

# Mouse Models of Atherosclerosis

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**ABSTRACT:** Atherosclerosis is a complex disease in which progressive cellular changes occur for decades before the acute manifestation of cardiovascular disease. Definition of atherogenic mechanisms in humans is hindered by the complexity and chronicity of the disease process, combined with the inability to sequentially characterize lesions in an individual patient because of shortcomings in noninvasive detection modalities. Therefore, there has been a reliance on animal models of the disease to define mechanistic pathways. Over the last decade, the mouse has become the predominant species used to create models of atherosclerosis. The initial interest was based on the great diversity of inbred strains with defined genetic backgrounds that provides a means of linking genes to the development of athero-

sclerosis. More recently, the ability to genetically modify mice to over or under express specific genes has facilitated the definition of pathways in the atherogenic process. All of the current mouse models of atherosclerosis are based on perturbations of lipoprotein metabolism through dietary and/or genetic manipulations. Although hyperlipidemia is necessary for the development of atherosclerosis, mouse models have demonstrated that many nonlipid factors can influence the severity and characteristics of lesions. This review selectively highlights some of the most commonly used mouse models of atherosclerosis and compare their lesions to those formed in the human disease. **KEY INDEXING TERMS:** Atherosclerosis; Mouse; Lipoproteins; Genetic manipulations. [*Am J Med Sci* 2002;323(1):3-10.]

**A**therosclerosis is a complex and progressive disease process in which the description of cellular and biochemical events has relied on complimentary data obtained from human and animal atherosclerotic lesions.<sup>1-3</sup> The disease starts in the form of a fatty streak that is characterized by a predominance of lipid-laden macrophages. Fatty streaks progress to fibro-lipid lesions containing an acellular lipid core encased by smooth muscle cell fibrous caps. This progression of the disease occurs in a clinically-silent manner over many decades before the disease is overtly manifested by occlusive events that are precipitated by rupture or erosion of atherosclerotic lesions.<sup>4,5</sup>

Until recently, the majority of atherosclerotic research focused on mechanisms in rabbits, with a lesser number of studies in pigs and nonhuman primates. These large animal models have provided invaluable insight. For example, the use of pig models of the disease initially revealed that monocyte infiltration was one of the primary cellular events in the atherogenic process.<sup>6</sup> Additionally, studies in monkeys and rabbits have been pivotal in defining the cellular events in the initiation and development of lesions.<sup>7,8</sup> In recent years, there has been an explosion in the number of in vivo studies that is

largely attributable to the use of mouse models to study atherogenic mechanisms.

## *The Use of Mice in Atherosclerosis Research*

The mouse has become an increasingly used species for biomedical investigations,<sup>9</sup> as is evident from their application in many disease areas including vascular pathology. This brief review will focus on the highlights of the most commonly used mouse models of atherosclerosis.

The use of mice in atherosclerosis research has several advantages over other species. A general advantage, exploited in many biological applications, is the extensive genetic information available on the numerous inbred strains. This knowledge permits the elucidation of specific genomic loci with a phenotype. Furthermore, the mouse is the most commonly used mammal for genetic manipulations. These genetically engineered changes include the random insertion of new genes, the insertion of specific mutants in place of the wild-type allele, and the disruption of a specific allele. All of the genetic manipulations offer highly specific changes that permit mechanistic insight to be derived. The use of mice also has a considerable advantage in studies on novel pharmacological agents. Because novel drugs can not be routinely synthesized in large amounts, the small size of mice offers considerable practical advantages to define anti-atherosclerotic efficacy.

In addition to the general advantage of the use of mice in biomedical research, the evolving mouse models offer the advantage of being able to generate

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a range of atherosclerotic lesions from those of simple macrophage foam cell morphology to more complex lesions consisting of acellular lipid cores, fibrous caps, and calcification. In common with all animal models of atherosclerosis, to date, no mouse model reliably exhibits lesion rupture or erosion that precipitates the acute cardiovascular events in humans.<sup>10</sup>

The small size of mice also facilitates the use of large group numbers when studying a specific atherogenic mechanism. This is particularly important in the studies of atherosclerosis, because the size of lesions seems to have a large degree of inherent variability. This variance is particularly surprising in mice, because they are genetically homogeneous in addition to being maintained in highly controlled environments. Therefore, large numbers are frequently required to develop statistically robust data.

Use of mice provides a convenient animal for bone marrow transplantation studies. This technique is now used extensively since the early reports in atherosclerosis research<sup>11,12</sup> and provides insight into the role of genetic manipulations that are specific for hematopoietic cells on the development of atherosclerosis.

The small size of mice has offered some technical challenges, such as the acquisition of sufficient blood to characterize cellular and chemical entities and characterization of small tissues. However, these are generally minor impediments compared with the significant advantages afforded by this species in atherosclerosis research.

### *Quantification of Atherosclerosis in Mice*

Quantification of atherosclerosis in humans and animal models provides considerable technical challenges due to the range of parameters that may be quantified. These parameters are limited not only to the physical size of lesions but also to the many cellular and chemical changes that occur in atherosclerosis. Further complexity arises from the differing characteristics of lesions that may occur in distinct regions of vasculature.

There are several methods for the quantification of atherosclerotic lesions in mice. The lesions formed in the initial atherosclerosis studies on mice were restricted to the aortic root. This arterial region remains the most commonly used in the quantification and characterization of atherosclerosis in these models.<sup>13</sup> The initial description of quantification in the aortic root described measurement of lesions in the ascending aorta 400  $\mu\text{m}$  above the coronary ostia. Many studies now also include lesions in the aortic sinus in the analysis.

An alternative method of analysis, often referred to as *en face*, involves the aorta being removed in its entirety and opened longitudinally. The aortas are pinned on a surface and the area of intima that is covered by lesions is determined. This can be per-

formed on either unstained tissue or aorta that has been stained with a lipid stain such as Sudan IV.<sup>14,15</sup>

The vascular regions of most interest to the clinical manifestation of atherosclerosis are the carotid and coronary beds. There has been recent interest in quantifying extent of atherosclerosis in the innominate artery.<sup>16</sup> However, although there have been frequent descriptions of lesions present in randomly selected coronary vessels, there currently has been no attempt to provide a systematic quantitative method for evaluation in this region.

### *Inbred Strain-Specific Susceptibility of Mice*

Inbred strains have provided considerable insight into mechanisms of selected diseases due to the well-defined genetics of these mice. No known inbred strains spontaneously develop atherosclerosis. However, extensive literature now exists on the differing susceptibilities of inbred mouse strains to develop atherosclerosis during feeding a modified diet that promotes hyperlipidemia. The modified diet used most commonly is enriched in saturated fat, cholesterol, and cholate. This diet is commonly referred to as the "Paigen" diet after the investigator who has contributed extensively to the early evolution of mice in atherosclerosis research. The inclusion of cholate in this diet is particularly controversial because it may produce inflammatory responses.<sup>5,6</sup> Feeding of modified diets increases plasma cholesterol concentrations by increasing the very-low-density lipoprotein (VLDL) fraction; mice fed a normal diet carry virtually all their cholesterol in the high-density lipoprotein (HDL) fraction.

Among the inbred strains, C57BL/6 mice are most susceptible to the development of diet-induced atherosclerosis. The C3H strain is among the most resistant to the development of atherosclerosis; the BALB/c strain is an example of intermediate susceptibility.<sup>17</sup> Even after prolonged feeding of hyperlipidemic diets, the lesions formed in C57BL/6 mice are restricted to the aortic root. These lesions are characterized by deposition of cholesterol, some in crystal form, and macrophages. After prolonged feeding, some investigators have noted more advanced lesions containing cellular debris and collagen.<sup>18</sup> However, in general, these lesions are small, restricted to the aortic root, and do not generally evolve beyond lesions containing lipid-laden macrophages. Also, it is interesting to note that, unlike the incidence of cardiovascular disease in humans, female mice generate larger lesions than male mice.<sup>17</sup>

A major benefit of using specific mouse strains for definition of atherogenic mechanisms is the ability to develop recombinant inbred (RI) strains. These are developed by the interbreeding of 2 parental strains. There are subsequent brother-sister breeding of the F2 strains. The offspring are subsequently interbred for multiple generations. This results in RI strains that have specific genetic mixtures be-

tween the parental strain. Therefore, after development of these RI strains, their propensity to develop atherosclerosis can be associated with a scan of genomic regions to identify alleles that are related to the phenotype. The first use of this approach demonstrated the presence of an *Ath1* gene that was localized on chromosome 1 near the apolipoprotein AII gene.<sup>19</sup> Subsequent studies have demonstrated several other loci associated with increased atherosclerosis, although these have not been related to specific genes.<sup>20</sup> However, there are some shortcomings in the use of quantitative trait analysis with a complex phenotype which may account for contradictory reports on the relationship of *Ath1* to the development of atherosclerosis.<sup>21</sup>

There have been extensive studies in inbred strains of mice to define a number of atherogenic mechanisms, such as acquired immunity<sup>22,23</sup> and inflammation.<sup>24-26</sup> Although the C57BL/6 inbred strain of mouse has been used extensively in atherosclerosis research, its use has diminished with the development of other mouse models of atherosclerosis that develop larger lesions of greater complexity when fed a more physiological diet. However, as discussed below, this strain has important consequences on mouse models of atherosclerosis that have been developed by gene targeting.

#### *Transgenic Models*

Several transgenic mice have been generated that develop atherosclerotic lesions. The enhanced susceptibility of transgenic mice to develop atherosclerosis has been provided by overexpressing an array of genes that have the common property of being involved in some step of regulating lipoprotein metabolism. Listed below is a selected list of some of these transgenic mice to provide an indication of the lipid abnormalities generated in these mice and the types of atherosclerotic lesions formed.

**Apolipoprotein B.** Apolipoprotein B (apoB) is the major protein directing the metabolic fate of both VLDL and LDL, and its increased plasma concentrations are associated with the development of cardiovascular diseases. Therefore, it may be expected that the increased presence of apoB in the plasma of transgenic mice would lead to enhanced atherosclerosis. The development of transgenic mice that express human apoB led to mice that had plasma concentrations similar to those of normolipidemic humans, but no pronounced vascular disease.<sup>27</sup> To promote atherosclerosis, the apoB transgenic mice were placed on a diet enriched in saturated fat and cholesterol.<sup>28</sup> Consistent with findings in C57BL/6 mice, the extent of atherosclerosis was greater in female mice than in male mice. The lesions formed in these mice have a simple morphology that primarily consists of macrophage foam cells.

**Mutant Forms of Apolipoprotein E.** Apolipoprotein E (apoE) is present on the surface of several lipoproteins, including chylomicrons, VLDL and HDL particles. It is an important modulator of lipoprotein interactions with several receptors, including low-density lipoprotein (LDL) receptors and LDL-related receptor.<sup>29,30</sup> In humans there are 3 major isoforms for apoE, which have been designated apoE2, -3, and -4; apoE3 is the most common allelic form. In addition, there are a number of mutant forms of apoE that impact the biological functions of the protein.<sup>31</sup>

Transgenic mice have been created to express the human apoE2 isoform, although the development of atherosclerosis in these mice has not been studied extensively. Lesion development has been defined more extensively in transgenic mice that express 2 more uncommon forms of apoE, apoE3-Leiden,<sup>32</sup> and human apoE2 (Arg112,Cys142).<sup>17</sup> Both these transgenic mice develop an abnormal  $\beta$ -migrating form of VLDL consistent with a prominent characteristic of type III hyperlipidemia. This occurs in these mice despite the continued presence of endogenous apoE.<sup>33,34</sup> These mutant apoE transgenic strains respond to diets enriched in saturated fat, cholesterol, and cholate with a hypercholesterolemic response and develop atherosclerosis in the aortic root. The lesions are initially rich in foam cells and progress to lesions that have fibrous caps.<sup>35,36</sup>

In addition to transgenic animals, an apoE-based mouse model of atherosclerosis has been created by gene replacement in a process that is commonly referred to as "knock-in." Unlike transgenic mice, in which it is common to express from a number of gene copies in various chromosomal locations, the technique of gene replacement converts an endogenous allele to a variant at the same position in the genome. This provides the same tissue-specific expression of the mutant gene as that of the endogenous gene. The development of mice that express apoE2 exhibited plasma lipoprotein characteristics that are equivalent to those of type III hyperlipidemic subjects, including the presence of  $\beta$ -migrating VLDL particles.<sup>37</sup> These mice spontaneously develop atherosclerotic lesions that are predominantly macrophage foam cells with small fibrous caps. These mice are responsive to supplementation of dietary saturated fats and cholesterol in generating a hypercholesterolemic response and augmenting atherosclerotic development. Lesions formed during the feeding of modified diets were also predominantly macrophage foam cells, although there was an increased incidence of fibrous caps, cholesterol clefts, and necrotic areas.

#### *Gene-Targeted Models.*

In gene-targeted mice, a specific allele is deleted that permits definition of a protein with very high specificity. Because these models are genetically en-

Table 1. Characteristics of Selected Mouse Models of Atherosclerosis

Model	Characteristics	Reference
Inbred Mice C57BL/6	Small lesions formed only in the aortic root	17
Transgenic/Gene Replacements apoB	Requires modified diet to form lesions of simple morphology only in the aortic root.	28
apoE variants	Requires modified diet to form lesions of simple morphology only in the aortic root.	35,43,74,75
Gene Targeted Apo E <sup>-/-</sup>	Mice are hyperlipidemic and also respond to modified diets. Lesions progress to an intermediate complexity of macrophage foam cells, necrotic cores, and fibrous caps. Lesions occur in several vascular beds.	46,47,76
LDL receptor <sup>-/-</sup>	Requires a modified diet to promote atherosclerosis. Lesions are representative of early lesions with a predominancy of macrophage foam cells.	43
Compound Genetic Manipulations apoBEC-1 <sup>-/-</sup> × LDL receptor <sup>-/-</sup>	Mice are hyperlipidemic and form pronounced atherosclerotic lesions in many vascular regions.	59
apoB transgenic × LDL receptor <sup>-/-</sup>	Mice are hyperlipidemic and form pronounced atherosclerotic lesions in many vascular regions.	60
apoCIII tg × LDL receptor <sup>-/-</sup>	Lesions require the feeding of a modified diet.	62

gineered to ablate a specific gene, these mice are commonly referred to by the colloquial term of "knock-out."

**LDL receptor<sup>-/-</sup>.** Deficiency of LDL receptors in humans leads to a dramatic phenotype of pronounced hypercholesterolemia.<sup>38</sup> Individuals afflicted with the homozygous forms of this disease usually succumb to a myocardial infarction during the second decade of life.<sup>39</sup> A naturally occurring mutation in rabbits also leads to greatly elevated plasma cholesterol concentrations and the development of atherosclerotic lesions.<sup>40</sup> In contrast, deletion of LDL receptors in mice leads to only a modest hypercholesterolemia when fed a normal diet.<sup>41</sup> However, these mice are very susceptible to atherosclerosis in these LDL receptor<sup>-/-</sup> mice, they were fed the diet developed by Paigen et al<sup>42</sup> that contains saturated fat, cholesterol, and cholate for prolonged intervals. This led to the appearance of atherosclerotic lesions throughout the aorta and large lesions in the aortic root and coronary arteries.<sup>43</sup> Subsequent studies have demonstrated that large atherosclerotic lesions are formed in these mice fed diets enriched in saturated fat without cholate.<sup>14</sup>

The lesions formed in these mice are of simple morphology consisting predominantly of lipid-laden macrophages.<sup>44</sup> Some of the features of advanced lesions, including necrotic cores and calcification, are generated only after prolonged feeding of high fat diets.

**ApoE<sup>-/-</sup>.** As noted above, apoE has a profound effect on lipoprotein metabolism. Therefore, as may be expected, deficiency of this apolipoprotein leads to hyperlipidemia, with an elevation of VLDL sized

particles. Three groups have developed apoE<sup>-/-</sup> mice by genetic engineering, and all have reported similar phenotype of hyperlipidemia and atherosclerosis, even when fed a normal diet.<sup>45-47</sup> Of all the models of mouse atherosclerosis, the apoE<sup>-/-</sup> mouse has been studied the most extensively for the effects of genetic and pharmacological interventions on the development of lesions.

The progression of lesions in this strain has been characterized during feeding of both normal and high-fat diets. Monocyte adhesion to intact endothelium, one of the earliest cellular events in atherogenesis, was detected in 2-month-old apoE<sup>-/-</sup> mice maintained on a normal diet. Lesions that predominantly contain macrophage foam cells occurred at 10 to 30 weeks, and intermediate and fibro-lipid lesions became more apparent from 15 weeks onward.<sup>48</sup> Complex lesions were also associated with localized media degeneration.<sup>49</sup> Feeding apoE<sup>-/-</sup> mice a diet enriched in saturated fat greatly accelerated the progression of these lesions. Complex lesions were formed in many vascular areas of apoE<sup>-/-</sup> mice on normal and modified diets. These included the aortic sinus, several regions of the aorta including the ascending region, the arch, and the renal area, and the innominate and coronary vessels.<sup>16,48,50</sup> Although intraplaque hemorrhage has been demonstrated in mature lesions in the innominate artery of apoE mice,<sup>16</sup> there has not been a demonstration of spontaneous rupture.

As mentioned previously, although wild-type C57BL/6 mice provide a limited model of the atherogenic process, this strain background also infers susceptibility of the disease in the genetically modified mice. Therefore, although the deficiency of

apoE leads to the development of hyperlipidemia, the severity of atherosclerosis formed is dependent on the strain of the mice.<sup>51-53</sup> This is dramatically shown in the comparison of apoE deficient mice that are either in a C57BL/6 or FVB/NJ background. ApoE deficiency in the FVB/NJ strain led to higher plasma cholesterol concentrations than in the C57BL/6 background. Despite the more pronounced hyperlipidemic response, atherosclerotic lesion area in the aortic root was approximately 8 times greater in the C57BL/6 strain. Therefore, other genetically determined factors are acting in concert with the effects of apoE deficiency to determine the severity of the disease process.

In addition to genetically engineered mice, a mouse strain has been identified with a natural mutation that leads to deficiency of apoE. This spontaneous hyperlipidemic mouse also develops severe hypercholesterolemia,<sup>54</sup> although the atherosclerosis is not as severe as in apoE  $-/-$  mice, possibly because of the strain background. The deficiency of apoE has consistently generated mice that are spontaneously hyperlipidemic and have lipoprotein profiles that resemble humans described with this defect.<sup>55</sup> A major shortcoming of apoE  $-/-$  mice is that their lipoprotein profiles are dissimilar from most human subjects. In these mice, most plasma cholesterol is carried in VLDL, rather than in LDL as in humans. Despite this aberrant lipoprotein profile, the characteristics of the atherosclerotic lesions formed in these mice have some of the closest similarities to humans of any available animal model.

#### *Compound Genetic Manipulations*

**ApoBEC-1  $-/-$  x LDL receptor  $-/-$ .** As noted above, LDL receptor  $-/-$  mice develop only modest hypercholesterolemia when maintained on normal laboratory diets. A potential reason for this lack of increased plasma cholesterol concentrations is the presence of the synthesis of apoB48 in the liver of mice, leading to a predominance of this form of apoB on circulating LDL. In humans, full-length apoB (referred to as apoB100) is synthesized in the liver, whereas the truncated form of apoB48 is generated only in the intestine. The presence of this truncated form of apoB on LDL would permit an enhanced binding of apoE and subsequent clearance with mechanisms other than the LDL receptor, such as LDL-related receptors.<sup>56</sup>

The truncated form of apoB arises because of an RNA editing mechanism in which a deamination at cytosine 6666 results in a translational stop codon. This editing is regulated by the enzyme termed apoBEC-1.<sup>57</sup> Deletion of this enzyme does not influence plasma cholesterol concentrations.<sup>58</sup> However, when apoBEC-1 deficiency is combined with ablation of LDL receptors, there is a large increase in plasma cholesterol concentrations, caused exclusively by increased LDL cholesterol.<sup>59</sup>

These mice develop extensive lesions throughout the aorta, including most of the branch points. Although the lesions that form in these mice have not been extensively described, they seem to range from simple lesion morphology of macrophage foam cells to more complex lesions containing smooth muscle cells and extracellular matrix.<sup>59</sup> Feeding a diet enriched in saturated fat enhances the progression of the disease.

**ApoB100 transgenic x LDL receptor  $-/-$ .** Both the apoB100 transgenic and LDL receptor  $-/-$  mouse models of atherosclerosis has been described above, and in both cases, feeding a modified diet is required for the development of significant atherosclerotic lesions. However, the combination of the apoB100 transgenic and LDL receptor  $-/-$  manipulations had a greater hypercholesterolemic response than the individual genetically manipulated mice when maintained on a normal diet.<sup>60</sup> In accord with the endogenous hyperlipidemia in apoB100 transgenic x LDL receptor  $-/-$  mice, there was a pronounced presence of atherosclerotic lesions throughout the aortic intimal surface.

**ApoCIII transgenic x LDL receptor  $-/-$ .** Familial combined hyperlipidemia is a relatively common lipid disorder and is associated with an enhanced incidence and severity of atherosclerotic diseases.<sup>61</sup> A mouse model that recapitulates some of the features of familial combined hyperlipidemia was generated by developing a mouse that expressed the human apolipoprotein CIII in combination with LDL receptor deficiency.<sup>62</sup> Formation of atherosclerotic lesions in the aortic root required the feeding a diet enriched in saturated fat and cholesterol. There is currently no description of the characteristics of the atherosclerotic lesions formed in these mice.

#### **Conclusions**

This is a selected review of some of the most commonly used mouse models of atherosclerosis. In each case, these models involve a defect in lipid metabolism to initiate the disease. However, there has been a wide range of mechanisms studied that could alter the extent and characteristics of the disease. This has mostly been achieved by interbreeding an atherosclerosis susceptible mouse to other knockout or transgenic animals. These interbreedings have studied the role of many factors in the development of atherosclerosis, for example, chemokines,<sup>63,64</sup> adhesion molecules,<sup>65</sup> acquired immunity,<sup>15,66</sup> and cytokines<sup>24</sup> (recently reviewed in Knowles and Maeda<sup>67</sup>). In addition, mouse models of atherosclerosis have been used in many pharmacological studies in multiple areas such as antioxidant therapy,<sup>68</sup> steroid hormone replacement,<sup>69</sup> and the inhibition of the renin angiotensin system.<sup>70,71</sup>

Mouse models are now used routinely in athero-

sclerosis research. How accurately these models mimic the process in humans is difficult to ascertain given our lack of detailed knowledge of the human disease. However, this is a generic concern that applies to all animal models of atherosclerosis. Certainly, the atherosclerotic lesions formed in mouse models do not progress to the stage in which lesions promote the clinical manifestations observed in humans. There have been few descriptions of myocardial infarction or stroke in mice as a consequence of atherosclerotic lesion formation,<sup>72</sup> despite the presence of extensive lesions that display characteristics similar to that of "vulnerable" lesions in humans.<sup>73</sup> However there are some striking similarities in the morphology of lesions formed in mice compared with specific stages of the human disease. Therefore, it is likely that mouse models will be increasingly used to define mechanisms in the atherogenic process.

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