

Changes in Plasma Membrane Properties and Phosphatidylcholine Subspecies of Insect Sf9 Cells Due to Expression of Scavenger Receptor Class B, Type I, and CD36*

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In mammalian cells scavenger receptor class B, type I (SR-BI), mediates the selective uptake of high density lipoprotein (HDL) cholesteryl ester into hepatic and steroidogenic cells. In addition, SR-BI has a variety of effects on plasma membrane properties including stimulation of the bidirectional flux of free cholesterol (FC) between cells and HDL and changes in the organization of plasma membrane FC as indicated by increased susceptibility to exogenous cholesterol oxidase. Recent studies in SR-BI-deficient mice and in SR-BI-expressing Sf9 insect cells showed that SR-BI has significant effects on plasma membrane ultrastructure. The present study was designed to test the range of SR-BI effects in Sf9 insect cells that typically have very low cholesterol content and a different phospholipid profile compared with mammalian cells. The results showed that, as in mammalian cells, SR-BI expression increased HDL cholesteryl ester selective uptake, cellular cholesterol mass, FC efflux to HDL, and the sensitivity of membrane FC to cholesterol oxidase. These activities were diminished or absent upon expression of the related scavenger receptor CD36. Thus, SR-BI has fundamental effects on cholesterol flux and membrane properties that occur in cells of evolutionarily divergent origins. Profiling of phospholipid species by electrospray ionization mass spectrometry showed that scavenger receptor expression led to the accumulation of phosphatidylcholine species with longer mono- or polyunsaturated acyl chains. These changes would be expected to decrease phosphatidylcholine/cholesterol interactions and thereby enhance cholesterol desorption from the membrane. Scavenger receptor-mediated changes in membrane phosphatidylcholine may contribute to the increased flux of cholesterol and other lipids elicited by these receptors.

Lipoproteins enable the efficient movement of lipids in an aqueous environment. The inverse correlation between high HDL¹ levels and the development of atherosclerosis and coronary artery disease has led to significant research in this field (1, 2). The basis for this atheroprotective effect of HDL, at least in part, is believed to be the transport of cholesterol from peripheral vascular tissues to the liver where cholesterol is excreted in the bile or converted to bile acids.

Scavenger receptor class B, type I (SR-BI), is a well characterized HDL receptor (3–5) that is highly expressed in tissues active in cholesterol metabolism such as the liver and steroidogenic tissues (6) and is expressed to a lesser extent in macrophages and vascular endothelium (7, 8). SR-BI plays a key role in systemic cholesterol transport by mediating the selective uptake of cholesteryl ester (CE) from HDL into the liver. Selective uptake is defined as the movement of CE from HDL particles into target cells without significant internalization and degradation of the HDL particle (9–14). This mechanism is distinct from LDL receptor-mediated lipid transport, where LDL is internalized and the LDL particle is degraded (15). The selective uptake pathway of HDL CE is a major pathway for the provision of substrate for steroid production in rodents; this pathway also exists in rabbits and is active in human hepatocytes, hepatomas, and ovarian granulosa cells (10–12, 16–22).

Although selective HDL CE uptake is a major function of SR-BI, other activities are also attributed to this receptor. SR-BI increases the bidirectional flux of free cholesterol (FC) between cells and HDL or phospholipid vesicle acceptors (23–27), an activity that may be responsible for net cholesterol removal from peripheral cells as well as the rapid hepatic clearance of HDL FC and its resultant secretion into bile (28, 29). Efflux of cholesterol from peripheral cells is the first step in reverse cholesterol transport, and recent findings show that SR-BI deficiency is associated with de-regulation of cholesterol homeostasis in the arterial wall (30) and that macrophage expression of SR-BI protects mice against aortic lesion development in atherosclerosis-susceptible mice (31, 32). These data strongly implicate SR-BI in the vascular wall, as well as in the liver, as a significant factor in suppressing atherosclerosis.

In addition to changes in the flux of FC and CE, SR-BI has effects on plasma membrane properties that may contribute to

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† We are deeply saddened by the sudden loss of our dear friend and colleague, Dr. David L. Williams, who passed away on July 16, 2004. Dave was a devoted scientist, teacher, and friend and will not be forgotten. His passing is a tremendous loss to all of us, and we will miss him dearly.

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¹ The abbreviations used are: HDL, high density lipoprotein; LDL, low density lipoprotein; SR-BI, scavenger receptor class B, type I; CE, cholesteryl ester; FC, free cholesterol; PC, phosphatidylcholine; PS, phosphatidylserine; SM, sphingomyelin; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PA, phosphatidic acid; COE, cholesteryl oleyl ether; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; h, human; gp, glycoprotein.

the mechanisms of cholesterol movement into and out of the membrane (3). For example, SR-BI expression increases the fraction of plasma membrane FC susceptible to oxidation by exogenous cholesterol oxidase (23) and increases the size of the fast kinetic pool of membrane FC for efflux to cyclodextrin acceptors (33). These findings suggest that SR-BI alters the molecular packing of FC in such a way as to enhance FC desorption from the membrane. Additionally, SR-BI has significant effects on membrane morphology as evidenced by SR-BI^{-/-} mice that have altered membrane ultrastructure and fail to form cell-surface microvillar channels on adrenal zona fasciculata cells (34). Thus, in addition to promoting cholesterol flux, SR-BI has multiple and, in some cases, dramatic effects on plasma membrane properties and structure.

In the present study SR-BI was expressed in Sf9 insect cells to determine whether the varied effects of SR-BI seen in mammalian cells also occur in a cell that has membranes with a very different lipid composition. Compared with mammalian cells (35), Sf9 cells have reduced levels of sphingomyelin and elevated levels of phosphatidylethanolamine (36, 37). Additionally, even when grown in mammalian serum, plasma membranes of Sf9 cells have very low cholesterol content and a correspondingly low cholesterol to phospholipid ratio (37, 38). Reaven *et al.* (39) showed that expression of SR-BI in insect Sf9 cells elicits the formation of double membrane structures that resemble the microvillar channels of steroidogenic tissues, indicating substantial changes in membrane morphology due to SR-BI. The present results show that, as in mammalian cells, SR-BI expression increased HDL CE-selective uptake, cellular cholesterol mass, FC efflux to HDL, and the sensitivity of membrane FC to cholesterol oxidase. These findings indicate that SR-BI has fundamental effects on cholesterol flux and membrane properties in cells of evolutionarily divergent origins with different lipid compositions. In addition, analysis of phospholipids by mass spectrometry showed substantial changes in phosphatidylcholine subspecies because of expression of SR-BI or the related class B scavenger receptor CD36.

EXPERIMENTAL PROCEDURES

Plasmids and Sequencing—PCR amplifications were performed by using a DNA Thermal Cycler 9700 (PerkinElmer Life Sciences). Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA). Plasmids used to produce the murine SR-BI and rat CD36-expressing baculoviruses were constructed as follows. For construction of *pFastBacI(SR-BI)*, primers 5'-GACCAATTGGCGGCCGCCGTCTCCTTCAGTCTCTGAGC-3' and 5'-GACCAGATCTTCTAGAGCGGACAGGTGTGACATCTGG-3' were employed to amplify the SR-BI coding region from a previously described vector, *pSG5(SR-BI)* (40). The resulting PCR products were digested with MfeI and XbaI and were ligated into the pFastBacI vector (Invitrogen), which had been restricted previously with EcoRI and XbaI. For construction of *pBakhis2A(CD36)*, the coding region of CD36 was restricted with BamHI and ligated into the BamHI site of pBakhis2A (Clontech). All plasmids were prepared using endotoxin-free maxi-prep kits (Qiagen, Valencia, CA) and sequenced throughout the coding region to confirm that no point mutations had been generated during the cloning procedures (40).

Production of Recombinant Baculoviruses in Sf9 Cells—Sf9 cells (*Spodoptera frugiperda*) were routinely maintained in Grace's supplemented insect medium (Invitrogen) containing 5–10% fetal bovine serum (Atlanta Biologicals, Norcross, GA), 50 units/ml penicillin, and 50 µg/ml streptomycin in a 27 °C incubator. Baculoviral expression of SR-BI in the Sf9 cells was accomplished using the Bac-to-Bac Baculovirus Expression System as described by the vendor (Invitrogen). Recombinant baculoviruses were harvested 72 h post-transfection and amplified three to four times by infection of Sf9 cells to produce high titer viral stocks.

Immunoblot Analysis—Baculovirus-infected Sf9 cells expressing SR-BI or CD36 (in 35-mm wells) were washed twice with phosphate-buffered saline (pH 7.4) and lysed with 300 µl of Nonidet P-40 cell lysis buffer (41, 42) containing 1 µg/ml pepstatin, 0.2 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, and 10 µg/ml aprotinin. Protein con-

centrations were determined by the method of Lowry *et al.* (43). Ten micrograms of cell lysate protein were analyzed by SDS-8% PAGE. Immunoblots were probed with antibodies directed to SR-BI (~82 kDa) and CD36 (~78 kDa) to confirm their expression in the Sf9 cells (44) (data not shown). Blots were also probed with antibody against the gp64 viral envelope protein (eBioscience).

Preparation of [¹²⁵I,³H]-hHDL—Human (h) HDL₃ (1.125 g/ml < ρ < 1.210 g/ml) (hereafter referred to as HDL) was isolated by sequential ultracentrifugation (45). HDL was labeled with [³H]cholesteryl oleyl ether ([³H]COE) or [³H]cholesteryl oleate ([³H]CE) (Amersham Biosciences) by using recombinant cholesteryl ester transfer protein as described (40, 46, 47). [³H]COE-HDL was labeled with [¹²⁵I]-dialcitol tyramine as described previously (40). 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 [¹²⁵I,³H]-HDL was 675 dpm/ng protein for [¹²⁵I] and 14 dpm/ng protein for [³H]COE.

HDL Cell Association, Selective CE Uptake, and Apolipoprotein Degradation—Sf9 cells (1 × 10⁶) were plated into 22-mm wells in growth medium and infected with 100 µl of high titer vector, SR-BI, or CD36 baculoviruses. Baculovirus-infected Sf9 cells, 72 h post-infection, were washed once with serum-free medium, 0.5% bovine serum albumin. [¹²⁵I,³H]-HDL particles were added at a concentration of 10 µg of protein/ml in serum-free medium, 0.5% bovine serum albumin and were incubated for 1.5 h at 27 °C. Values for cell-associated HDL apolipoprotein (expressed as HDL CE) and the selective uptake of HDL CE were obtained as described previously (40).

Cholesterol Efflux, Cholesterol Oxidase, and Cholesteryl Ester Hydrolysis Assays—Sf9 cells (1 × 10⁶) were plated into 22-mm wells in growth medium at 27 °C and infected with 100 µl of high titer vector, SR-BI, or CD36 baculoviruses. After 48 h, cells were labeled for 18 h with 3 µCi/ml [³H]cholesterol (PerkinElmer Life Sciences) in Grace's insect medium containing 10% fetal calf serum. Cells were washed twice and equilibrated for 2 h in Grace's insect medium. [³H]cholesterol efflux was measured after 0.5, 1, 2, and 4 h at 27 °C in triplicate using HDL as an acceptor as described previously (23). Fractional efflux values were corrected for the small amount of radioactivity released in the absence of HDL. Cholesterol oxidase assays were performed 72 h post-infection as described by Smart *et al.* (48), and modified by Kellner-Weibel *et al.* (33). For measurement of [³H]CE-HDL hydrolysis, at 72 h post-infection [³H]CE-HDL was added (10 µg of protein/ml) in DMEM containing 0.5% bovine serum albumin. After incubation at 37 °C for 4 h, the medium was removed, and the monolayers were washed three times with phosphate-buffered saline, and the distribution of ³H between FC and CE was determined (47). Free and total cholesterol mass (and CE by difference) was measured at 72 h post-infection by gas-liquid chromatography (49).

Phospholipid Analysis—Phospholipids were analyzed by nanospray electrospray ionization on a QuattroLC tandem mass spectrometer (50). Internal standards for PA (591 *m/z*, 28:0), PI (835 *m/z*, 34:1), PE (678 *m/z*, 28:0), PS (634 *m/z*, 28:0), SM (703 *m/z*, 32:1), and PC (678 *m/z*, 28:0) were purchased from Avanti. Standards (1 pmol/µl) were sprayed from nanovials in 2:1 methanol and chloroform with the source maintained at 80 °C. A counter-current flow of nitrogen emerging around the cone (250 liters/h) was used to promote solvent evaporation from the sprayed droplets. A nanovial, loaded with 1 µl of standard or sample, produced a stable spray for more than 1 h allowing both survey mass spectra and daughter ion analysis for each lipid present. For CID experiments, argon was used in the collision cell. All CID parameters were optimized for each molecular ion in the positive and negative ion modes. Sf9 cells were lysed using nitrogen decompression, centrifuged at 1000 × *g* to remove nuclei and debris, and centrifuged again at 100,000 × *g* to collect membranes. Internal standards were added prior to lipid extraction. The membrane lipids were extracted in chloroform/methanol and dried under nitrogen. Samples were diluted 1:5,000 in methanol/chloroform (2:1) for analysis. A survey scan from *m/z* 600 to *m/z* 1000 (4 s) was taken to establish the phospholipid peaks present in each sample. Constant neutral loss and precursor ion scans were then performed using optimized conditions determined for each phospholipid as described (50). Where appropriate, fatty acyl tail composition was determined by tandem mass spectrometry analysis in the negative ion mode.

For each phospholipid, the percent distribution of species with various acyl tail lengths was determined within each analysis based on the sum of the fatty acyl carbons in the *sn*-1 + *sn*-2 positions. Percent distributions from seven independent samples were then averaged. Statistical comparisons were made by a two-tailed Student's *t* test between pairs of the four treatment groups with correction made for multiple comparisons as indicated in the table legends. Determinations

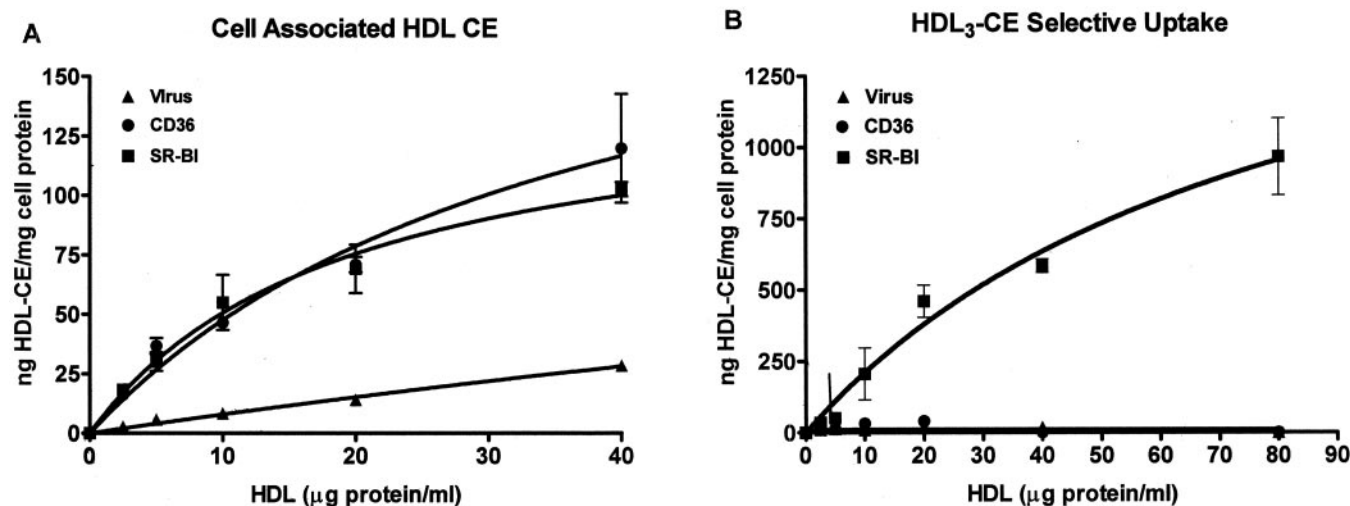


FIG. 1. Cell association of HDL- and HDL CE-selective uptake mediated by CD36 and SR-BI. Sf9 cells expressing rat CD36, murine SR-BI, and uninfected control were incubated at 27 °C for 1.5 h with 10 $\mu\text{g/ml}$ ^{125}I -labeled HDL₃, after which cells were processed to determine cell-associated HDL CE (A) and HDL COE-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 HDL₃.

and analyses of the total double bonds in the fatty acyl chains of each phospholipid were done in the same fashion.

RESULTS

HDL Binding and Selective Uptake of Cholesteryl Ester in Sf9 Cells—To test whether binding of HDL to high affinity receptors will induce CE-selective uptake, Sf9 cells were infected with CD36, a class B scavenger receptor with 30% homology to SR-BI, or SR-BI baculovirus and binding studies were performed as described under “Experimental Procedures.” Fig. 1A shows that HDL binds to both CD36- and SR-BI-expressing Sf9 cells and confirms that both receptors were expressed on the cell surface. The apparent K_d values for HDL binding by CD36 and SR-BI were 37.2 and 19.2 $\mu\text{g/ml}$ of HDL protein, respectively. Note that in this and other comparisons of SR-BI and CD36, Sf9 cells were infected with amounts of each virus that produced equivalent levels of HDL binding activity. The ability of SR-BI-expressing Sf9 cells to mediate selective HDL COE uptake has been demonstrated previously (39). The results in Fig. 1B confirm that SR-BI-expressing cells mediate efficient and selective uptake of HDL CE and show that CD36-expressing cells do not. Most interestingly, the ability of CD36 to mediate HDL COE-selective uptake is markedly less than in mammalian cells in which CD36 has about 15% of the activity of SR-BI (40, 51). After confirming SR-BI expression and function at the plasma membrane, we tested for other activities shown by SR-BI in mammalian cells.

Cholesterol Content of SR-BI-expressing Sf9 Cells—SR-BI expression in COS-7 and WI38 cells increases cellular cholesterol content of both free and esterified cholesterol (44, 52). Because Sf9 cells typically have low cholesterol content, we tested whether SR-BI expression would alter cholesterol content. After 72 h of infection with either control, SR-BI, or CD36 baculovirus, cells were extracted, and their cholesterol content was determined. As shown in Fig. 2, the expression of SR-BI significantly increased the total cholesterol content of Sf9 cells including both free and esterified cholesterol, whereas uninfected cells contain only free cholesterol. Cells infected with control baculovirus or CD36 virus showed no change in free cholesterol although a small amount of esterified cholesterol was detected.

The esterified cholesterol in SR-BI-expressing Sf9 cells is most likely derived from serum lipoproteins. To confirm that esterified cholesterol was introduced from serum and not by

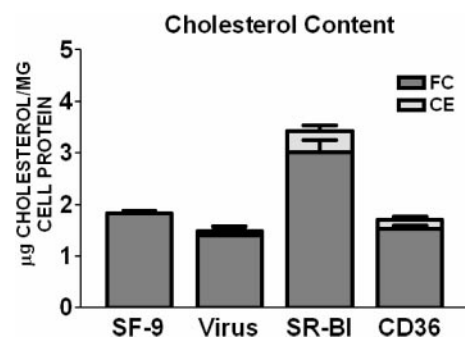


FIG. 2. Cholesterol content of cells expressing SR-BI and CD36 receptors. Sf9 cells expressing rat CD36, murine SR-BI, and uninfected control were prepared as described. Lipids were extracted, and cholesterol mass was determined by gas chromatography, normalized to cell protein, and expressed as μg of lipid/mg of cell protein. Values represent the mean of triplicate determinations.

conversion from FC, cells were incubated with ^3H cholesterol for 24 h, and the amount of ^3H CE formed was determined. As shown in Fig. 3A, only 0.2% of ^3H cholesterol was esterified in SR-BI-expressing cells with no substantial difference from cells that were infected with control or CD36-expressing baculovirus. For comparison, with COS-7 cells incubated with ^3H cholesterol in the same manner, 18% of the ^3H cholesterol was converted to ^3H CE with SR-BI expression versus 4% with CD36 expression or transfection with vector (44). This is consistent with other data showing that Sf9 cells do not have acyl-coenzyme A cholesterol acyltransferase activity (37).

In mammalian cells SR-BI expression facilitates HDL CE hydrolysis; typically, 80% of ^3H CE is rapidly hydrolyzed after uptake from HDL, whereas only 30% of ^3H CE is hydrolyzed after uptake into vector-transfected cells (47). To test this activity in the insect cell system, Sf9 cells were incubated with ^3H CE-labeled HDL, and the distribution of cellular ^3H CE and ^3H FC was determined. As shown in Fig. 3, despite a 2.5-fold increase in HDL ^3H CE uptake (Fig. 3B), SR-BI-expressing cells hydrolyzed only 30% of ^3H CE (Fig. 3C), the same percentage as control Sf9 cells. This indicates that SR-BI does not facilitate HDL CE hydrolysis in this insect cell system.

SR-BI-mediated FC Efflux—To test the ability of SR-BI-expressing Sf9 cells to mediate cholesterol efflux, cells were labeled with ^3H FC for 24 h, and FC efflux was determined by

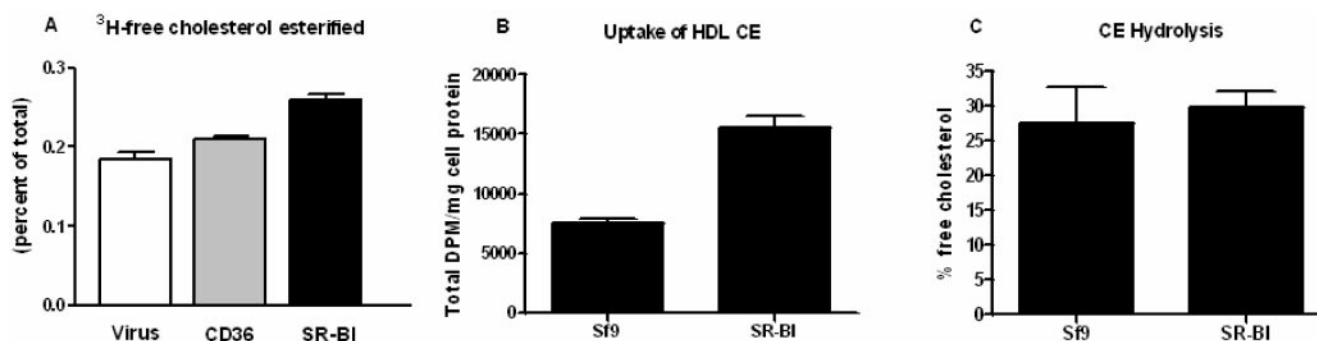


FIG. 3. Disposition of FC and HDL CE in SR-BI- and CD36-expressing Sf9 cells. A, at 48 h post-infection, Sf9 cells expressing rat CD36, murine SR-BI, or control virus were labeled for 24 h with 10% FBS/DMEM containing 3 μ Ci/ml [3 H]cholesterol. The amount of esterified [3 H]cholesterol was determined and expressed as the percent of total [3 H]cholesterol. Values represent the mean of triplicate determinations. B and C, at 72 h post infection murine SR-BI-expressing or uninfected Sf9 cells were incubated with [3 H]CE-HDL (10 μ g of protein/ml) in DMEM containing 0.5% bovine serum albumin. After incubation for 4 h, total cellular radioactivity (B) and the distribution of radiolabel between FC and CE (C) were determined. Values represent the mean of triplicate determinations.

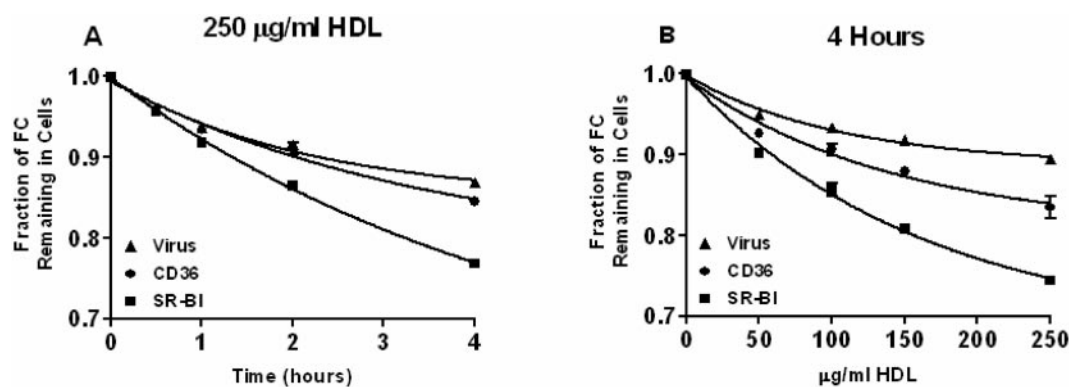


FIG. 4. Free cholesterol efflux mediated by SR-BI and CD36. Control virus-infected Sf9 cells or cells expressing rat CD36 or murine SR-BI were incubated for 24 h with 10% FBS Grace's containing 3 μ Ci/ml [3 H]cholesterol. After incubation with HDL₃, media were collected to determine the amount of [3 H]cholesterol released. A, cells were incubated for the indicated times at 27 $^{\circ}$ C with HDL₃ (250 μ g of protein/ml). B, cells were incubated for 4 h with the indicated HDL₃ concentrations. Values represent the mean of triplicate determinations.

measuring the fraction of [3 H]FC transferred to medium containing HDL. Without an acceptor there was virtually no efflux in 4 h (data not shown). In the presence of HDL (250 μ g/ml) there was significant cholesterol efflux in 4 h from Sf9 cells expressing SR-BI (Fig 4A). CD36-expressing cells showed enhanced FC efflux compared with virus-infected cells (Fig. 4A), although the effect was less than that seen with SR-BI over a range of HDL concentrations (Fig. 4B).

Cholesterol Susceptibility to Cholesterol Oxidase—In mammalian cells SR-BI alters the organization or distribution of membrane free cholesterol as judged by an increased susceptibility to exogenous cholesterol oxidase (23, 33). To test whether SR-BI has this activity in insect cells, Sf9 cells were infected with control baculovirus or baculovirus encoding CD36 or SR-BI for 48 h, followed by 24 h labeling with [3 H]cholesterol. After exposing the cells to cholesterol oxidase for 4 h, lipids were extracted, and cholesterol oxidation was determined. Fig. 5 shows a dramatic increase in cholesterol oxidase susceptibility in Sf9 cells expressing SR-BI when compared with baculovirus-infected or CD36-expressing cells. This is similar to results in mammalian cells. Most interestingly, CD36-expressing cells also showed a substantial 2-fold increase in cholesterol oxidase susceptibility compared with baculovirus-infected cells, although this was much less than with SR-BI.

Membrane Phospholipids in SR-BI-expressing Sf9 Cells—Stable expression of SR-BI in the human lung fibroblast cell line WI38-VA13 increased both FC and phospholipid mass (52). To determine whether phospholipids were altered upon SR-BI expression in Sf9, we utilized electrospray ionization mass spectrometry to quantify and profile phospholipids. Internal

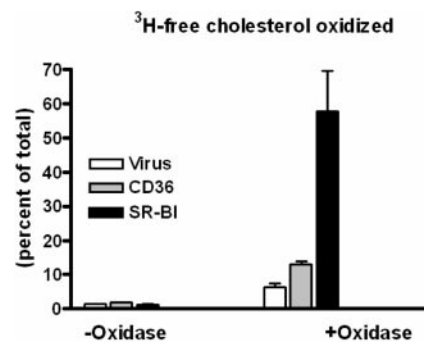


FIG. 5. Cholesterol oxidase sensitivity of membrane free cholesterol. At 48 h post-infection, Sf9 cells expressing rat CD36, murine SR-BI, or control virus were labeled for 24 h with 10% FBS/DMEM containing 3 μ Ci/ml [3 H]cholesterol. The percent of [3 H]cholesterol converted to [3 H]cholestenone was determined after 4 h of incubation with or without exogenous cholesterol oxidase. Values represent the mean of triplicate determinations.

standards for each phospholipid class were used to determine phospholipid/protein ratios. For each phospholipid, the percent distribution of species with various acyl tail lengths was determined within each analysis based on the sum of the fatty acyl carbons in the *sn*-1 + *sn*-2 positions. Determinations of total double bonds in the fatty acyl chains of each phospholipid were done in the same fashion. To eliminate changes that could result from viral infection, Sf9 cells were infected with control baculovirus at the same level of infection as with the SR-BI virus and CD36 virus. Equivalence of infection was judged by Western blot analysis of the virus envelope protein gp64 (Fig. 6A, inset).

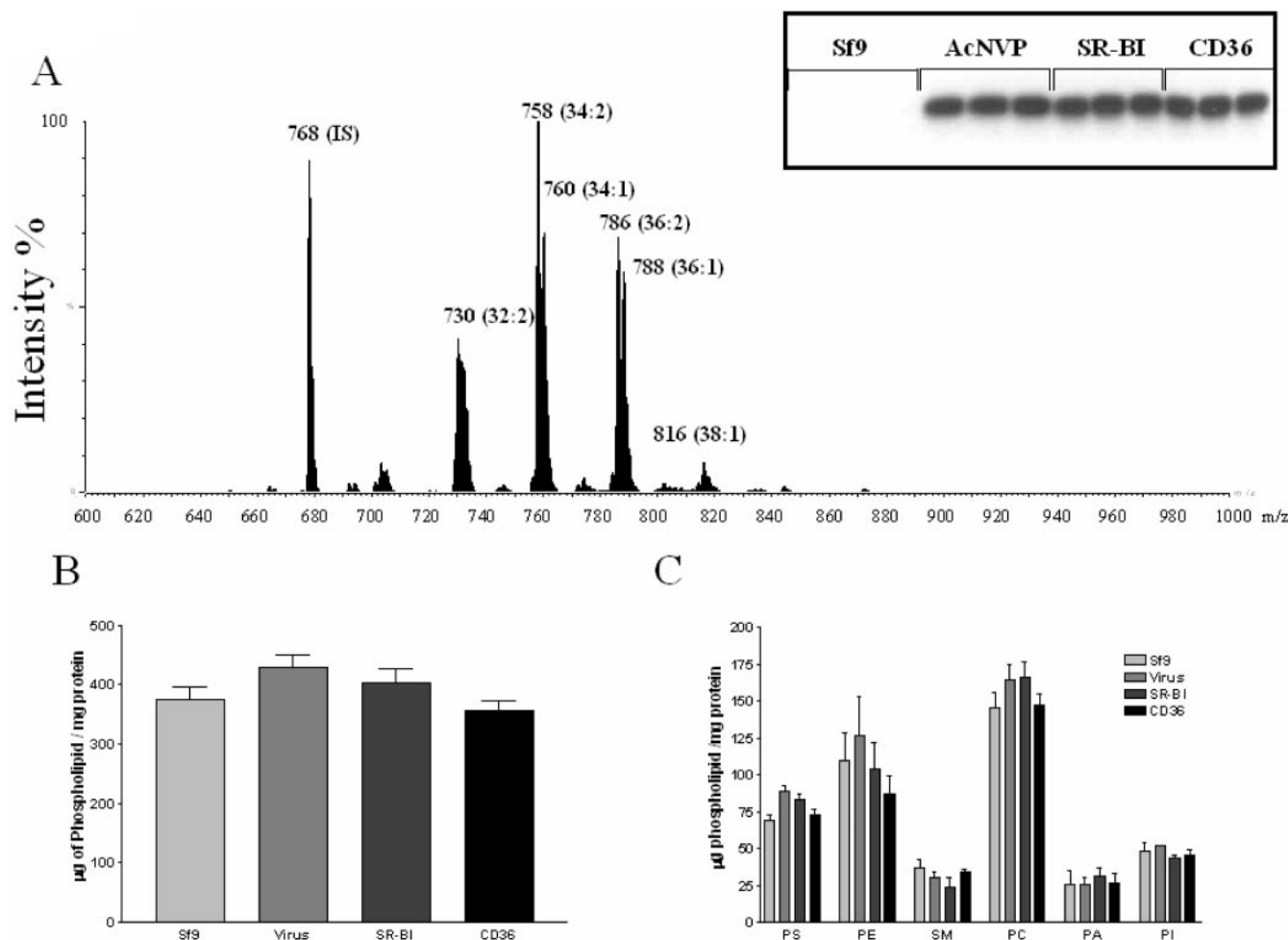


FIG. 6. Effect of scavenger receptor on phospholipid composition of Sf9 cells. At 72 h post-infection, lipids were extracted and analyzed by mass spectrometry as described under "Experimental Procedures." A, representative mass scan of PC species in SR-BI-expressing cells. Numbers above each peak indicate the PC mass. Numbers in parentheses indicate the total acyl tail carbons:double bonds. Inset, Western blot analysis of the virus envelope protein gp64 in triplicate samples of uninfected cells (*Sf9*), control virus-infected cells (*AcNVP*), SR-BI virus-infected cells (*SR-BI*), or CD36 virus-infected cells (*CD36*). B, total phospholipid content as determined by the sum of PC, PS, PI, PA, PE, and SM. Mass measurements are based on internal standards run with each sample. C, content of individual phospholipid species. Values represent the means \pm S.E. of samples from seven experiments.

Fig. 6A depicts the PC species determined by functional scan analysis; peaks are labeled with the mass of the particular PC species and the total carbon and double bonds it represents. Functional scans were performed for all phospholipids, as described under "Experimental Procedures." The results show that total phospholipid content was changed little by SR-BI or CD36 expression or control virus infection (Fig. 6B). Fig. 6C shows that PC and PE are the most abundant phospholipids in Sf9 cells grown in calf serum with the order of abundance being PC > PE > PS > PI > PA = SM. The absence of major changes in phospholipid content upon baculovirus infection is similar to results from previous studies (36–38). The abundance of individual phospholipids in Sf9 cells (Fig. 6C) is also similar to previous results (36, 37) with the exception that PI abundance was much higher in one study (37).

Although major changes in total phospholipid mass were not seen, changes within specific phospholipids were significant. For example, the data in Table I show the percent distribution of acyl tail lengths in PC species upon SR-BI or CD36 expression compared with uninfected or virus-infected cells. In this case a substantial decrease in PC with 32 fatty acid carbons was seen in SR-BI- and CD36-expressing cells. As compared with control virus-infected cells, SR-BI-expressing cells showed a 29% decrease in the relative abundance of PC 32. No change

was seen in PC 34, but SR-BI expression led to significant increases in the relative abundance of PC species with 36, 38, and 40 carbons with most of the increase occurring in PC 36 that showed a 14% increase in relative abundance. Most interestingly, the changes in PC 32 and PC 36 occurred primarily in PC 32:1 and PC 36:1 although the mono- and di-unsaturated species of each were of similar abundance in virus-infected control cells (PC 32:1, 8.3% of total PC; PC 32:2, 8%; PC 36:1 14%; and PC 36:2, 16%) (data not shown). CD36-expressing cells showed similar changes. Thus, scavenger receptor expression in Sf9 cells leads to the accumulation of PC species with longer acyl tails primarily because of changes in PC 32:1 and PC 36:1. Note that as shown in Table II, SR-BI or CD36 expression had no effect on the overall distribution of PC species with 0–4 double bonds. Furthermore, as compared with uninfected cells, baculovirus infection had no effect on double bond distribution or PC acyl tail length with the exception of a relative increase in the abundance of PC 40.

No significant changes were seen in acyl tail length distribution in PE, PI, PS, PA, or sphingomyelin when SR-BI or CD36 were compared with virus-infected cells (Table III). The acyl tail length distribution in PE was nearly identical in all groups. PA showed a trend toward a reduced abundance of PA 34 and an increase in PA 38 compared with virus-infected cells;

TABLE I
Comparison of phosphatidylcholine distribution with varying carbon lengths

Data show the percent distribution among the various PC species in each of the four groups. Significant differences as determined by Student's *t* test corrected for multiple comparisons among all groups ($p < 0.0127$) are indicated as compared with Sf9 cells (SF) or virus-infected cells (V). Data represent mean \pm S.D. of seven experiments.

Phospholipid	Carbons	SR-BI	CD36	Virus	Sf9
PC	30	1.3 \pm 1.2	1.2 \pm 1.2	1.3 \pm 1.4	2.5 \pm 2.4
	32	12.3 \pm 4.8 SF/V	13.0 \pm 4.5 SF/V	17.4 \pm 3.1	20.2 \pm 5.5
	34	41.7 \pm 2.6	42.2 \pm 2.2	42.4 \pm 2.5	42.1 \pm 4.9
	36	39.3 \pm 4.5 SF/V	38.6 \pm 4.6 SF/V ^a	34.6 \pm 2.0	31.7 \pm 5.4
	38	4.5 \pm 0.4 SF/V	4.1 \pm 0.5 SF/V	3.5 \pm 0.5	3.2 \pm 0.7
	40	0.9 \pm 0.2 SF/V	1.0 \pm 0.3 SF/V ^a	0.7 \pm 0.2 SF	0.4 \pm 0.1

^a V indicates significant difference when corrected for multiple comparisons among SR-BI, CD36, and virus groups ($p < 0.017$) but slightly above significance ($0.0127 < p < 0.0141$) when compared among all groups.

TABLE II
Comparison of phosphatidylcholine distribution with varying double bonds

Data show the percent distribution among the various PC species in each of the four groups. Significant differences as determined by Student's *t* test corrected for multiple comparisons among all groups ($p < 0.0127$) are indicated as compared with Sf9 cells (SF) or virus-infected cells (V). Data represent mean \pm S.D. of seven experiments.

Phospholipid	Double bonds	SR-BI	CD36	Virus	Sf9
PC	4	1.1 \pm 0.6	0.9 \pm 0.4	0.6 \pm 0.3	0.6 \pm 0.3
	3	3.3 \pm 0.4	3.0 \pm 0.3	2.9 \pm 0.5	3.5 \pm 1.1
	2	45.4 \pm 4.8	46.7 \pm 4.7	47.4 \pm 2.4	49.5 \pm 4.9
	1	40.4 \pm 5.4	39.5 \pm 5.0	40.3 \pm 2.5	36.9 \pm 4.0
	0	9.8 \pm 1.5	9.9 \pm 3.1	8.7 \pm 0.9	9.5 \pm 2.4

TABLE III
Comparison of phospholipid distribution with varying carbon lengths

Phospholipid	Carbons	SR-BI	CD36	Virus	Sf9
PE	30	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
	32	8.0 \pm 1.5	7.7 \pm 1.8	7.3 \pm 1.5	7.5 \pm 1.8
	34	34.6 \pm 4.2	37.8 \pm 7.2	36.6 \pm 6.5	38.5 \pm 7.6
	36	43.5 \pm 3.4	39.9 \pm 8.6	41.2 \pm 6.0	41.7 \pm 7.0
	38	8.8 \pm 2.2	8.3 \pm 2.8	9.2 \pm 3.3	7.5 \pm 2.2
	40	5.1 \pm 1.8	6.3 \pm 2.4	5.7 \pm 2.8	4.7 \pm 3.1
PA	30	3.1 \pm 1.9	3.0 \pm 1.9	1.6 \pm 1.0	1.4 \pm 1.0
	32	6.1 \pm 3.3	6.5 \pm 2.2	6.3 \pm 2.3	5.6 \pm 2.0
	34	31.3 \pm 7.0 SF	35.4 \pm 6.5	40.9 \pm 11	42.5 \pm 5.2
	36	28.9 \pm 4.5	29.4 \pm 5.9	27.7 \pm 7.7	28.6 \pm 7.3
	38	15.2 \pm 4.3 SF	14.2 \pm 3.1 SF	12.8 \pm 5.0	9.7 \pm 2.5
	40	15.4 \pm 5.5	11.5 \pm 4.9	10.7 \pm 3.4	12.0 \pm 7.5
PI	30	2.3 \pm 1.2	4.0 \pm 1.7 SF	3.6 \pm 1.7	1.8 \pm 1.5
	32	10.8 \pm 2.5	10.2 \pm 3.9	8.5 \pm 1.9	8.4 \pm 2.0
	34	38.1 \pm 3.8	35.1 \pm 4.5 SF	37.8 \pm 2.5 SF	43.4 \pm 5.3
	36	29.6 \pm 5.4	28.4 \pm 7.9	31.8 \pm 6.6	32.2 \pm 5.3
	38	14.3 \pm 4.9	15.4 \pm 6.3	13.3 \pm 5.0	10.7 \pm 3.9
	40	4.9 \pm 2.2	6.9 \pm 3.4	4.9 \pm 2.4	3.6 \pm 2.9
PS	30	3.1 \pm 1.4	3.3 \pm 1.8	4.2 \pm 1.8	4.9 \pm 2.5
	32	18.9 \pm 3.1	20.5 \pm 3.7	21.2 \pm 2.6	25.3 \pm 6.2
	34	40.4 \pm 3.6	42.3 \pm 3.5	41.0 \pm 3.2	42.0 \pm 3.8
	36	31.6 \pm 4.2 SF	28.6 \pm 5.7	28.4 \pm 4.3	23.0 \pm 5.5
	38	4.9 \pm 2.2	3.6 \pm 1.4	4.2 \pm 1.6	3.8 \pm 2.1
	40	1.2 \pm 0.7	1.7 \pm 1.0	1.0 \pm 0.5	1.0 \pm 0.7
SM ^a	32	5.9 \pm 3.2	7.1 \pm 3.1	5.7 \pm 5.0	5.1 \pm 4.6
	34	19.4 \pm 3.1 SF	19.4 \pm 3.8 SF	20.2 \pm 6.4 SF	28.3 \pm 4.1
	36	40.3 \pm 7.9	37.5 \pm 9.5	37.5 \pm 9.2	37.8 \pm 8.8
	38	16.8 \pm 4.0	16.4 \pm 2.5	19.7 \pm 6.0	16.3 \pm 2.6
	40	11.1 \pm 5.5	12.2 \pm 4.8	11.0 \pm 5.3	8.3 \pm 2.3
	42	6.5 \pm 3.1	7.4 \pm 4.6	6.0 \pm 3.8	4.1 \pm 3.3

^a Represents carbons from (sphingoid base + acyl chain). Data show the percent distribution among the various phospholipid species in each of the four groups. Significant differences as determined by Student's *t* test corrected for multiple comparisons among all groups ($p < 0.0127$) are indicated as compared with Sf9 cells (SF). Data represent mean \pm S.D. of seven experiments.

these changes were significant compared with uninfected cells. No changes or trends were observed in PA 32 and PA 36 which were the PC acyl tail lengths showing the largest changes upon SR-BI expression. Similarly, no changes in acyl tail length distribution were seen for SR-BI cells compared with virus-infected Sf9 cells for PI, PS, or sphingomyelin (Table III).

SR-BI cells showed a relative increase in PS 36 and a decrease in SM 34 compared with uninfected Sf9 cells, but these changes were also seen as a trend (PS) or significant (SM) when comparing virus-infected with uninfected cells. The only other difference attributed to baculovirus infection was a relative decrease in the abundance of PI 34. Changes in the distribution of

TABLE IV
Comparison of phospholipid distribution with varying double bonds

Phospholipid	Double bonds	SR-BI	CD36	Virus	Sf9
PE	4	6.7 ± 2.5	8.0 ± 3.2	8.5 ± 6.0	6.8 ± 4.4
	3	6.7 ± 4.5	10.6 ± 11	8.6 ± 7.7	7.8 ± 8.1
	2	43.4 ± 5.6	40.0 ± 9.0	41.3 ± 5.4	42.5 ± 10.9
	1	37.2 ± 3.7	33.8 ± 6.9	34.8 ± 5.3	35.3 ± 5.7
	0	6.1 ± 2.0	7.6 ± 3.7	6.8 ± 2.8	7.6 ± 3.3
PA	4	6.9 ± 2.7	6.8 ± 1.2 SF	6.1 ± 3.3	4.3 ± 2.1
	3	5.9 ± 1.1	6.2 ± 1.7	6.5 ± 3.1	4.9 ± 2.4
	2	41.5 ± 5.5	43.2 ± 7.5	45.8 ± 11	44.6 ± 4.4
	1	32.2 ± 5.3	32.6 ± 5.5	29.8 ± 8.7	32.0 ± 6.5
	0	13.5 ± 4.4	11.2 ± 3.3	11.8 ± 3.3	14.2 ± 4.7
PI	4	9.3 ± 3.5	8.4 ± 3.3	8.4 ± 4.5	6.8 ± 5.9
	3	8.0 ± 3.9	9.3 ± 4.8	7.0 ± 3.1	5.8 ± 3.4
	2	16.8 ± 2.6	18.4 ± 3.0	15.3 ± 3.0	15.7 ± 3.2
	1	53.0 ± 7.6	50.8 ± 8.2	56.0 ± 7.0	58.5 ± 8.2
	0	12.8 ± 1.5	13.1 ± 2.2	13.3 ± 2.5	13.1 ± 3.4
PS	4	1.5 ± 0.8	1.0 ± 0.6	0.9 ± 0.4	1.0 ± 0.5
	3	5.5 ± 1.4	5.1 ± 2.0	4.9 ± 1.2	6.5 ± 2.9
	2	47.2 ± 4.7	48.5 ± 4.9	52.0 ± 2.8	53.7 ± 7.2
	1	38.3 ± 4.1 SF	39.6 ± 6.2 SF	35.8 ± 2.6	31.7 ± 5.5
	0	7.5 ± 2.8	5.8 ± 2.1	6.4 ± 2.0	7.1 ± 2.6
SM ^a	4	5.1 ± 2.5	5.4 ± 3.5	4.6 ± 2.7	3.5 ± 2.4
	3	17.3 ± 3.4 SF	16.8 ± 3.9 SF	18.8 ± 5.5	22.9 ± 4.2
	2	43.3 ± 7.5	40.1 ± 8.6	36.0 ± 9.8	37.0 ± 12.2
	1	24.5 ± 6.5	26.1 ± 5.3	31.1 ± 11	28.8 ± 9.4
	0	9.8 ± 3.2	11.5 ± 4.5	9.5 ± 4.8	7.8 ± 3.5

^a Represents double bonds from (sphingoid base + acyl chain). Data show the percent distribution among the various phospholipid species in each of the four groups. Significant differences as determined by Student's *t* test corrected for multiple comparisons among all groups (*p* < 0.0127) are indicated as compared with Sf9 cells (SF). Data represent mean ± S.D of seven experiments.

double bonds in PE, PI, PS, PA, or sphingomyelin in SR-BI- or CD36-expressing cells were also very limited and only significant in comparison to uninfected Sf9 cells (Table IV).

DISCUSSION

In this study we used Sf9 cells, a primitive insect cell line, to study SR-BI because these cells do not have lipoprotein receptors and, although grown in calf serum, maintain a much lower cholesterol content and have a different phospholipid profile compared with mammalian cells. Thus, these cells provide an interesting system to test the effects of SR-BI on plasma membrane properties. Previous studies showed that SR-BI-expressing Sf9 cells exhibit HDL CE-selective uptake (39), a finding confirmed in the present study. Additionally, SR-BI expression led to large increases in FC accumulation and in the susceptibility of plasma membrane FC to cholesterol oxidase. SR-BI also enhanced FC efflux to HDL. These results suggest that SR-BI facilitates FC accumulation in the membrane, organizes membrane cholesterol, and facilitates the flux of membrane FC in Sf9 cells similarly to mammalian cells. That these membrane changes occur in the evolutionarily distant Sf9 cell suggests that these are inherent properties of SR-BI that may not require interaction with other membrane proteins.

The mechanistic basis for the change in the organization of membrane cholesterol due to SR-BI is not well understood, but the present study provides new information that bears on this issue. The SR-BI-mediated increase in sensitivity of membrane FC to exogenous cholesterol oxidase is seen in both mammalian and insect cells suggesting that this effect does not depend on a particular phospholipid composition or FC content because these differ greatly between Sf9 and mammalian cells. Additionally, in mammalian cells, SR-BI-enhanced cholesterol oxidase sensitivity is not dependent on increased membrane FC content because SR-BI-expressing cells cultured in lipoprotein-deficient serum have increased cholesterol oxidase sensitivity compared with control cells that have the same cholesterol

content (52). Thus, it is not the absolute content of FC but how it is disposed in the membrane that is detected by cholesterol oxidase in SR-BI-expressing cells. A variety of studies with model membranes indicates that cholesterol oxidase activity reflects the ease with which cholesterol desorbs from the lipid bilayer (53, 54). Recent studies with recombinant cholesterol oxidase support this view that an increase in enzymatic activity reflects an increase in the escape of cholesterol from the membrane whether this is because of altered lipid packing density or to phase changes in the membrane (55). In relation to membrane lipid organization, cholesterol oxidase shows greater reactivity with cholesterol in liquid-disordered as opposed to liquid-ordered domains because of the greater rate of cholesterol desorption from the former domain (55). This is relevant to SR-BI because this receptor appears to be present in disordered membrane domains compared with more ordered cholesterol- and sphingolipid-rich membrane rafts as judged by solubilities in nonionic detergents (56, 57). The presence of SR-BI in such domains may facilitate cholesterol flux by localizing HDL particles to membrane regions with favorable energetics for cholesterol movement into and out of the membrane.

An additional feature that is expected to influence cholesterol desorption from the membrane is the interaction of cholesterol with phospholipid acyl tails. Studies in membrane vesicles measuring cholesterol exchange (58–61) or cholesterol oxidase sensitivity (54, 60) show that cholesterol interaction with phosphatidylcholine *versus* sphingomyelin increases cholesterol efflux from the membrane. Additionally, with PC bilayers cholesterol efflux is enhanced by longer chain acyl tails and by a greater degree of unsaturation. These features of phosphatidylcholine acyl tails are believed to weaken the interaction with cholesterol thereby facilitating cholesterol efflux from the membrane. This effect of acyl tail length is of particular interest in light of the present results. In mixed monolayer systems minimal cholesterol desorption occurred with unsat-

urated PC acyl chains of 14–17 carbons, whereas PC acyl chains of 18 and 20 carbons enhanced cholesterol desorption (62). In the present study, SR-BI expression led to decreased abundance of PC 32 species and increased abundance of PC 36, PC 38, and PC 40. Almost all of the changes in PC species with longer acyl chains occurred in species with mono- or polyunsaturated acyl chains. As judged by studies with model membranes, the increased abundance of PC with longer unsaturated acyl tails would be expected to decrease PC/cholesterol interactions and thereby enhance cholesterol desorption from the membrane (58–60). These results suggest that SR-BI expression alters the plasma membrane PC composition in a manner that is expected to facilitate cholesterol flux into and out of the membrane. How these SR-BI-mediated changes in membrane PC occur or are distributed in the membrane is unknown, but we speculate that they occur preferentially in domains on plasma membrane microvillar extensions where clusters of SR-BI are found (56).

In agreement with previous studies, we observed no significant changes in phospholipid content or phospholipid composition in Sf9 cells because of baculovirus infection (36–38). The distributions of acyl tail lengths and double bonds among the individual phospholipids were not much affected by virus infection. Additionally, the observed changes in acyl tail lengths upon SR-BI or CD36 expression were largely limited to PC. The distributions of acyl tail lengths in PE, for example, were identical among the four groups of Sf9 cells. These results support the conclusion that the changes in PC acyl tail lengths in the scavenger receptor-expressing cells are specific to SR-BI or CD36 and are not because of baculovirus infection. Although we consider it unlikely, we cannot rule out the possibility that the changes in PC acyl tail length in SR-BI- or CD36-expressing cells require both scavenger receptor expression and baculovirus infection.

As expressed in mammalian cells (40, 51), the related class B scavenger receptor CD36 binds HDL with high affinity but mediates little HDL CE-selective uptake in Sf9 cells. CD36 also fails to increase the cholesterol content of Sf9 cells. Most interestingly, CD36 expression has similar effects to SR-BI on the distribution of PC species including the decrease in PC 32 and the increases in PC 36, PC 38, and PC40. As with SR-BI these changes occur primarily in species with mono- or polyunsaturated acyl tails. These results suggest that CD36 may also alter the plasma membrane in a manner that will facilitate cholesterol flux. This suggestion is supported by the finding that CD36 expression enhanced FC efflux to HDL but less efficiently than SR-BI. Similarly, CD36 increased the cholesterol oxidase-sensitive pool of membrane FC, but much less so than SR-BI. These results indicate that CD36 has modest, but measurable, effects on FC flux but no detectable effect on HDL CE-selective uptake. One interpretation of these results is that the modest effects of CD36 on FC flux primarily reflect changes in membrane PC species, whereas the much greater effects of SR-BI are because of receptor-facilitated FC transfer that occurs in addition to the changes in membrane PC.

Do the changes in PC species because of CD36 expression have a physiological role relevant to the function of CD36? CD36 has a variety of functions including activity as a fatty acid translocase that facilitates uptake of long chain fatty acids into adipocytes, skeletal muscle, and heart (63, 64). In the absence of protein-mediated transport, the rate-determining step for fatty acid movement across a phospholipid bilayer is desorption of fatty acid from the bilayer to the aqueous phase (65–67). The rate of desorption of long chain fatty acids decreases with increasing chain length and increases with the degree of unsaturation; the latter effect reflects reduced hydro-

phobic interactions with phospholipid acyl tails. Similarly, an increase in the membrane fatty acid desorption rate would be expected if the degree of PC unsaturation were increased because this would also disrupt the packing of fatty acids with PC acyl tails. Although speculative, one possibility is that a CD36-mediated increase in membrane PC with unsaturated acyl tails may contribute to the transport of long chain fatty acids by CD36.

Although SR-BI expression in Sf9 cells showed many similarities to mammalian cells, two effects were different. The first is that the increase in cellular cholesterol mass occurred primarily in free and not esterified cholesterol, a result likely explained by the lack of cholesterol esterification activity in Sf9 cells (Fig. 3) (37). The second is that SR-BI did not enhance the hydrolysis of HDL CE as occurs in mammalian cells (Fig. 3) (47). This suggests that SR-BI does not facilitate delivery of CE to a metabolically active pool in Sf9 cells or these cells lack a hydrolase that can interact with SR-BI. This result also supports the view that SR-BI itself does not have CE hydrolase activity (47).

In summary, this study demonstrates that SR-BI has many of the same activities in insect cells as in mammalian cells but also shows interesting differences. SR-BI increases FC accumulation in Sf9 cells and dramatically increases the susceptibility of membrane FC to cholesterol oxidase. This result as well as the demonstration of SR-BI-induced morphological changes in Sf9 cells (39) indicates that the ability of SR-BI to alter membrane organization is a highly conserved property. The accumulation of PC species with longer mono- or polyunsaturated acyl chains because of SR-BI or CD36 expression may explain some of the changes in membrane properties in Sf9 cells and may contribute to the increased flux of cholesterol and other lipids elicited by these receptors.

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