Animal Models of Atherosclerosis

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Abstract—Atherosclerosis is a chronic inflammatory disorder that is the underlying cause of most cardiovascular disease. Both cells of the vessel wall and cells of the immune system participate in atherogenesis. This process is heavily influenced by plasma lipoproteins, genetics, and the hemodynamics of the blood flow in the artery. A variety of small and large animal models have been used to study the atherogenic process. No model is ideal as each has its own advantages and limitations with respect to manipulation of the atherogenic process and modeling human atherosclerosis or lipoprotein profile. Useful large animal models include pigs, rabbits, and nonhuman primates. Due in large part to the relative ease of genetic manipulation and the relatively short time frame for the development of atherosclerosis, murine models are currently the most extensively used. Although not all aspects of murine atherosclerosis are identical to humans, studies using murine models have suggested potential biological processes and interactions that underlie this process. As it becomes clear that different factors may influence different stages of lesion development, the use of mouse models with the ability to turn on or delete proteins or cells in tissue specific and temporal manner will be very valuable. (Arterioscler Thromb Vasc Biol. 2012;32:1104-1115.)

Key Words: atherosclerosis ■ genetically altered mice

Atherosclerosis is a chronic inflammatory disease affecting the large and medium-size arteries. Both the innate and adaptive immune systems are involved and are often activated in response to hyperlipidemia. Atherosclerosis is at the core of cardiovascular disease leading to myocardial infarctions, stroke, and peripheral vascular disease encountered in most human populations, except where caloric intake is suboptimal. Animal models of atherosclerosis have greatly increased our understanding of this chronic inflammatory disease. In this review we will describe the utility of several of these models that further this understanding.

Hypercholesterolemia and variations in arterial vessel hemodynamics are 2 important variables influencing atherogenesis. Even within susceptible arteries, atherosclerosis is a regional inflammation largely determined by variations in local hemodynamics. Atherosusceptible sites in arteries are regions of disturbed flow or low shear stress, whereas atheroresistant sites are regions of laminar flow and relatively high shear stress. These 2 regions are subject to the same systemic risk profiles; however they respond differently and, in addition, lesion growth at different susceptible sites can vary greatly. This caveat makes it obligatory to examine more than one site of lesion development in animal models in order to clarify the subtle relationships between hemodynamics and the cell and cellular factors that influence the atherogenic process.

Animal Models of Atherosclerosis

Of Mice and Men
Currently the mouse is the most frequently used species for atherosclerosis studies. Mice and humans differ in several parameters that may influence atherogenesis (Table I in the online-only Data Supplement). In addition, their lesion distribution is not identical. Lesions occur more frequently in humans in the coronary arteries, carotids, and peripheral vessels, such as the iliac artery, and in mice more frequently in the aortic root, aortic arch, and innominate artery. Nonetheless many of the critical features of the atherosclerotic process are shared. The primary advantages of the mouse for the study of atherogenesis and its complications rests on its relatively low cost of purchase and maintenance, ease of breeding, ease of genetic manipulation, and the ability to monitor atherogenesis in a reasonable time frame (Table I and Table I in the online-only Data Supplement). The relatively large number of inbred mouse strains with different susceptibility to development of atherosclerosis has great potential for the identification of genes involved in the determination of sensitivity or resistance to atherosclerosis, especially when crossed into genetically manipulated atherosclerosis models. Murine atherosclerotic models depend on generating a non-HDL based hypercholesterolemia. This is most readily accomplished by the genetic ablation of apoE or the LDL receptor (LDLR). Although apoE deficiency in humans is rare, the absence of a functional LDLR in humans results in familial hypercholesterolemia that is notable for its increased risk of cardiovascular disease. The arterial lesions in human familial hypercholesterolemia have some of the characteristics of the mouse lesion, including the presence of lesions in the aortic valves and, in some autopsy studies, in the aortic root.

Although studies in murine models have significantly contributed to our understanding of the mechanisms of atherogenesis, the extent to which the mouse serves as an accurate model of the human disease is open to discussion. Most murine models...
do not manifest the unstable atherosclerotic plaque with overly- ing thrombosis, the lesion most often associated with clinically significant acute cardiovascular episodes. Murine lesions do not characteristically develop the thick fibrous cap seen in chronic human atherosclerosis. In addition, the number of lamellae in the normal arterial media layer is small and the characteristic medial vasa vasora seen in the large arteries of humans is not observed. Unlike humans, mice seldom develop atherosclerosis in the coronary arteries but readily develop atherosclerosis in the aortic root. The much more rapid heart rate of the mouse and hence disturbed blood flow probably accounts for the atherosclerosis predilection at this site. The small size of the mouse complicates some of the analysis of atherosclerotic vessels, though recent technological advances and immunochemical approaches have mitigated this to some extent. The wild type mouse is an “HDL” animal (ie, HDL is the primary lipoprotein), which may contribute to the relative atheroresistance even in atherosusceptible wild type mouse strains. Furthermore, mice do not exhibit the same range of HDL subsets as is found in humans. Inasmuch as subclasses of HDL have been claimed to exhibit different levels of atheroprotection, this could be important. Finally, wild type mice do not express cholesteryl ester transfer protein (CETP), a plasma protein that has occasioned much interest as a potential target for atheroprotection in humans.

**Large Animal Models of Atherosclerosis**

In contrast to the mouse, larger animals are more expensive to purchase, feed, and maintain in conditions appropriate to modern animal husbandry. Additionally, the development of complex atherosclerotic lesions usually involves a longer period of time than in the mouse. Nevertheless, some large animal models have particular advantages for certain types of studies. Most studied are pigs, rabbits, and nonhuman primates.

**Experimental Atherosclerosis in Pigs**

Pigs spontaneously develop atherosclerosis and this is accelerated by feeding an atherogenic diet. The pig is large enough to allow for noninvasive measurements of arteries and for harvesting sufficient arterial tissue for analysis, has a human-like lipoprotein profile, and develops lesions in the coronary arteries. In part because of its size, the pig has been useful for the elucidation of the heterogeneous interaction of hemodynamics and the responses of the arterial endothelial layer. Atherosclerotic lesions generally occur at arterial bends, characterized by flow separation and low shear stress. A number of seminal studies on the pig were carried out by Gerrity and colleagues. Even pigs on a low fat chow diet were shown to exhibit areas of either enhanced or limited aortic permeability (demonstrated using Evans blue staining), which corresponded respectively to atherosclerosis sensitive or resistant areas of the aortic arch. Subsequently, Davies and colleagues have characterized the endothelial transcriptome at various atherosclerosis susceptible and resistant sites of the porcine arteries and have found that the endothelial gene expression phenotype is distinct at these 2 sites. Feeding pigs a 1.5% cholesterol, 19.5% lard diet for up to 30 weeks induced a hypercholesterolemia involving mostly LDL and generated predominantly foam cell atherosclerotic lesions in atherosusceptible areas of the aorta.

**Table 1. Advantages and Disadvantages of Various Animal Models of Atherosclerosis**

<table>
<thead>
<tr>
<th>Species</th>
<th>Advantages</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>Mice</td>
<td>Ease of breeding/size of litters</td>
<td>No coronary lesions</td>
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<tr>
<td></td>
<td>Multiple inbred strains</td>
<td>Limited complexity of lesions and medial vasa vasora</td>
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<tr>
<td></td>
<td>Ease of genetic manipulations to study role of cell types or proteins on atherosclerosis</td>
<td>Model of atherogenesis not plaque instability</td>
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<tr>
<td></td>
<td>Relatively low cost of maintenance</td>
<td>Limited tissue availability and technical difficulties due to small size</td>
</tr>
<tr>
<td></td>
<td>Atherosclerosis develops over relatively short period of time</td>
<td>Wild type relatively atherosclerosis resistant</td>
</tr>
<tr>
<td></td>
<td>Smaller amounts of compounds needed for studies due to small size</td>
<td>Monotypic HDL</td>
</tr>
<tr>
<td>Pig</td>
<td>Size facilitates tissue availability and non-invasive measurements</td>
<td>Expense</td>
</tr>
<tr>
<td></td>
<td>Human-like lipoprotein profile except for human HDL subclasses</td>
<td>No genetic modifications</td>
</tr>
<tr>
<td></td>
<td>Moderately atherosclerosis sensitive on normal diet</td>
<td>Limited genetic models</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Cholesterol sensitive</td>
<td>Largely foam cell lesions</td>
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<tr>
<td></td>
<td>Expresses CETP</td>
<td>Hepatic lipase deficient</td>
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<tr>
<td></td>
<td>WHHL genetic model</td>
<td>Modest genetic modifications</td>
</tr>
<tr>
<td>Nonhuman primates</td>
<td>Humanoid lipoproteins and lesions</td>
<td>Expense</td>
</tr>
<tr>
<td></td>
<td>Develop coronary lesions</td>
<td>No genetic modifications</td>
</tr>
<tr>
<td></td>
<td>Social and behavioral studies</td>
<td>Limited genetic information</td>
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</tbody>
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CETP indicates cholesteryl ester transfer protein; WHHL, Watanabe hereditary hypercholesterolemic.
the finding that the ratio of lipid-rich macrophage foam cells to lipoid-free monocytes associated with the endothelial surface of nonprogressing atherosusceptible arterial sites increased with increased duration of fat feeding.20 Extracts of the atherosusceptible aortic areas of the hyperlipidemic pigs exhibited monocyte chemotactic activity, unlike extracts of normal aorta or atheroresistant areas of the aorta in the hyperlipidemic pigs.21 The superimposition of streptozotocin-induced diabetes onto the high-fat diet feeding of pigs induces complex lesions in the first few centimeters of the coronary arteries without a major effect on plasma cholesterol levels, although plasma triglyceride levels increase.22 These lesions resemble human coronary lesions in complexity, with a thick fibrous cap, a necrotic lipid core, calcification, and hemorrhaging. Porcine models of familial hypercholesterolemia have been shown to develop complex atherosclerotic lesions.23 The genetic basis for the hypercholesterolemia is the inheritance of variants in apoB100 resulting in delayed LDL clearance. However, the size and composition of the LDL differ in the high-fat diet fed pigs and the familial hypercholesterolemic pigs.24

Experimental Atherosclerosis in Rabbits

The rabbit is the species perhaps most sensitive to dietary cholesterol overload.25 In the cholesterol-fed New Zealand white (NZW) rabbit, remnant lipoproteins predominate and the lesions are composed largely of macrophage-derived foam cells.26 Rabbits have many of the same advantages as the pig but, although they express CETP, they have very low plasma levels of hepatic lipase and lack apoA-Ⅱ. The modulation of the lipoprotein and atherosclerotic response in rabbits as a function of feeding cholesterol diets containing different dietary fats has been examined in an extensive series of studies by Kritchevsky.27 It was in the rabbit that the impact of lipoproteins of different sizes on atherosclerosis was first studied. For example, it was noted early on that alloxa induces diabetes surprisingly inhibited cholesterol-induced atherosclerosis in rabbits.28 This appears to be attributable to the fact that in this diabetic model very large triglyceride-rich lipoproteins (>75 nm diameter) accumulate in the plasma and the vascular wall has extremely limited permeability for these particles.29

Like the pig, rabbit strains that exhibit familial hypercholesterolemia have been identified. The Watanabe hereditary hypercholesterolemic (WHHL) rabbit has a defect in the LDLR.30 This model was carefully studied at about the time that Goldstein and Brown first identified the LDLR as the defective protein underlying human familial hypercholesterolemia. In contrast to the cholesterol-fed NZW rabbit, the predominant lipoprotein in the WHHL rabbit is LDL. Lesions progress in WHHL rabbits to advanced lesions over time, with many of the foam cells in the lesions being of smooth muscle cell (SMC) origin.30,31 WHHL heterozygous rabbits fed 1% cholesterol for 11 to 12 months develop lesions very similar to those seen in homozygous familial hypercholesterolemic patients and include areas of necrosis, cholesterol clefts, and calcification.32 With selective breeding, Watanabe was able to develop a substrain of WHHL males with robust coronary atherosclerosis.33 Transgenic rabbits have been generated.34 However, overall the rabbit does not lend itself to the ease and types of genetic manipulation that are possible in mice.

Atherosclerosis in the Non-Human Primate

Atherosclerosis and lipoprotein metabolism has also been studied in nonhuman primates. Old World monkeys were studied by Portman and Andrus.36 They found that there were differences in atherosclerosis susceptibility among Cebus, woolly, and squirrel monkeys, ranking in that order from low to higher susceptibility. Other studied primate models include African Green, Rhesus, and Cynomolgus monkeys and baboons.37–40 They all have the advantage of having a humanoid lipoprotein metabolism with a predominance of non-HDL lipoproteins, human-like HDL subclasses, and expression of CETP. On feeding a high-fat diet, males develop more atherosclerosis than female animals, as is also the case in humans. Rhesus monkeys fed a high-fat, high cholesterol diet develop complex coronary artery lesions41 with an increase in the density of vasa vasora in the media and thickened intima.42 This species was among the first to reveal the regression of coronary atherosclerosis consequent on the return to a low-fat diet.43 Regression was associated with a reduction in cholesterol content of the lesion, consistent with a reduction in either the number of foam cells or their lipid content. Davis and Wissler compared the development of atherosclerosis over time in Rhesus and Cynomolgus monkeys fed a high-fat (12.5% coconut oil and 12.5% butter fat) diet containing 2% cholesterol.39,44 The Cynomolgus monkeys were more responsive to the diet. They had approximately twice as high plasma cholesterol levels, developed more skin xanthomata, and had more lipophages (ie, lipid loaded monocytes) in their blood than the Rhesus monkeys. Atherosclerosis developed more rapidly in the Cynomolgus monkeys, and their lesions were much richer in foam cells. Cynomolgus monkeys have been used for extensive studies of the influence of social status on atherosclerosis.45–47 Atherosclerosis in females was particularly dependent on social status, with submissive females developing almost as much atherosclerosis as in male animals. In contrast, competitive females have less atherosclerosis. This difference could be abrogated by ovariectomy. Baboons exhibit lesions, albeit fatty streaks for the most part, in both the wild and captive state with some fibrocellular lesions in the aorta and coronary arteries.48,49 When challenged with high cholesterol, high-fat diets producing relatively modest hyperlipidemia, there is a good deal of variability in the lipoprotein and atherosclerosis response among individual baboons.50 This led to the selective breeding of animals for high and low VLDL and LDL levels in response to diet.51 Some responders exhibit a large HDL1 lipoprotein when fed a saturated fat diet and there appears to be a negative correlation between the extent of atherosclerosis and HDL1 levels. The analysis of members of a pedigree from crosses between olive and yellow baboons showed that the expression of arterial endothelial cell adhesion molecules in response to TNFα stimulation was highly heritable.52 The size, the expense of their upkeep, and the length of time required for development of atherosclerosis, as well as the difficulties of genetic studies, have limited the utility of nonhuman primates for atherogenesis research.
Limited studies have been done in such other species such as rats, hamsters, pigeons, and chickens. Space limitations do not permit the review of atherosclerosis in these species.

**Use of Genetically Modified Mice to Study Atherosclerosis**

Despite its limitations, the mouse remains the favored species for atherosclerosis investigation. The ease of genetic manipulation allows for transgenesis, for gene knockout and knockin, and for temporal and tissue specific conditional knockout or expression of genes. Genetic modifications influencing either the level or function of specific immune cells (eg, MCSF1−/− decreasing monocytes) have played a major role in dissecting the cellular and molecular mechanism of the role of the immune system on atherogenesis (Table II in the online-only Data Supplement). The relative ease of breeding mice allows for the simultaneous modification of more than one gene in a single animal model. As atherogenesis is influenced by multiple genes often acting in concert (eg, see Ref. 53), this represents an important advantage.

The 2 most frequently used models of mouse atherosclerosis are the apoE−/− model and the LDLR−/− model. They differ in their dietary needs for developing atherosclerosis (Table II). The apoE−/− model is perhaps the most widely used. The advantage of the apoE−/− model is that complex vascular lesions readily develop in animals fed the normal low-fat rodent chow (Table II), even when plasma cholesterol levels are between 300 to 500 mg/dL. These lesions are comparable to human lesions. The rate of atherogenesis can be notably accelerated by the feeding of a high-fat diet (WTD) (typically 0.2% cholesterol, 21% milk fat), resulting in significant increases in plasma lipid levels (>1,000 mg/dL). Examples of lesions in chow or WTD-fed apoE−/− mice exposed to approximately the same lifetime burden of plasma cholesterol are shown in Figure I in the online-only Data Supplement. The lesions in mice fed WTD are more enriched in foam cells, whereas the lesions in the chow-fed animals are more complex and cellular. It is worth noting that the effect of various treatments or gene manipulations on the atherosclerosis phenotype is not necessarily the same when chow or a high-fat diet is fed. For example, when chow is fed to apoE−/− mice and immune deficient apoE−/− RAG−/−, atherosclerosis is attenuated in the aortic root in the immune deficient animals, but no such attenuation is observed when a high fat diet is fed to the same animals. (The recombination activation genes RAG1 and 2 are involved in the recombination of immunoglobulin gene sequences and T cell receptor gene sequences, which are the hallmark of B and T cells, respectively. Thus in the absence of this recombination all cells of the adaptive immune system are lacking.) A thorough pathological analysis of the evolution of atherosclerosis in apoE−/− mice fed either chow or WTD represents the best systematic analysis of lesion development in mice. Lesions developed in the aortic root, the innominate artery (brachiocephalic) and other branches of the aorta, as well as the pulmonary and carotid arteries. While fed chow, foam cell lesions first appear at 8 to 10 weeks of age. After 15 weeks intermediate lesions are present, containing spindle shaped cells (mostly SMC) and beyond 20 weeks, fibrous plaques are evident containing SMC, extracellular matrix, and an overlying fibrous cap. This time course mainly refers to the aortic root, as lesions develop at different rates at each of the atherosclerosis prone sites. This time course is accelerated by the feeding of the WTD, with the more advanced lesions in these mice containing obvious cholesterol crystals, necrotic core, and calcification. The adhesion molecules, VCAM-1 and ICAM-1, are expressed early in the lesion prone areas. In older apoE−/− animals, hemorrhage has been observed in lesions suggesting some degree of lesion instability. The aortic root has 3 sinuses that develop lesions at different rates (unpublished observation), with the sinuses associated with the left coronary artery usually developing lesions first.

Despite the widespread use of the apoE−/− model, it has several disadvantages. Plasma cholesterol is mostly carried on lipoprotein remnants (containing apoB48 as the main apoB protein) rather than the LDL, which is the most frequent “offending” particle in human atherosclerosis. The hyperlipoproteinemia of apoE−/− mice is largely attributable to the absence of the lipoprotein ligand for the major cell surface receptors responsible for high affinity plasma lipoprotein clearance (LDLR and LRP). However, apoE has other functions affecting macrophage biology, immune function, and adipose tissue biology, each of which could have an impact on atherosclerosis independent of plasma lipid levels. For example, apoE is expressed in bone marrow derived cells (mostly the macrophage/monocyte lineage) and the transfer of bone marrow from apoE−/− mice into wild type mice increased aortic root atherosclerosis independent of effects on plasma lipid levels. This suggests that macrophage derived apoE has an independent role in lesion development, perhaps related to the promotion of cholesterol efflux from the macrophage foam

<table>
<thead>
<tr>
<th>Table 2. Some Differences in the Phenotype and Response of LDLR−/− and apoE−/− Mice LDLR, apoE, and Ref. columns</th>
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<tbody>
<tr>
<td>Develops atherosclerosis on chow</td>
</tr>
<tr>
<td>Prominent lipoproteins</td>
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<tr>
<td>Significant independent effect upon transplantation of bone marrow</td>
</tr>
<tr>
<td>Effect of natural killer T-cell deficiency on atherosclerosis</td>
</tr>
<tr>
<td>LIGHT overexpression in T cells in female mice</td>
</tr>
<tr>
<td>Effect of FXR deficiency</td>
</tr>
<tr>
<td>Effect of hepatic lipase deficiency</td>
</tr>
<tr>
<td>Correlation VLDL levels with aortic root atherosclerosis</td>
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</table>
cells. On the other hand, very low levels of apoE selectively expressed in the adrenal cells can also attenuate atherosclerosis in the apoE−/− animal without affecting plasma lipid levels. A major disadvantage to the use of this model is that the transfer of bone marrow from a mouse expressing apoE into an apoE−/− recipient reduces atherosclerosis but also significantly reduces plasma lipid levels. The apoE mediated reduction in plasma lipids can itself contribute to the reduction in atherosclerosis.

The advantage of the LDLR−/− model is that, as important as is the LDLR for lipoprotein homeostasis, it does not have the multitude of functions as described for apoE. The absence of the LDLR mainly influences lipoprotein uptake and clearance, resulting in a greater preponderance of LDL as the cholesterol carrying plasma lipoprotein in chow-fed animals. However on chow, limited lesions only develop in older LDLR−/− animals. Significant lesion development requires the feeding of an atherogenic diet containing high fat with or without cholest erol. A variety of high-fat diets with different levels of cholesterol have been used, so that studies with this model are often not precisely standardized or comparable from one laboratory to another (Table 3). With high-fat feeding, there is an accumulation of larger VLDL/remnant lipoproteins and with most of these diets, plasma total cholesterol levels are much higher than in the chow-fed apoE−/− mice. This profile can be modified by feeding a high cholesterol, low-fat diet in which the level of the VLDL/remnants are substantially reduced and the insulin resistance often associated with the feeding of the typical WTD is substantially attenuated. Lesions in the LDLR−/− model have a greater preponderance of foam cells than in chow-fed apoE−/− mice. Unlike the apoE−/− mice, no systematic pathological analysis of lesion development in the LDLR−/− mice has been reported. An advantage of this model is the expression of the LDLR on bone marrow derived cells in WTD diet-fed LDLR−/− mice does not have a large impact on atherosclerosis or plasma lipids as it does in the apoE-deficient model.

Table 3. Some Commonly Used Atherogenic Diets in Murine Atherosclerosis Studies

<table>
<thead>
<tr>
<th>Diet name</th>
<th>Diet composition</th>
<th>Comments</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Western type</td>
<td>21% milk fat,</td>
<td>Most widely used diet</td>
<td></td>
</tr>
<tr>
<td>diet (WTD)</td>
<td>0.2% total cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified WTD</td>
<td>4.4% fat,</td>
<td>Reduced insulin resistance</td>
<td>Relative to WTD</td>
</tr>
<tr>
<td></td>
<td>1.0% cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified WTD</td>
<td>15.8% fat,</td>
<td>Higher cholesterol diet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.25% cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palm oil diet</td>
<td>10% palm oil,</td>
<td>Equal amounts of monounsaturated and saturated fatty acids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1% cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semisynthetic diets (low and high fat)</td>
<td>2–18% fat, 0–1.25% cholesterol</td>
<td>Used to examine effects of cholesterol and types of dietary fat on atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>Paigen diet</td>
<td>15% cocoa butter, 1.25% cholesterol, 1% corn oil, 0.5% cholic acid</td>
<td>Cholic acid has been shown to induce inflammation</td>
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LDLR deficiency has been coupled with a deficiency of the apoB100 editing enzyme apoBec-1 or human apoB100 transgene to yield mouse models that develop atherosclerosis while being fed chow. In both models apoB100 containing lipoproteins predominate. Some groups use the apoE*Leiden mice that are transgenic for an apoE variant with low affinity for the LDLR. These mice, are less hyperlipidemic than apoE−/− mice on chow and an atherogenic diet and develop lesions when fed the atherogenic diet. As mice lack CETP and CETP influences HDL levels in humans, models in which a human CETP transgene has been expressed in high-fat diet fed LDLR−/−, apoB100 transgenic, and apoE*Leiden have been used to study atherosclerosis. On expression of CETP, HDL levels are decreased and VLDL and LDL levels increased.

Neither the apoE−/− nor the LDLR−/− models manifests significant coronary artery lesions. However, there are more complex models in which these lesions have been observed. For example, mice deficient in both apoE and the scavenger receptor SR-BI exhibit dramatic obstructive coronary artery atherosclerosis with myocardial infarction. The detailed mechanisms of this phenotype are not clear, though SR-BI affects the function of platelets. Also double knockout mice lacking both apoE and the LDLR fed a WTD develop coronary artery atherosclerosis and myocardial infarction especially when subject to stress.

In the mouse there is only a single apoE isoform, whereas in humans apoE exists in 3 major isoforms, E2, E3, and E4, which have different effects on lipoprotein homeostasis and atherosclerosis. Maeda and colleagues have “humanized” mice with respect to the apoE isoform genotype. The apoE2 knockin mice spontaneously develop atherosclerosis. These apoE isoform knockin mice are very useful to explore the mechanisms by which the apoE isoforms influence lipid metabolism and atherosclerosis. Besides the genetic ablation of apoE and the LDLR for atherogenesis studies, the manipulation of apoA-I, the major apoprotein of HDL, both by transgenesis and by ablation has furnished some of the best evidence of the atheroprotective effects of HDL. Other lipoprotein-related genes (eg, apoA-IV, apoA-II, hepatic lipase, lecithin:cholesterol acyltransferase, and phospholipid transfer protein) have been studied though the precise mechanism of the impact of these proteins on atherosclerosis remains to be clarified.

Its limitations aside, the mouse has been of inestimable value in dissecting the putative mechanisms of atherogenesis. The development of atherosclerosis involves the interplay of genetic factors operating on plasma lipoproteins, on the cells participating in atherogenesis and on the vascular phenotype. Several cell populations participate in the evolution of the atherosclerotic lesion. Activated endothelial cells express adhesion molecules that attract blood cells into the vessel wall. Of these, the most prominent are the monocyte, which on migration into the intima differentiate into macrophage/dendritic cell. The macrophages take up sequestered modified apoB containing lipoproteins with their cholesterol load to form the canonical foam cell, the hallmark cell of the lesion. These cells present antigens to activate other immune cells and signal to other cellular participants in the plaque through the secretion of a variety of proteins including cytokines and growth factors. During the evolution of the plaque the macrophages may...
proliferate and undergo apoptosis and phagocytosis of the apoptotic cells (efferocytosis). Depending on the efficiency of efferocytosis, apoptotic cells may be removed reducing the size of lesions or they may accumulate and be subject to secondary necrosis, producing the necrotic core characteristic of advanced plaques. Mouse atherogenic models have been useful in examining the role of subsets of monocytes and macrophage in the genesis and evolution of the plaque. The extent of cholesterol loading in the lesion foam cells is the result of lipoprotein uptake and lipid efflux. Lipid efflux is a favorite target for attempts to reduce lesion size and complement of foam cells. Macrophages may also migrate out of the lesion during regression regimes (see below). Although the macrophage foam cells are the most abundant leukocytes in the lesion, studies in murine models have implicated other leukocytes including neutrophils, a variety of T and B cell subsets, and mast cells in the atherogenic process. These cells, though contributing little to the lesion mass, mainly influence atherosclerosis by their secretion of a variety of proteins that regulate other cells or components of the plaque. Studies in mice have demonstrated that among the T-cell subsets TH1 cells and natural killer T cells are notably proatherogenic, whereas the role of TH2 and TH17 cells are less certain. On the other hand, regulatory T cells largely inhibit murine atherosclerosis. The effects of the T cells are mediated largely by the cytokines they secrete. Within the B-cell subtypes, B1 cells, which produce natural IgM antibodies directed at oxidized LDL, are believed to be atheroprotective, whereas B2 cells, which produce IgG antibodies, have been shown to be antiatherogenic as well as proatherogenic. The balance between the proatherogenic and the inhibitory immune cells will influence the vessel wall phenotype. The report by Lichtman and colleagues on the decline of anti-inflammatory regulatory T cells in mice with extended exposure to hyperlipidemia illustrates well the dynamic balance of the cells of the adaptive immune cells during prolonged hyperlipidemia and the evolution of the atherosclerotic lesions. This balance may also explain why innominate artery atherosclerosis is increased in immune incompetent apoE−/− RAG−/− mice, which lack both T and B cells compared to apoE−/− mice with comparable plasma lipids (unpublished). A related result was reported some years ago by Elhage in the CD4-deficient animal when they observed an increment in aortic lesion. None of these cells of the adaptive immune system are obligatorily required for the development of mature atherosclerotic lesions as is evident from the robust atherosclerotic lesions seen in both apoE−/− or LDLR−/− mice crossed with RAG−/− mice. The mature atherosclerotic plaque contains a significant mass of SMC in the intima. These cells are mostly derived from the migration of medial SMC that is largely mediated by the secretion of platelet-derived growth factor by macrophages. The SMC are mostly responsible for the elaboration of matrix molecules and for the formation of the fibrous cap. The fibrous cap protects the core of the plaque from the circulating cells of the blood, especially platelets that are responsible for the thrombosis associated with ruptured plaques. Among the agents that destabilize the plaque are macrophage-derived proteases, especially metalloproteases. It is worth noting that the SMCs have different developmental origin for different regions of the macrovasculature and this could contribute to some of the site-specific atherosclerosis responses.

The transplantation of bone marrow into the atherogenic mouse models has been very useful in elucidating the role of blood cells, including monocytes and cells of the adaptive immune system on atherogenesis. This approach has also been useful in identifying chemokines and cytokines mediating the recruitment and development of these cells in the artery wall. The irradiation that precedes bone marrow transplantation destroys the recipient’s bone marrow containing the precursors of these immune cells, which is reconstituted by either normal or genetically modified bone marrow from the donor animals. This procedure can be used to differentiate the contribution of circulating blood cells from those of endothelial cells and SMC in the evolution of the atherosclerotic plaque. Fixed macrophages, eg, Kupffer cells are not completely eliminated by the irradiation. Elimination and reconstitution of these cells can be achieved by treatment with the macrophage toxin clodronate. As mentioned earlier, apoE−/− and LDLR−/− animals are differently sensitive to the apoE or LDLR presence in the donor bone marrow.

The adoptive transfer of specific cell types into hosts deficient in cells of a particular type is another strategy for the evaluation of the individual role of immune cell in atherogenesis. One example is the adoptive transfer of splenocytes containing relatively large numbers of natural killer T cells into immune deficient hosts, showing the proatherogenic effect of invariant natural killer T cells. Also labeled cells can be transferred to follow their short-term recruitment into the vessel wall and lesions.

A third strategy is to attenuate the function of particular genes in specific cells of the atherosclerotic plaque via the Cre recombinase mediated excision of a portion of the target gene that has been flanked by loxP sequences resulting in the inactivation of the gene. Selectivity is achieved by the use of cell-type specific promoters to drive the expression of the Cre recombinase. Two recent examples illustrate this approach. The elimination of PPARY specifically in SMC using Cre recombinase driven by the SM22 promoter revealed that pioglitazone-induced reduction in atherosclerosis is mediated via its effects on the activity of PPARY expressed in SMC. The elimination of the estrogen receptor in endothelial cells via Cre recombinase driven by the Tie 2 promoter revealed the importance of the expression of this receptor in these cells in accounting for the atheroprotective effect of estradiol in LDLR−/− mice. Cre recombinase driven by the LysM or aP2 promoter has been used to specifically eliminate genes in macrophage, although these promoters are not as cell specific (eg, aP2 is also expressed in adipocytes).

Although the above systems regulate tissue-specific expression, usually throughout the animals’ lifespan, they do not specifically address the temporal regulation of expression of genes during the evolution of the plaque. Such systems take advantage of the responsiveness of promoters to drug administration, with tetracycline being most frequently used for this approach. A tetracycline-controlled transactivator activates or represses the transcription of genes whose promoter contains a tetracycline responsive element in a tetracycline dependent manner. A recent elaboration of this system involves replacing...
the coding sequence of the endogenous gene of interest with a tetracycline transactivator gene and inserting the cDNA for the gene of interest into a specific genomic locus that allows low basal transcriptional activity but high inducible expression. The cDNA is under the control of a tetracycline responsive element containing promoter and is expressed or not expressed depending on the presence or absence of doxycycline (tetracycline analog) in the diet. As a proof of principle, the expression of the apoE gene was specifically and rapidly induced resulting in decreased plasma lipids and regression of established atherosclerotic lesions. Finally, in those cases where the expression of genes in the liver are influential for lipoprotein homeostasis and consequent effects on atherosclerosis, recombinant adenovirus or especially adeno-associated virus may serve as vectors to increase gene expression. These viruses generally home to the liver and can be introduced into mice at various times during the atherogenic process.

Genetics of Vascular Lesion Development

Inbred strains of mice with varying sensitivity to atherosclerosis have been valuable in identifying new genes involved in this pathogenesis. C57BL/6 is the most sensitive mouse strain. Cross breeding with more resistant strains, such as C3H, FVB, and Balb/c, have helped to localize regions within chromosomes harboring genes that influence atherosclerosis in the F1 and F2 generations in wild type mice and in crossing either the apoE or LDLR null gene into different genetic backgrounds. Some of the operative genes have been identified. The value of comparative genomic approaches involving human GWA studies and mice QTL studies to identify and validate potential atherosclerosis or lipoprotein modifying genes in mouse models has been recently reviewed. The involvement of the genetics of the vessel wall is indicated by 2 studies in mouse models. Using bone marrow transplantation, it was demonstrated that nonbone marrow derived cells (perhaps endothelial cells) accounts for the relative resistance of the C3H mouse to atherosclerosis. Very recently the transplant of carotid arteries between apoE−/− mice in the C57BL/6 and FVB genetic background has shown that genetic susceptibility of the vascular wall itself contributes to the differences in atherosclerosis among these 2 strains in this arterial bed.

Maeda and colleagues have recently described an interesting model that dramatically indicates the importance of geometry and hemodynamics influencing atherosclerosis. They compared atherosclerosis in apoE−/− mice in the C57BL/6 and 129S6/SvEvTac genetic backgrounds. The atherosclerosis resistant 129 strain exhibited a selective increase in aortic arch atherosclerosis. This appears to be attributable to the sharper curvature of the bend in the aortic arch and they have identified QTLs associated with the phenotype.

Murine Models of Atherosclerosis Regression and Plaque Dynamics

There had been a tendency to regard the atherosclerotic plaque as an inexorable steadily progressing chronic inflammation. However more recent work has emphasized the dynamic nature of the lesion whose net outcome is the result of monocyte recruitment, lipid loading to form foam cells, and macrophage apoptosis and emigration to regional lymph nodes. The nature of the emigrating cells and the signals that elicit this behavior are of interest. The dynamic nature of the plaque is best illustrated in aortic transplant experiments in which aortic segments bearing lesions from an atherosclerotic animal are transplanted into a normolipidemic host, resulting in a decrease in the macrophage content of the transplanted vessel. Such reduction is promoted by overexpression of apoA-I in the recipient. A more recent study indicates that this reduction can be achieved by limiting monocyte recruitment into lesions in the face of ongoing macrophage apoptosis.

Finally, 2 interesting models of lesion regression have recently been developed. Microsomal triglyceride transfer protein is required for the assembly of the apoB-containing lipoproteins. Conditional deletion of this gene in high-fat-fed apoB100 transgenic mice along with switching to a low-fat diet lowers plasma lipoproteins and allows for the analysis of the properties of lesions during regression. This model highlights again the importance of lowering plasma levels of apoB-containing lipoproteins for the regression of atherosclerosis. Additionally, when conditional hypomorphic apoE expressing mice or tetracycline transactivator controlled apoE are induced to express apoE, established lesions are reversed.

Reverse cholesterol transport, that is moving cholesterol from lipid loaded cells in the lesion to the liver, has received much attention as a way of limiting atherogenesis or even reversing established lesions. The 2 major transporters ABCA1 and ABCG1 collaborate in promoting cellular cholesterol efflux from lipid-loaded macrophages. The increased expression of these transporters is mediated by the activation of the nuclear hormone receptor LXR (perhaps by cholesterol derivatives generated following cholesterol loading of cells), which activates not only the genes involved in cholesterol transport, but also counter regulatory anti-inflammatory products. Alterations in cholesterol homeostasis mediated by the ABC transporters affects other cells in the body such as the insulin-producing cells of the pancreatic islets and the growth potential of neutrophil and monocyte precursors in the bone marrow. Thus, monocytes accompanies hypercholesterolemia and provides a pool of monocytes for recruitment to growing atherosclerotic plaques. Monocytes may also be recruited from the spleen stores for population of the growing plaques.

Atherosclerosis Associated With Other Chronic Diseases

It is well known that the incidence and nature of atherosclerosis in humans is affected by the coexistence of other inflammatory diseases such as the autoimmune diseases systemic lupus erythematosus and rheumatoid arthritis and the more prevalent chronic diseases such as obesity, diabetes, and chronic renal disease. Mouse models that have been developed to examine many of these chronic disorders will be useful to reveal the underlying basis for increased atherosclerosis. A review of mouse models to examine the accelerated atherosclerosis associated with systemic lupus erythematosus has recently been published.
Diabetes

The coexistence of diabetes is often associated with an exacerbation of cardiovascular disease and atherosclerosis in particular. We referred above to the exacerbation of complex coronary atherosclerosis in streptozotocin-treated pigs fed a high-fat diet. Murine models involving alteration of insulin levels or insulin signaling have also been developed to study the impact of diabetes on atherosclerosis. Though streptozotocin has also been used in mice, it has its disadvantages, including other organ toxicity and differential susceptibility of genders and mouse strains. In 1 model of type 1 diabetes, infection of LDLR−/− mice expressing the lymphocytic choriomeningitis viral glycoprotein driven by the insulin promoter leads to an immune response that destroys the beta cells of the islets. When these mice are fed a cholesterol-free diet there is an increase in lesion area without a change in plasma lipids. This phenotype can be corrected by insulin therapy. However, when cholesterol is added to the diet, lesion area of diabetic and nondiabetic mice with comparable plasma cholesterol levels are similar, suggesting that the resultant hypercholesterolemia accounts for the increased atherosclerosis. Studies in animal models suggest a relationship between the hyperglycemia and hyperlipidemia. The induction of diabetes in this transgenic model when advanced atherosclerotic lesions are present leads to plaque disruption. Very recently a new murine model of type 1 diabetes that accelerates atherosclerosis in LDLR−/− mice has been described. It involves a dominant mutation of the insulin 2 gene resulting in a disruption of the folding of proinsulin. A new model that is useful for the study of the impact of insulin resistance on atherosclerosis has also been recently described. WTD-fed apoE−/− mice that are heterozygous for the insulin receptor and the insulin receptor substrate 1 are hyperinsulinemic and exhibit enhanced atherosclerosis in the absence of overt diabetes or changes in plasma lipids.

Apart from the systemic risk factors, there is an effect of insulin resistance on the macrophage apoptosis/necrosis network. This may provide an important link between insulin resistance and plaque necrosis, which is prominent in the lesions of diabetic humans. Macrophages have an insulin signaling pathway. Hepatic insulin signaling also regulates VLDL secretion, which may account for the hyperlipidemia of type 2 diabetes and the associated increased atherogenesis.

Chronic Renal Disease

Glomerulonephritis accompanies systemic lupus erythematosus and may contribute to its phenotype. Partial nephrectomy has been used to study the relationship between kidney function and atherosclerosis. ApoE−/− mice that have been subject to 5/6th nephrectomy develop uremia, hypercholesterolemia, and increased atherosclerosis that is correlated with the first 2 plasma parameters. The response is intermediate when only unilateral nephrectomy is performed. The full understanding of the mechanism by which kidney function accounts for these changes is not clear. One mechanism that has been implicated relates to cholesterol homeostasis in the macrophage foam cells. This is mostly attributable to reduced ABCA1 function and also to an increase of the migration of monocytes in response to an MCP1 gradient. Clearly much further work on this is required.

Conclusions

Atherogenesis is a relatively slow chronic inflammatory interaction that proceeds at different rates at different sites and is largely attributable to the specific imprinting of the vessel wall by its hemodynamic differentiation. Despite limitations of the murine atherogenic models, knowledge of the atherosclerosis process has been proceeding apace thanks to the power of mouse genetics and the ability to take advantage of the rapid generation of mouse cohorts of defined genetic makeup. The applicability of the mouse findings to the human patient has to be tested; a substantial undertaking, though facilitated by the rapid pace of advances in human genetic analysis. What the mouse studies do accomplish is to catalogue the biological processes and interactions that have potential to point to mechanisms that are likely to be operative in the patient. This is an enormously powerful approach. Exemplifying the mouse to human application is a recent study of germ-free mice. This study points to the importance of dietary lecithin as a risk factor for atherosclerosis in both species and highlights the critical role of the intestinal microbiome in regulating plasma lipoprotein homeostasis, a new microenvironment that has to be taken into account.

Atherogenesis is influenced by many genes and gene interactions. The manipulation of single genes including the manipulation of these genes in specific cell types has been very valuable, but it is gene interactions that will be the focus of ongoing research. Most of the cell type conditional modification of gene expression has involved such modification throughout the life of the animal. The increased use of temporal modification of gene expression will add considerably to the appreciation of cellular mechanisms operating in different phases of the plaque evolution. However, it is clear that this is insufficient given the implication that many factors and components operate to different extents as the lesion evolves. Hitherto much of the mechanistic analysis has depended on gene expression and immunochemistry of the lesion, but we can look forward to the application of more quantitative proteomic analysis at various stages of lesion development. A major advance would be the development of biomarkers that can be followed in experimental models in vivo so that a better picture of lesion evolution can be obtained in real time. Also the rapid development of real-time imaging of tissues and cells in evolving atherosclerotic plaques in animals will add to our understanding of the process.

Given the complexity of the evolution of plaques, especially to unstable plaques, one can anticipate an increased emphasis on network and system analysis over the duration of lesion maturation. It is clear that atherosclerosis is a regional vascular disorder mostly driven by hemodynamics, although other developmental factors could also be at play. But there is a necessity for investigators not to generalize about the response too broadly from the study of single vascular sites and at single times. Thus, it will be necessary to use much more complex conditional genetic reagents in the future.

We mostly think of the translation of findings in animals to the understanding of human atherosclerosis. However, mice...
may be used to facilitate the understanding of human translational studies, such as the elucidation of the mechanisms of action of those agents in use in clinical therapy or clinical trials, and detailed appreciation of the action of genes identified as potential agents in GWAS experiments in human populations. The use of animals for atherosclerosis studies will be of increasing interest for the development of more powerful clinical therapies for this widespread pathology.

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References
Animal Models of Atherosclerosis


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Supplemental Material

<table>
<thead>
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<th>Mice</th>
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<tr>
<td>Heart rate</td>
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<tr>
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<tr>
<td>Major lipoprotein</td>
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<tr>
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<td>-</td>
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<tr>
<td>Atherosclerosis generation time</td>
<td>Months (genetically modified mice)</td>
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<tr>
<td>Sites of atherosclerosis</td>
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<tr>
<td></td>
<td>Aortic arch</td>
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<tr>
<td></td>
<td>Innominate artery</td>
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Table I. Atherosclerosis relevant differences between mice and humans

<table>
<thead>
<tr>
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<tbody>
<tr>
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<tr>
<td>T cells</td>
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<td>B cells</td>
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<td>Cytokines</td>
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</table>

Table II. Mouse models have been instrumental in examining the role of the immune system on atherosclerosis.
Literature Cited.


Figure Legend.

Figure I. Atherosclerotic lesions in the innominate artery and aortic root sinus associated with the left coronary artery (LCS) in mice exposed to approximately the same lifetime burden of plasma cholesterol (9,377-12,523 mg/dl). The chow fed apoE-/- mice were sacrificed at 27 weeks of age and had average plasma cholesterol levels of 464 mg/dl. The apoE-/- and LDLR-/- mice fed WTD for 6 weeks were switched to the diet at 8 weeks of age (14 weeks at time of sacrifice). The average plasma cholesterol levels on WTD were 1,095 mg/dl for apoE-/- mice and 1,255 mg/dl for the LDLR-/- mice. Note that the lesions in the WTD fed mice are richer in macrophage foam cells, especially in the case of the LDLR-/- mice, while the lesions in the chow fed apoE-/- mice are much more complex and cellular, with notable necrotic cores and presence of cholesterol crystals.