

Atheroprotection in the Absence of “Caves”: Is it the Fat, the Vessels, or Both?

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The protein caveolin-1 is the major coat protein for the organelle termed caveolae. Caveolae (or “small caves”) can be isolated from cells as cholesterol and sphingomyelin-enriched microdomains that are traditionally found decorating both apical and/or basolateral membranes in terminally differentiated cells. Caveolae are the primary plasmalemmal vesicle type found in vascular cells and are implicated in several basic processes, including endocytosis, potocytosis, cellular signaling, and cholesterol homeostasis.¹ Work by many laboratories has placed caveolae and their less complex siblings, lipid rafts, on front and center stage in a variety of broad biology fields, including vascular biology, immunology, microbiology, and cancer biology. The article by Frank et al² in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* sheds light on several fundamental issues regarding the role of caveolae-type vesicles in cholesterol homeostasis and atherogenesis.

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Frank et al² examine the hypothesis that caveolae-type organelles are critical for atherosclerotic lesion progression in mice. To this end, mice deficient in caveolin-1 (Cav-1^{-/-}) and, subsequently, plasmalemmal-type caveolae^{3,4} were bred to mice deficient in apolipoprotein E (ApoE^{-/-}), defective in hepatic LDL cholesterol clearance. Presumptively, it was difficult to predict the net outcome on lipoprotein metabolism and lesion development in this model given the cellular data that (1) caveolin-1 is a cholesterol binding protein that can transport cholesterol from the endoplasmic reticulum to the plasma membrane⁵ and (2) a major receptor for HDL, scavenger receptor SR-B1, and a scavenger receptor for modified forms of LDL, CD36, can reside in and signal in caveolae-type microdomains.⁶ Whether caveolin-1/caveolae are required for cholesterol transport onto HDL or modified LDL is debatable and probably dependent on the abundance of caveolae in the cell types studied. A hint that something interesting may occur in Cav-1^{-/-} mice was alluded to in the initial characterization of the mice, where there was a

large increase in triglyceride content and a slight increase in cholesterol content of VLDL/IDL lipoproteins, but no change in HDL or total cholesterol levels.⁷ Interestingly, breeding Cav-1^{-/-} mice to the ApoE^{-/-} background (ApoE^{-/-}/Cav-1^{-/-}) significantly increased the levels of total cholesterol and triglycerides compared with ApoE^{-/-} alone when the mice were fed normal chow. On feeding these strains a Western diet high in fat, similar qualitative increases in cholesterol, triglycerides, and VLDL/IDL/LDL cholesterol were seen, with no change in HDL levels. Thus, the genetic loss of Cav-1 in an ApoE-deficient background resulted in a proatherogenic lipid profile similar to that seen in CD36-null mice bred to an ApoE background.^{8,9}

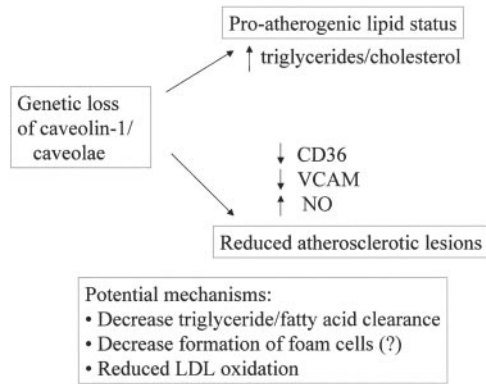
The coup de grace occurred on histological examination of atheromatous lesions in the aortae of these mice. In mice fed normal chow or a Western diet, the genetic loss of Cav-1 resulted in a 70% to 80% reduction in lesion burden despite an unfavorable, proatherogenic lipid status. These data suggest that the loss of Cav-1 severely impairs LDL-mediated vascular dysfunction, inflammation, and lesion progression. The authors suggested this may be due to two potential mechanisms: (1) a decrease in stability of the scavenger receptor for oxidized or modified LDL, CD36, and (2) an increase in nitric oxide production, which would reduce inflammation. Indeed, the protein levels of CD36 and vascular cell adhesion molecule-1 (VCAM-1) (as a surrogate for inflammation) were markedly reduced in aortic extracts from ApoE/Cav-1^{-/-} mice compared with ApoE^{-/-} alone, consistent with the former hypothesis (Figure). These remarkable findings unequivocally support the importance of caveolin-1/caveolae in the pathogenesis of atherosclerosis in mice.

These provocative findings raise several interesting questions regarding the potential mechanisms to explain the lipoprotein defects as well as the marked reduction in atherosclerotic lesions. With regard to the altered lipoproteins status, the shift to a proatherogenic lipid profile suggests that the metabolism of LDL cholesterol, oxidized LDL, or fatty acids is impaired. Because hepatic metabolism of LDL cholesterol is clearly a clathrin-mediated pathway, the shift to a proatherogenic lipid status in the Cav-1/ApoE-null mice likely reflects impaired triglyceride or fatty acid clearance.⁷ Indeed a decrease in triglyceride and fatty acid metabolism due to the loss of CD36 or impaired lipoprotein lipase function in skeletal muscle or adipose tissue¹⁰ may result in increased VLDL/LDL subfractions and may partially explain the phenomenon of insulin resistance observed in these mice.¹¹ However, key experiments examining if defects in lipoprotein status

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Pro- and anti-atherosclerotic phenotypes associated with the loss of caveolin-1.

reflect changes in lipid accumulation in peripheral cells (vasculature, adipose, and skeletal muscle) need to be performed in Cav-1/ApoE-null mice to clearly address this issue.

With respect to the reduced lesion size in Cav-1/ApoE-null mice, the data are consistent with the idea that CD36 requires caveolin-1 to stabilize its trafficking to the plasma membrane¹² as shown by the decrease in CD36 protein expression in vessels from the Cav-1/ApoE-null samples. This idea is further strengthened by data showing that CD36-null mice bred to ApoE^{-/-} phenocopy the changes in plasma lipoproteins as well as the reduction in lesions observed in the Cav-1/ApoE nulls.^{8,9} However several issues need to be resolved before completely linking the phenotypes of the CD36 and Cav-1 deficient mice. For example, CD36 is a key receptor for macrophage uptake of modified LDL, and modified LDL uptake is indeed impaired in macrophages isolated from CD36^{-/-} mice, thus reducing foam cell formation. However, it is not clear if modified LDL uptake is impaired in macrophages deficient in caveolin-1. Moreover, much controversy surrounds if macrophages even express caveolins or have caveolae-type organelles.¹³ In addition, CD36 is primarily found on microvascular endothelial cells, monocytes/macrophages, platelets, adipocytes,^{14,15} and perhaps smooth muscle,¹⁶ but not on macrovascular endothelial cells lining the large vessels typically influenced by atherosclerosis. Therefore, the loss of CD36 in the aortae of Cav-1/ApoE-null mice found by Frank et al² is likely due to the mixed cell populations in the aorta. Another possible explanation for the reduced lesion size in the Cav-1/ApoE mice may be due to activation of endothelial nitric oxide synthase (eNOS), which will exert many atheroprotective actions, including reducing VCAM-1 expression as documented in the aortae of the Cav-1/ApoE nulls. Previous work has shown that Cav-1^{-/-} mice produce more eNOS-derived NO than do littermates,^{3,4} consistent with the concept that Cav-1 is a negative regulator of NO production.¹⁷ Indeed, breeding of eNOS^{-/-} mice to ApoE mice increases lesion area,^{18,19} supporting the atheroprotective role of NO in the setting of high levels of cholesterol. Whether hyperactivation of eNOS due to the loss of Cav-1 delays endothelial

dysfunction in the setting of hypercholesterolemia is not known. Finally, it is possible that the loss of caveolin somehow prevents the initial oxidation of LDL in the vessel wall, perhaps by NO buffering of oxidative stress, thus reducing CD36-dependent foam cell formation and the ensuing inflammatory response.

Regardless of the precise molecular mechanisms to explain the lipoprotein alterations or reduced lesions in the Cav-1/ApoE null mice, the paper by Frank et al² importantly documents for the first time in vivo that caveolin-1 and/or caveolae are important for cholesterol homeostasis and the progression of atherosclerosis. Detailed analysis of lipid metabolism, macrophage function, and vascular signaling in these mice will shed light on the role of “little caves” in atherogenesis.

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