Caveolin-1 does not affect SR-BI-mediated cholesterol efflux or selective uptake of cholesteryl ester in two cell lines

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Abstract  Free cholesterol (FC) has been reported to efflux from cells through caveolae, which are 50–100 nm plasma membrane pits. The 22 kDa protein caveolin-1 is concentrated in caveolae and is required for their formation. The HDL scavenger receptor BI (SR-BI), which stimulates both FC efflux and selective uptake of HDL-derived cholesteryl ester (CE), has been reported to be concentrated in caveolae, suggesting that this localization facilitates flux of FC and CE across the membrane. However, we found that overexpression of caveolin-1 in Fischer rat thyroid (FRT) cells, which lack caveolin-1 and caveolae, or HEK 293 cells, which normally express very low levels of caveolin-1, did not affect FC efflux to HDL or liposomes. Transient expression of SR-B1 did not affect this result. Similarly, caveolin-1 expression did not affect selective uptake of CE from labeled HDL particles in FRT or HEK 293 cells transduced with SR-BI.1 We conclude that basal and SR-BI-stimulated FC efflux to HDL and liposomes and SR-BI-mediated selective uptake of HDL CE are not affected by caveolin-1 expression in HEK 293 or FRT cells.—Wang, L., M. A. Connelly, A. G. Ostermeyer, H-h. Chen, D. L. Williams, and D. A. Brown. Caveolin-1 does not affect SR-BI-mediated cholesterol efflux or selective uptake of cholesteryl ester in two cell lines. J. Lipid Res. 2003. 44: 807–815.

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Maintaining proper free cholesterol (FC) homeostasis is crucial for cell viability and for preventing atherosclerosis in humans. To this end, FC synthesis and metabolism of dietary FC are tightly regulated in cells (1). FC homeostasis is maintained at the organismal level by reverse cholesterol transport (RCT), which is transport of cholesterol from peripheral tissues to the liver for excretion into the bile and to steroidogenic cells for synthesis of steroid hormones (2).

RCT is mediated by HDL, which removes FC from peripheral cells and also delivers cholesteryl ester (CE) to the liver and steroidogenic cells (3). These cells can take up HDL CE in either of two ways (4). The first, the nonselective pathway, occurs through endocytosis of HDL particles and delivery to lysosomes via the classical endocytic pathway. Endocytosis of HDL is inefficient, and is a relatively minor pathway. Instead, most HDL CE uptake occurs by selective uptake without concomitant uptake of HDL protein. Although the mechanism of selective uptake is just starting to be elucidated (5, 6), it is known to be strongly enhanced by the scavenger receptor BI (SR-BI), which binds HDL (2, 7, 8). As expected, SR-BI is expressed at high levels in the liver and steroidogenic cells. Transgenic hepatic overexpression of SR-BI markedly reduces plasma HDL levels in mice (9). SR-BI overexpression can limit atherosclerosis and cardiovascular pathophysiology in mice (10, 11), demonstrating the physiological importance of RCT.

In addition to its role in selective uptake of HDL CE, SR-BI stimulates the bidirectional flux of FC between cells and lipoproteins (3, 5, 12–14). This activity may be responsible for the rapid hepatic clearance of FC from plasma HDL and its resultant secretion into bile (15). In addition, it suggests that SR-BI expression in peripheral tissues may facilitate uptake of FC by nascent HDL particles. Supporting this idea, the rates of FC efflux from a panel of cultured cell types correlated well with SR-BI expression levels (12).

Abbreviations: CE, cholesteryl ester; DOPC, dioleoyl phosphatidylcholine; FC, free cholesterol; FRT, Fischer rat thyroid; RCT, reverse cholesterol transport; SR-BI, scavenger receptor BI.
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Both FC efflux to HDL or liposome acceptors (16, 17) and selective uptake of CE (18) have been reported to occur through caveolae. Consistent with this idea, SR-BI shows a partial colocalization with caveolin-1 by immunofluorescence (19). Caveolin-1 (20–22) is an important structural component of caveolae and is required for their formation (23, 24). Thus, caveolin-1 levels might be expected to be high in cells undergoing high rates of FC efflux or CE selective uptake. Surprisingly, treatment of rat ovarian granulosa cells with dibutyryl cAMP or follicle-stimulating hormone down-regulated caveolin-1, though both SR-BI and selective uptake of CE were strongly induced (25). Caveolin-1 expression was not stimulated by similar hormone treatment of murine ovarian granulosa cells, although SR-BI expression and CE selective uptake were both induced (26). Similarly, in preliminary experiments, we found that though adrenocorticotropin hormone treatment of Y1 mouse adrenocortical cells stimulated SR-BI expression and CE selective uptake, caveolin-1 expression was not induced and may even have been reduced (M. A. Connelly and D. L. Williams, unpublished observations).

Recent studies directly examining the effect of caveolin-1 expression on FC efflux or selective uptake have not yielded a consistent picture. Lisanti and colleagues found that caveolin-1 inhibited FC efflux to HDL in cultured fibroblasts (27), and that the protein also inhibited SR-BI-mediated selective uptake in transfected hepatocytes (28). Smart and colleagues saw no effect of caveolin-1 expression on FC efflux to HDL in macrophages, but found that caveolin-1 inhibited SR-BI-mediated selective uptake in transfected macrophages and fibroblasts (29). Results of three other groups suggest that caveolin-1 can sometimes stimulate FC efflux. Overexpression of caveolin-1 in synchronized fibroblasts stimulated FC efflux to plasma (17). A dominant-negative caveolin-1 mutant decreased FC efflux to serum in fibroblasts (30), while suppression of caveolin-1 in macrophages reduced FC efflux to apolipoprotein A1 (apo-A1) (31).

We have now examined the effect of caveolin-1 expression on FC efflux and SR-BI-mediated CE selective uptake in two cultured cell lines. Caveolin-1 is not required for viability of cultured cells, and certain cell lines express little or no caveolin-1 and do not contain invaginated caveolae. Exogenous expression of caveolin-1 in these cells can induce formation of caveolae (23, 24). In this paper, we examined FC efflux and selective uptake in two cell lines, HEK 293 and Fischer rat thyroid (FRT), that express little or no caveolin-1, and also in stable caveolin-1 transfectants of the same lines.

MATERIALS AND METHODS

Cells

HEK 293 human embryonic kidney cells and stable transfectants of these cells expressing myc- and HA-tagged canine caveolin-1 (32), referred to here as HEK 293-Cav, were the gifts of D. Lublin (Washington University, St. Louis, MO). Caveolin-1 was detectable in a punctate cell-surface pattern in HEK 293-Cav cells, but not HEK 293 cells, by immunofluorescence microscopy (data not shown). The doubly-tagged protein has the same topology as wild-type caveolin, and associates with caveolar rafts biochemically (Dietzen, 1995 #3782). HEK 293 and Madin Darby canine kidney (MDCK) cells (33), COS-1 monkey kidney cells, NIH 3T3 mouse fibroblasts, and MEB-4 mouse melanoma cells (34) were maintained in DMEM with 10% fetal calf serum (FCS), 50 U/ml penicillin, and 50 μg/ml streptomycin. Stably transfected cells were maintained with 200 μg/ml G418. FRT cells (35) were the gift of E. Rodriguez-Boulan (Cornell University Medical College, New York, NY). We stably transfected these cells with a G418 resistance marker in an empty vector by the calcium phosphate method (36) to generate a cloned line called FRT-Vec. An FRT cell line stably transfected with myc-tagged canine caveolin-1 and a G418 resistance marker (24), referred to here as FRT-Cav, was the gift of C. Zurzolo (University of Naples, Naples, Italy). These cells, but not the parental FRT cells, exhibit cell surface caveolae (24). We confirmed that in our hands, caveolin-1 in these cells was localized in a punctate caveolae-like plasma membrane pattern by immunofluorescence microscopy (data not shown). All FRT cells were maintained in Ham’s F12 medium with Coon’s modification containing 10% FCS, 50 U/ml penicillin, and 50 μg/ml streptomycin. Transfectants were maintained with 200 μg/ml G418.

Other reagents

A plasmid encoding murine SR-BI was previously described (37). Transient transfections using Lipofectamine Plus or Lipofectamine 2000 (Life Technologies) were performed under conditions recommended by the supplier. Rabbit polyclonal antibodies to the C-terminal domain of SR-BI (the gift of S. Azhar, Veteran Affairs Palo Alto Health Care System, Palo Alto, CA) have been described (25). Rabbit polyclonal antibodies to caveolin-1 were from BD Transduction Laboratories (Lexington, KY). Horseradish peroxidase-coupled goat anti-rabbit or anti-mouse IgG secondary antibodies were from Jackson Laboratories (West Grove, PA). Chemiluminescence reagents were from Pierce (Rockford, IL) or New England Nuclear. Purified recombinant human CE transfer protein was kindly provided by Mark Bamberger and Ronald Clark (Pﬁzer Central Research, Groton, CT).

To prepare liposomes, 10 mg of dioleoyl phosphatidylcholine (DOPC) or egg PC (Avanti Polar Lipids, Alabaster, AL) dissolved in chloroform-methanol, 1:1 (v/v), or ethanol was dried under nitrogen gas and then lyophilized for 30–45 min, suspended in 1 ml sterile water by vortexing, and sonicated to clarity (about 45 min). One milliliter sterile 2× DMEM and 100 μl 7.5% sodium carbonate solution were added and the volume adjusted to 10 ml with DMEM.

Preparation of human HDL3 and [125I,3H]HDL

Human HDL3 (1.125 g/ml < ρ < 1.210 g/ml; referred to as HDL3), isolated from normal human serum by sequential ultracentrifugation (38), was the gift of M. Phillips and G. Rothblat (Children’s Hospital of Philadelphia, Philadelphia, PA). HDL was used directly for FC efflux experiments. For selective uptake experiments, HDL was labeled with [3H]cholesterol oleoyl ether ([3H]CE), a nonhydrolyzable analog of CE, using recombinant CE transfer protein as previously described (39), with the following modifications. The CE transfer protein was incubated with [3H]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 column (Pharmacia, Peapack, NJ). The HDL was then labeled with [125I]dilactitol.
tyramine as previously described (40). Particles were dialyzed in four changes of 150 mM NaCl, 10 mM K$_2$HPO$_4$, and 1 mM EDTA (pH 7.4), and stored at 4°C under argon. The specific activities of the [125I]$^3$H]HDL particles were 66–281 dpm/ng protein for 125I and 33–45 dpm/ng protein for $^3$H.

**FC efflux**

The method was adapted from Rothblat et al. (41). Cells were seeded at 4–5 $\times$ 10$^5$ per 10 cm dish on Day 1. When appropriate, cells were transfected with SR-BI on Day 2. On Day 3, cells were washed once with DMEM and then incubated 48 h with 10 ml labeling medium. (To prepare labeling medium, $[^3]$H]cholersterol was added to DMEM containing 10% FCS to 1 $\mu$g/ml, filter sterilized, and incubated at 37°C overnight before use.) On Day 5, cells in each 10 cm dish were trypsinized, resuspended in DMEM containing 1% BSA, and divided evenly among three wells of a 6-well dish, plated essentially at confluency. On Day 6 or 7, the medium was removed and cells were washed once with DMEM. Two milliliters of DMEM, with or without DOPC or egg PC liposomes (1 mg/ml or HDL (200 $\mu$g protein/ml) as acceptor, were added to each well. At each time point, two 50 $\mu$l (FRT and FRT-Cav) or 100 $\mu$l (HEK 293 and HEK 293-Cav) aliquots of media were removed and subjected to centrifugation for 5 min at top speed in a microfuge to pellet any cell debris. Radioactivity in the supernatant was measured by scintillation counting. Media remaining at the end of the assay was discarded and cells were lysed in 1 ml 2% SDS, DNA in the lysates was sheared by passage through a 25 gauge needle, and radioactivity in duplicate 100 $\mu$l aliquots was measured by scintillation counting. The total amount of radioactivity released to the media at each time, the total amount of radioactivity in cells+media, and the fraction of total radioactivity in the media at each time were calculated. Values shown are averages of duplicate aliquots taken from each of duplicate dishes.

In a pilot experiment, lipids were extracted from HEK 293 cells labeled by this method and separated by high-performance thin layer chromatography (33). The FC and CE bands were visualized with iodine vapor, scraped from the plate, and radioactivity was determined by scintillation counting. Less than 3% of the $^3$H radioactivity in the lysates and the known specific $^3$H activity in the HDL particles. Selective CE uptake was determined by subtracting the amount of FC taken up nonselectively from the total. SR-BI-dependent selective uptake was determined by subtracting selective uptake by vector-transfected cells from selective uptake by SR-BI-transfected cells. Finally, SR-BI-dependent selective uptake efficiency was determined by normalizing selective uptake to the amount of SR-BI-dependent cell-associated intact HDL (determined from the amount of TCA-insoluble 125I radioactivity in cell lysates), taken as a measure of cell surface receptor expression. The relative amounts of HDL bound by different cell types corresponded well with the amount of SR-BI expressed detected by Western blotting (data not shown).

**Quantitation of SR-BI and caveolin-1 proteins**

Immunoblot analysis was as previously reported (43) or as follows: cells were washed twice with PBS (pH 7.4) and lysed with 300 $\mu$l NP-40 cell lysis buffer (37, 42) containing 1 $\mu$g/ml peptatin, 0.2 mM phenylmethylsulfonfyl fluoride, 1 $\mu$g/ml leupeptin, and 10 $\mu$g/ml aprotinin. Protein concentrations were determined as described (44). Proteins (10 $\mu$g) were resolved on an SDS-8% PAGE gel, transferred to a nitrocellulose membrane, and probed with antibodies directed against SR-BI or caveolin-1 followed by appropriate secondary antibodies. Proteins were detected by chemiluminescence using conditions recommended by the suppliers.

**RESULTS**

**Caveolin-1 expression varies among cultured cell lines**

Caveolin-1 levels can vary widely between cultured cell lines (23, 24, 45). We reasoned that overexpression of caveolin-1 in cells that normally express little or none of the protein should maximize our chances of detecting an effect on FC efflux or selective uptake of HDL CE. For this reason, we first examined caveolin-1 expression in several cultured cell lines. As shown in Fig. 1, caveolin-1 was expressed at similar high levels in MDCK and NIH-3T3 cells at a moderate level in COS-1 cells, and at a much lower level in HEK 293 cells. (We do not know why NIH-3T3 caveolin-1 had a lower mobility on gels than that from the other cell lines, as murine, canine, and human caveolin-1a proteins have the same number of amino acids. Differences in constitutive posttranslational modifications, such as phosphorylation, may have been responsible.) As shown previously (24), caveolin-1 was not detectable in FRT cells. However, exogenous expression of caveolin-1 in these cells induces formation of caveolae (24), showing that the protein can be assembled correctly. As we found previously (34), caveolin-1 was also undetectable in the MEB-4 subline of the B16 melanoma cells, even when 80 $\mu$g of cell protein was analyzed (not shown). HEK 293 cells have been previously reported to express low but
physiologically important levels of caveolin-1 (46). Thus, overexpression of caveolin-1 in these cells might significantly enhance caveolin-1-dependent function. For these reasons, we examined FC efflux and selective uptake of HDL CE in FRT cells, HEK 293 cells, and stable caveolin-1 transfectants of both lines. As shown previously (24, 32), caveolin-1 was expressed efficiently in both HEK 293-Cav and FRT-Cav cells (Fig. 1C). HEK 293 cells do not exhibit cell-surface caveolae. Transfected caveolin-1 is localized to the plasma membrane of these cells, though it is not known if the protein induces formation of caveolae. FRT cells do not contain caveolae, but caveolae are present in the caveolin-1 overexpressing line examined here (24).

**Caveolin-1 overexpression in HEK 293 cells does not affect FC efflux**

We first examined efflux of FC from HEK 293 or HEK 293-Cav cells using DOPC or egg PC liposomes as acceptors. Cells were labeled for 48 h with [3H]cholesterol, trypsinized, and reseeded in fresh dishes before addition of liposomes. Aliquots of media were taken at various times for quantitation of radioactivity in a scintillation counter. As shown in Fig. 2, overexpression of caveolin-1 did not affect efflux. Efflux rates were very similar both at early times (Fig. 2A) and after more prolonged incubation (Fig. 2B). The apparently slightly greater rate of efflux to DOPC than egg PC liposomes at early times observed in Fig. 2B was not reproducible (see Fig. 2A). Although initial rates of efflux to the two acceptors were similar, for unknown reasons, efflux to DOPC was slower than to egg PC after longer times. As shown in Fig. 2B, efflux to media lacking acceptors was very low in all experiments.

To determine whether stimulation of FC efflux by caveolin-1 might require SR-BI, we expressed SR-BI transiently in both HEK 293 and HEK 293-Cav cell (Fig. 3). SR-BI was not detectable in either line before transfection, but was expressed at comparable levels in the two lines after transfection (Fig. 3C). We then examined FC efflux using HDL as an acceptor (Fig. 3A). As expected, expression of SR-BI stimulated FC efflux in both cell types. However, caveolin-1 overexpression did not further enhance efflux. SR-BI also stimulated FC efflux when DOPC liposomes were used as acceptors (Fig. 3B), as we have previously observed for POPC liposomes (5). This suggests that the effect of SR-BI on efflux is at least partially independent of direct HDL binding. However, caveolin-1 overexpression did not further enhance SR-BI-stimulated FC efflux to DOPC (Fig. 3B).

**Caveolin-1 expression does not affect FC efflux in FRT cells**

To exclude the unlikely possibility that caveolin-1 can enhance FC efflux, but that the low level of caveolin-1 expressed in HEK 293 cells saturates this effect, we repeated the experiments using FRT-Cav and FRT-Vec cells.

FC efflux to both DOPC and HDL acceptors occurred at identical rates from FRT-Vec and FRT-Cav cells (Fig. 4A). We next determined whether caveolin-1 enhanced SR-BI-stimulated FC efflux to HDL by transiently expressing SR-BI in both lines to generate FRT-Vec/SR-BI and FRT-Cav/SR-BI. SR-BI was not detectable by immunoblotting in FRT or FRT-Cav cells (see Fig. 5C), and was expressed at comparable levels in FRT-Vec/SR-BI and FRT-Cav/SR-BI cells when measured at the time of the assay.
Although SR-BI expression caused the expected stimulation of FC efflux, caveolin-1 expression did not affect this stimulation (Fig. 4B).

We conclude that caveolin-1 does not enhance basal or SR-BI-stimulated FC efflux in HEK 293 or FRT cells.

**Caveolin-1 does not affect HDL CE uptake**

We next examined HDL CE uptake in FRT and FRT-Cav cells (Fig. 5). As expected, in each case about 90% of total uptake was selective (compare scales of Figs. 5A and B with C). Also as expected, transient expression of SR-BI enhanced selective uptake in both FRT and FRT-Cav cells (Fig. 5C). However, selective uptake was much greater in FRT/SR-BI cells (and FRT-Vec/SR-BI, not shown) than in FRT-Cav/SR-BI cells (Fig. 5C). This resulted from the fact that for unknown reasons SR-BI expression was consistently higher in FRT than in FRT-Cav cells when measured 2 days after transfection when the selective uptake assay was performed (Fig. 5E). This difference disappeared by 6 days after transfection, when the cholesterol efflux assay was performed (Fig. 4C). Because of the difference in SR-BI expression, we normalized HDL CE selective uptake in the two cell lines (Fig. 5C) to the level of cell surface SR-BI expression (Fig. 5A) to determine the selective uptake efficiency. This was very similar for FRT/SR-BI and FRT-Cav/SR-BI (Fig. 5D).

SR-BI expression increased the amount of HDL degraded in lysosomes in FRT cells (Fig. 5B, bars 1 and 2), consistent with enhanced HDL binding to cells. Surprisingly, however, SR-BI expression in FRT-Cav cells did not enhance HDL degradation (Fig. 5B, bar 3 vs. bar 4). As most HDL CE uptake was selective, however, this unexplained behavior does not affect our conclusions regarding selective uptake.

We next examined CE selective uptake in HEK 293 and HEK 293-Cav cells. As in FRT cells, SR-BI expression increased HDL CE selective uptake in HEK 293 cells regardless of caveolin-1 expression, though the magnitude of the SR-BI-dependent increase was smaller than in FRT cells (Fig. 6C). In contrast to what was observed in FRT cells, the level of transient SR-BI expression was not reproducibly different in HEK 293 and HEK 293-Cav cells. Instead, SR-BI expression levels in both cell lines showed only slight random fluctuations between experiments (Fig. 6E). Normalizing total SR-BI-mediated HDL CE selective uptake (Fig. 6C) for cell surface SR-BI expression showed that caveolin-1 expression did not significantly affect selective uptake efficiency in HEK 293 cells (Fig. 6D).
Caveolin-1 binds to FC (47) and associates with FC-dependent lipid rafts. These properties have suggested a role of caveolin-1 and caveolae in cholesterol homeostasis. The fact that caveolin-1-null mice are viable and have normal serum cholesterol levels (48) shows that this role is not essential. However, as outlined previously, results of a number of investigators have not resolved the question of whether caveolin-1 and caveolae play a more subtle role in cholesterol homeostasis.

Three pathways of FC efflux from cells have been described (3). The first, basal FC efflux to lipoprotein acceptors occurs via aqueous diffusion of FC from the plasma membrane. SR-BI mediates the second pathway by which FC moves between cells and phospholipid-rich lipoproteins or vesicles. A third pathway, mediated by the ABC transporter ABCA1, involves FC and phospholipid efflux from cells to lipid-poor or lipid-free apolipoproteins, but not to phospholipid-rich acceptors. In the present study, we found no effect of caveolin-1 expression on basal or SR-BI-stimulated FC efflux to HDL or phospholipid vesicles, but this may explain this discrepancy.

Pol et al. found that expression of a dominant-negative form of caveolin-1 reduced cholesterol efflux from COS-1 cells to serum (30). This mutant had complex effects on intracellular FC trafficking, and may have had pleiotropic effects on cholesterol homeostasis. Arakawa et al. found that caveolin-1 stimulated efflux to apoA-1 in macrophages (31). FC efflux to apoA-1 is mediated primarily by the ABCA1 transporter in a manner that is induced by FC.
enrichment of cells (50). Very little of the efflux measured in our assay was likely to occur by this pathway. FC efflux to liposomes from cells not expressing SR-BI or FC efflux to HDL or liposomes in cells expressing SR-BI is known to be independent of ABCA1. It is possible that ABCA1 made a small contribution to FC efflux to HDL from cells not expressing SR-BI, as a small amount of apoA1 may have dissociated from HDL. As our cells were not treated to upregulate ABCA1, the contribution of this pathway to the observed efflux was probably very minor.

We found no effect of caveolin-1 expression on SR-BI-mediated selective uptake of CE in either FRT or HEK 293 cells. These findings suggest that the previously reported caveolar localization of SR-BI (19) does not affect its function. These results differ from those of Matveev et al. (29) and Frank et al. (28, 49), as both groups found that caveolin-1 expression inhibited selective uptake.

The most recent study (49) is particularly relevant, as the authors examined selective uptake in the same caveolin-1-transfected HEK 293 cell line that we used here. In contrast to our results, these authors reported a modest (30–40%) inhibition of SR-BI-dependent selective uptake in the caveolin-1-overexpressing line. However, in that study, selective uptake was not corrected for the level of cell surface SR-BI expression. In contrast to the situation in FRT cells, we found no reproducible difference between the level of transient SR-BI expression in HEK 293 and HEK 293-Cav cells. However, expression levels fluctuated somewhat between experiments, as shown in Fig. 6. This fluctuation could explain the modest inhibition of selective uptake by caveolin-1 reported by Frank et al. (49). When we corrected for differences in surface expression of SR-BI, we found no difference between the parental and caveolin-1 expressing lines. However, a difference in SR-BI expression levels would not account for the discrepancy between our results and those of Matveev et al. (29), as those investigators reported slightly increased cell surface HDL binding upon caveolin-1 overexpression in the CHO cell line d1A7[SRC-BI]. Cell type-specific factors may explain the difference between our results and theirs.

Our studies may be reconciled with early studies showing that FC efflux (16) and CE selective uptake (18) occur in caveolae. These studies identified caveolae by biochemical isolation or by the increased sensitivity of caveolar FC to cholesterol oxidase. These methods may not distinguish between caveolae and membrane rafts, which are sphingolipid- and cholesterol-rich membrane microdomains suggested to be in the liquid-ordered phase (51–53). Although rafts are probably concentrated in caveolae, raft formation does not require caveolin-1, and rafts are present in cells that do not contain invaginated caveolae. Thus, FC efflux to HDL and SR-BI-mediated CE selective uptake may occur selectively in rafts without a specific requirement for caveolin-1 or invaginated caveolae. In addition, early work suggested that the FC content of caveolae is important in cholesterol flux across the membrane (16, 17). Overexpressing caveolin-1 has been shown to induce formation of morphologically normal caveolae in several cell types (23, 24). However, it is possible that simply over-expressing caveolin-1 is not sufficient to increase the FC content of these caveolae.

We showed here that caveolin-1 does not affect the stimulation of FC efflux and HDL CE selective uptake by SR-BI. Our recent results suggest that another activity of SR-BI is also independent of caveolin-1. We previously showed that SR-BI expression in COS-7 cells enhances the sensitivity of membrane FC to oxidation by exogenous cholesterol oxidase (5, 54) and alters the distribution of membrane FC between the slow and fast kinetic pools for FC efflux from the cell (54). These results suggest that SR-BI may somehow alter plasma membrane lipid organization. We have also shown that SR-BI expression in HEK 293 cells yields a similar increase in sensitivity to cholesterol oxidase treatment, despite the fact that these cells express very little caveolin-1 (M. de la Llera-Moya, M. A. Connelly, S. A. Klein, G. H. Rothblat, and D. L. Williams, unpublished data). Thus, SR-BI-mediated changes in plasma membrane lipid organization may also occur independently of caveolin-1 and caveolae.

It is clear that caveolin-1 does not affect the ability of SR-BI to mediate HDL CE selective uptake and FC efflux in the two cell lines examined in the present study. This raises an interesting question about the distribution of SR-BI on the plasma membrane. Previous studies showed that SR-BI is localized heterogeneously-sized punctate clusters on the cell surface of Chinese hamster ovary and COS-7 cells (19, 55). We have observed a very similar pattern in caveolin-1-negative FRT cells (Y. Peng and D. L. Williams, unpublished data), implying that the punctate distribution of SR-BI does not result from localization to caveolae. Additional studies will be required to better define these punctate structures and to determine whether they are related to cholesterol and sphingomyelin-rich rafts.

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