5 μCi/mL [^{3}H]-cholesterol (New England Nuclear Life Sciences) in Dulbecco's minimal essential medium containing 10% calf serum immediately after reseeding. Cells were washed, and [^{3}H]-cholesterol efflux was measured at 4 h in triplicate using two different concentrations of HDL$_3$ acceptor (25 and 250 μg/mL protein) as previously described (*28*). The release of radioactive cholesterol to HDL$_3$ was measured by scintillation counting of filtered aliquots of acceptor-containing medium and expressed as the fraction of the total 2-propanol-soluble label in the cells plus the label that was released into the medium. Fractional efflux values were corrected for the small amount of radioactivity released in the absence of HDL$_3$.

Cholesterol influx was measured with transfected cells prepared in exactly the same way as for the efflux assays except the cells were not labeled with [^{3}H]-cholesterol. Instead, HDL$_3$ was labeled with radioactive FC by exchanging [^{3}H]-cholesterol from glass fiber filters onto which radioactive cholesterol had been dried under N_2 (*28*). Cells were incubated for 2 h with 10 μg/mL [^{3}H]-cholesterol-labeled HDL$_3$, and the influx of HDL FC was measured as previously described (*28*).

To normalize the data for FC efflux and influx to the amount of cell surface receptor expressed in the transient transfections, modified HDL cell association assays were performed in parallel with the FC flux studies. COS-7 cells (in 22 mm wells) were washed once with serum-free medium/0.5% BSA, and [^{125}I, ^{3}H]hHDL$_3$ was added at 10 μg of protein/mL in serum-free medium/0.5% BSA. After incubation for 1 h at 37 °C, medium was removed, cells were washed 3 times with PBS/0.1% BSA (pH 7.4) and 2 times with PBS (pH 7.4), cells were lysed with 0.5 mL of 0.1 N NaOH, the wells were washed with an additional 0.5 mL of 0.1 NaOH, and the wash plus lysate was counted for γ-radiation. After counting, an aliquot was removed for protein determination (*37*).

Cholesterol Oxidase Assays, [^{3}H]-Cholesterol Esterification, and Cholesterol Mass. Transiently transfected COS-7 cells were replated in 22 mm wells and labeled with 5 μCi/mL [^{3}H]-cholesterol as described above. Cholesterol oxidase assays were performed 24 h post-labeling as described by Smart et al. (*40*), as modified by Kellner-Weibel et al. (*34*). Following cholesterol oxidase treatment for 4 or 8 h at 37 °C, the cell monolayers were extracted with 2-propanol, and the [^{3}H]-cholesterol and [^{3}H]-cholestanone were quantitated after separation by thin-layer chromatography (*34*). The time zero samples (after the 24 h labeling period) were also analyzed by thin-layer chromatrography to determine the distribution of label in FC and CE (*34*).

For quantification of cholesterol content, transiently transfected COS-7 cells were replated in 35 mm wells and incubated 24 h in Dulbecco's minimal essential medium containing 10% calf serum. Lipids were extracted with 2-propanol, containing cholesteryl methyl ether as an internal standard. Free and total cholesterol (and ester cholesterol by difference) were measured by gas−liquid chromatography (*41*).

Immunofluorescence. Transiently transfected COS-7 cells were replated 24 h post-transfection into a 10 cm dish containing a glass microscope slide. After 24 h, the slide was placed in a two-well immunofluorescence chamber (Electron Microscopy Sciences) and processed as described by Berrios (*42*). Briefly, the medium was removed, and the cells were fixed for 4 min with 4% (w/v) paraformaldehyde in 18 mM $MgSO_4$, 5 mM $CaCl_2$, 41 mM KCl, 24 mM NaCl, 5 mM PIPES, pH 7.5. Cells were washed at room temper-

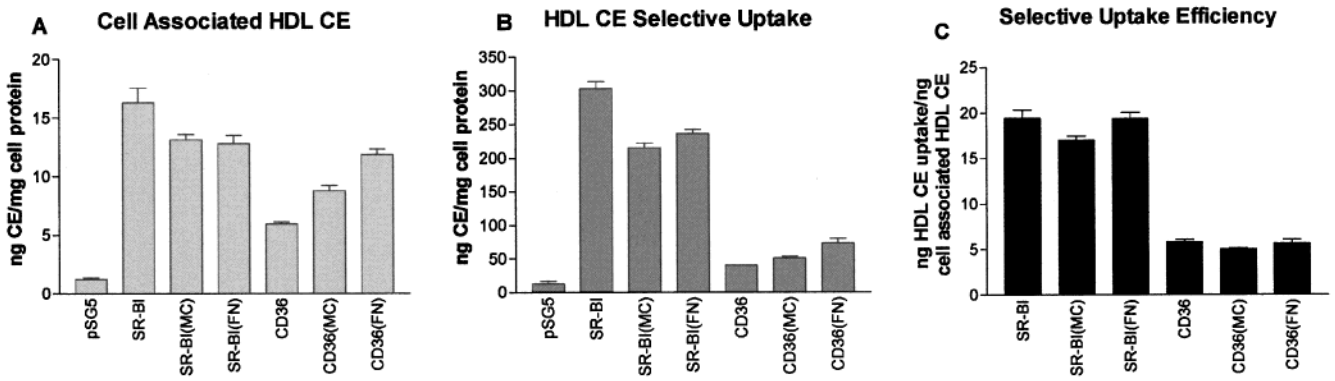

FIGURE 1: Cell association of HDL and HDL CE selective uptake mediated by wild-type and epitope-tagged CD36 and SR-BI. COS-7 cells transfected with vector pSG5 or expressing CD36, CD36(MC), CD36(FN), SR-BI, SR-BI(MC), or SR-BI(FN) were incubated at 37 °C for 1.5 h with 10 μg/mL [^{125}I, ^{3}H]-HDL$_3$, after which cells were processed to determine cell-associated HDL CE (A) and HDL CE selective uptake (B). Values represent the mean of triplicate determinations after subtraction of values obtained with the addition of a 40-fold excess of unlabeled HDL$_3$. (C) The efficiency of HDL CE selective uptake was determined by subtracting the values from vector-transfected cells, and normalizing the amount of HDL CE selective uptake to the amount of cell-associated HDL particles.

ature twice with the same buffer without or with detergent (0.5% Triton X-100, 0.5% Nonidet P-40) for nonpermeabilized or permeabilized conditions, respectively. The primary antibody was added with or without detergent for 30 min at 37 °C. Cells were washed as above and incubated with Texas Red-conjugated secondary antibody for 30 min at 37 °C. Cells were washed as above and additionally with 140 mM NaCl, 0.1% Triton X-100, 0.02% SDS, 0.01 M potassium phosphate, pH 7.5, as modified from Fisher et al. (*43*). A drop of Antifade (Molecular Probes) was added, and a coverslip was sealed with clear nail polish. Specimens were examined with a Zeiss Ultraphot or a Nikon E800 microscope, each equipped with a Nikon 60 × 1.4 NA phase/oil immersion objective lens and a cooled CCD interline camera (Princeton).

RESULTS

Membrane Orientation of the N- and C-Termini of SR-BI and CD36. SR-BI and CD36 are members of the CD36 gene family that show considerable amino acid sequence identity, glycosylation patterns, and predicted membrane topology (*15*, *18*, *23*). Both receptors show a predicted topology with a short N-terminal cytoplasmic tail, a transmembrane domain, a single large extracellular domain, a second transmembrane domain, and a short C-terminal cytoplasmic tail. Studies of CD36 suggest that the predicted topology is correct (*44*, *45*). In the case of SR-BI, immunostaining (*23*) and antibody inhibition studies (*14*) support the predicted orientation of the C-terminal cytoplasmic tail and extracellular domains of SR-BI, but the membrane orientation of the N-terminus has not been addressed. To this end, we produced plasmids encoding SR-BI and CD36 each with a Flag epitope appended to the N-terminus or a c-*myc* epitope appended to the C-terminus. To ensure that the addition of the epitope did not affect the function of these proteins, the epitope-tagged receptors were transiently expressed in COS-7 cells and tested for their ability to bind HDL particles and to promote the selective uptake of HDL CE. As shown in Figure 1, wild-type and epitope-tagged SR-BI and CD36 each bound HDL (panel A) and mediated HDL CE selective uptake (panel B). When the background values from pSG5 vector-transfected cells were subtracted, and the HDL CE selective uptake activities (panel B) were normalized to the cell surface expression levels of the respective receptor (panel A), the epitope-tagged receptors showed the same selective uptake efficiency (panel C) as their wild-type counterparts. These data indicate that the tagged receptors were expressed on the cell surface and that their function was not impaired by the addition of the epitope tags.

Immunofluorescence analysis, without and with detergent treatment to permeabilize the cell membrane, was used to test the predicted membrane orientation of the N- and C-termini of SR-BI. COS-7 cells expressing the tagged versions of SR-BI were stained with antibodies directed either against the extracellular domain of SR-BI (#356) or against epitopes at the N-terminus (Flag) or C-terminus (c-*myc*). In the presence of detergent, all three antibodies detected SR-BI (Figure 2, left panels, +). In the absence of detergent (Figure 2, left panels, −), only the antibody directed against the extracellular domain of SR-BI stained the cells. No staining of vector-transfected COS-7 cells was seen with the above antibodies (data not shown). An anti-histone antibody, used as a control for membrane permeabilization, stained nuclei only in the presence of detergent (data not shown). These results indicate that the N- and C-termini of SR-BI are cytoplasmically oriented and not accessible to antibodies unless the plasma membrane is permeabilized.

Similar experiments were performed with the tagged CD36 receptor to confirm its topology in the transiently transfected COS-7 cells. In the presence of detergent, all three antibodies (anti-CD36, anti-Flag, and anti-*myc*) detected abundant CD36 expression (Figure 2, right panels, +). In the absence of detergent (Figure 2, right panels, −), only the antibody directed against the extracellular domain detected CD36 expression. No staining of vector-transfected COS-7 cells was seen with the above antibodies (data not shown). Thus, these data confirm the topology of CD36 (*44*, *45*). Based on these results, previous data (*23*), and the predicted topologies of both receptors, Figure 3 shows the membrane orientations of wild-type SR-BI and CD36 and the mutant and chimeric receptors analyzed in this study. The amino acid delineations of these receptors are given in the figure legend.

Role of the SR-BI C-Terminal Cytoplasmic Tail in HDL CE Selective Uptake. SR-BII is identical to SR-BI except that it contains a different C-terminal cytoplasmic tail as a result of alternate splicing of SR-BI pre-mRNA (*20*, *46*).

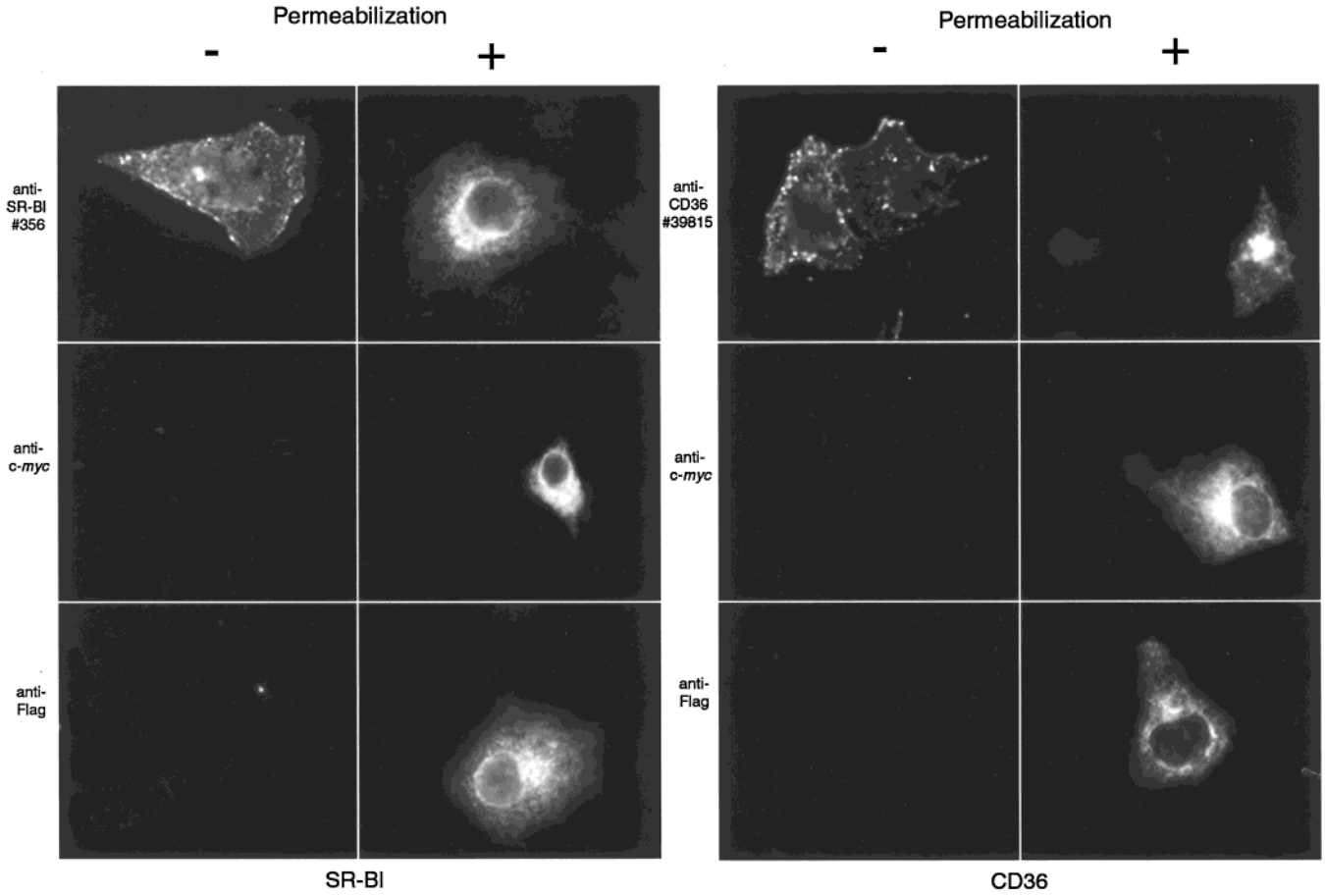

FIGURE 2: Immunofluorescence with epitope-tagged SR-BI and CD36. COS-7 cells expressing SR-BI or CD36 (upper panels), SR-BI(MC) or CD36(MC) (middle panels), or SR-BI(FN) or CD36(FN) (lower panels) were processed for immunofluorescence in the absence or presence of detergent for permeabilization of the plasma membrane as indicated (− or +). The cells were stained with primary antibodies directed against either the extracellular domain of SR-BI (#356) or CD36 (39815) or tags at the N-terminus (anti-Flag) or C-terminus (anti-*myc*) as indicated. Texas Red sulfonyl chloride-conjugated donkey anti-rabbit or anti-mouse secondary IgG was used for visualization by fluorescence microscopy. Note that much of the plasma membrane staining of SR-BI and CD36 is lost upon detergent permeabilization of the cells whereas the Golgi staining is preserved.

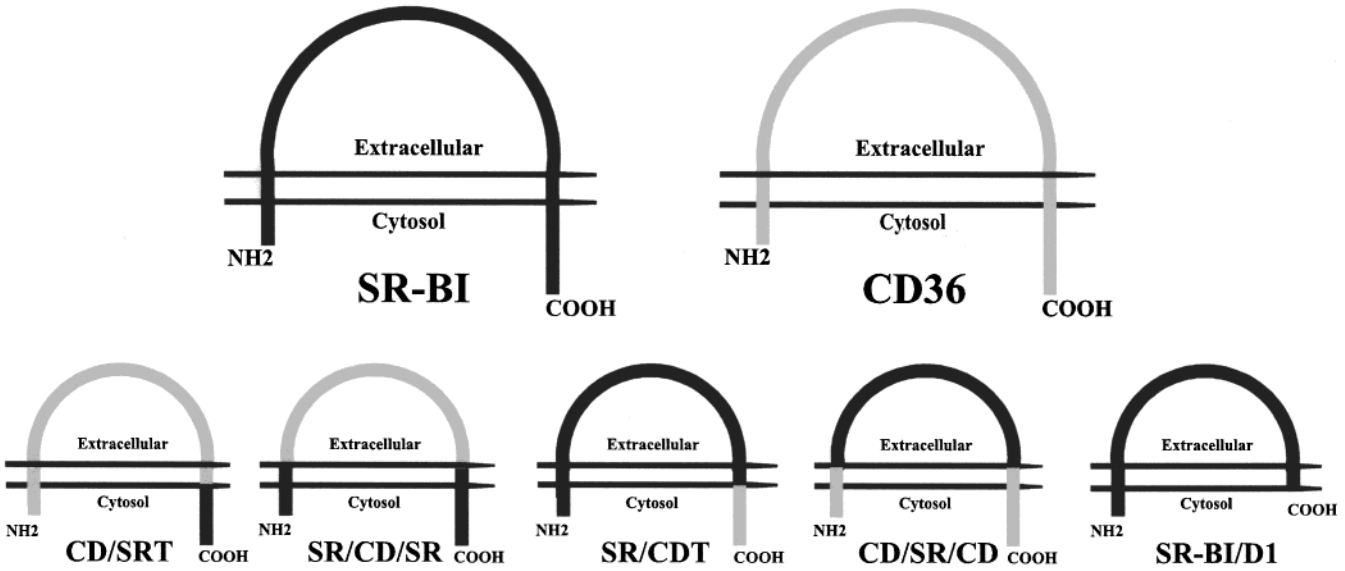

FIGURE 3: Schematic diagram of SR-BI, CD36, and chimeric receptors. This diagram illustrates the topology of SR-BI and CD36 in comparison to the chimeric receptors. The amino acid delineations of the chimeras are as follows: CD/SRT = CD36 a.a. 1−458 and SR-BI a.a. 464−509; SR/CD/SR = CD36 a.a. 44−435 and SR-BI a.a. 1−41 and 436−509; SR/CDT = SR-BI a.a. 1−467 and CD36 a.a. 459−472; CD/SR/CD = CD36 a.a. 1−40 and 441−472 and SR-BI a.a. 43−440; and SR-BI/D1 = SR-BI a.a. 1−467.

Previous studies showed that SR-BII binds HDL particles with the same affinity as SR-BI, but shows 5−7-fold reduced HDL CE selective uptake activity compared to SR-BI (*25*, *46*). This result suggests either that the SR-BI C-terminal

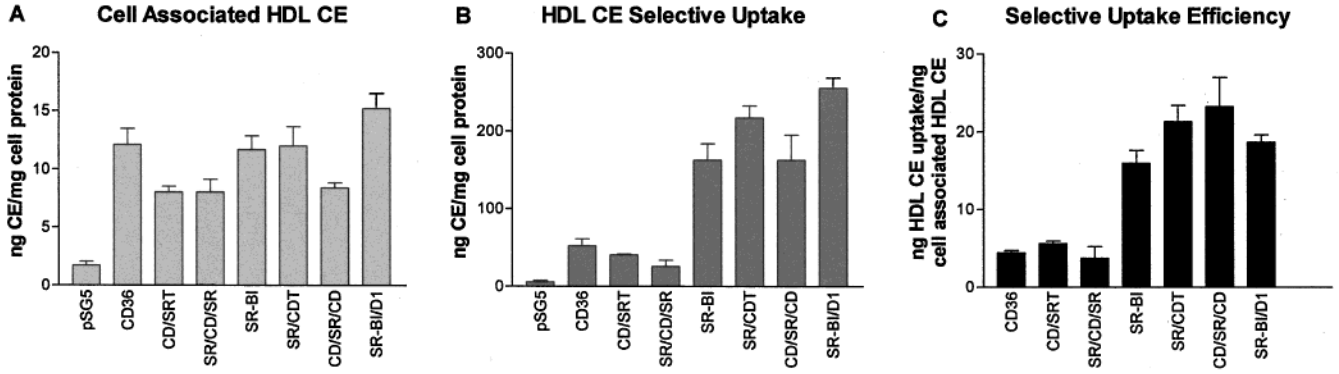

FIGURE 4: Cell-associated HDL CE and HDL CE selective uptake mediated by SR-BI, CD36, or chimeric receptors. COS-7 cells transfected with vector pSG5 or transiently expressing CD36, CD/SRT, SR/CD/SR, SR-BI, SR/CDT, CD/SR/CD, or SR-BI/D1 (Figure 3) were incubated at 37 °C for 1.5 h with 10 μg/mL [^{125}I, ^{3}H]-HDL$_3$, after which cells were processed to determine cell-associated HDL CE (A) and HDL CE selective uptake (B). Values represent the mean of triplicate determinations after subtraction of values obtained with the addition of a 40-fold excess of unlabeled HDL$_3$. (C) The efficiency of HDL CE selective uptake was determined by subtracting the values from vector-transfected cells, and normalizing the amount of HDL CE selective uptake to the amount of cell-associated HDL particles.

tail is essential for high-efficiency HDL CE selective uptake or that the SR-BII C-terminal tail is in some way inhibitory to this process. To distinguish between these possibilities, the C-terminal tail of SR-BI was deleted to produce SR-BI/D1 (Figure 3). This receptor is truncated at residue Q467, which is three residues C-terminal to the transmembrane domain and corresponds to the last common amino acid between SR-BI and SR-BII. As shown in Figure 4, analysis of SR-BI/D1 for HDL cell association (panel A), HDL CE selective uptake (panel B), and selective uptake efficiency (normalized to the amount of receptor-bound HDL) (panel C) showed that the receptor lacking the C-terminal tail was fully active and equivalent to wild-type SR-BI. In addition, the chimeric receptor, SR/CDT, in which the C-terminal tail of CD36 was swapped for that of SR-BI, was as active as wild-type SR-BI and SR-BI/D1. Note also that the various chimeric receptors containing the extracellular domain of CD36 showed the same low level of HDL CE selective uptake activity as CD36 (Figure 4) irrespective of whether they contained the cytoplasmic N- and C-terminal tails or the transmembrane domains of SR-BI. In contrast, the chimeric receptors containing the extracellular domain of SR-BI showed the enhanced HDL CE selective uptake activity characteristic of SR-BI irrespective of whether they contained the C-terminal tail or the N- and C-terminal tails plus the transmembrane domains of SR-BI or CD36. Thus, these data confirm and extend the results (*25*, *26*) indicating that HDL CE selective uptake activity is an inherent property of the SR-BI extracellular domain.

Domains of SR-BI Required for the Bi-directional Flux of Free Cholesterol. In addition to mediating HDL CE selective uptake, SR-BI accelerates the efflux of free cholesterol from cells to HDL (*28*−*30*, *34*). In contrast, CD36, which binds HDL with high affinity (*24*−*26*), does not significantly increase FC efflux to HDL (*28*, *34*). Similarly, SR-BI increases the influx of HDL FC into cells (*28*, *29*, *31*, *34*) whereas CD36 does not (*28*, *34*), despite the fact that both receptors bind HDL to the cell surface and both receptors are localized to cholesterol-rich caveolar fractions of the plasma membrane (*21*−*23*, *47*). These studies led to the conclusion that simply binding HDL particles to cholesterol-rich domains of the plasma membrane is not sufficient to enhance the bi-directional movement of FC. In addition to binding HDL, SR-BI must provide a specific facilitation of FC movement into and out of the plasma membrane (*28*).

In the present study, chimeric receptors (Figure 3) composed of different domains of SR-BI and CD36 were used to determine which domains of SR-BI are essential for the enhancement of FC flux between cells and HDL and to test which domains of CD36 can functionally substitute for the corresponding domains in SR-BI. Accordingly, COS-7 cells were transfected with the SR-BI/CD36 chimeric receptors, wild-type SR-BI, CD36, or vector alone, and cellular FC efflux was measured to 25 or 250 μg/mL HDL$_3$. Parallel dishes of the same cells were assayed for binding of radiolabeled HDL to estimate cell surface expression of each receptor in order to normalize FC efflux to the receptor expression level. As illustrated by the representative experiment in Figure 5, all receptors which contain the extracellular domain of SR-BI stimulated FC efflux to HDL$_3$ (panels B and C) irrespective of whether the cytoplasmic and transmembrane domains were derived from SR-BI or from CD36. In addition, the SR-BI mutant lacking the C-terminal tail (SR-BI/D1) showed full FC efflux activity.

Similar experiments were carried out to examine the activity of the chimeric receptors to stimulate the influx of FC from HDL to the cell membrane. As shown in Figure 6 (panel B), FC influx from HDL behaved similarly to FC efflux in that receptors containing the extracellular domain of SR-BI showed enhanced activity compared to receptors containing the extracellular domain of CD36. This difference in influx activity between SR-BI and CD36 was seen irrespective of whether the transmembrane and cytoplasmic domains were derived from SR-BI or CD36. Additionally, deletion of the C-terminal cytoplasmic tail in SR-BI/D1 did not significantly alter FC influx activity.

Domains of SR-BI Required for the Alteration of Membrane Cholesterol Content and Distribution. Previous studies showed that SR-BI expression alters the content and distribution of cholesterol among plasma membrane domains as judged by several experimental parameters. The SR-BI-mediated increase in membrane cholesterol content was detected as a net increase in cellular FC mass and as a disproportionately enhanced fractional esterification of newly influxed HDL ^{3}H-FC (*34*). The latter effect can be explained by the sigmoidal cholesterol substrate dependence of acyl-

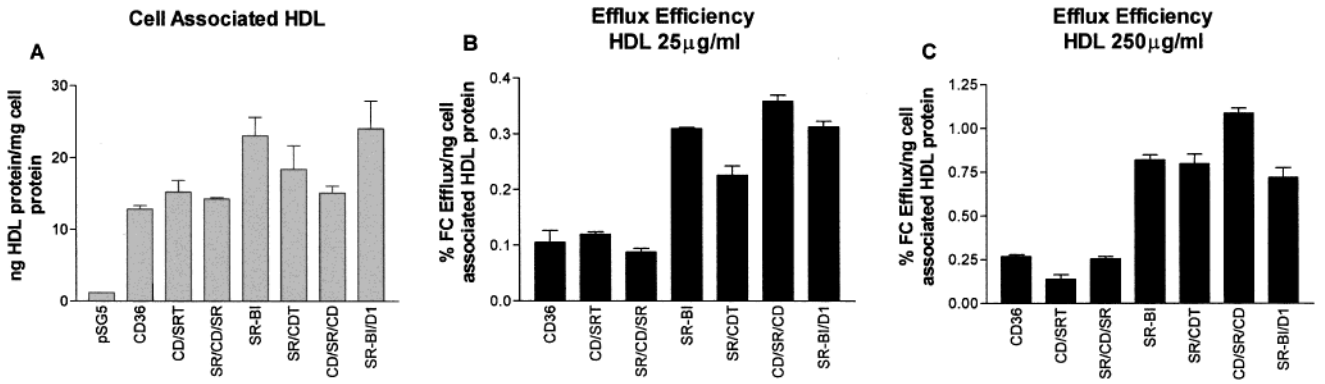

FIGURE 5: Cell-associated HDL and FC efflux mediated by SR-BI, CD36, or chimeric receptors. (A) COS-7 cells transfected with vector pSG5 or transiently expressing CD36, CD/SRT, SR/CD/SR, SR-BI, SR/CDT, CD/SR/CD, or SR-BI/D1 (Figure 3) were incubated at 37 °C for 1 h with [^{125}I, ^{3}H]-hHDL$_3$ to determine cell surface receptor expression as described under Experimental Procedures. Parallel wells of cells that had been prelabeled with [^{3}H]-cholesterol were incubated with 25 μg of protein/mL or 250 μg of protein/mL of HDL$_3$ for 4 h to measure the efflux of [^{3}H]-cholesterol. After incubation, cells were harvested to determine the amount of [^{3}H]-cholesterol released from the cells. Values are the mean of triplicate determinations. The efficiency of FC efflux to 25 μg/mL HDL$_3$ (B) or 250 μg/mL HDL$_3$ (C) was determined by subtracting the values from vector-transfected cells, and normalizing the percent FC efflux to the amount of cell-associated HDL particles determined in (A).

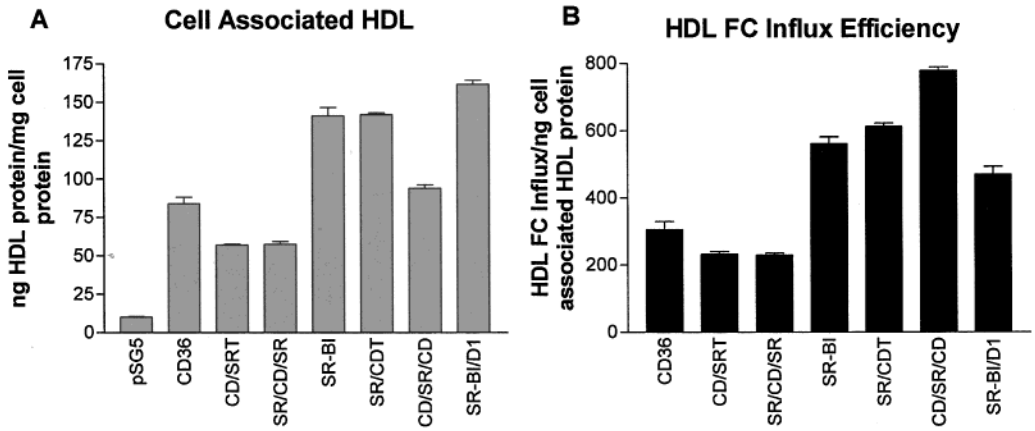

FIGURE 6: Cell-associated HDL and HDL FC influx mediated by SR-BI, CD36, or chimeric receptors. (A) COS-7 cells transfected with vector pSG5 or transiently expressing CD36, CD/SRT, SR/CD/SR, SR-BI, SR/CDT, CD/SR/CD, or SR-BI/D1 (Figure 3) were incubated at 37 °C for 1 h with [^{125}I, ^{3}H]-hHDL$_3$ to determine cell surface receptor expression as described under Experimental Procedures. (B) Parallel wells of cells were incubated at 37 °C for 2 h with 10 μg/mL [^{3}H]-cholesterol-labeled HDL$_3$ to measure FC influx [μg of FC h^{-1} (mg of cell protein)$^{-1}$]. The efficiency of FC influx was determined by subtracting the values from vector-transfected cells and normalizing the amount of HDL FC influx to the amount of cell-associated HDL particles determined in (A). Values are the mean of triplicate determinations.

CoA cholesterol acyltransferase (*48*) which gives rise to an apparent threshold in plasma membrane cholesterol content that is necessary to drive esterification (*49*). The altered distribution of cholesterol among membrane domains was detected by an SR-BI-dependent increase in the sensitivity of membrane cholesterol to treatment of cells with extracellular cholesterol oxidase (*28*, *34*) and by an increase in the size of the fast kinetic pool for cellular cholesterol efflux to cyclodextrin acceptors (*34*). To determine the SR-BI domains responsible for these parameters of cholesterol metabolism, chimeric receptors were first tested for their effect on cellular cholesterol mass when cells were grown in serum-containing medium. The data in Table 1 show that cells expressing SR-BI and chimeric receptors containing the extracellular domain of SR-BI had increased cellular total cholesterol mass that was due to increases in both free cholesterol and cholesteryl ester. In contrast, no increase in cellular cholesterol mass was seen in cells expressing CD36 or chimeric receptors containing the extracellular domain of CD36 irrespective of whether the other receptor domains were derived from SR-BI or CD36. Thus, the enhanced cellular free cholesterol content is specific for SR-BI and is due to its extracellular domain.

Table 1: Cholesterol Mass in COS-7 Cells Expressing SR-BI, CD36, or Chimeric Receptors[a]

receptor	total cholesterol	free cholesterol (μg/mg of cell protein)	ester cholesterol
PSG5 vector	26.4 ± 0.2	22.8 ± 0.8	3.6 ± 0.7
CD36	26.8 ± 0.8	23.8 ± 1.1	3.0 ± 0.9
CD/SRT	28.0 ± 0.3	23.4 ± 1.0	4.5 ± 0.8
SR/CD/SR	27.2 ± 1.7	23.3 ± 1.3	3.9 ± 0.4
SR-BI	40.5 ± 0.8	30.5 ± 0.2	10.0 ± 0.9
SR/CDT	42.9 ± 0.4	31.5 ± 0.2	11.5 ± 0.2
CD/SR/CD	40.3 ± 2.4	30.1 ± 1.8	10.2 ± 1.5
SR-BI/D1	42.2 ± 1.0	30.6 ± 1.0	11.6 ± 0.2

[a] COS-7 cells transfected with vector pSG5 or transiently expressing CD36, CD/SRT, SR/CD/SR, SR-BI, SR/CDT, CD/SR/CD, or SR-BI/D1 (Figure 3) were incubated 24 h in Dulbecco's minimal essential medium containing 10% calf serum. Lipids were extracted, and cholesterol mass was measured by gas chromatography. Values are the mean (±standard deviation) of triplicate determinations.

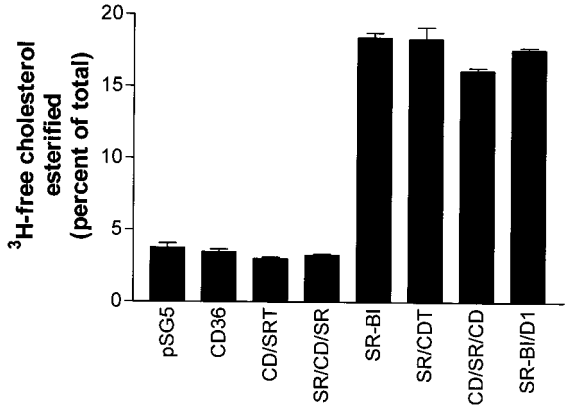

FIGURE 7: Esterification of newly influxed [^{3}H]-cholesterol in cells expressing SR-BI, CD36, or chimeric receptors. (A) COS-7 cells transfected with vector pSG5 or transiently expressing CD36, CD/SRT, SR/CD/SR, SR-BI, SR/CDT, CD/SR/CD, or SR-BI/D1 (Figure 3) were incubated for 24 h with 5 μCi/mL [^{3}H]-cholesterol in serum-containing medium as described under Experimental Procedures. Cells were then harvested, and the percent distribution of [^{3}H]-cholesterol between free and esterified cholesterol was determined by thin-layer chromatography of the lipid extract. Values are means of triplicate determinations.

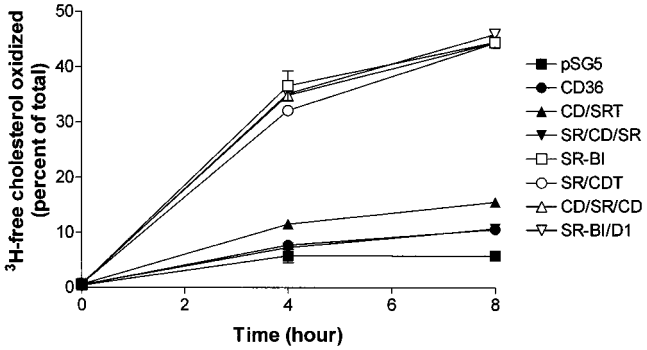

FIGURE 8: Cholesterol oxidase sensitivity of membrane cholesterol in cells expressing SR-BI, CD36, or chimeric receptors. COS-7 cells transfected with vector pSG5 or transiently expressing CD36, CD/SRT, SR/CD/SR, SR-BI, SR/CDT, CD/SR/CD, or SR-BI/D1 (Figure 3) were incubated for 24 h with 5 μCi/mL [^{3}H]-cholesterol in serum-containing medium as described under Experimental Procedures. After washing, cells were incubated with exogenous cholesterol oxidase for 4 or 8 h, and the percent of cellular [^{3}H]-cholesterol oxidized was determined. Values are the mean of triplicate determinations.

The effect of SR-BI on the fractional esterification of newly influxed cholesterol was tested by incubating transfected cells in serum-containing medium with ^{3}H-FC followed by lipid extraction and determination of the distribution of ^{3}H-FC between free and esterified cholesterol by thin-layer chromatography. The data in Figure 7 show that the fractional esterification of ^{3}H-FC was enhanced nearly 6-fold by SR-BI compared to CD36. Cells expressing chimeric receptors containing the extracellular domain of SR-BI showed a similar enhancement in ^{3}H-FC esterification irrespective of whether the other receptor domains were derived from SR-BI or CD36. Similarly, cells expressing chimeric receptors containing the extracellular domain of CD36 showed reduced fractional esterification activity even when the other domains of the receptor were derived from SR-BI. As in the case of the parameters measured above, deletion of the C-terminal cytoplasmic tail of SR-BI had no effect on the fractional esterification activity.

The distribution of membrane cholesterol in cells expressing wild-type and chimeric receptors was tested by determining the sensitivity of membrane cholesterol in unfixed cells to extracellular cholesterol oxidase (*34*). This protocol has been reported to primarily oxidize cholesterol localized to plasma membrane caveolae (*40*). The data in Figure 8 show the percent of cholesterol oxidized at two time intervals of oxidase treatment in cells transfected with vector or with wild-type or chimeric receptors. As is evident, the enhanced sensitivity to extracellular cholesterol oxidase was seen with wild-type SR-BI and with all chimeric receptors containing the extracellular domain of SR-BI irrespective of whether the other receptor domains were derived from SR-BI or CD36. In addition, deletion of the C-terminal tail of SR-BI had no effect on this activity. The transmembrane and cytoplasmic domains of SR-BI did not confer the enhanced sensitivity to cholesterol oxidase upon the extracellular domain of CD36.

DISCUSSION

Topology of SR-BI and CD36. Secondary structure predictions suggest two membrane-spanning regions in each of the structurally related receptors, SR-BI and CD36. These putative transmembrane domains reside near the amino- and carboxyl-terminal ends of these proteins. In the case of SR-BI, a previous immunofluorescence study with antibody directed to the C-terminal tail supported the cytoplasmic location of this domain (*23*) although the membrane disposition of the N-terminus had not been tested. In addition, Cys462 and Cys470 in the predicted C-terminal cytoplasmic domain were shown to be fatty acylated (*26*). In the present study, we appended epitope tags to either end of SR-BI and showed by immunofluorescence that detection of the N- or C-terminus required detergent permeabilization of the membrane. Thus, both N- and C-termini of SR-BI are cytoplasmically oriented. In the case of CD36, our results confirm the similar membrane orientation of the N- and C-termini of this receptor (*44, 45*). Thus, these results further support the models that SR-BI and CD36 have similar topologies with two transmembrane domains, a single large extracellular domain, and the N- and C-termini located on the cytoplasmic side of the membrane (Figure 3). The presence of a single large extracellular domain in SR-BI is also supported by experimental data showing that epitope tags inserted after amino acids 48, 80, 124, 178, 192, 283, 289, 341, 345, 388, or 417 were all detectable by flow cytometry without membrane permeablization when expressed in COS-7 cells (Connelly, M. A., and Williams, D. L., data not shown). These data, although supportive of the proposed topology, must be qualified in that none of the receptors tagged in the extracellular domain, despite expression on the cell surface, had full HDL binding activity and some were completely inactive (data not shown). In contrast, the receptors tagged at the N- and C-termini were fully active.

Domains of SR-BI Required for Free Cholesterol and Cholesteryl Ester Flux and Perturbation of Membrane Cholesterol Content and Distribution. Previous studies showed that the extracellular domain of SR-BI, in addition to its role in binding HDL particles, is required for the efficient selective uptake of HDL CE (*25, 26*). The reduced efficiency of HDL CE selective uptake with SR-BII (*25, 46*), however, suggested that the SR-BI C-terminal tail is essential

for high-efficiency HDL CE selective uptake, an activity that could be shared by the corresponding domain of CD36. Alternatively, the SR-BII C-terminal tail could be inhibitory to the selective uptake process. The present analysis of the SR-BI/D1 receptor lacking the C-terminal tail showed that this receptor has full HDL CE selective uptake activity. Thus, the C-terminal tail of SR-BI does not impart an enhanced HDL CE selective uptake activity. Rather, the C-terminal tail of SR-BII inhibits HDL CE uptake activity. The mechanism of this inhibition is unclear, but it presumably does not reflect a difference in targeting to plasma membrane domains since both SR-BI and SR-BII have been localized to caveolar fractions in biochemical fractionation experiments (*46*).

Although the transmembrane domains and the N-terminal tail of SR-BI appear to provide no specific function in HDL CE selective uptake, these domains likely serve for the proper targeting of the receptor to the plasma membrane. As noted above, the C-terminal tail of SR-BI is not required for either CE transfer or correct membrane targeting. Also, as shown in the experiments reported here, SR-BI lacking the C-terminal tail showed no impairment in mediating the bidirectional flux of free cholesterol, in the accumulation of cellular free cholesterol and cholesteryl ester mass, or in the enhancement of cholesterol oxidase sensitivity. Although the C-terminal tail of SR-BI appears to have no discernible function in these various aspects of cellular cholesterol metabolism, a recent report identified a PDZ-domain-containing protein, CLAMP, that interacts specifically with the SR-BI C-terminal tail when expressed in CHO cells (*50*). This protein appears to yield higher expression levels of SR-BI in transfected cells, suggesting that the C-terminal tail may be important for SR-BI processing or turnover. These authors also suggested that CLAMP may interact with the C-terminal tail of SR-BI to inhibit the hydrolysis of HDL CE.

The present analysis of wild-type and chimeric receptors for free cholesterol efflux and influx showed that these activities required the extracellular domain of SR-BI, and that the transmembrane and cytoplasmic domains of either SR-BI or CD36 could provide full activity. Similarly, the transmembrane and cytoplasmic domains of SR-BI did not confer these functions on the extracellular domain of CD36 despite its ability to bind HDL particles with high affinity. A similar localization of function to the extracellular domain of SR-BI was seen also in parameters that reflect the cholesterol content of the membrane. Thus, both the SR-BI-mediated increase in cellular free cholesterol mass (Table 1) and the SR-BI-mediated enhancement in the esterification of newly influxed free cholesterol (Figure 7) tracked closely with the extracellular domain.

This localization of function to the extracellular domain of SR-BI was also observed in the sensitivity of SR-BI-expressing cells to extracellular cholesterol oxidase (Figure 8). Under these assay conditions, cholesterol oxidase sensitivity appears to reflect the cholesterol content of membrane caveolae as judged by biochemical fractionation studies (*40*). Previous studies showed that a major fraction of SR-BI is present in caveolar domains of transfected CHO cells and the Y1-BS1 adrenocortical cell (*23*). More recent work has shown a similar localization of both SR-BI and CD36 to caveolar domains of vascular endothelial cells (*47*). In this case, SR-BI has been shown to maintain the cholesterol content of caveolae by mediating cholesterol uptake from HDL. This activity appears to be necessary for the localization and activation of endothelial nitric oxide synthase in caveloae (*47*). The SR-BI-mediated increase in membrane free cholesterol and the enhanced sensitivity to extracellular cholesterol oxidase in transfected COS-7 cells may reflect a similar role for SR-BI in maintaining caveolar cholesterol content and function.

Localization of SR-BI Function to the Extracellular Domain. The present studies as well as those reported earlier on HDL CE selective uptake (*25, 26*) indicate that a spectrum of rather distinct SR-BI-mediated activities is localized to the extracellular domain of this receptor. The transmembrane domains and the N-terminal tail of SR-BI appear to provide no specific function for this panel of activities other than proper targeting of the receptor to the plasma membrane. The C-terminal tail of SR-BI is dispensable for membrane targeting as well as the function of the receptor, at least for those facets of cholesterol metabolism that have been tested thus far. Given the distinct nature of some of these SR-BI-mediated effects, it would be reasonable to suspect that some effects would require the extracellular domain of the receptor while others might not. HDL CE selective uptake, for example, appears to involve the docking of HDL particles to SR-BI via direct interaction with apoA-I (*51*) followed by the selective transfer of HDL core lipids into the plasma membrane of the cell (*11, 52*). In this process, HDL cholesteryl esters move down their concentration gradient to the plasma membrane through a postulated hydrophobic "channel" that is formed by or requires the extracellular domain of the receptor (*52*). This process appears to be an inherent property of the SR-BI extracellular domain as it occurs in the absence of the C-terminal tail and occurs with the CD36 transmembrane domains substituted for these domains in SR-BI. In contrast to this process of CE transfer from the HDL particle, the SR-BI-mediated enhancement in cholesterol oxidase sensitivity occurs in the absence of HDL binding, is correlated with the accumulation of free cholesterol in the plasma membrane, and appears to reflect a redistribution of cholesterol among membrane domains (*34*). Surprisingly, this membrane organizational effect also is localized to the extracellular domain of SR-BI, does not require the C-terminal cytoplasmic domain, and occurs with the CD36 transmembrane domains substituted for these domains in SR-BI. Two hypotheses may be suggested to account for these results.

First, the extracellular domain of SR-BI may mediate multiple and distinct activities by virtue of discrete functional subdomains or via interaction with other membrane proteins. Evidence such as the nonreciprocal cross-competition between HDL and LDL (*9*), identification of mutations that disrupt HDL but not LDL binding (*53, 54*), and the observation that SR-BI exhibits both high- and low-affinity binding of HDL particles (*14, 51, 52*) points to the presence of multiple binding sites on SR-BI. In addition, a recent study showed that antibody to the extracellular domain of SR-BI blocked HDL binding without altering the SR-BI-mediated increase in cholesterol oxidase sensitivity (*53, 54*). This result, as well, is suggestive of multiple functional domains in the extracellular loop of SR-BI. Further dissection of the extracellular domain by mutagenesis will serve to test

whether the extracellular loop of SR-BI contains subdomains that mediate distinct activities.

The second hypothesis is that the apparently distinct functional activities of SR-BI are, in fact, secondary to, and driven by, changes in membrane cholesterol content. In this case, loading cholesterol into the membrane via the selective uptake of cholesteryl ester or the influx of free cholesterol may provoke reorganization of membrane lipid domains that secondarily give rise to increased cholesterol flux and enhanced sensitivity to cholesterol oxidase. The reorganization of membrane lipids, as reflected by enhanced cholesterol oxidase sensitivity and an enlarged fast kinetic pool for FC efflux to cyclodextrins (*28*, *34*), may be due to cholesterol−phospholipid interactions that result in membrane raft or caveola formation (*55*) or modulate the chemical activity of cholesterol in the plasma membrane (*56*). Mutagenesis studies may prove useful in testing this hypothesis, but it will be equally important in future studies to more carefully define how SR-BI alters the composition and organization of plasma membrane lipids.

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REFERENCES

1. Miller, G. J., and Miller, N. E. (1975) *Lancet 1*, 16.
2. Glomset, J. A. (1968) *J. Lipid Res. 9*, 155.
3. Glass, C., Pittman, R. C., Civen, M., and Steinberg, D. (1985) *J. Biol. Chem. 260*, 744.
4. Reaven, E., Chen, Y.-D. I., Spicher, M., and Azhar, S. (1984) *J. Clin. Invest. 74*, 1384.
5. Gwynne, J. T., and Hess, B. (1980) *J. Biol. Chem. 255*, 10875.
6. Glass, C., Pittman, R. C., Weinstein, D. B., and Steinberg, D. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 5435.
7. Stein, Y., Dabach, Y., Hollander, G., Halperin, G., and Stein, O. (1983) *Biochim. Biophys. Acta 752*, 98.
8. Pittman, R. C., Knecht, T. P., Rosenbaum, M. S., and Taylor, C. A., Jr. (1987) *J. Biol. Chem. 262*, 2443.
9. Acton, S., Rigotti, A., Landschulz, K. T., Xu, S., Hobbs, H. H., and Krieger, M. (1996) *Science 271*, 518.
10. Krieger, M. (1999) *Annu. Rev. Biochem. 68*, 523.
11. Williams, D. L., Connelly, M. A., Temel, R. E., Swarnakar, S., Phillips, M. C., de la Llera-Moya, M., and Rothblat, G. H. (1999) *Curr. Opin. Lipidol. 10*, 329.
12. Landschulz, K. T., Pathak, R. K., Rigotti, A., Krieger, M., and Hobbs, H. H. (1996) *J. Clin. Invest. 98*, 984.
13. Rigotti, A., Edelman, E. R., Seifert, P., Iqbal, S. N., DeMattos, R. B., Temel, R. E., Krieger, M., and Williams, D. L. (1996) *J. Biol. Chem. 271*, 33545.
14. Temel, R. E., Trigatti, B., DeMattos, R. B., Azhar, S., Krieger, M., and Williams, D. L. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 13600.
15. Rigotti, A., Trigatti, B., Babitt, J., Penman, M., Xu, S., and Krieger, M. (1997) *Curr. Opin. Lipidol. 8*, 181.
16. Rigotti, A., Trigatti, B. L., Penman, M., Rayburn, H., Herz, J., and Krieger, M. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 12610.
17. Calvo, D., and Vega, M. A. (1993) *J. Biol. Chem. 268*, 18929.
18. Acton, S. L., Scherer, P. E., Lodish, H. F., and Krieger, M. (1994) *J. Biol. Chem. 269*, 21003.
19. Calvo, D., Dopazo, J., and Vega, M. A. (1995) *Genomics 25*, 100.
20. Webb, N. R., de Villiers, W. J., Connell, P. M., De Beer, F. C., and van der Westhuyzen, D. R. (1997) *J. Lipid Res. 38*, 1490.
21. Lisanti, M. P., Scherer, P. E., Vidugiriene, J., Tang, Z., Hermanowski-Vosatka, A., Tu, Y.-H., Cook, R. F., and Sargiacomo, M. (1994) *J. Cell Biol. 126*, 111.
22. Dorahy, D. J., Lincz, L. F., Meldrum, C. J., and Burns, G. F. (1996) *Biochem. J. 319*, 67.
23. Babitt, J., Trigatti, B., Rigotti, A., Smart, E., Anderson, R. G. W., Xu, S., and Krieger, M. (1997) *J. Biol. Chem. 272*, 13242.
24. Calvo, D., Gomez-Coronado, D., Suarez, Y., Lasuncion, M. A., and Vega, M. A. (1998) *J. Lipid Res. 39*, 777.
25. Connelly, M. A., Klein, S. M., Azhar, S., Abumrad, N. A., and Williams, D. L. (1999) *J. Biol. Chem. 274*, 41.
26. Gu, X., Trigatti, B., Xu, S., Acton, S., Babitt, J., and Krieger, M. (1998) *J. Biol. Chem. 273*, 26338.
27. Rothblat, G. H., de la Llera-Moya, M., Atger, V., Kellner-Weiber, G., Williams, D. L., and Phillips, M. C. (1999) *J. Lipid Res. 40*, 781.
28. de la Llera-Moya, M., Rothblat, G. H., Connelly, M. A., Kellner-Weiber, G., Sakar, S. W., Phillips, M. C., and Williams, D. L. (1999) *J. Lipid Res. 40*, 575.
29. Ji, Y., Jian, B., Wang, N., Sun, Y., de la Llera Moya, M., Phillips, M. C., Rothblat, G. H., Swaney, J. B., and Tall, A. R. (1997) *J. Biol. Chem. 272*, 20982.
30. Jian, B., de la Llera-Moya, M., Ji, Y., Wang, N., Phillips, M. C., Swaney, J. B., Tall, A. R., and Rothblat, G. H. (1998) *J. Biol. Chem. 273*, 5599.
31. Stangl, H., Hyatt, M., and Hobbs, H. H. (1999) *J. Biol. Chem. 274*, 32692.
32. Ji, Y., Wang, N., Ramakrishnan, R., Sehayek, E., Huszar, D., Breslow, J. L., and Tall, A. R. (1999) *J. Biol. Chem. 274*, 33398.
33. Kozarsky, K. F., Donahee, M. H., Rigotti, A., Iqbal, S. N., Edelman, E. R., and Krieger, M. (1997) *Nature 387*, 414.
34. Kellner-Weiber, G., de la Llera-Moya, M., Connelly, M. A., Stoudt, G., Christian, A. E., Haynes, M. P., Williams, D. L., and Rothblat, G. H. (2000) *Biochemistry 39*, 221.
35. Azhar, S., Nomoto, A., Leers-Sucheta, S., and Reaven, E. (1998) *J. Lipid Res. 39*, 1616.
36. Baillie, A. G. S., Coburn, C. T., and Abumrad, N. A. (1996) *J. Membr. Biol. 153*, 75.
37. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.
38. Havel, R. J., Eder, H. A., and Bragdon, J. H. (1955) *J. Clin. Invest. 34*, 1345.
39. Francone, O. L., Haghpassand, M., Bennett, J. A., Royer, L., and McNeish, J. (1997) *J. Lipid Res. 38*, 813.
40. Smart, E. J., Ying, Y. S., Conrad, P. A., and Anderson, R. G. W. (1994) *J. Cell Biol. 127*, 1185.
41. Klansek, J. J., Yancey, P., St. Clair, R. W., Fischer, R. T., Johnson, W. J., and Glick, J. M. (1995) *J. Lipid Res. 36*, 2261.
42. Berrios, M. (1989) *Med. Lab. Sci. 46*, 276.
43. Fisher, P. A., Berrios, M., and Blobel, G. (1982) *J. Cell Biol. 92*, 674.
44. Gruarin, P., Thorne, R. F., Dorahy, D. J., Burns, G. F., Sitia, R., and Alessio, M. (2000) *Biochem. Biophys. Res. Commun. 275*, 446.
45. Tao, N., Wagner, S. J., and Lublin, D. M. (1996) *J. Biol. Chem. 271*, 22315.
46. Webb, N. R., Connell, P. M., Graf, G. A., Smart, E. J., de Villiers, W. J. S., De Beer, F. C., and van der Westhuyzen, D. (1998) *J. Biol. Chem. 273*, 15241.
47. Uittenbogaard, A., Shaul, P. W., Yuhanna, I. S., Blair, A., and Smart, E. J. (2000) *J. Biol. Chem. 275*, 11278.
48. Chang, C. C., Lee, C. Y., Chang, E. T., Cruz, J. C., Levesque, M. C., and Chang, T. Y. (1998) *J. Biol. Chem. 273*, 35132.

49. Xu, X.-X., and Tabas, I. (1991) *J. Biol. Chem. 266*, 17040.
50. Ikemoto, M., Arai, H., Feng, D., Tanaka, K., Aoki, J., Dohmae, N., Takio, K., Adachi, H., Tsujimoto, M., and Inoue, K. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 6538.
51. Williams, D. L., de la Llera-Moya, M., Thuahnai, S. T., Lund-Katz, S., Connelly, M. A., Azhar, S., Anantharamaiah, G. M., and Phillips, M. C. (2000) *J. Biol. Chem. 275*, 18897.
52. Rodrigueza, W. V., Thuahnai, S. T., Temel, R. E., Lund-Katz, S., Phillips, M. C., and Williams, D. L. (1999) *J. Biol. Chem. 274*, 20344.
53. Gu, X., Kozarsky, K., and Krieger, M. (2000) *J. Biol. Chem. 275*, 29993.
54. Gu, X., Lawrence, R., and Krieger, M. (2000) *J. Biol. Chem. 275*, 9120.
55. Brown, D. A., and London, E. (1998) *Annu. Rev. Cell Dev. Biol. 14*, 111.
56. Radhakrishnan, A., Anderson, T. G., and McConnell, H. M. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 12422.

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