Caveolin-1 and regulation of cellular cholesterol homeostasis

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Caveolae are 50- to 100-nm cell surface plasma membrane invaginations present in terminally differentiated cells. They are characterized by the presence of caveolin-1, sphingolipids, and cholesterol. Caveolin-1 is thought to play an important role in the regulation of cellular cholesterol homeostasis, a process that needs to be properly controlled to limit and prevent cholesterol accumulation and eventually atherosclerosis. We have recently generated caveolin-1-deficient [Cav-1(−/−)] mice, which caveolae organelles are completely eliminated from all cell types, except cardiac and skeletal muscle. In the present study, we examined the metabolism of cholesterol in wild-type (WT) and Cav-1(−/−) mouse embryonic fibroblasts (MEFs) and mouse peritoneal macrophages (MPMs). We observed that Cav-1(−/−) MEFs are enriched in esterified cholesterol but depleted of free cholesterol compared with their wild-type counterparts. Similarly, Cav-1(−/−) MPMs also contained less free cholesterol and were enriched in esterified cholesterol on cholesterol loading. In agreement with this finding, caveolin-1 deficiency was associated with reduced free cholesterol synthesis but increased acyl-CoA:cholesterol acyltransferase (ACAT) activity. In wild-type MPMs, we observed that caveolin-1 was markedly upregulated on cholesterol loading. Despite these differences, cellular cholesterol efflux from MEFs and MPMs to HDL was not affected in the Cav-1-deficient cells. Neither ATP-binding cassette transporter G1 (ABCG1)- nor scavenger receptor class B type I (SR-BI)-mediated cholesterol efflux was affected. Cellular cholesterol efflux to apolipoprotein A-I was not significantly reduced in Cav-1(−/−) MPMs compared with wild-type MPMs. However, ABCA1-mediated cholesterol efflux was clearly more sensitive to the inhibitory effects of glyburide in Cav-1(−/−) MPMs versus WT MPMs. Taken together, these findings suggest that caveolin-1 plays an important role in the regulation of intracellular cholesterol homeostasis and can modulate the activity of other proteins that are involved in the regulation of intracellular cholesterol homeostasis.

High-density lipoprotein; lipoproteins; macrophages; atherosclerosis

EXPERIMENTAL PROCEDURES

Materials. Antibodies and their sources were as follows: anti-caveolin-1 IgG (mAb 2297; gift of Dr. Roberto Campos-Gonzalez, Department of Cancer Biology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania).

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Cells were seeded in a six-well plate and labeled with 5 by scintillation counting. Animals. Cav-1(+/−) mice were as we previously described. All animals used in these studies were in the C57Bl/6J genetic background and were genotyped by PCR, as previously described (43). Housing and maintenance was provided by the Albert Einstein College of Medicine barrier facility; mice were kept on a 12-h:12-h light/dark cycle and had ad libitum access to food and water. All animal protocols used in this study were preapproved by the Albert Einstein College of Medicine Institute for Animal Studies.

Isolation and analysis of mouse peritoneal macrophages. Elicited mouse peritoneal macrophages (using sodium thioglycollate) were obtained and isolated as described by others (22a). Before incubation with appropriate media, the cells were washed with PBS. Then, the isolated macrophages were either incubated with DMEM-10% FBS or with DMEM containing 0.2% BSA and 75 μg/ml acetylated low-density lipoprotein (LDL) for 48 h.

Determination of cellular cholesterol content. Cellular cholesterol was extracted from cells by using isopropanol. Cholesterol content was determined by using colorimetric tests for total cholesterol (Sigma Aldrich, St. Louis, MO) and free cholesterol (Wako Chemical, Richmond, VA).

Cholesterol synthesis and esterification determination. Cells were seeded in six-well plates the day before the experiment. Labeling was performed for 6 h at 37°C using DMEM 10% FBS containing 5 μCi/well [1-14C]sodium acetate (American Radiolabeled Chemicals, St. Louis, MO) or 1 μCi/well [9,10-3H(N)]sodium oleate (PerkinElmer, Boston, MA). After the incubation, plates were placed on ice, and cells were washed with PBS and solubilized with 0.2 N NaOH overnight on a rocker. Lipids were extracted following the Bligh and Dyer method (4). The resulting extract was analyzed by thin-layer chromatography on ITLC SG plates (Gelman Sciences, Ann Arbor, MI) using a solvent system composed of hexane-diethylther-acetic acid (90:10:1 vol/vol/vol). The areas of the plates containing cholesterol and CE were scraped into vials, and radioactivity was determined by scintillation counting.

Efflux experiments. Mouse embryonic fibroblasts were prepared and cultured as we previously described (43). For cholesterol efflux experiments, MEFs were labeled as previously described (13). Briefly, cells were seeded in a six-well plate and labeled with 5 μCi [3H]cholesterol per well the following day. Cells were washed and incubated 24 h later with DMEM-0.2% BSA for 12 h. For the efflux experiments, cells were washed with DMEM-0.2% BSA and DMEM containing HDL3 (50 μg/ml) or lipid-free apoA-I (50 μg/ml) was added to the cells. Medium was collected as previously described (13). For macrophages, they were labeled during the cholesterol-loading period with 5 μCi [3H]cholesterol per well dispersed in 0.1% ethanol (% final volume of medium) for 24 h. After labeling was completed, a 12-h incubation period (DMEM containing 0.2% fatty acid free BSA) was performed to allow equilibration of the labeled cholesterol with intracellular cholesterol pools. For efflux experiments, cells were washed twice with DMEM alone, and efflux medium was added (50 μg/ml HDL) to the cells. Media aliquots were taken at different times of incubation and treated as previously described (13). At the end of the experiment, cells were solubilized in 0.5 N NaOH to determine protein and [3H]cholesterol content. Results presented are expressed as the percentage of labeled cholesterol remaining in the cells as a function of time.

Western blot analyses. The protein concentration was measured using the BCA protein assay (Bio-Rad Laboratories, Hercules, CA), with BSA as the protein standard. Equal amounts of protein for each sample were loaded and run on SDS-polyacrylamide 12% gels. After transfer to nitrocellulose, the expression levels of caveolin-1, SR-BI, ABCG1, and ABCA1 were examined by using specific antibodies. For SREBP-1 analysis, 2 h before the end of the experiment, cells were treated with N-acetyl-Leu-Leu-Nle-CHO (ALLN, 25 μg/ml). AJP-Heart Circ Physiol • VOL 291 • AUGUST 2006 • www.ajpheart.org
demonstrated in primary cultures of macrophages (18, 25). As such, caveolin-1 may be involved in the regulation of cellular cholesterol metabolism. Therefore, we have examined the expression levels of ABCA1 in macrophages obtained from WT and Cav-1(-/-) mice. The results presented in Fig. 3 indicate that although ABCA1 levels remain lower in Cav-1(-/-) cells, cholesterol loading of macrophages stimulates ABCA1 expression to a similar extent in both WT and Cav-1(-/-) cells. SR-BI expression has also been associated with the ability of HDL to promote cholesterol efflux from cells. In these studies (Fig. 3), we show that SR-BI and ABCG1 expression are not affected by a caveolin-1 deficiency. It is important to note that the SR-BI protein was not detected in any of the cholesterol-loaded MPMs. In addition, ABCG1 upregulation in the presence of acetylated low-density lipoprotein (AcLDL) was similar in both WT and Cav-1(-/-) MPMs, as previously observed in the case of human macrophages (26).

Cellular cholesterol content of MEFs and peritoneal macrophages. Because caveolin-1 may affect cholesterol trafficking within cells, we next examined the cellular cholesterol composition of both WT and Cav-1(-/-) MEFs, under normal conditions (cells incubated with 10% serum only), or in cells loaded with cholesterol (in the presence of 10% serum and 50 μg/ml cholesterol complexed with cyclodextrin). Under both

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**Fig. 1.** Immunofluorescent localization of caveolin-1 (Cav-1) and caveolin-2 (Cav-2) in wild-type (WT) and Cav-1(-/-) mouse embryonic fibroblasts (MEF) cells loaded with varying amounts of cholesterol. MEFs were seeded into 12-well plates containing glass coverslips. The following day, cells were incubated with or without 50 μg/ml CD-cholesterol (cholesterol complexed with methyl-β-cyclodextrin). Cells were then immunostained with antibodies directed against Cav-1 or Cav-2 and visualized by confocal fluorescence microscopy. Cholesterol-loaded cells were also stained with (BODIPY 493/503). Note that the pattern of Cav-1 localization is dramatically affected by cellular cholesterol loading: A: Cav-1 and Cav-2 in WT MEFs; B: Cav-1 and Bodipy in cholesterol-loaded WT MEFs; C: Cav-2 and Bodipy in cholesterol-loaded WT MEFs; D: Cav-2 in Cav-1(-/-) MEFs; E: Cav-2 and Bodipy in cholesterol-loaded Cav-1(-/-) MEFs. For merged image, inset shows a higher magnification of cell identified by the arrow.

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conditions (Table 1), we observed that Cav-1(−/−) MEFs had reduced free cholesterol but a slightly increased esterified-cholesterol content. These results suggest that caveolin-1 can regulate cholesterol trafficking within the cell, possibly by modifying the accessibility of cholesterol to acyl-CoA:cholesterol acyl-transferase (ACAT) or by affecting its function.

In accordance with our results with Cav-1(−/−) MEFs, the free cholesterol content of Cav-1(−/−) macrophages (both control incubated with 10% serum or cholesterol loaded using AcLDL) was decreased compared with WT macrophages (Table 2). In addition, the esterified-cholesterol content was increased in Cav-1(−/−) macrophages. However, when cholesterol loaded using AcLDL, Cav-1(−/−) macrophages showed an increased total cellular cholesterol content. This difference observed between MEFs and macrophages may be due to the absence of serum, i.e., MEFs were loaded with CD-cholesterol (cholesterol complexed with methyl-β-cyclodextrin) in the presence of serum.

**Cholesterol synthesis and esterification in MEFs.** To determine whether cholesterol synthesis and/or cholesterol esterification were affected in Cav-1(−/−) MEFs, cells were labeled with [1-14C]sodium acetate or [9,10-3H(N)]sodium oleate for the quantification of cholesterol synthesis and cholesterol esterification, respectively. Cholesterol synthesis was reduced and cholesterol esterification was increased in Cav-1(−/−) cells (Fig. 4). These data suggest that caveolin-1 deficiency is

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**Fig. 2. Immunofluorescent localization of Cav-1 and Cav-2 in WT and Cav-1(−/−) macrophages (MPMs).** MPMs were seeded into 12-well plates containing glass coverslips. The following day, cells were incubated with or without of 75 μg/ml acetylated low-density lipoprotein (AcLDL). Cells were then immunostained with antibodies directed against Cav-1 or Cav-2 and visualized by confocal fluorescence microscopy. Cholesterol-loaded cells were also stained with Bodipy. Note that the pattern of caveolin-1 localization is dramatically affected by cellular cholesterol loading. A: Cav-1 and Cav-2 in WT MPMs; B: Cav-1 and Cav-2 in Cav-1(−/−) MPMs; C: Cav-1 and Cav-2 in cholesterol-loaded WT MPMs; D: Cav-1 and Cav-2 in cholesterol-loaded Cav-1(−/−) MPMs.
Cholesterol content of MEFs

Table 1.

<table>
<thead>
<tr>
<th>Fibroblast Genotypes</th>
<th>Total Cholesterol</th>
<th>Free Cholesterol, µg/mg of cell protein</th>
<th>Esterified Cholesterol, µg/mg of cell protein</th>
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<tr>
<td>Basal cholesterol levels</td>
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<tr>
<td>Cav-1 (+/+ )</td>
<td>27.2 (SD 2.0)</td>
<td>23.8 (SD 1.3)</td>
<td>3.4 (SD 0.9)</td>
</tr>
<tr>
<td>Cav-1 (−/−)</td>
<td>25.0 (SD 2.3)</td>
<td>19.5 (SD 2.0)*</td>
<td>5.5 (SD 0.3)*</td>
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<tr>
<td>After loading with CD cholesterol</td>
<td></td>
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<tr>
<td>Cav-1 (+/+ )</td>
<td>43.3 (SD 2.4)</td>
<td>34.2 (SD 2.2)</td>
<td>9.1 (SD 0.4)</td>
</tr>
<tr>
<td>Cav-1 (−/−)</td>
<td>41.1 (SD 3.0)</td>
<td>28.8 (SD 1.1)*</td>
<td>12.3 (SD 2.8)*</td>
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</table>

Values are means (±SD). Mouse embryonic fibroblasts (MEFs) were incubated with 50 µg/ml of cholesterol [complexed to cyclodextrin (CD)] for 48 h. *Significant differences between the wild-type (WT) and Caveolin-1 (Cav-1) knockout MEF cells.

Cav-1: WT KO WT KO

Fig. 3. Expression of Cav-1, scavenger receptor class B type 1 (SR-BI), ATP-binding cassette transporter G1 (ABCG1), and ATP-binding cassette transporter A1 (ABCA1) in mouse peritoneal macrophages (MPMs). MPMs were prepared and grown in the presence of M-CSF. MPMs from WT and Cav-1(−/−) were then cultured in DMEM 10% FBS, with or without 75 µg/ml AcLDL. Forty-eight hours after the incubation, cells were solubilized and 30 µg of protein were separated by SDS-PAGE. Cav-1, ABCA1, ABCG1, and SR-BI levels were assessed using specific antibodies.

-associated with deregulation of the control of cholesterol homeostasis.

Determination of endogenous SREBP-1 levels in MEFs. Previous studies have shown that caveolin-1 could mediate the transfer of newly synthesized cholesterol from the ER to the plasma membrane (48). SREBP-1 is a member of a family of transcription factors (6) that regulate the transcription of genes involved in cellular lipid homeostasis (23). Before being activated, full-length SREBP (125 kDa) is located in the ER but requires proteolysis for activation in the nucleus. This process necessitates the participation of the SREBP cleavage activation protein (SCAP) (6). SCAP is a sterol sensor protein that initiates the SREBP proteolytic cascade, leading to the transfer of the basic-helix-loop-helix-leucine zipper active fragment (68 kDa) to the nucleus. Therefore, a change in ER free-cholesterol content would be associated with a change in both the 125-kDa and the 67-kDa relative proportion in Cav-1(−/−) cells. Our results (Fig. 5) demonstrate that Cav-1(−/−) cells show increased levels of the full-length SREBP-1 form but reduced levels of the active nuclear SREBP-1 form. The data are consistent with an increase in the ER free cholesterol content in Cav-1(−/−) cells.

Cellular cholesterol efflux from MEFs. We have previously shown that caveolin-1 does not affect SR-BI-mediated cellular cholesterol efflux in HEK-293T cells (16). In the present study, we examined the ability of HDL3 to promote cellular cholesterol efflux from WT and Cav-1(−/−) MEFs. Interestingly, our observations suggest that loss of caveolin-1 expression does not affect the ability of HDL3 to promote cellular cholesterol efflux from MEF (Fig. 6).

Cellular cholesterol efflux from MPMs. We have also examined the ability of HDL and apoA-I to promote cellular cholesterol efflux from macrophages. Figure 7 indicates that HDL can promote cellular cholesterol efflux equally well from both WT and Cav-1(−/−) macrophages. The same observation was made in cholesterol-loaded cells (data not shown). This finding is in agreement with the absence of effect of caveolin-1 on SR-BI expression in macrophages (Fig. 3). Because apoA-I has been shown to promote specific ABCA1-mediated cholesterol efflux, we next examined the ability of apoA-I to promote cellular cholesterol efflux from both WT and Cav-1(−/−) macrophages under normal or cholesterol-loaded conditions (Fig. 8). Whereas there were no differences between cells loaded with cholesterol, apoA-I-mediated cellular cholesterol efflux was slightly reduced under basal conditions (macrophages not loaded with cholesterol). These results correlate well with the expression of ABCA1 in the different conditions: maximum cholesterol efflux is observed with the highest levels of ABCA1 expression. Additionally, caveolin-1 regulates the expression of ABCA1 and may, therefore, directly affect cellular cholesterol efflux to apoA-I.

To determine the contribution of ABCA1 to apoA-I-mediated cholesterol efflux in MPMs, cholesterol-loaded cells were treated with the ABCA1 inhibitor glyburide (53). Under these conditions, we found that Cav-1(−/−) MPMs showed a more profound reduction in apoA-I-mediated cholesterol efflux (Fig. 9). These data suggest that caveolin-1 may normally participate, either directly or indirectly, in apoA-I-mediated cholesterol efflux from macrophages.

DISCUSSION

In the present study, we have shown that a caveolin-1 deficiency leads to the dysregulation of the intracellular flux of cellular cholesterol. We show that a caveolin-1 deficiency is
CAEVOLIN-1 AND CHOLESTEROL HOMEOSTASIS

SREBP-1 Isoforms in MEFs

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<td>Full-Length</td>
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Fig. 5. Sterol regulatory element binding protein-1 (SREBP-1) levels in WT and Cav-1(-/-) MEFs. MEFs were grown in DMEM 10% FBS. Two hours before the MEFs were solubilized, the cells were treated with N-acetyl-Leu-Leu-Leu-Nle-CHO (ALLN, 25 μg/ml). After solubilization, 30 μg of protein were separated using 8% SDS-PAGE. Levels of the full length (125 kDa) and of the activated (67 kDa) forms were then determined by Western blot analysis.

Fig. 6. Effects of a Cav-1 deficiency on cellular cholesterol efflux mediated by HDL3. MEFs were labeled in the presence of [3H]cholesterol. After an overnight equilibration in DMEM 0.2% BSA, HDL3 (50 μg/ml) was incubated with the cells. Aliquots of media were taken at indicated times and counted. Efflux is expressed as the percentage of [3H]cholesterol remaining in cells as a function of time. Experiments were performed in triplicate.

Regulation of caveolin-1 expression by cellular cholesterol. It is now quite clear that caveolin-1 is regulated by cellular cholesterol levels. The CAV-1 promoter contains two sterol regulatory element-like sequences that can bind SREBP-1 and inhibit CAV-1 transcription (3). In addition, it has recently been shown that CAV-1 transcription can be regulated by the nuclear receptors peroxisome proliferator-activated receptor γ and liver X receptor (28). Consistent with these findings, several groups have now shown that cellular cholesterol levels affect caveolin-1 protein levels, whereas cholesterol loading of certain cell lines can lead to the upregulation of caveolin-1 protein levels (9, 16, 22), inhibition of cholesterol synthesis or reduction of cellular cholesterol levels with an extracellular acceptor are associated with lowered caveolin-1 protein levels (13, 22). Our current data validate these conclusions with macrophages loaded with cholesterol using AcLDL. Caveolin-1 expression has previously been demonstrated in macrophages (2, 18, 30). We now show for the first time that caveolin-1 expression is upregulated in cholesterol-loaded macrophages. In the case of MPMs, this finding is especially important because caveolin-1 protein levels are upregulated >10 times on cholesterol loading (Fig. 3). This finding may suggest an important role for caveolin-1 in the regulation of cellular cholesterol homeostasis. Indeed, numerous studies have now explored a potential role for caveolin-1 in the regulation of cellular cholesterol metabolism. Earlier studies suggested a role for caveolin-1 in the associated with reduced free cholesterol and increased esterified cholesterol. However, caveolin-1 deficiency does not have a major effect on cellular cholesterol efflux. Thus caveolin-1 may act as an intracellular cholesterol transporter, as previously suggested (48). In addition, a caveolin-1 deficiency also affects the subcellular localization of caveolin-2, in accordance with our previous findings (43).
regulation of cellular cholesterol efflux to HDL (9, 11). However, later studies have not drawn the same conclusions (13, 16, 29, 51). The specificity of the cholesterol labeling, the type of extracellular cholesterol acceptors used, and/or the cell types used may explain some of the conflicts observed in the different studies. According to our results, we can conclude that caveolin-1 may play a role in the regulation of intracellular cholesterol metabolism and transport.

**Caveolin-1 and the intracellular movement of free cholesterol.** Previous studies have shown that caveolin-1 may mediate the transfer of newly synthesized cholesterol from the ER to the plasma membrane (48). This implies that a deficiency in caveolin-1 would be associated with the accumulation of cholesterol in the ER and potentially the reduced transfer of newly synthesized cholesterol from the ER to the plasma membrane. Accumulation of ER cholesterol would lead to increased ACAT activity and reduced free cholesterol synthesis. These predictions are in fact what we observed in Cav-1(-/-) cells (Tables 1 and 2, and Fig. 4). As a consequence, a caveolin-1 deficiency is associated with a significant increase in esterified-cholesterol (EC) and reduced free cholesterol (FC) under basal conditions (without cholesterol loading) and in cholesterol-loaded cells. This change in the EC-to-FC ratio may explain the reduced ABCA1 expression that we observed in Cav-1(-/-) macrophages (Fig. 3). A reduction in the transfer of cholesterol from the ER to the plasma membrane would also be associated with an inhibition of SREBP-1-mediated transcription of genes involved the metabolism of cholesterol. This is indeed what we observed in Cav-1(-/-) cells, which display reduced free cholesterol synthesis and increased ACAT activity. These findings are, in part, reminiscent of the work presented by another group (40). Pol et al. (40) showed that in BHK cells transfected with a dominant negative mutant of caveolin-3 (cav-3DGV), reduced free cholesterol synthesis was observed as well as reduced plasma membrane accumulation. However, they did not observe any increases in esterified-cholesterol levels. This last observation may be related to the fact the mutant protein could reduce access of ACAT to free cholesterol because caveolin presents a high affinity for cholesterol. In general, we believe that movement of cholesterol (newly synthesized or not) from the ER is affected in Cav-1(-/-) cells because increased esterified cholesterol was observed in Cav-1(-/-) cells incubated with CD-cholesterol. This hypothesis is also consistent with the association between caveolin-1 and SCP-2 recently demonstrated by Zhou et al. (55). Caveolin-1 may enhance the transfer of cholesterol to the plasma membrane already observed with SCP-2 alone (41).

**Fig. 7.** Effects of a Cav-1 deficiency on cellular cholesterol efflux mediated by HDL3. MPMs were labeled in the presence of [3H]cholesterol. After an overnight equilibration in DMEM 0.2% BSA, HDL3 (50 μg/ml) was incubated with the cells. Aliquots of media were taken at indicated times and counted. Efflux is expressed as the percentage of [3H]cholesterol remaining in cells as a function of time. Experiments were performed in triplicate.

**Fig. 8.** Effects of a Cav-1 deficiency on cellular cholesterol efflux mediated by apolipoprotein A-I (apoA-I). Mouse peritoneal macrophages were labeled in the presence or absence of AcLDL (CTL) and with [3H]cholesterol. After an overnight equilibration in DMEM 0.2% BSA, apoA-I (50 μg/ml) was incubated with the cells. Aliquots of media were taken at indicated times and counted. Efflux is expressed as the percentage of [3H]cholesterol remaining in cells as a function of time. Experiments were performed in triplicate.

**Fig. 9.** Inhibition of ABCA-1-mediated cholesterol efflux in cholesterol-loaded macrophages. MPMs were incubated in the absence or presence of AcLDL and labeled with [3H]cholesterol. After an overnight equilibration in DMEM 0.2% BSA, apoA-I (50 μg/ml) was incubated with the cells. Aliquots of media were taken at indicated times and counted. Efflux is expressed as the percentage of efflux observed under control conditions. Experiments were performed in triplicate. *Statistical significance.
Role of caveolin-1 in the regulation of cellular cholesterol efflux. In MEFs, caveolin-1 deficiency had no effect on the regulation of cellular cholesterol mediated by HDL, Two important proteins that regulate cholesteryl efflux from macrophages to HDL have been identified. They are SR-BI and ABCG1 (24, 34, 52). Because SR-BI is not detected in cholesterol-loaded macrophages, cholesteryl efflux to HDL is more likely to be dependent on ABCG1 under these conditions. HDL-mediated cholesteryl efflux from cholesterol-loaded macrophages was not affected in Cav-1(−/−) macrophages (not shown). This finding suggests that ABCG1-mediated cholesteryl efflux is not affected in Cav-1(−/−) MPM. ABCG1 expression is in fact not affected in Cav-1(−/−) MPMs (Fig. 3). We also show that cholesterol efflux to HDL is unaffected in normal MPMs (Fig. 7). Taken together, these data suggest that a caveolin-1 deficiency does not affect HDL-mediated cholesteryl efflux (via ABCG1 and SR-BI) in MEFs nor MPMs.

In macrophages, ABCA1 expression is in part regulated by cellular cholesterol levels (27). This observation may explain why, in Cav-1(−/−) MPMs, ABCA1 expression levels are reduced relative to those observed in WT MPMs. However, reduced ABCA1 expression in Cav-1(−/−) MPMs is not associated with a reduced ability of apoA-I to promote cellular cholesteryl efflux from MPMs (difference not significant). Under these conditions, caveolin-1 would have minimal effects, because its levels are relatively low (Fig. 3) and ABCA1 levels are therefore rate limiting for the efflux process. On the other hand, ABCA1 expression levels are upregulated in both WT and Cav-1(−/−) MPMs upon cholesterol-loading, although ABCA1 levels remain lower in Cav-1(−/−) MPMs. Cholesterol loading of macrophages stimulates ABCA1 expression to a similar extent in both WT and Cav-1(−/−) cells. However, cellular cholesteryl efflux to apoA-I is not affected. This finding suggest that caveolin-1, which is remarkably upregulated in WT MPMs, can regulate ABCA1-mediated cellular cholesteryl efflux to apoA-I because higher levels of ABCA1 are required in WT than in Cav-1(−/−) MPMs to promote equivalent levels of cellular cholesteryl efflux to apoA-I. We also performed additional experiments in the presence of glyburide (Fig. 9). Our results show that, in Cav-1(−/−) MPMs, ABCA1 is more sensitive to the effects of glyburide than in WT cells. It is possible that caveolin-1 regulates ABCA1 function in WT cells. In this case, caveolin-1 may either directly participate in apoA-I-mediated cellular cholesteryl efflux or may protect ABCA1, directly or indirectly, from the effect of glyburide.

The minor differences observed in terms of cholesteryl efflux indicate a minimal role for caveolin-1 in the regulation of cellular cholesteryl efflux to apoA-I and HDL. These findings are consistent with the fact that plasma HDL-cholesterol levels in Cav-1(−/−) mice remain similar to those observed in WT animals (42). This is in marked contrast to the findings made with ABCA1(−/−) mice, in which HDL particles are almost completely absent (31, 36). In addition, cholesteryl efflux from fibroblasts obtained from Tangier disease patients (12, 44, 50) or from macrophages isolated from ABCA1(−/−) mice (36) have demonstrated a reduced ability to efflux cholesteryl to apolipoprotein A-I.

Caveolin-1 and the formation of lipid droplets. Under specific conditions, caveolin-1 has been shown to interact with lipid droplets (17, 37, 40). Our current study also suggests that caveolin-2 may play a role in the formation of esterified-cholesterol containing lipid droplets, as previously suggested (17, 37, 40). Its role in this compartment has yet to be determined but, in the absence of caveolin-1, it may help facilitate the accessibility of esterified cholesterol to cholesterol ester hydrolase and, therefore, compensate for the absence of caveolin-1 during cholesteryl efflux.

Contrary to a previous study (18), we show that caveolin-2 localizes to the plasma membrane in the presence of caveolin-1. Our current study is also consistent with previous reports using nonmacrophage cell types (5, 32, 38, 43) and demonstrates that in the absence of caveolin-1, caveolin-2 remains in the Golgi complex (Figs. 1 and 2). Therefore, caveolin-2 requires the presence of caveolin-1 to be properly targeted to the plasma membrane in MEFs and MPMs.

Overall these findings suggest that caveolin-1 plays an important role in the regulation of intracellular cholesterol homeostasis, but that its role in the regulation of cellular cholesterol efflux is not as critical as previously suggested. These conclusions are also in agreement with our previous findings showing that caveolin-1-deficient mice are less susceptible to atherosclerosis than WT mice, both in the ApoE(−/−) background (14). Caveolin-1 deficiency in macrophages is, therefore, unlikely to play a crucial role in the protection against atherosclerosis observed in these mice.

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