Do the ApoE$^{-/-}$ and Ldlr$^{-/-}$ Mice Yield the Same Insight on Atherogenesis?

Godfrey S. Getz, Catherine A. Reardon

Abstract—Murine models of atherosclerosis are useful for investigating the environmental and genetic influences on lesion formation and composition. ApoE$^{-/-}$ and Ldlr$^{-/-}$ mice are the 2 most extensively used models. The models differ in important ways with respect to the precise mechanism by which their absence enhances atherosclerosis, including differences in plasma lipoproteins. The majority of the gene function studies have utilized only 1 model, with the results being generalized to atherogenic mechanisms. In only a relatively few cases have studies been conducted in both atherogenic murine models. This review will discuss important differences between the 2 atherogenic models and will point out studies that have been performed in the 2 models where results are comparable and those where different results were obtained. (Arterioscler Thromb Vasc Biol. 2016;36:1734-1741. DOI: 10.1161/ATVBAHA.116.306874.)

Key Words: atherosclerosis ■ diet ■ genetic background ■ lipoprotein ■ mice

Atherosclerosis is a complex inflammatory process involving the large blood vessels, a process that evolves throughout the course of its progression. The difficulty of obtaining repeated estimates of the progression of this vascular inflammation in human studies has placed heavy emphasis on preclinical experiments to elicit improved understanding of the process. The early 1990s ushered in a new approach to the preclinical study of atherogenesis with the description of the apoprotein E–deficient mouse (ApoE$^{-/-}$) by Zhang et al$^{1}$ and by Plump et al$^{2}$ in the same year and the description of the low-density lipoprotein (LDL) receptor–deficient mouse (Ldlr$^{-/-}$) by Ishibashi et al$^{3}$ in the following year. These 2 models in the atherosclerosis susceptible C57BL/6 genetic background are widely used to study atherosclerosis with a variety of physiological and genetic interventions, although the use of ApoE$^{-/-}$ mice is more frequent than the Ldlr$^{-/-}$ mice. It is not surprising that investigators have generalized findings obtained with either of these models to fashion the overall picture of atherogenesis. This may be legitimate in many cases, but what we outline below gives some pause about the universality of these generalizations. The question posed in the title of this review may seem straightforward, although a detailed perusal of the literature indicates that in a relatively small number of cases have the same interventions been studied in the same way with the 2 models, and in an even more limited number of examples is the comparison made in the same laboratory. This latter caveat has assumed increasing importance with the recognition that the metabolism of dietary components by gut flora may exert a substantial influence on metabolic outcomes. The microbial environment likely differ from one vivarium to another, and the nature and composition of the diet may have an impact, especially as studies in ApoE$^{-/-}$ mice can be performed using low-fat chow diet or high-cholesterol, high-fat atherogenic diet. In an interesting comparison of the ApoE$^{-/-}$ and Ldlr$^{-/-}$ mice by the same laboratory, several morphological differences were observed. Both sets of knockouts were fed the same high-cholesterol (1.25%) diet containing cholic acid for 1, 2, or 3 months. The ApoE$^{-/-}$ mice exhibited higher plasma cholesterol and larger aortic root lesions with larger necrotic cores, more chondrocytes and bone formation and more smooth muscle cells and matrix at 3 months of diet than did the Ldlr$^{-/-}$ mice.$^{4}$ Whether these distinctions were attributable to the lipoproteins driving the inflammatory process or were intrinsic to the functions of apolipoprotein E (apoE) versus the LDL receptor remains to be established. In atherogenic diet–fed animals, the larger atherosclerotic lesions in ApoE$^{-/-}$ mice compared with those in Ldlr$^{-/-}$ mice are also seen in the atherosclerosis-resistant FVB genetic background, although lesion area was lower than that in the C57BL/6 background.$^{5,6}$ It is of interest that a semisynthetic diet lacking added cholesterol is also capable of inducing atherosclerosis.$^{5}$

Lipoproteins in ApoE$^{-/-}$ and Ldlr$^{-/-}$ Mice

In both models, atherogenesis is driven, at least in part, by non–high-density lipoprotein hyperlipidemia. Among its many functions, apoE serves as a major ligand for the uptake of chylomicron and very low–density lipoprotein (VLDL) remnants into hepatocytes by the LDL receptor, the LDL receptor–related protein 1, and cell surface heparan sulfate proteoglycans. As a result of the critical role of apoE for...
lipoprotein remnant clearance, Apoe<sup>−/−</sup> mice are hyperlipidemic even when fed normal chow, although this is accentuated by feeding a high-fat, high-cholesterol diet. The chylomicron or VLDL remnants that accumulate are predominantly apoB48-containing cholesteryl ester–rich particles. Apoe<sup>−/−</sup> mice also have a low level of high-density lipoprotein cholesterol compared with wild-type and Ldlr<sup>−/−</sup> mice. However, the predominant lipoprotein in the chow-fed Ldlr<sup>−/−</sup> mice is the apoB100-containing LDL. On chow diet, these mice do not readily develop atherosclerosis and, thus, a high-cholesterol diet with or without a high fat is needed to provide the hyperlipidemic drive for atherogenesis. The major accumulating lipoprotein in the plasma of Ldlr<sup>−/−</sup> mice fed a high-cholesterol, low-fat diet is LDL<sup>7</sup> and with the high-cholesterol, high-fat diet VLDL is also elevated.<sup>8</sup> The VLDL in this model is much more triglyceride rich than is the case for the Apoe<sup>−/−</sup> mice. The extent to which these differences in lipoproteins influence atherosclerosis will be explored below. However, the analysis of a large pool of Ldlr<sup>−/−</sup> mice fed the Western type diet for 12 weeks in our laboratory reveals a good correlation between aortic root lesion size and VLDL cholesterol concentration.<sup>9</sup> This was not the case for Apoe<sup>−/−</sup> mice maintained on standard chow for 27 weeks. In these latter animals, the best predictor of aortic root lesion size was the inverse of high-density lipoprotein cholesterol. These correlations were not operative for the size of the innominate lesions in either model, suggesting that lipoproteins do not drive atherogenesis in the same way at the 2 vascular sites.

Lipoproteins and the Initiation of Atherogenesis

One of the earliest features of atherogenesis is the influx of apoB-containing lipoproteins into the subendothelial space at atherosusceptible vascular sites, where they are retained and aggregated.<sup>10</sup> This occurs well before the evidence of macrophage accumulation in these locales.<sup>11</sup> Ultrastructural analysis of this subendothelial space in the arterial wall in Apoe<sup>−/−</sup> mice detected clusters of lipoprotein particles each of which ranges in size from 33 to 66 nm in diameter for individual particles—the sizes of chylomicron remnants and intermediate-density lipoproteins. Lipoprotein size, permeability, composition, and association with matrix components determine their retention in the subendothelial space. It is difficult to tease apart the effect of plasma cholesterol levels, apoprotein content, and lipoprotein size between the 2 models. To help resolve this difficulty, Véniant et al.<sup>12</sup> engineered a series of models in which this could be at least partially resolved. They generated Apoe<sup>−/−</sup> and Ldlr<sup>−/−</sup> mice expressing only apoB100 (apoB<sup>B000/100</sup>). As it turned out, female Ldlr<sup>−/−</sup> apoB<sup>B000/100</sup> and Apoe<sup>−/−</sup> apoB<sup>B000/100</sup> mice fed standard chow exhibited almost identical plasma cholesterol levels, although the size of their lipoproteins differed significantly with the former having LDL with a mean diameter of 24 nm as the predominant plasma lipoprotein and the latter being made up of mostly larger VLDL size particles of mean diameter of 63 nm.<sup>13</sup> Therefore, for a given plasma cholesterol level, there were more particles in the LDL size range in the Ldlr<sup>−/−</sup> apoB<sup>B000/100</sup> mice and more VLDL-sized particles in the Apoe<sup>−/−</sup> apoB<sup>B000/100</sup> mice. The extent of atherosclerosis in the whole aorta was higher in the Ldlr<sup>−/−</sup> apoB<sup>B000/100</sup> mice. This suggests that although the VLDL particles in the Apoe<sup>−/−</sup> apoB<sup>B000/100</sup> mice may carry more cholesterol per particle to the artery wall, the higher number of LDL-sized particles in Ldlr<sup>−/−</sup> apoB<sup>B000/100</sup> mice or increased vascular wall permeability of the LDL-sized particles or their retention through interaction with matrix components is more atherogenic. The digestion of lipoproteins by sphingomyelinase secreted from endothelial cells or macrophages results in protein aggregation and interaction with matrix proteins and proteoglycans.<sup>14</sup> Although the lipoproteins in Apoe<sup>−/−</sup> mice relative to the lipoproteins in Ldlr<sup>−/−</sup> mice are enriched in sphingomyelin,<sup>15</sup> the deletion of sphingomyelinase results in similarly reduced lesion formation in both the Apoe<sup>−/−</sup> and the Ldlr<sup>−/−</sup> models.<sup>16</sup> However, the regions of apoB that mediate the association of the accumulating lipoproteins with proteoglycans in the intima differ between the 2 models. In the Ldlr<sup>−/−</sup> mice, the LDL predominantly associates with heparin sulfate proteoglycans via interaction with apoB100 residues 3359–3369.<sup>17,18</sup> This sequence is absent in apoB48, the predominant apoB species in the Apoe<sup>−/−</sup> mice. In the case of apoB48-containing lipoproteins, residues 84 to 94 are responsible for their binding to proteoglycans, a site that may be masked in apoB100-containing lipoproteins.<sup>19</sup>

Macrophages in Atherogenesis

Macrophages are the first and critical cells to accumulate in the developing lesions, forming the characteristic foam cells that fuel further signaling as the lesions mature. These cells express both apoE and the LDL receptor, but only apoE is a secreted protein and, thus, can function extracellularly. Macrophage-derived apoE has the capacity to rescue the phenotype of Apoe<sup>−/−</sup> mice, reducing plasma lipoprotein levels and atherosclerosis. This was initially demonstrated with the transplantation of wild-type bone marrow that express apoE into Apoe<sup>−/−</sup> recipients.<sup>20,21</sup> Bone marrow transplantations are frequently performed to determine whether the gene of interest is operating mainly in bone marrow–derived cells (eg, macrophages) or in nonhematopoietic vascular cells. Because bone marrow–derived macrophage apoE has on its own a profound impact on atherosclerosis, bone marrow transplantation studies are generally performed in Ldlr<sup>−/−</sup> recipients to avoid these compounding effects. The LDL receptor is downregulated in the presence of hyperlipidemia, and the transplantation of LDL receptor–expressing bone marrow has little if any effect per se on atherosclerosis in Ldlr<sup>−/−</sup> mice.<sup>22</sup> In addition to bone marrow transplantation, the macrophage selective repair of the hypomorphic Apoe gene<sup>23</sup> also is atheroprotective. In these experiments, the reduction in plasma lipoproteins and atherosclerosis by apoE go hand in hand. But this is not always the case. Macrophage-specific expression of a human apoE transgene in Apoe<sup>−/−</sup> mice reduces atherosclerosis<sup>24</sup> even in animals matched for plasma cholesterol levels. Furthermore, mice that are transgenic for adrenal apoE expression at different levels show that at low apoprotein levels atherosclerosis may be reduced with little or no effect on plasma lipids.<sup>25</sup>

These studies highlight 2 features of the extrahepatic production of apoE in securing atheroprotection. First,
macrophages are not unique in their capacity to rescue the apoE-deficient phenotype. Second, apoE has additional atheroprotective functions beyond its capacity to lower plasma lipids. ApoE has been shown to exhibit anti-inflammatory properties, polarizing macrophages from the proinflammatory M1 subset to the anti-inflammatory M2 subset. ApoE also has antioxidative activity, which could contribute to its capacity to reduce atherosclerosis. It also promotes cellular cholesterol efflux from macrophages. None of these properties is shared by the bone marrow or extrahepatic expression of the LDL receptor. A phenomenon that may be important in the context of this discussion is that peritoneal macrophages isolated from Ldlr−/− mice fed a Western type diet show a reduced secretion of apoE. How this relates to the mechanisms promoting atherogenesis in Ldlr−/− mice is unclear. If peritoneal macrophages reflect the phenotype of lesional macrophages, then reduced apoE production in aortic macrophages may also contribute to the development of atherosclerosis in the Ldlr−/− mice.

Macrophages are but one of the cells derived from the bone marrow that populate the atherosclerotic lesion and modulate its evolution. There is a tendency to infer that effects of bone marrow transplantation are attributable to the macrophage lineage in the marrow. Although this may often be the case, it is not always so. For example, myeloid-specific knockout of ABCA1 (ATP-binding cassette A1) in the Ldlr−/− background had little impact on atherosclerosis, although earlier studies using bone marrow transplantation from global Abca1-deficient donors had suggested that the transporter was atheroprotective.

Macrophages in lesions are derived from the influx of blood monocytes, although recent work has highlighted the importance of local proliferation, especially in advanced lesions, to the macrophage content of the lesion. The level of blood monocytes and neutrophils is a risk factor for atherosclerosis, and their level can be regulated by cholesterol homeostasis in the progenitor cells of the bone marrow. In hyperlipidemic or Abca1/Abcg1-deficient animals, the plasma membranes of these progenitor cells are enriched in lipid rafts, where signaling receptors, including interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors, are concentrated. This makes the cells more sensitive to growth factor stimulation, resulting in increased numbers of circulating leukocytes. Cell surface apoE bound to heparan sulfate proteoglycans functions in a cell autonomous fashion in the progenitor cells to facilitate the removal of cellular cholesterol. This property of apoE probably accounts for the higher leukocytosis in Western type diet-fed Apoe−/− animals than in Ldlr−/− animals. The cell autonomous apoE may also function in peripheral macrophages such as those in the lesions to enhance cholesterol efflux from macrophage foam cells.

**Lymphocytes in Atherogenesis**

Although monocytes/macrophages are required for the development of atherosclerosis, this is not the case for T and B cells. Animals lacking these latter cells develop fairly robust atherosclerotic lesions. However, this conclusion is not as straightforward as it suggests because there are several types of T and B cells that could exert either proinflammatory or anti-inflammatory effects. Thus, Th1 and invariant natural killer T cells are proinflammatory, whereas regulatory T cells (Tregs) and possibly Th2 cells are anti-inflammatory. Conflicting results have been reported for the role of Th17 in atherosclerosis. The relative contribution of these cells to the atherogenic process may differ by arterial site at which lesions develop. For example, deficiency of the adaptive immune system in Apoe−/− mice and Ldlr−/− mice reduces lesion formation at the aortic root but not at the innominate artery. Several studies indicate the complexity of these counterbalances. T-cell density in maturing lesions in both the models declines between 1 and 3 months on a cholate-containing atherogenic diet. However, this affects the Apoe−/− animals more profoundly than the Ldlr−/− animals, which have a higher T-cell density at all times. However, when effector T cells and Tregs were separately examined in Ldlr−/− mice fed a cholate-free atherogenic diet, effector T cells increased in lesions with time on diet, whereas circulating and lesion Tregs peaked at 4 weeks of diet and dropped dramatically thereafter. Thus, the ratio of the lesion effector T/Treg cells was profoundly reduced by the end of 20 weeks of diet. This illustrates how sensitive the lesion composition is to the time at which lesions are sampled.

Oxidation of lipoproteins is thought to be a major mediator of atherogenesis. Inasmuch as apoE has antioxidative properties, the oxidation of lipoproteins may be more prominent in the Apoe−/− model. These oxidatively modified lipoproteins serve as autoantigens, eliciting the production of autoantibodies. It is, therefore, perhaps not surprising that the B1 cell produced antibody EO6/T15, which recognizes phosphocholine head group of oxidized phospholipids, was cloned from the Apoe−/− mouse given that antibodies to oxidized LDL epitopes are especially high in this model. Accumulating evidence suggests that these autoantibodies are atheroprotective.

An unusual differential effect of T cells in these 2 animal models is seen in their response to apoA-I deficiency. The Ldlr−/− Apoal−/− mice fed with palm oil and modest levels of dietary cholesterol exhibit obvious dermatitis, lymphadenopathy with an increase in the number, and steroid loading of T cells. However, peritoneal macrophages from the double-knockout mice did not have an increased load of cholesterol compared with Ldlr−/− mice, perhaps because the plasma cholesterol was substantially lower in the double knockouts. With Apoe−/− Apoa1−/− mice fed a Western type diet, no obvious skin lesions were noted, and the peritoneal macrophages isolated at 6 weeks of diet had twice the cholesterol load compared with their single Apoe−/− counterparts. The basis for these distinctions is not clear. Whether apoE, either exogenous or cell autonomous, contributes to the equilibrium of cholesterol in skin and peritoneal macrophages in these models needs further study.

**Atherosclerosis-Related Gene Expression in the 2 Models**

Both the Apoe−/− and the Ldlr−/− models have been used as platforms to assess the role of a large variety of genes in atherogenesis. The majority of the gene functions examined have utilized only 1 model, with most studies using the Apoe−/− mice. In most studies, this involved generating double knockouts of
the gene of interest, although in some cases, especially with the Ldlr<sup>−/−</sup> model, bone marrow transplantation was used. To obtain a true direct sense of how these genes influence atherogenesis, atherosclerosis should be examined with both models subject to the same gene knockout in the same vivarium (ie, microbiome effects), subject to the same diet or the same plasma cholesterol load, and sampled at multiple arterial sites of both males and females and at several times during the evolution of lesions given the highly dynamic inflammatory process associated with atherogenesis. Satisfying these requirements is certainly difficult and may be impossible in view of the differences in plasma lipoproteins in the 2 models. It is perhaps not surprising, therefore, that few studies approach these ideal comparisons. In any event, these considerations must be borne in mind in evaluating questions of the similarity of insight into the atherogenic process provided by the 2 models. In addition, it is necessary to take account of the possibility of false-positive or false-negative results, which, if present, would compound the interpretation of the differences discussed below. Unfortunately, the competitive nature of publication decisions does not make for ready publication of replicate studies, which would be important to demonstrate reproducibility of results, especially in the light of the many experimental differences that may influence the study outcomes.

As tabulated by Hopkins, several gene functions have been examined in the 2 models, with atherosclerosis responding in similar direction (either increase or decrease of lesions or no effect; Table). In most cases, changes in lesion area or coverage are evaluated. Whether this is attributable to a change in the number of cells in the lesion or some cell intrinsic changes is uncertain, partly because few investigators set out to make this distinction.

One study examining apoptosis in the aortic root in Apoe<sup>−/−</sup> Chop<sup>−/−</sup> and Ldlr<sup>−/−</sup> Chop<sup>−/−</sup> male mice performed in the same laboratory comes close to meeting the above-specified requirements. A similar reduction in lesion area and necrotic plaque area was observed in both models, suggesting that the differences in the 2 models do not affect responsiveness to the removal of CHOP (C/EBP homologous protein) function. The findings are more complex in Ifng<sup>−/−</sup> mice. Male and female Ldlr<sup>−/−</sup> and Ldlr<sup>−/−</sup> Ifng<sup>−/−</sup> mice were fed a cholesterol-enriched diet for either 8 or 20 weeks. Lesions in the aortic arch and ascending aorta were reduced in the absence of interferon-γ. Macrophages and smooth muscle cells were reduced in the lesions after 8 weeks of diet but not at 20 weeks, and there was marked decline in lesional T cells between 8 and 20 weeks regardless of the presence of the cytokine. In the Apoe<sup>−/−</sup> Ifng<sup>−/−</sup> mice, although both genders were examined, only male mice exhibited a reduction in lesions (in the aortic arch and ascending aorta) when fed a chow diet or Western type diet. Only in the male mice on Western type diet was there a complex interaction between the cytokine and the sex between the 2 models.

There are also gene knockouts that exhibit different phenotypes in the 2 models (Table). Farnesoid X receptor (FXR) is a bile acid–activated nuclear receptor. Female Apoe<sup>−/−</sup> Fxr<sup>−/−</sup> mice fed a high-fat diet developed less aortic root atherosclerosis than did Apoe<sup>−/−</sup> control mice. However, double-knockout mice in the Ldlr<sup>−/−</sup> background fed Western type diet also had a lower lesion area (as measured by en face analysis) but only in male mice. Hepatic lipase is a plasma lipase that not only hydrolyses plasma lipoprotein phospholipids but can also function as a ligand for the uptake of lipoproteins in the liver. Knocking out this function is associated with increased lesion formation in Ldlr<sup>−/−</sup> mice and decreased lesion formation in Apoe<sup>−/−</sup> mice.

In the Ldlr<sup>−/−</sup> model, the removal of IL-6 function had no impact on plasma lipids or atherosclerotic lesions in the aortic root, whereas in the Apoe<sup>−/−</sup> model, loss of this function was found to be associated with an increment in lesions in the whole aorta and the aortic arch and a decline in IL-10 levels. It is possible that changes in VLDL and LDL levels in the Apoe<sup>−/−</sup> Hif<sup>−/−</sup> model contributed to the lesion enhancement.

Results with abrogation of CD40, a costimulatory molecule, are complex. The lesions in Ldlr<sup>−/−</sup> Cd40<sup>−/−</sup> mice are similar in size to the lesions in Ldlr<sup>−/−</sup> mice, although the transplantation of bone marrow from CD40-deficient mice into Ldlr<sup>−/−</sup> recipients fed the Western diet had reduced lesions. However, Apoe<sup>−/−</sup> Cd40<sup>−/−</sup> mice fed chow have notably reduced lesions, apparently as a result of impaired signaling via tumor necrosis factor receptor–associated factor 6. The basis for the difference between these 2 studies is not clear.

Although platelet–endothelial cell adhesion molecule 1 (PECAM-1) knockout results in a reduction in aortic arch atherosclerosis, particularly the inner curvature, in both murine models, there was a difference in the atherosclerotic response in other vascular sites. No difference was noted in the extent of atherosclerosis in the thoracic descending and abdominal aortas of Apoe<sup>−/−</sup> Pecam1<sup>−/−</sup> and Apoe<sup>−/−</sup> mice fed chow, but the atherosclerotic lesions at these arterial sites were increased in the double-knockout mice compared with Apoe<sup>−/−</sup> mice when the animals were fed the Western type diet for 13 weeks in 1 study but not in another. Similarly, these lesions were increased in Western type diet-fed Ldlr<sup>−/−</sup> Pecam1<sup>−/−</sup> mice. Bone marrow transplantation experiments indicated that PECAM-1 expressed on endothelial cells and hematopoietic cells (leukocytes and platelets) inhibits lesion formation in the thoracic and abdominal aortas in Apoe<sup>−/−</sup> mice. Thus, PECAM-1 regulation of lesion formation was subject to complex influences by model and site of aorta examined. Much further work is required to understand the mechanistic basis of these differences.

Complex results were also seen with the knockout of Gmcsf in the 2 models, although in both models, the plaque phenotype is apparently independent of the growth promoting action of GM-CSF. In the Ldlr<sup>−/−</sup> model, mice fed the Western diet for 12 weeks revealed no change in lesion size in the aortic root, although there was a reduction in macrophage apoptosis and plaque necrosis in the absence of GM-CSF. This plaque phenotype is apparently attributable to reduced GM-CSF induction of the expression of IL-23, leading to increased levels of Bel-2 in the lesions that likely account for the attenuated apoptosis. In contrast, Apoe<sup>−/−</sup> mice lacking
GM-CSF exhibited increased size of the aortic root lesions with increased macrophage content and reduced PPARγ (peroxisome proliferator-activated receptor gamma) and ABCA1 expression.\(^6^6\) PPARγ and ABCA1 expression in the aortic root were not affected by Gmcsf deficiency in Ldlr\(^−/−\) mice.\(^6^5\)

Signaling via the GM-CSF receptor and the IL-3 receptor in Apoe\(^−/−\) bone marrow transplanted into Ldlr\(^−/−\) mice also did not affect lesion size, although the lesions had fewer macrophages and increased necrosis that was accompanied by reduced blood monocytes and neutrophils and their precursors in the bone marrow.\(^6^7\)

**Coronary Artery Atherosclerosis**

Neither Apoe\(^−/−\) nor Ldlr\(^−/−\) mice are characterized by significant coronary artery atherosclerosis. However, models based on these backgrounds have been developed in which coronary artery atherosclerosis is a prominent feature. Mice that are doubly deficient in apoE and the LDL receptor develop obstructive coronary artery atherosclerosis and myocardial infarction, associated with hyperlipidemia when fed a Western type diet.\(^6^8\) Macrophage-specific overexpression of urokinase on an Apoe\(^−/−\) background also results in obstructive lipid-rich coronary lesions associated with myocardial infarction.\(^6^9\)

Perhaps, the most dramatic model involving premature mortality resulting from occlusive coronary artery atherosclerosis leading to myocardial infarction and cardiomegaly is seen in chow-fed mice lacking both apoE and scavenger receptor B1 (SR-B1).\(^7^0\) The hypomorphic apoE (Apoe\(^hb\)) Srb1\(^−/−\) mice fed an atherogenic diet containing cholate produces a similar coronary artery and cardiac phenotype.\(^7^1\) The binding of high-density lipoprotein to SR-B1 triggers activation of the adapter protein PDZK1 (PDZ domain-containing 1), AKT1, and endothelial nitric oxide synthase in endothelial cells. Indeed, mice with Pdzk1, Akt1, or Enos deficiency in the Apoe\(^−/−\) background develop coronary atherosclerosis,\(^7^2\) but an atherogenic diet is required to observe the phenotype. In the case of Akt1, bone marrow transplantation studies indicate that the expression of this protein in the vasculature, probably endothelial cells, is more important for coronary artery atheroprotection than its expression on hematopoietic cells.\(^7^3\) Akt has at least

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CHOP indicates C/EBP homologous protein; DKO, double knockout; ICAM, intracellular adhesion molecule 1; IFN-γ, interferon-γ; and IL, interleukin.

*If no information on sex is included, it means either only 1 sex was studied or the sex of the animals was not identified.
2 isoforms, Akt1 and Akt3. Unlike the atherosclerosis phenotype of Akt1 deficiency in the Apoe<sup>−/−</sup> background, Akt3 deficiency in this background is associated with an increase in atherosclerosis. This seems to be attributable to deficient expression of this isoform in macrophages and increased macrophage lipoprotein uptake, likely mediated by pinocytosis. This is in contrast to the attenuation of this phenotype in atherosclerosis. This suggests that SR-B1 expressed in either macrophages or endothelial cells may contribute in a complex way to the coronary artery lesions. Coronary artery lesions are also seen in Ldlr<sup>−/−</sup> Srb1<sup>−/−</sup> mice, but again only if fed an atherogenic diet.

**Conclusion and Future Perspectives**

The title of this review poses a question that is not readily answered. This is due largely to the difficulty of normalizing the influences on atherosclerosis as reflected by the level and nature of the apoB-containing lipoproteins in the 2 models and difference in experimental design including the duration of the experiment, the dietary composition, the sex of the mice, the protocol of arterial sampling for the assessment of lesions at ≥1 arterial sites, and the possible influence of the intestinal microbiome that may vary between vivariums. There are few reports in which these influences are the same or similar. What is common to both models is the need to induce hypercholesterolemia. For the Ldlr<sup>−/−</sup> model, it is largely the defect in hepatic receptor that accounts for the hypercholesterolemia, whereas deficiencies in apoE in hepatocytes or other cells, notably macrophages, may contribute to the hyperlipidemia. Although the deficiency of the hepatic LDL receptor is critical for the development of hypercholesterolemia, the possible influence of the receptor on other relevant vascular and immune cells has not been intensively studied. Recent work has drawn important attention to risk factors other than hypercholesterolemia per se. Most notable of these is the blood leukocytes, especially monocytes, which seem to be differentially affected by cell autonomous apoE. Cholesterol efflux, which may play an important atheroprotective role, may also respond to cell autonomous apoE in the macrophages in the atherosclerotic lesions. Thus, apoE may contribute to atheroprotection separately from its role in controlling hypercholesterolemia.

Many investigators have used one or other model in which to explore environmental or genetic influences on lesion formation, composition, and stability. These experiments are often performed without regard to whether each atherosusceptible model would yield comparable results. Even in the few instances where the 2 models have been closely contrasted, the precise mechanistic impact of the nature of the hypercholesterolemia and the absence of apoE or LDL receptor on the evolution of lesions because of the genetic modification has not been dissected.

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**Disclosures**

None.

**References**


Ideally, atherosclerosis should be examined with both murine models subject to the same genetic manipulation in the same vivarium, subject to the same diet or the same plasma cholesterol load, and sampled at multiple arterial sites of both males and females and at several times during the evolution of lesions.

The role of gene products on the atherogenic process is often inferred based on studies in only 1 model and often in only 1 sex. These inferences may not be borne out in the other model or sex. Sex differences have also been observed.

Although there are studies where the genetic manipulation was examined in both models and similar effects on atherosclerosis were observed, there are also studies where the same genetic manipulation in the 2 models yielded different results. Sex differences have also been observed.

Ideally, atherosclerosis should be examined with both murine models subject to the same genetic manipulation in the same vivarium, subject to the same diet or the same plasma cholesterol load, and sampled at multiple arterial sites of both males and females and at several times during the evolution of lesions.
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