Genetic Ablation of Caveolin-1 Confers Protection Against Atherosclerosis

Philippe G. Frank, Hyangkyu Lee, David S. Park, Narendra N. Tandon, Phillip E. Scherer, Michael P. Lisanti

Objective—The development of atherosclerosis is a process characterized by the accumulation of lipids in the form of modified lipoproteins in the subendothelial space. This initiating step is followed by the subsequent recruitment and proliferation of other cell types, including monocytes/macrophages and smooth muscle cells. Here, we evaluate the potential role of caveola membrane domains in the pathogenesis of atherosclerosis by using apolipoprotein E–deficient (ApoE−/−) mice as a model system.

Methods and Results—Caveolin-1 (Cav-1) is a principal structural protein component of caveolae membrane domains. To directly assess the in vivo role of caveolae and Cav-1 in atherosclerosis, we interbred Cav-1−/− mice with ApoE−/− background. However, despite this hypercholesterolemia, we found that loss of Cav-1 gene expression was clearly protective against the development of aortic atheromas, with up to an ≈70% reduction in atherosclerotic lesion area. Mechanistically, we demonstrated that loss of Cav-1 resulted in the dramatic downregulation of certain proatherogenic molecules, namely, CD36 and vascular cell adhesion molecule-1.

Conclusions—Taken together, our results indicate that loss of Cav-1 can counteract the detrimental effects of atherogenic lipoproteins. Thus, Cav-1 is a novel target for drug development in the pharmacologic prevention of atheroma formation. Our current data also provide the first molecular genetic evidence to support the hypothesis that caveolar transcytosis of modified lipoproteins (from the blood to the sub-endothelial space) is a critical initiating step in atherosclerosis.

Key Words: caveolin ■ cholesterol ■ lipoproteins ■ HDL

Atherosclerosis is a leading cause of death in the United States and the Western world. The development of atherosclerosis is a process characterized by the accumulation of lipids in the form of modified lipoproteins in the subendothelial space. Bioactive lipids generated can then induce an endothelial cell response (ie, the expression of monocyte-specific adhesion molecules and monocyte colony-stimulating factors, among others) that causes the attraction of monocytes and their subsequent transmigration into the subendothelial space. Monocytes can, after differentiation into macrophages, take up modified lipoproteins via cell surface receptors for modified lipoproteins, termed scavenger receptors. This uptake by macrophages results in the synthesis of several factors that stimulate the migration and proliferation of smooth muscle cells. Eventually, this process generates the formation of foam cells and an atheroma.1

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Caveolae (ie, plasmalemmal vesicles) are 50- to 100-nm cell surface plasma membrane invaginations that are found in terminally differentiated cells. They are especially abundant in endothelial cells, smooth muscle cells, fibroblasts, and adipocytes. For example, in endothelial cells, it has been estimated that there are ≈5000 to 10 000 caveolae per cell.2 Caveolin-1 (Cav-1) has been identified as the major caveolae marker protein in non-muscle cells. It is a 178–amino acid integral plasma membrane protein that drives caveolae formation through oligomerization (with itself and with Cav-2) and by interacting with cholesterol.3–5 The formation of this functional assembly unit facilitates targeting of numerous constituents to caveolae, including proteins involved in signal transduction (mitogen-activated protein kinase, adenylyl cyclase, protein kinase Cα, and integrins), membrane receptors (insulin receptor, receptor for advanced glycosylated end products, platelet-derived growth factor and epidermal growth factor receptors, CD36, scavenger receptor class B type I, and endothelin receptor), membrane transporters (porin), and structural molecules and enzymes (annexin II and endothelial [eNOS] and neural nitric oxide synthases). (For a more extensive list, see Razani and Lisanti,6 Anderson,7 and Smart et al.8)
Cav-1 has been suggested to play a critical role in the development of atherosclerosis.9–12 Electron microscopic studies have clearly shown that endothelial cell caveolae function in the uptake and transcytosis of native and/or oxidized LDL, and, therefore, in the initiation of atherosclerosis.13,14 In further support of this hypothesis, CD36 (a class B scavenger receptor that binds both native and modified LDL) is expressed in endothelial cells, has been localized to caveolae, and interacts with Cav-1.9,11,15

We and others have recently reported on the generation of Cav-1–deficient (Cav-1−/−) mice by using standard homologous recombination techniques.16,17 Interestingly, Cav-1−/− fibroblasts, adipocytes, endothelial cells, and smooth muscle cells all lack morphologically identifiable caveola organelles. Despite the absence of Cav-1 and caveolae, Cav-1−/− aortic endothelial cells present with a normal morphology, and the aortic endothelial cell barrier does not show any evidence of fibrosis or abnormal cellular proliferation.18

In addition, Cav-1 functions in the metabolism of lipoproteins and, as such, could shift the distribution of lipoproteins toward a more atherogenic profile, as suggested by preliminary studies in Cav-1–deficient mice.19 Interestingly, Cav-1−/− mice develop hypertriglyceridemia (elevated VLDL/chylomicrons) but have normal plasma cholesterol levels.19 Finally, many investigators have now shown that Cav-1 can negatively regulate eNOS activity.20-22 This negative regulation is especially important in view of the fact that eNOS activation has been associated with a protective effect against the development of atherosclerosis.23,24

The current study was initiated to gain new insights into the role of Cav-1 in the development of atherosclerosis. For this purpose, we interbred Cav-1−/− mice with atherosclerosis-prone mice (apolipoprotein E–null [ApoE−/− mice]) to generate ApoE/Cav-1 double-knockout (dKO) mice. Interestingly, our results indicate that genetic ablation of Cav-1 confers dramatic protection against atherosclerosis. This protective effect occurs despite an abnormal lipoprotein profile, with elevated plasma cholesterol levels. Mechanistically, we show that these findings are likely due to defects in expression of certain proatherogenic molecules, such as CD36 and vascular cell adhesion molecule-1 (VCAM-1), in dKO mice.

Methods

Materials
Antibodies and their sources were as follows: anti-Cav-1 IgG (monoclonal antibody 2297; a gift of Dr Roberto Campos-Gonzalez, BD Transduction Laboratories, Lexington, KY);25 anti-Cav-2 IgG (monoclonal antibody 65; a gift of Dr. Roberto Campos-Gonzalez);26 rabbit anti-Cav-1 IgG and rabbit anti-VCAM-1 IgG (Santa Cruz Biotechnology, Inc); and rabbit anti-mouse apoA-I IgG (Biodesign International). A monoclonal antibody (clone Mo25) directed against CD36 was previously described;27 importantly, this antibody also recognizes murine CD36. A rabbit polyclonal antibody directed against murine GDI-1, a cytosolic protein, was as previously described.28 All other reagents were analytical grade.

Animals
Cav-1−/− mice were generated as previously described.16 All animals used in the current studies were in the C57BL/6J genetic background and were genotyped by polymerase chain reaction.16 Holding and maintenance were provided by the Albert Einstein College of Medicine barrier facility; mice were kept on a 12-hour light/dark cycle and, except where noted, had ad libitum access to food and water. All animal protocols used in this study were approved by the Albert Einstein College of Medicine Institute for Animal Studies. ApoE-deficient (ApoE−/−) mice in the C57BL/6J genetic background were as previously described29–32 and were obtained from the Jackson Laboratory (JAX mice; Bar Harbor, ME). Cav-1−/− mice and ApoE−/− mice were interbred, and the genotypes of the offspring were determined by polymerase chain reaction, as detailed by the Jackson Laboratory. Female ApoE−/−/Cav-1+/+ mice and female ApoE−/−/Cav-1−/− mice were fed a high-cholesterol diet (Western-type diet: 21% fat, wt/wt, and 0.15% cholesterol, wt/wt; Teklad adjusted-calories diet No. 88137) or a control diet (PicoLab Rodent 20, No. 5053). Similar experiments were also carried out with male mice.

Analysis of Lipoprotein Profiles
Fasted blood samples were collected into tripotassium EDTA–containing evacuated tubes after laparotomy and subsequent clamping of the descending aorta. Plasma obtained from 2 mice of the same genotype (150 μL) was pooled and loaded onto 2 Superox 6 columns (analytical grade, Amersham Pharmacia Biotech) connected in series to achieve a total bed volume of ~50 mL and a void volume of 15 mL. Plasma was passed over the columns at a flow rate of 0.5 mL/min, and 0.3-mL fractions were collected. The total cholesterol content of each fraction was determined and plotted against elution volume (Sigma total cholesterol kit). Plasma cholesterol and triglyceride levels were determined by a colorimetric assay system (Sigma cholesterol and triglyceride determination kits).

Tissue Preparation and Quantitation of Atherosclerosis
Collection of aortas and analysis of atherosclerosis were performed essentially as described by Palinski et al.31 After the mice were killed by CO₂ asphyxiation, the aortic tree was perfused with a solution containing evacuated tubes after laparotomy and subsequent clamping of the aorta. Aortas were collected from the heart to the iliac bifurcation and dissected to remove any adventitial tissue. After a short wash in 70% ethanol, the aortas were pinned flat on a black wax surface. Images were collected and analyzed with the ImageJ program developed by Dr Wayne Rasband (National Institutes of Health, Bethesda, Md; available at http://www.rsb.info.nih.gov/ij).

Western Blot Analysis
Protein concentrations were measured with the bicinchoninic acid protein assay (Bio-Rad Laboratories) with bovine serum albumin as the protein standard. Equal amounts of protein for each sample were loaded and run on sodium dodecyl sulfate–polyacrylamide 12% gels. After transfer to nitrocellulose, the expression levels of Cav-1, CD36, VCAM-1, and GDI-1 were examined by using specific antibodies.

Statistical Analyses
Values are reported as mean±SD. Comparisons between control and Cav-1−/− mice were performed with the Student’s t test when appropriate or the Mann-Whitney test (for comparison of aortic lesion areas).

Results
ApoE−/− mice spontaneously develop atherosclerotic lesions.30,31 Therefore, they are a valuable tool to study the development of atherosclerosis and the genes implicated in
Fasting Plasma Cholesterol and Triglyceride Levels Observed in Cav-1 (−/−) and Cav-1 (+/++) Animals, Both in the ApoE (−/−) Background

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normal Chow Diet</th>
<th>Western-Type Diet</th>
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<tbody>
<tr>
<td></td>
<td>Cholesterol, mg/dL</td>
<td>Cholesterol, mg/dL</td>
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<tr>
<td>ApoE (−/−)/Cav-1 (+/+)</td>
<td>540.0±120.1</td>
<td>864.9±336.8</td>
</tr>
<tr>
<td>ApoE (−/−)/Cav-1 (−/−)</td>
<td>703.7±56.5*</td>
<td>1303.5±146.6*</td>
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<td></td>
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<tr>
<td>Plasma triglycerides, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoE (−/−)/Cav-1 (+/)</td>
<td>138.1±45.6</td>
<td>123.4±37.8</td>
</tr>
<tr>
<td>ApoE (−/−)/Cav-1 (−/)</td>
<td>322.8±62.9*</td>
<td>283.3±133.3*</td>
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<tr>
<td>Total body weight, g</td>
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</tr>
<tr>
<td>ApoE (−/−)/Cav-1 (+/)</td>
<td>26.5±1.8</td>
<td>25.8±4.0</td>
</tr>
<tr>
<td>ApoE (−/−)/Cav-1 (−/)</td>
<td>26.1±2.0</td>
<td>28.2±1.5</td>
</tr>
</tbody>
</table>

Values are the average±SD.

*Significant difference, as compared with control animals (P<0.05), n=5 animals for each experimental group. Importantly, the weights of the mice used in this study did not significantly differ on either type of diet used (see above). Note that Cav-1 (−/−) mice show an ≈1.3- to 1.5-fold increase in plasma cholesterol levels, as compared with Cav-1 (+/+) mice. Similarly, Cav-1 (−/) mice show a ≈2.3-fold increase in plasma triglyceride levels, as compared with Cav-1 (+/+) mice.

this process. Previous studies have shown that ApoE−/− mice present a markedly altered lipoprotein profile.30 In particular, they show important increases in plasma cholesterol levels because of increased VLDL-sized and remnant lipoprotein fractions.30 Recent studies from our laboratory have shown that Cav-1 (−/−) mice also show an abnormal lipoprotein profile. However, in Cav-1 (−/−) mice, the VLDL/chylomicron-sized fraction and consequently, plasma triglyceride levels, were markedly increased9; total plasma cholesterol levels remained normal. Here, we investigated the role of Cav-1 in the pathogenesis of atherosclerosis by using Cav-1 (−/−) mice as a model system. More specifically, we generated mice that were deficient in both ApoE and Cav-1 (dKO mice); they were compared with mice that were deficient in ApoE alone (ApoE−/−/Cav-1 (+/++) mice).

Cav-1 Deficiency Induces the Development of a More ‘Proatherogenic’ Lipoprotein Profile in ApoE−/− Mice

ApoE−/−/Cav-1 (+/+) and dKO female mice were fed either a high-cholesterol diet (Western-type diet, as described earlier) or a normal control diet (PicoLab Rodent 20, as described earlier) for 5 months. Interestingly, the Table shows that loss of Cav-1 resulted in an ≈1.3- to 1.5-fold increase in plasma cholesterol levels and an ≈2.3-fold increase in plasma triglyceride levels. For example, fasting plasma cholesterol levels were ≈ 1300 mg/dL in dKO mice fed the Western-type diet.

We next examined their lipoprotein profiles in detail by gel filtration chromatography (Figure 1A and 1B). The lipoprotein profiles of animals fed a normal chow diet are shown in Figure 1A. Note that there is a slight increase in the large apoB-containing and remnant lipoproteins (VLDL-sized and IDL/LDL-sized fractions) in Cav-1−/− deficient animals. However, no changes were observed in the HDL fraction or in the levels of plasma apoA-I, the main protein marker of HDL (Figure 2).

Figure 1B shows the lipoprotein profiles of animals fed a Western-type diet. Interestingly, there was a marked increase (>2-fold) in the large, apoB-containing and remnant lipoproteins (VLDL-sized and IDL/LDL-sized fractions) in Cav-1−/− deficient animals. In addition, we observed that the plasma levels of apoA-I were remarkably reduced by >80% in Cav-1−/− deficient animals (Figure 2). Taken together, these results indicate that loss of Cav-1, in the context of the ApoE−/− genetic background, leads to the appearance of a more “proatherogenic” lipoprotein profile.

Cav-1 Deficiency Dramatically Reduces the Extent of Atherosclerosis in ApoE−/− Mice

To directly examine the role of Cav-1 in the pathogenesis of atherosclerosis, aortas were harvested from 11-month-old
female mice fed a normal chow diet or 6-month-old female mice fed a Western-type diet for 5 months (ie, ApoE\(-/-\)/Cav-1\(+/-\) vs dKO mice). Atherosclerosis was analyzed by en face quantification with Sudan IV to positively stain the lipid-laden, atheromatous lesions. Representative images of these stained aortas are shown in Figures 3 and 4 (A panels).

Note that in animals fed a normal chow diet, Cav-1 deficiency led to a dramatic reduction of \(\approx 70\%\) in the total area occupied by these atherosclerotic lesions (Figure 3; lesion area, 0.092\(\pm\)0.037 vs 0.029\(\pm\)0.017 cm\(^2\)). Similarly, in animals fed a Western-type diet, loss of Cav-1 also markedly reduced the total area of atherosclerotic lesions by \(\approx 70\%\) (Figure 4; lesion area, 0.175\(\pm\)0.019 vs 0.052\(\pm\)0.017 cm\(^2\)). It is important to note that this protective effect occurred despite a >2-fold elevation in plasma LDL cholesterol levels in the dKO mice compared with the ApoE\(-/-\)/Cav-1\(+/-\) mice (Figure 1B).

Interestingly, ApoE\(-/-\)/Cav-1\(+/-\) mice fed a Western-type diet had relatively similar fasting plasma cholesterol levels compared with dKO mice fed a normal chow diet (865\(\pm\)337 vs 704\(\pm\)57 mg/dL). When we compared the extent of atherosclerosis in these 2 mouse strains with similar total cholesterol levels but fed different diets, we found that loss of Cav-1 resulted in an \(\approx 83\%\) reduction in the total area of atherosclerotic lesions (lesion area, 0.175\(\pm\)0.019 vs 0.029\(\pm\)0.017 cm\(^2\)). In fact, this amount of protection might even be an underestimate, because measurements in dKO mice fed a normal chow diet were made after 11 months, whereas measurements taken from ApoE\(-/-\)/Cav-1\(+/-\) mice fed a Western-type diet were performed after only 5 months on the diet.

In addition, we obtained very similar results with 6-month-old male mice fed a Western-type diet for 5 months. In this case, Cav-1 deficiency led to an \(\approx 65\%\) reduction in the amount of fatty streak formation observed in ApoE\(-/-\) mice

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In addition, we obtained very similar results with 6-month-old male mice fed a Western-type diet for 5 months. In this case, Cav-1 deficiency led to an \(\approx 65\%\) reduction in the amount of fatty streak formation observed in ApoE\(-/-\) mice

Figure 2. Cav-1-deficient animals fed a Western-type diet show markedly reduced plasma levels of apoA-I. Levels of apoA-I, the main protein marker of HDL, were determined by Western blot analysis. Results with 2 representative animals are shown for each genotype. Equal volumes of plasma were loaded in each lane. Importantly, plasma apoA-I levels were similar in animals fed a normal chow diet (top). However, Cav-1-deficient animals fed a Western-type diet showed markedly reduced plasma levels of apoA-I (bottom) compared with control animals. These results indicate that loss of Cav-1, in the context of the ApoE\(-/-\) genetic background, leads to the appearance of a more "proatherogenic" lipoprotein profile.

Figure 3. Cav-1 deficiency reduces the extent of atheromatous lesions in ApoE\(-/-\) mice (normal chow diet). A, En face visualization of aortas, which were harvested from ApoE\(-/-\)/Cav-1\(+/-\) and dKO mice fed a normal chow diet. After dissection and cleaning, aortas were positively stained with Sudan IV to visualize lipid-laden, atheromatous lesions (fatty streaks). B, Quantitation. Images were collected and analyzed with the ImageJ program to determine total area occupied by atherosclerotic lesions per aorta. Note that in animals fed a normal chow diet, Cav-1 deficiency led to a dramatic reduction of \(\approx 70\%\) in total area occupied by these atherosclerotic lesions (lesion area, 0.092\(\pm\)0.037 vs 0.029\(\pm\)0.017 cm\(^2\)). *Statistically significant (P<0.05, n=5) for each group of animals.

Cav-1 Deficiency Reduces Aortic Expression of Proatherogenic Molecules CD36 and VCAM-1 in ApoE\(-/-\) Mice

CD36 is a member of the class B scavenger receptor family and has been shown to localize to caveolae membranes and...
interact with Cav-1. CD36 is a proatherogenic molecule that recognizes both LDL and modified forms of LDL and is abundantly expressed in endothelial cells, smooth muscle cells, and macrophages. We have recently examined the association between Cav-1 and CD36 by using transient expression in cultured Cos-7 and HEK-293T cells. Our results indicated that in the absence of Cav-1, CD36 was expressed at relatively low levels and was retained intracellularly in a perinuclear compartment that we identified as the Golgi complex. In contrast, when CD36 was coexpressed with Cav-1, CD36 was targeted to the plasma membrane, and its expression levels were dramatically upregulated by several fold. These results indicate that Cav-1 expression might be required for the normal functioning, cell surface transport, and stable expression of CD36. In addition, genetic ablation of CD36 expression is protective against the development of atherosclerosis in ApoE−/− mice, thereby reducing the area of atheromatous lesions by ≈ 45% to 75%. Thus, we next examined the expression levels of CD36 in aortas isolated from female ApoE−/−/Cav-1+/+ versus dKO mice.

Figure 6 (first panel) shows that loss of Cav-1 gene expression led to dramatic reductions in CD36 expression levels (≈85% to 90% reduced). These data provide the first in vivo confirmation of the notion that Cav-1 indeed functions to stabilize CD36. As such, loss of CD36 protein expression might contribute to the atherosclerosis-resistant phenotype of dKO mice.

Another possible mechanism that could explain the results presented in this study is the effect mediated by eNOS activation on certain cell adhesion molecules. Adhesion molecules have been shown to play a major role in the initiation of atherosclerosis. These molecules are highly expressed at the surfaces of endothelial cells at sites prone to atherosclerotic lesions. Adhesion molecules are responsible

Figure 4. Cav-1 deficiency reduces the extent of atheromatous lesions in female ApoE−/− mice (Western-type diet). A, En face visualization of aortas, which were harvested from ApoE−/−/Cav-1+/+ and dKO female mice fed a Western-type diet. After dissection and cleaning, aortas were positively stained with Sudan IV to visualize lipid-laden, atheromatous lesions (fatty streaks). B, Quantitation. Images were collected and analyzed with the ImageJ program to determine total area occupied by atherosclerotic lesions per aorta. Note that in animals fed a Western-type diet, loss of Cav-1 markedly reduced the total area of atherosclerotic lesions by ≈ 70% (lesion area, 0.175±0.019 vs 0.052±0.017 cm²). Note that total lesion area was higher in animals fed a high-cholesterol (Western-type) diet, as expected. *Statistically significant (P<0.05, n=5) for each group of animals.

Figure 5. Cav-1 deficiency reduces the extent of atheromatous lesions in male ApoE−/− mice (Western-type diet). A, En face visualization of aortas, which were harvested from ApoE−/−/Cav-1+/+ and dKO male mice fed a Western-type diet. After dissection and cleaning, aortas were positively stained with Sudan IV to visualize lipid-laden, atheromatous lesions (fatty streaks). B, Quantitation. Images were collected and analyzed with the ImageJ program to determine total area occupied by atherosclerotic lesions per aorta. Note that in animals fed a Western-type diet, loss of Cav-1 markedly reduced the total area of atherosclerotic lesions by ≈ 65% (lesion area, 0.210±0.061 vs 0.078±0.061 cm²). Note that total lesion area was higher in animals fed a high-cholesterol (Western-type) diet, as expected. *Statistically significant (P<0.05, n=7) for each group of animals.
for the attachment of monocytes to the endothelium. This step is followed by the transmigration of monocytes into the subendothelial space of the vessels.

Among these proteins, VCAM-1 appears to play a major role in atherosclerosis.39 Interestingly, Kawashima et al40 have shown that eNOS overexpression leads to decreased VCAM-1 protein levels in aortic endothelial cells. Because Cav-1 is a natural, endogenous inhibitor of eNOS activity41 and loss of Cav-1 gene expression in Cav-1−/− deficient mice, which have cavity domains 4−deficient mice, which have demonstrated an important role for VCAM-1 in the regulation of cell adhesion molecules51 and its ability to activate eNOS via the scavenger receptor class B type I (SR-BI).52 In addition, several independent studies have now shown that HDL can inhibit adhesion molecule expression in animal models.53−55 Taken together, these observations suggest that HDL could normally play a major role in the downregulation of Cav-1 expression and its subcellular localization.

We have previously shown that CD36, a known scavenger receptor that recognizes both native and oxidized LDL, normally requires coexpression with Cav-1 for its proper trafficking to the plasma membrane and its stable expression.15 The observations presented in this study suggest that Cav-1 deficiency could lead to decreased uptake and, therefore, decreased transcytosis of proatherogenic lipoproteins. Another possible explanation of this phenomenon would be that atherogenic lipoprotein uptake by macrophages is reduced in Cav-1−/− mice. These results suggest that one or more of the very early events involved in the development of atherosclerosis are impaired in Cav-1−/− animals. Several studies have now proposed a role for caveolae in the transcytosis of certain serum macromolecules, such as albumin,44 insulin,45 and native LDL (as well as modified LDL).13,14 In support of this assertion, we have recently demonstrated that Cav-1−/− endothelial cells show defects in the caveolae-mediated uptake of serum albumin, both in vitro and in vivo.19 In the case of LDL, this hypothesis remains to be examined in our animal model.

Oxidation of atherogenic lipoproteins is another important event involved in the development of atherosclerosis.46 In this study, oxidized LDL or isoprostane levels were not measured, but previous studies have suggested that increased eNOS activity can increase LDL oxidation.47 However, this effect does not appear to play an important role in dKO animals because these mice are less susceptible to fatty streak formation. However, increased eNOS activity might still play an important role in the findings presented in this article. Increased eNOS activity can downregulate VCAM-1 expression48 and therefore, might reduce monocyte adhesion to the endothelium and subsequent transmigration into the subendothelial space. VCAM-1 expression in endothelial cells has been found to be associated with lesion-prone sites in ApoE−/− mice but is almost absent in wild-type animals.49 On the other hand, expression of another cell adhesion molecule, intercellular adhesion molecule-1 (ICAM-1), was increased in lesion-prone sites of the aortic arch in both wild-type and ApoE−/− mice. In addition, VCAM-1 expression appears to precede lesion formation,48 suggesting an important role for this adhesion molecule in the initiation of atherosclerotic lesions. These findings are consistent with studies in VCAM-1 domain 4−deficient mice, which have demonstrated an important role for VCAM-1 but not ICAM-1 in the regulation of monocyte recruitment to the arterial intima.50,51 We have previously shown that HDL treatment can downregulate Cav-1 expression in endothelial cells.52 This observation is also in agreement with the effects of HDL on the regulation of cell adhesion molecules53 and its ability to activate eNOS via the scavenger receptor class B type I (SR-BI).54 In addition, several independent studies have now shown that HDL can inhibit adhesion molecule expression in animal models.55,55 Taken together, these observations suggest that HDL could normally play a major role in the downregulation of Cav-1 expression and its subcellular localization.
duced in dKO mice compared with ApoE−/−/Cav-1+/+ mice. In Cav-1−/− deficient animals, we also observed a major increase in the amount of VLDL-like particles. This finding might indeed be related to the inability of these large lipoproteins to enter the arterial wall. Finally, our current observations suggest that caveolae and Cav-1 should be the focus of future drug development in the pharmacologic prevention of atheroma formation.

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References


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