assess risk in familial cases where the specific mutation is not yet known.

Unfortunately, sensitive genetic testing of genetically heterogeneous disorders is not yet practical for patients who are not members of families with a defined mutation. For example, mutations in at least five different genes can cause LQT, yet only three of these genes have been identified. Moreover, 40 mutations have already been defined in the three known genes. Analysis of risk in an individual who is not part of an LQT family would require mutational analysis of all LQT-causing genes. Advances in the sensitivity and efficiency of genetic testing, coupled with continued molecular genetic discoveries, will enable more reliable and cost-effective analysis of risk for cardiovascular disorders in the general population.

Risk stratification is another important application of molecular genetics. In FHC, for example, certain mutations of the gene encoding β cardiac myosin heavy chain carry substantially greater risk of sudden death (*32*). Substitution of Glu for Gly at position 256 is associated with a disease penetrance of only 56% and a benign prognosis, whereas substitution of a Gln for Arg at position 403 is associated with 100% disease penetrance and a high risk of sudden death (*33*). Similarly, physiologic studies of LQT-associated mutations in HERG indicate a spectrum of HERG K^+ channel dysfunction, which ranges from a partial loss of function to complete dominant negative suppression. Although the power of a single piece of genetic information can be limited by modification of genetic and environmental factors, this prognostic information can nevertheless be quite useful in inherited cardiovascular disorders. This is particularly true when one can select from a spectrum of therapies that are increasingly aggressive, such as medical therapy versus implantation of an internal defibrillator for FHC or LQT.

Thus, in less than a decade, the techniques of molecular genetics have contributed dramatically to our understanding of cardiovascular disease pathogenesis. Many genes that have a major effect on cardiovascular risk have already been identified, and genetic diagnosis, prognosis, and mechanism-based therapy are available in some cases. Continued genetic discoveries and technological advances are likely to make genetic testing and genotype-based therapy a routine part of clinical care in the future.

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Mouse Models of Atherosclerosis

Jan L. Breslow

As a species the mouse is highly resistant to atherosclerosis. However, through induced mutations it has been possible to develop lines of mice that are susceptible to this disease. For example, mice that are deficient in apolipoprotein E, a ligand important in lipoprotein clearance, develop atherosclerotic lesions resembling those observed in humans. These lesions are exacerbated when the mice are fed a high-cholesterol, high-fat, Western-type diet. Other promising models are mice that are deficient in the low density lipoprotein receptor and transgenic mice that express human apolipoprotein B and transdominant mutant forms of apolipoprotein E. These models are now being used to study the pathogenesis of atherosclerotic lesions, as well as the influence of genetics, environment, hormones, and drugs on lesion development.

Atherosclerotic cardiovascular disease is the major cause of morbidity and mortality in much of the world. Atherogenesis is a complex process in which the lumen of a blood vessel becomes narrowed by cellular and extracellular substances to the point of obstruction. Lesions tend to form at the branch points of arterial blood vessels and progress through three stages (Fig. 1). The first stage is the fatty streak lesion, which is characterized by the presence of lipid-filled macrophages (foam cells) in the subendothelial space. The second stage is the fibrous plaque, which consists of a central acellular area of lipid, derived from necrotic foam cells, covered by a fibrous cap containing smooth muscle cells and collagen. The final stage is the complex lesion, which shows evidence of thrombus formation with deposition of fibrin and platelets.

Researchers in vascular biology are

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working to identify the important cells and molecules involved in each stage of atherogenesis, as well as the environmental and genetic factors that promote lesion formation. These are complex questions that require in vivo models that mimic the human disease. Experimental approaches that deviate widely from the human disease or rely too heavily on in vitro systems could be misleading. Until recently, atherogenesis had been studied mainly in primates and in low density lipoprotein (LDL) receptor–deficient rabbits. Unfortunately, these systems cannot provide sufficiently large numbers of animals, nor do they lend themselves to genetic analysis.

Development of Mouse Models

About 10 years ago, several laboratories attempted to produce atherosclerosis in mice in order to identify potential modifier genes. Mice are highly resistant to atherosclerosis. On a low-cholesterol, low-fat diet, they typically have cholesterol levels of <100 mg/dl, mostly contained in the antiatherogenic high density lipoprotein (HDL) fraction, and do not develop lesions. However, when mice were fed a very high cholesterol, high-fat diet that also contained cholic acid, their cholesterol levels rose by a factor of two to three, with the majority now in the non-HDL fraction. After many months on this diet, certain inbred strains of mice, such as C57BL/6, developed several layers of foam cells in the subendothelial space in a circumscribed area near the aortic valve leaflets, whereas other inbred strains, such as C3H/HeJ, did not (*1*). Crosses between susceptible and resistant strains were used to identify potential genetic susceptibility loci. The most carefully studied of these loci, *ath1*, was mapped to chromosome 1 in the region of the gene that codes for the second most abundant HDL apolipoprotein, apolipoprotein A-II (apoA-II) (*2*).

Although initially promising, the model had two problems. First, in contrast to human lesions, which occur at branch points of major vessels and progress to the fibrous plaque stage, the mouse lesions were small, occurred only in the region of the aortic valve leaflets, and did not progress. Second, the diet required to produce the lesions was unphysiological, as it contained 10 to 20 times the cholesterol of a Western-type diet plus the unnatural dietary constituent cholic acid. This diet caused a chronic inflammatory state in the atherosclerosis-susceptible C57BL/6 strain but not in the atherosclerosis-resistant C3H/HeJ strain (*3*), which raised the possibility that genetic differences between the strains might relate to diet-induced inflammation rather than to atherosclerosis. This is a valid concern, because diet-induced perturbation of the inflammatory process could obscure the more subtle interplay of immune cells and cytokines involved in atherogenesis.

In 1992, two laboratories used gene knockout technology to generate mice deficient in apolipoprotein E (apoE) (*4*). ApoE, which is made primarily in the liver, is a surface constituent of lipoprotein particles and a ligand for lipoprotein recognition and clearance by lipoprotein receptors. ApoE-deficient mice have delayed clearance of lipoproteins, and on a low-cholesterol, low-fat diet, their cholesterol levels reach 400 to 600 mg/dl as a result of accumulation of chylomicron and very low density lipoprotein (VLDL) remnants enriched in esterified and free cholesterol (*5*). Notably, these mice develop not only fatty streaks but also widespread fibrous plaque lesions at vascular sites typically affected in human atherosclerosis (*6*, *7*). Lesions form at the base of the aorta and the lesser curvature of the thoracic aorta; at the branch points of the carotid, intercostal, mesentéric, renal, and iliac arteries; and in the proximal coronary, carotid, femoral, subclavian, and brachiocephalic arteries. Lesions begin at 5 to 6 weeks of age with monocyte attachment to the endothelium in lesion-prone areas and transendothelial migration. Fatty streak lesions begin to appear at 10 weeks, and intermediate lesions containing foam cells and spindle-shaped smooth muscle cells appear at 15 weeks. Fibrous plaques appear after 20 weeks; these consist of a necrotic core covered by a fibrous cap of smooth muscle cells surrounded by elastic fibers and collagen. In older mice, fibrous plaques progress. In some advanced lesions there is partial destruction of underlying medial cells with occasional aneurysm formation, and in others calcification occurs in the fibrous tissue. Extensive fibroproliferation can narrow the lumen, even to the point of occlusion of vessels. Complicated lesions characterized by thrombosis have not been found.

One of the hallmarks of atherosclerosis is its exacerbation by high-cholesterol, high-fat diets. This effect is mimicked in apoE-deficient mice (*6*). When these mice were fed a Western-type diet (containing 0.15% cholesterol and 21% fat, derived mainly from milk fat), their cholesterol levels rose to three to four times the levels on the low-cholesterol, low-fat diet, and their lesions increased in size and rate of progression.

Additional mouse models of atherosclerosis have been created by introducing other mutations that also alter lipoprotein profiles. In type III hyperlipoproteinemia, a human dyslipidemia associated with atherosclerosis, patients have mutant forms of apoE that interfere with normal clearance of chylomicron and VLDL remnant lipoproteins. Transgenic mice have been created that express two of these mutant forms of apoE that act in a transdominant manner, apoE Leiden and apoE R142C (Arg^{142} → Cys) (*8*). ApoE Leiden transgenic mice that were fed a very high cholesterol diet containing cholic acid had cholesterol levels of 1600 to 2000 mg/dl and developed fatty streak and fibrous plaque lesions. Lesions were not observed when the mice were fed a low-cholesterol, low-fat diet. ApoE R142C transgenic mice that were fed the very high cholesterol–cholic acid diet had cholesterol levels of 370 mg/dl and developed lesions that were mainly of the fatty streak variety. Lesions were not observed when these mice were fed a low-cholesterol, low-fat diet.

The atherogenic stimuli in apoE-deficient and apoE mutant mice are chylomicron and VLDL remnants. Additional mouse models have attempted to address other atherogenic stimuli. Cell surface LDL receptors recognize apolipoprotein B (apoB) on LDL and apoE on intermediate density lipoprotein (IDL) and, through a high-affinity process of binding and internalization, remove these lipoproteins from the circulation. LDL receptor–deficient mice have been created to induce high plasma levels of these atherogenic lipoproteins (*9*). On a low-cholesterol, low-fat diet, these mice have twice the normal level of

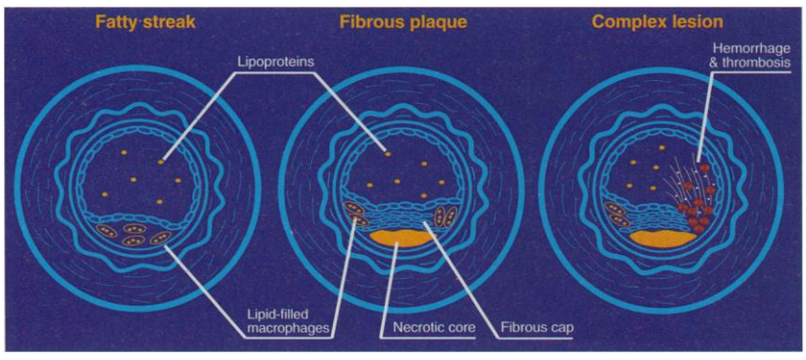

Fig. 1. Schematic drawing of the three stages of atherosclerotic lesion formation.

plasma cholesterol, with most of the increase in the IDL and LDL lipoprotein fractions. This perturbation of the lipoprotein system is insufficient to cause atherosclerosis. However, when LDL receptor–deficient mice were fed a very high cholesterol diet containing cholic acid, they developed cholesterol levels of >1500 mg/dl and had massive fatty streak lesions. Lesions consisting of a lipid-filled necrotic core capped with foam cells were also observed, but there was no evidence of fibrous plaques. LDL receptor knockout mice that were fed the Western-type diet developed cholesterol levels of 1200 mg/dl and had lesions that were mainly fatty streaks (*10*).

Transgenic mice expressing the human *apoB* gene (HuBTg mice) have also been developed as an atherosclerosis model (*11*, *12*). ApoB is the sole protein component in LDL and, as noted above, is the ligand for LDL receptor–mediated removal of LDL from the circulation. On a low-cholesterol, low-fat diet, HuBTg mice have cholesterol levels of 100 to 200 mg/dl and do not develop lesions. However, when these mice were fed a very high cholesterol–cholic acid diet, cholesterol levels rose to 300 to 500 mg/dl and fatty streak lesions occurred. Some of the lesions showed cholesterol crystals in a necrotic core, but fibrous caps were not demonstrated.

LDL receptor–deficient mice and HuBTg mice provide interesting alternatives to apoE-deficient mice as models of atherosclerosis. Unlike apoE-deficient mice, LDL receptor–deficient mice and HuBTg mice do not develop lesions on a low-cholesterol, low-fat diet. The initial reliance on the very high cholesterol–cholic acid diet to produce lesions is problematic (as is true for the C57BL/6 model), as is the uncertainty about progression to the fibrous plaque lesion stage. It is encouraging that LDL receptor–deficient mice develop lesions on a Western-type diet, and perhaps with longer feeding periods these mice will develop the same type of fibrous plaque lesions seen in humans.

Applications of the Mouse Models

In principle, the mouse models of atherosclerosis (summarized in Table 1) can provide insights into lesion pathogenesis, genetic modifiers, and the influence of environment, hormones, and drugs on the disease. Although this work is largely in its infancy, the studies reported to date are promising. With regard to lesion pathogenesis, it is now possible to study the molecular events involved in monocyte attachment to endothelium, monocyte transmigration to the subendothelial space, subintimal foam cell formation, foam cell necrosis and the ensuing fibroproliferative reaction, and the roles of immune cells, cytokines, and their receptors in lesion progression. These studies can be carried out by documenting the molecules and cell types present in the lesions, crossbreeding the atherosclerotic mice with other mutant mice harboring specific defects in the same molecules or cells, and then noting suppression or enhancement of the atherosclerosis phenotype.

The foam cell lesions of both the apoE-deficient and LDL receptor–deficient mice contain oxidized epitopes of lipoprotein particles, accompanied by very high plasma levels of autoantibodies to oxidized lipoproteins (*10*, *13*). On the basis of in vitro studies and lesion immunohistochemistry, lipoprotein oxidation in the subendothelium has been hypothesized to be necessary for foam cell formation. These mouse models can be used to test this hypothesis in vivo. Support for the hypothesis has come from a recent pharmacological study in which an antioxidant, *N,N′*-diphenyl-1,4-phenylenediamine, was shown to decrease lesion area in apoE-deficient mice without affecting cholesterol levels (*14*). The mouse atherosclerosis models can also be used to identify molecules involved in lipoprotein oxidation through crossbreeding with other appropriate mutant mice. Similarly, experiments could be designed to test whether autoantibodies to oxidized lipoproteins participate in lesion pathogenesis or are a response to lesion development.

In another study, the role of the macrophage in lesion development was tested by crossbreeding apoE-deficient mice with mice that have the op mutation, a mutation in the gene that codes for macrophage colony stimulating factor (MCSF). MCSF influences monocyte and macrophage development, and mice with the op mutation have reduced levels of blood monocytes and tissue macrophages (*15*). Macrophages in the subendothelium have been hypothesized to protect against atherosclerosis by scavenging noxious materials such as oxidized lipoproteins, but they have also been hypothesized to contribute to foam cell formation and, by their death, to lesion progression. Lesions in mice that were doubly mutant (that is, apoE-deficient mice that also had the op mutation) were one-seventh the size of lesions in apoE-deficient mice, with almost no progression to the fibroproliferative stage, even though cholesterol levels in the doubly mutant mice were two to three times those in the apoE-deficient mice. These results strongly suggest that the net effect of the macrophage is proatherogenic. This hypothesis can be further tested by crossbreeding the apoE-deficient mice with mice that have mutations in other aspects of monocyte function.

The availability of mouse atherosclerosis models allows the use of a variety of techniques to identify genes that enhance or suppress the phenotype. In early studies, genes that might influence lipoprotein levels were expressed in transgenic mice on the C57BL/6 background. The mice were fed a very high cholesterol–cholic acid diet, and the effect of the transgene on the size of the lesions that formed at the aortic leaflets was assessed. In this model, expression of the human *apoA-I* gene, which codes for the major protein of HDL, led to raised HDL levels and reduced lesion area; coexpression of the human *apoA-I* gene and the human

Table 1. Summary of current mouse models of atherosclerosis.

Model	Atherogenic stimulus	Cholesterol level (diet)	Lesion type
C57BL/6	VLDL, LDL	200 to 300 (very high cholesterol, cholic acid)	Fatty streak (aortic leaflet)
ApoE deficiency	Chylomicron and VLDL remnants	400 to 600 (low cholesterol, low fat)	Fatty streak, progressing to fibrous plaque, at branch points of major vessels
		1500 to 2000 (Western type)	Same pattern as for low-cholesterol, low-fat diet, but with larger lesions and faster progression
ApoE Leiden	Chylomicron and VLDL remnants	1600 to 2400 (very high cholesterol, cholic acid)	Fatty streak, fibrous plaque
ApoE R142C	Chylomicron and VLDL remnants	370 (very high cholesterol, cholic acid)	Fatty streak
LDL receptor deficiency	IDL, LDL	1500 (very high cholesterol, cholic acid)	Fatty streak, progressing to necrotic core but without fibrous cap
		1200 (Western type)	Fatty streak
HuBTg	LDL	300 to 500 (very high cholesterol, cholic acid)	Fatty streak, progressing to necrotic core but without fibrous cap

apoA-II gene, which codes for the second most abundant HDL protein, was less protective (*16*). These results suggest that both the amount and the apolipoprotein content of HDL influence susceptibility to atherosclerosis. In another study, outbred transgenic mice expressing mouse *apoA-II* developed lesions when they were fed the very high cholesterol–cholic acid diet, whereas control mice did not; this result again suggested that overexpression of *apoA-II* is proatherogenic (*17*). More recently, mice expressing the human *apoA-I* transgene were bred with apoE-deficient mice, and the offspring were fed a low-cholesterol, low-fat diet; at 4 months of age, fatty streak lesions were almost totally suppressed, whereas at 8 months some small fatty streaks had appeared but fibrous plaques were suppressed (*18*). Thus, *apoA-I* expression led to decreased lesion area and inhibited lesion progression. These same mice showed a strong inverse correlation between HDL cholesterol levels and lesion size. This effect of HDL was independent of the effect of non-HDL cholesterol levels on lesion size.

The relation between low HDL cholesterol levels and increased atherosclerosis observed in human epidemiological studies has been attributed largely to the association of low HDL cholesterol levels with high levels of atherogenic apoB-containing lipoproteins, such as VLDL, IDL, small dense LDL, and postprandial particles. These mouse studies suggest that HDL has an additional independent protective effect, perhaps by accelerating reverse cholesterol transport or directly protecting the vessel wall against noxious atherogenic stimuli. Evidence for one or both of these proposed mechanisms of HDL action can now be sought in the mouse atherosclerosis models.

Another potential atherosclerosis modifier is the cholesterol ester transfer protein (*CETP*) gene. This gene codes for a plasma protein that mediates the exchange of triglycerides in VLDL for cholesterol esters in HDL. HDL cholesterol levels have been inversely correlated with plasma CETP activity in a variety of clinical studies. Although mice (unlike humans) do not have plasma CETP activity, transgenic mouse lines have been created with either the human or monkey *CETP* genes, and the effects of this gene on atherosclerosis in mouse models have been evaluated. Expression of the monkey *CETP* gene in C57BL/6 mice that were fed the very high cholesterol–cholic acid diet led to reduced HDL cholesterol levels, raised non-HDL cholesterol levels, and increased lesion size (*19*). However, expression of the human *CETP* gene in transgenic mice that were made hypertriglyceridemic by expression of the human *apoC-III* gene resulted in reduced lesion size (*20*). These experiments suggest that CETP can be proatherogenic or antiatherogenic, depending on other factors that determine the lipoprotein profile.

Another candidate gene that has been assessed for its role in atherogenesis is *apo(a)*. Apo(a) is a large glycoprotein that forms a disulfide bond with the apoB moiety of LDL; the resulting particle is called Lp(a). Clinical studies have yielded conflicting data concerning the role of Lp(a) levels in coronary heart disease susceptibility. When outbred transgenic mice expressing *apo(a)* were fed the very high cholesterol–cholic acid diet, they developed small fatty streaks in the region of the aortic valve leaflets; these lesions were not present in control mice (*21*). Because human apo(a) does not bind to mouse apoB and because the apo(a) in these mice was not lipoprotein-associated, these results were interpreted to mean that apo(a) is directly atherogenic. More recently, *apo(a)* transgenic mice were crossbred with HuBTg mice (*12*, *22*). Lp(a) does form in these mice, and when they were fed the very high cholesterol–cholic acid diet, they developed lesions that were twice the size of those in the HuBTg mice. These studies indicate that apo(a) and Lp(a) are marginally atherogenic. However, further studies with transgenic mice expressing higher levels and different isoforms of apo(a) are needed, as are tests of atherogenicity in the context of more natural diets.

Induced mutant mouse models have been used to test the atherogenicity of apoE itself. For example, mice with only one inactivated allele of apoE developed atherosclerosis when they were fed the very high cholesterol– cholic acid diet; this finding suggests that even at half its normal production, apoE is atherogenic in the face of a severe dietary challenge (*23*). In other studies, bone marrow transplantation into apoE-deficient mice has been shown to correct the hyperlipidemia and prevent atherosclerosis, which indicates that reconstitution of macrophage apoE is sufficient to correct the metabolic defect (*24*). Finally, local production of apoE by expression of a transgene in macrophages or in the blood vessel wall was found to diminish atherosclerosis independent of its cholesterol-lowering effect (*25*). Thus, local production of apoE may play a special role in the prevention of atherosclerosis.

The mouse atherosclerosis models can also be used to test the effects of environment, hormones, and drugs on atherogenesis. In the apoE-deficient and LDL receptor–deficient mice, lesion size increases as the diet is changed from low cholesterol–low fat to high cholesterol–high fat (*6*, *10*). Popular, but as yet unproved, theories about the effects of various macro- and micronutrients on atherogenesis can also be tested. With regard to hormonal effects on atherosclerosis, among the LDL receptor–deficient mice on the very high cholesterol–cholic acid diet, the lesion area throughout the vascular tree is apparently greater in males than in females; this model may prove useful in studying the mechanisms by which sex hormones affect atherogenesis (*26*). Finally, these mouse models can be used to test for drugs that inhibit atherogenesis. The availability of large numbers of atherosclerosis-prone mice will allow relatively inexpensive and thorough preclinical testing of new candidate drugs.

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