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Genetic Modifiers of Atherosclerosis in Mice

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Abstract

Atherosclerosis is a complex, multifactorial disease with both genetic and environmental determinants. Experimental investigation of the effects of these determinants on the development and progression of atherosclerosis has been greatly facilitated by the use of targeted mouse models of the disease, particularly those resulting from the absence of functional genes for apolipoprotein E or the low density lipoprotein receptor (LDLR). This review focuses on the influence on atherosclerosis of combining apoE or LDLR deficiencies with factors affecting atherogenesis, including (1) inflammatory processes, (2) glucose metabolism, (3) blood pressure, and (4) coagulation and fibrinolysis. We also discuss the general problem of using the mouse to test the effects on atherogenesis of human polymorphic variations and future ways of enhancing the usefulness of these mouse models.

Keywords

apoE; LDL receptor; hypertension; inflammation; diabetes

The Mouse as a Model System for Studying Atherosclerosis

Ten years ago, no strains of mice were available that spontaneously developed complex atherosclerotic lesions, although small lesions of the fatty streak type could be induced in strain C57BL/6 mice by feeding them high-fat/high-cholesterol diets (see References 1 through 3). The advent of gene targeting4–6 to modify genes in a predetermined manner changed the situation dramatically by allowing the generation of mice having targeted inactivation of the apolipoprotein E (*Apoe*) gene in 19927–9 and of the LDL receptor (*Ldlr*) gene in 1993.10 Both of these animals, under appropriate conditions, develop complex atherosclerotic lesions and provide practical atherosclerotic mouse models.11–13

ApoE is an amphipathic protein that plays a pivotal role in lipoprotein trafficking. ApoE is a constituent of chylomicrons, VLDL, and HDL and acts as a ligand for the receptor-mediated clearance of these particles.14 Mice lacking apoE have plasma cholesterol levels that are 4 to 5 times normal and develop atherosclerotic lesions spontaneously, even when fed a normal chow diet, which is low in fat and cholesterol. The lesions resemble human lesions

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and progress over time from an initial fatty streak to a complex lesion with a fibrous cap,15,16 and lesion development can be accelerated by a high-fat, high-cholesterol diet.17 Mice lacking the LDLR have less overt disease, with a modest 2 times normal plasma cholesterol level when maintained on a normal chow diet, and they develop atherosclerosis only slowly.18 However, in response to a high-fat, high-cholesterol diet, LDLR-deficient mice exhibit massive elevations in plasma cholesterol and rapidly develop atherosclerotic lesions throughout the aorta.19 There is much less published data on the kinetics of lesion development in LDLR-deficient mice than in apoE-deficient mice. Nevertheless, the lesions that develop in *Apoe*- and *Ldlr*-deficient mice are generally the same, with the plaques developing in a time-dependent manner, starting from the proximal aorta and spreading toward the distal aorta, and particularly involving locations where blood flow is disturbed. Although the merits of each model and of different methods of assessing the extent of atherosclerosis are still debated, results with the 2 models are generally comparable and largely independent of whether the quantification is based on the lipid content of the aorta, the surface area of lesions in the aortic tree, cross-sectional plaque size in the proximal aorta, or cellular composition of plaque materials. The predictable development of plaques in these mutants, along with other more general advantages of mice, such as their small size, short generation time, and relative ease of care, have quickly made the mouse a very effective and practical model for the study of atherosclerosis. However, the most important advantage is the availability of genetically defined inbred and mutant strains and the well-established means of using these strains to manipulate the mouse genome.

In humans, current evidence suggests that susceptibility to atherosclerosis is most likely due to unfavorable combinations of mutations affecting genes in several pathways, but our knowledge about which genes are involved is limited.20 Genetic analysis in mice provides a powerful approach toward identifying the genes and pathways involved. For example, crosses between inbred strains of mice have led to the identification of several atherosclerotic quantitative trait loci (QTL) controlling strain-specific differences in diet-induced atherosclerosis susceptibility.3,21 Likewise, valuable information about atherosclerotic modifiers has been obtained by studying crosses of $Apoe^{-/-}$ or $Ldlr^{-/-}$ mice with mice carrying other mutations.

The use of $Apoe^{-/-}$ and $Ldlr^{-/-}$ mice to develop an understanding of the genetic factors that modify atherogenesis provides the theme for this review. Other atherogenic mouse models and the effects of modifying genes related to lipid metabolism are well studied and have recently been reviewed.11–13,22 Consequently, in the present review, we consider how atherosclerosis is modified by 4 other variables: inflammation, disturbances in glucose metabolism, hypertension (HTN), and coagulation/fibrinolysis. These conditions are a source of continuous injurious stimuli that can trigger the early stages of atherosclerosis. We include several examples showing how the mouse has been used to test human polymorphisms as potential atherosclerotic modifiers based on prior epidemiological studies. Finally, we discuss some of the current limitations of mouse models of atherosclerosis and suggest where future improvements might be made.

Atherosclerosis and Inflammation

One approach to identifying modifiers of atherogenesis related to inflammation is to start from the knowledge that a product already implicated in inflammation occurs in a notable way in atherosclerotic plaques. For example, finding a specific cytokine in atherosclerotic plaques but not in normal vessels is circumstantial evidence that the cytokine may be involved in the pathogenesis of atherosclerosis, although this association does not reveal whether the molecule (1) reduces the atherogenic process, (2) accelerates it, or (3) is merely secondary to atherosclerosis. However, by using genetic or other manipulations to alter the level of the potentially important product, some discrimination among these alternatives is possible.

In 1993, Ross20 suggested in his landmark review that atherosclerosis can largely be viewed as a self-perpetuating inflammatory disease. Accordingly, we begin by considering the genes listed in Table 1 that are known to affect inflammation and that have been tested for involvement in atherogenesis by using *Apoe*$^{-/-}$ and/or *Ldlr*$^{-/-}$ mice. As Ross elaborated in his review, the atherosclerotic inflammatory response progresses in discrete stages, which are so characteristic that the presence of certain inflammatory cell types can be used to define the progression of atherosclerotic lesions.

In the early stages, the lesions are fatty streaks composed of lipid-rich macrophages. At this stage, molecules involved in leukocyte (and particularly, monocyte/macrophage) function, recruitment, rolling, adherence, transendothelial migration, and activation are likely to play key roles. The importance of monocytes/macrophages in the pathogenesis of atherosclerosis has been confirmed by experiments affecting molecules in these pathways. For example, osteopetrotic (*op*) mice have a mutation in the gene for macrophage colony stimulating factor (MCSF) that causes a severe decrease in the number of monocytes/macrophages in the mutant animals. ApoE-deficient mice crossed with *op* mice produce offspring that develop much smaller lesions than do control apoE-deficient mice.23 In fact, the absence of MCSF causes the single, largest decrease in lesion size of all of the genes that have been tested to date (Table 1, line 14). Importantly, there is a gene-dosage effect, as MCSF heterozygotes also have reduced lesions. Likewise, administration of an antibody against the receptor for MCSF reduces lesion size in apoE-deficient mice.24 Cell adhesion molecules that facilitate monocyte rolling and adherence also influence atherogenesis (see lines 10 to 13 of Table 1),25–28 as do cytokines that affect monocyte recruitment and activation (reviewed in Reference 29). Monocyte chemoattractant protein-1 (MCP-1) is a cytokine that acts through its receptor, CC chemokine receptor 2 (CCR2), on monocytes, macrophages, and T lymphocytes. The absence of MCP-1 dramatically decreases lesion size in LDLR-deficient mice30 (see line 1, Table 1). Similarly, the absence or decrease of CCR2 causes a reduction in lesion size in apoE-deficient mice31,32 (see lines 2 and 3, Table 1). In the opposite direction, irradiated *Apoe*$^{-/-}$ mice, in which the bone marrow has been replaced with cells that overexpress MCP-1, have an increased atherosclerotic lesion size33 (see line 4, Table 1).

Although these alterations in the MCSF and MCP-1 pathways have marked effects on the progression of atherosclerosis, they do not completely eliminate macrophage-derived foam

cell development and fatty streak formation. Furthermore, the plaques in these mice still progress with time to more complex lesions. Additionally, although most of the evidence implicates macrophages as proatherosclerotic mediators, there is some evidence that they have some atheroprotective effects.34

The fatty streak progresses to intermediate lesions that contain monoclonal expansions of smooth muscle cells as well as increased numbers of macrophages and T cells. The potential role of T and B lymphocytes has been extensively evaluated, revealing a complex picture. Mice deficient in either recombinase-activating gene 1 or 2 (RAG1 or RAG2) do not produce functional T or B cells owing to a defect in V(D)J recombination. Experiments with *Apoe*$^{-/-}$ mice on a high-fat diet showed that the total absence of T and B cells caused by the absence of RAG1 or RAG2 did not affect lesion development.35,36 However, on a normal chow diet, *Apoe*$^{-/-}$ mice deficient in RAG1 have a modest decrease in lesion size compared with *Apoe*$^{-/-}$ controls (see Table 1, lines 15 and 16).35 The role of T cells in atherogenesis has been investigated by studying genetic alterations of the CD40-CD154 interaction37 or by the administration of antibodies against CD154.38 CD40, a cell surface receptor found on many immune cells, shares homology with tumor necrosis factor receptors. CD154, the ligand for CD40, is thought to be restricted to CD4+ T lymphocytes. When *Ldlr*$^{-/-}$ mice are fed a high-fat diet, treatment with antibodies to CD154 reduces expression of adhesion molecules and lesion size.37 Consistent with this observation, *Apoe*$^{-/-}$ mice that are also deficient in CD154 have a dramatic 5-fold decrease in lesion size, and the plaques in these mice also have a more stable, collagen-rich plaque phenotype with a reduced T cell/ macrophage content (see line 5, Table 1). Similar plaque phenotypes have been seen in mice lacking the interferon-g receptor (IFN-γR)39 (line 6, Table 1).

The small-molecule mediators of acute inflammation, such as histamine, prostaglandins, leukotrienes, and thromboxanes, have not been extensively studied as atherosclerotic modifiers despite the observation that some anti-inflammatory agents such as aspirin clearly reduce the risk of atherosclerotically mediated cardiac events.40,41 Two experiments are, however, relevant: disruption of the 12/15-lipoxygenase gene in *Apoe*$^{-/-}$ mice decreases atherosclerotic lesions,42 and transgenically overexpressing group IIa phospholipase A_2 ($sPLA_2$) in C57BL/6 mice fed a high-fat diet increases lesion size.43 Thus, arachidonic acid metabolites have demonstrable effects on lesion development.

Although macrophages and T cells are important in atherogenesis, neutrophils appear to be less important. Neutrophils are not notable in atherosclerotic lesions, even in CCR2-deficient, *Apoe*$^{-/-}$ mice, which have persistent neutrophilia in other tissues after inflammatory stimulation.44 The C-X-C chemokine receptor 2 (CXCR2) is the receptor for interleukin-8 and growth-regulated oncogene (GROα) and is predominantly, though not exclusively, expressed in neutrophils. Irradiated *Ldlr*$^{-/-}$ mice, whose bone marrow is replaced with cells lacking the mouse homolog to CXCR2, develop smaller lesions45 (see line 7, Table 1), indicating that this pathway is important in atherogenesis. However, as the authors suggest, the CXCR2 pathway may be enhancing atherogenesis by promoting monocyte adhesion, recruitment, and activation rather than through neutrophil actions.29,45

Hyperglycemia and Atherosclerosis

In humans, hyperglycemia is a strong risk factor for atherosclerotic cardiovascular disease.46 Patients with diabetes have a greater incidence of myocardial infarction (MI) and stroke,47 and only part of the increased risk can be explained by their other risk factors such as HTN or hyperlipidemia.48 The mechanism behind these interacting effects is poorly understood and may be due to any number of factors, including mitogenic effects of hyperinsulinemia or endothelial cell dysfunction caused by hyperglycemia.49 Recent work with streptozocin-treated mouse models of atherosclerosis is shedding light on a possible mechanism of hyperglycemic vascular injury and particularly, on the role of advanced glycation end products (AGEs). AGEs are a by-product of hyperglycemia that result from the nonenzymatic glycation of proteins, followed by oxidation-mediated irreversible rearrangements.50 Interaction of AGEs with the receptor for AGEs (RAGE)51 induces several inflammatory markers52 that could increase atherosclerosis. Moderate hyperglycemia, achieved by giving supplemental insulin to streptozocintreated, cholesterol-fed *Ldlr*$^{-/-}$ mice of mixed genetic background, did not increase atherosclerotic lesion size53 (see Table 2, line 4). However, more fulminant hyperglycemia in streptozocin-treated, back-crossed apoE-deficient mice resulted in large increases in plasma glucose and atherosclerotic lesion size54,55 (see Table 2, lines 1 and 2). The experiments of Park et al55 support the importance of AGEs by demonstrating that this increase in lesions could be suppressed in a dose-dependent manner by daily injections of the soluble receptor for advanced glycation end products (sRAGE), which acts as a molecular “sink” for AGEs (see Table 2, line 3). Whether all of the enhancement of atherosclerosis in diabetes can be explained by the AGEs will have to wait for further experiments. Efforts to understand the link between hyperglycemia and atherosclerosis will be greatly enhanced by the development of animal models with both sustained hyperglycemia and atherosclerosis solely due to genetic factors.53,56

In humans and mice, diabetes is clearly a polygenic disorder,57–59 and although some chromosomal regions have been linked to diabetes, identification of the genes involved has proven difficult. New diabetic mouse models promise to change this situation. The insulin receptor (IR) is a receptor tyrosine kinase, and binding of insulin to the IR stimulates phosphorylation of insulin receptor substrates 1 and 2 (IRS-1 and IRS-2), which then activate other signaling molecules in the insulin signaling cascade. Mice homozygously deficient in either IR or IRS-2 die prematurely,60,61 whereas mice deficient in IRS-1 have severe growth retardation.62,63 In contrast, compound heterozygotes, which are heterozygous for a normal copy and a disrupted copy of IR, IRS-1, and/or IRS-2, survive and develop insulin-resistant diabetes.64,65 The insulin-responsive glucose transporter, GLUT4, is important in postprandial glucose metabolism as a facilitative transporter in skeletal muscle and adipose tissue. Mice heterozygous for disruption of *GLUT4* develop hyperinsulinemia and hyperglycemia as they age.66 The phenotypes in these mice are complex, because both the IRS-1–deficient and *GLUT4* heterozygous animals also have markedly elevated blood pressure.66,67 Nevertheless, exciting and informative results relating diabetes and atherosclerosis are likely to follow when these new diabetic models are combined with apoE or LDLR deficiencies.

Interaction Between HTN and Atherosclerosis

HTN (defined as a blood pressure >140/90 mm Hg) and atherosclerosis are leading causes of morbidity and mortality in the developed world,68 and a considerable body of evidence suggests that HTN contributes to the development and progression of atherosclerosis. For instance, atherosclerosis occurs in high-pressure arteries but not in low-pressure veins, and lesions tend to be localized at areas of high wall stress.69 People with HTN are 3 times more likely to develop atherosclerosis than normotensive people,70 and antihypertensive treatment reduces the risk of death from atherosclerotically mediated cardiovascular events.71,72 Complex genetics and confounding variables make the study of HTN plus atherosclerosis difficult in humans. In addition, proving a direct causal role for HTN in the pathogenesis of atherosclerosis is complicated by the powerful homeostatic mechanisms that control blood pressure. Thus, alterations in blood pressure invariably cause compensatory changes in various vasoactive mediators, and changes in these circulating vasoactive mediators not only affect blood pressure but also act locally to affect atherosclerosis. This makes it difficult to separate the mechanical effects of blood pressure in atherogenesis from the local effects of vasoactive mediators.

Many of the animal model studies of HTN plus atherosclerosis have been made by using surgical treatments to induce chronic coarctation of the abdominal aorta. Aortic constriction increases both the pressure and the lesions proximal to the constriction in hypercholesterolemic rabbits.73,74 Surgical constriction of the renal artery also increases atherosclerosis in rabbits.75 To date, these surgical approaches have not been applied to study HTN and atherogenesis in the mouse. Another approach has utilized drugs to raise or lower blood pressure.76 For instance, infusion of agents that increase blood pressure, like angiotensin II or N^{G}-nitro-L-arginine methyl ester, increases atherosclerosis in some models,77–79 whereas treatment of HTN with various antihypertensive drugs decreases atherosclerosis in several animal models (reviewed in Reference 76). Antihypertensive drug treatment studies in atherogenic mouse models have yielded conflicting data. Some studies have shown atheroprotective effects of antihypertension therapy without demonstrably lowering the blood pressure.80–82 However, in 1 of the most interesting studies, Makaritsis et al83 showed that neither a-adrenergic nor angiotensin receptor blockade alone lowered blood pressure or decreased lesions in *Apoe*$^{-/-}$ mice, but *simultaneous* blockade decreased both blood pressure and lesion size. Furthermore, we observed that chronic treatment with the angiotensin-converting enzyme (ACE) inhibitor enalapril did not significantly reduce atherosclerosis or lower the blood pressure of apoE-deficient mice, which are inherently normotensive.84

Currently, there are only a few genetic models with combined atherosclerosis and HTN. In one study, mice that express both a human renin transgene and a human angiotensinogen transgene were generated on a C57BL/6 genetic background. When fed a high-cholesterol diet, these mice have an elevated blood pressure (by 20 mm Hg) and develop larger lesions than equivalent nontransgenic mice.85 Recently, we have developed a genetic model in which HTN is combined with atherosclerosis by crossing apoE-deficient mice with endothelial nitric oxide synthase (*eNOS*$^{-/-}$)–deficient mice.84 Similar results were obtained in *eNOS*$^{-/-}$, *Apoe*$^{-/-}$ mice maintained on a high-fat diet.86 eNOS serves important basal

regulatory functions in the vasculature. In response to stimuli such as shear stress or acetylcholine, eNOS catalyzes the production of NO, which diffuses across the endothelial cell membrane into smooth muscle cells, inducing vasodilation. It also acts locally to prevent platelet and leukocyte adhesion.87 In comparison with the *Apoe*$^{-/-}$ controls, doubly deficient *eNOS*$^{-/-}$, *Apoe*$^{-/-}$ mice are hypertensive (by 20 mm Hg), have atherosclerotic lesions twice the size, and also develop kidney damage (see Table 3, lines 1 and 2). These deleterious effects of eNOS deficiency were reduced by chronic treatment with enalapril at the same dose that was ineffective in *Apoe*$^{-/-}$ mice that were *eNOS*$^{+/+}$ and had a normal blood pressure.84 These experiments are also notable because they demonstrate that in mice, the atheroprotective effects of enalapril are independent of NO production through eNOS and that a measurable component of increased atherosclerosis is due to an increase in blood pressure.

The distribution of atherosclerotic plaques appears to be different in *eNOS*$^{-/-}$ *Apoe*$^{-/-}$ mice than in *Apoe*$^{-/-}$ mice, potentially shedding light on one mechanism that may determine the localization of atheromas. Plaques develop in the descending and abdominal aortas of the double-knockout mice even by 4 months of age. In contrast, in apoE-deficient mice, lesions do not become prominent in these regions until 8 months of age. As previously mentioned, atherosclerotic plaques tend to develop in areas where blood flow is turbulent and where flow-induced shear stress is low but where pressure-induced vascular wall strain is high.69,88,89 It is possible that normal eNOS function prevents the development of plaques in areas of high shear stress, so that an absence of eNOS leads to plaque development in these areas. Cross-breeding *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ mice with other genetic models of HTN is desirable, because analysis with various antihypertensive treatments may clarify the causative link between increased blood pressure and enhanced atherosclerosis and perhaps identify better treatment strategies.

A powerful approach to confirm genes that modify the severity of atherosclerosis is to design experiments in mice to test whether gene polymorphisms identified in human epidemiological association studies actually affect atherosclerosis. There are many challenges in studying complex diseases such as atherosclerosis in humans. Not only are the environmental and genetic backgrounds of individuals diverse, but also there is a high likelihood that differences will occur in other unsuspected genes tightly linked to a candidate gene. Effects due to linked differences can therefore easily be misinterpreted as due to differences in the candidate gene. Well-designed experiments in mice can eliminate these complications, although only a few such tests have yet been done.

The human *ACE* gene provides an example. This gene has 2 common alleles, *I* and *D*, which differ by the presence (*I*)or absence (*D*) of an Alu sequence in intron 16. Individuals homozygous for the *I* allele have an ≈35% lower ACE activity than do individuals homozygous for the *D* allele.90 Several large studies have examined the frequencies of these *ACE* gene variants in case-control subjects for atherosclerosis or MI. Association of the *ACE* genotype with disease was found in some studies but not in others.91–93 We have therefore examined the effect of plasma ACE levels on diet-induced atherosclerosis by using *Apoe*-heterozygous mice combined with 1 (±) or 2(+/+) copies of the *Ace* gene. The 2-copy animals have normal plasma ACE levels, whereas the 1-copy animals have half-normal

levels (the genetic equivalent of 50% inhibition by a converting-enzyme inhibitor). The mice were all from an F_1 generation between C57BL/6 and 129 mice, so that their genetic backgrounds were completely identical except for their *Ace* gene status. The results showed that plasma ACE activity differences similar to those seen in humans did not lead to differences in blood pressure or in atherosclerotic lesion size. These results suggest that variation in the *ACE* gene in humans is unlikely to affect the development of atherosclerosis or HTN in humans (see Table 3, line 3).94,95

Coagulation, Fibrinolysis, and Atherosclerosis

In humans, blood clots and thromboses play a major role in the morbidity and mortality associated with cardiovascular disease.68 Most ischemic cardiovascular events are due to thrombotic occlusion, and the risk of MI and stroke is decreased by anticoagulant and fibrinolytic therapy. However, evidence that hemostatic pathways affect the development and progression of atherosclerosis is circumstantial. For example, elevated fibrinogen levels in plasma are a risk factor for cardiovascular disease,96 but it is not clear whether the elevated plasma fibrinogen enhances atherosclerosis or is a response to a chronic inflammatory condition, as fibrinogen is an acute-phase protein. To test whether reduced plasma fibrinogen levels alter the development of atherosclerosis, Xiao et al97 crossed fibrinogen-deficient mice to *Apoe*$^{-/-}$ mice on a 129/B6 mixed genetic background. In these mice, fibrinogen deficiency did not alter lesion size (see Table 3, line 4). In contrast, in mice that were more mildly atherosclerotic as a consequence of expressing an apo (a) transgene, fibrinogen deficiency decreased both atherosclerotic lesions and apo (a) accumulation in the vessel wall.98 The authors suggested that the high plasma cholesterol in *Apoe*$^{-/-}$ mice may have masked the smaller effect of fibrinogen deficiency.

Plasminogen activator inhibitor-1 (PAI-1) is another member of the fibrin/fibrinolytic pathway whose association with coronary heart disease has been extensively tested in humans, with mixed results.99–101 PAI-1 is the primary inhibitor of the conversion of plasminogen to plasmin. Plasminogen is a proenzyme that is converted to its active form, plasmin, by physiological activators such as tissue plasminogen activator and urokinase plasminogen activator. Plasmin-mediated proteolysis is critical to the dissolution of fibrin matrixes in arterial and venous thrombi. The balance between plasminogen activation/inactivation may be critical in the development of atherosclerosis. A common polymorphism in the promoter region of the *PAI-1* gene, which causes varied expression of the gene, is consequently a candidate for affecting atherosclerosis. Experiments by Sjoland et al102 in both *Apoe*- and *Ldlr*-deficient mice on a C57BL/6 background have shown that neither the absence of PAI-1 nor its overexpression by a transgene affects lesion development, suggesting that the levels of PAI-1 do not affect the development of atherosclerosis (see Table 3, lines 6 and 7). Nevertheless, the absence of plasminogen greatly increases atherosclerotic lesion size in *Apoe*$^{-/-}$ mice of a hybrid C57BL/6/NIH Black Swiss/129 background103 (see Table 3, line 5). To reconcile these experiments, the authors proposed that alternative inhibitors of plasminogen activators may exist in mice, so that the effects of manipulating the *PAI-1* locus are masked, or that the increased infectious complications that are present in plasminogen-null mice may affect the development of atherosclerosis.102

Other Considerations

A general caveat is required in determining causative links between gene mutations and atherosclerosis. Current evaluations of atherosclerosis in mice focus on the development of plaques and their progression to complex lesions. Plaque rupture and subsequent thrombosis, which precipitate clinical events like MI, are very rare in these mouse models and are only seen in extreme circumstances (eg, *Apoe*$^{-/-}$-*Ldlr*$^{-/-}$ doubly mutant mice on a high-fat diet).104 Consequently, although the *ACE* or *PAI-1* polymorphisms do not affect plaque development in mice, as described above, their involvement in later stages of vascular disease cannot be excluded.

In evaluating causative links between gene mutations and atherosclerosis, the choice between *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ mice is not of crucial importance, as there is no compelling evidence that either of these models is inherently better than the other. We have not found any examples in the literature in which the same gene, either overexpressed or knocked out in both *Apoe*-deficient and *Ldlr*-deficient mice, gives different results (see Table 1, lines 1 to 3 and 10 and Table 3, lines 6 and 7). However, it is important that analyses of these mouse models be well controlled to ensure that the genetic backgrounds of the parental strains, in most cases C57BL/6 as opposed to 129, does not bias the results. Presently, this complication is avoided through repeated back-crossing to the C57BL/6 genetic background. Less rigorous studies use littermates to control for genetic variability, on the assumption that genetic differences not linked to the locus of interest will be randomly distributed among the offspring. However, if only a small number of littermates are used, nonrandom segregation of any nonlinked differences may bias the results. The effects of genes linked to the mutation are more difficult to eliminate unless other strict breeding strategies are employed (reviewed in References 11 and 105). Thus, special caution is necessary when multiple genetic alterations (often available only on a 129 genetic background) are combined and bred with an atherosclerotic model (often on a C57BL/6 genetic background). The situation is particularly difficult when the combined mutations cause decreased lesions, because every altered locus generated in 129 embryonic stem cells carries with it linked DNA from the 129 strain, which is an atherosclerosis-resistant strain when compared with C57BL/6. A spurious decrease in lesion size is consequently more likely than a spurious increase in lesion size.

Atherosclerotic modifiers differ between mouse strains. Thus, plaque development caused by the absence of apoE is different on an FBV or a C3H genetic background compared with the C57BL/6 background.106,107 Also, a naturally occurring apoE deficiency in *Mus musculus molossinius*, with severe xanthomas and a shortened life span, was recently identified, but these mice have relatively small aortic plaques when compared with *Apoe*$^{-/-}$ mice on a C57BL/6 background.108 Identification of the loci that contribute to strain differences in atherosclerotic susceptibility will likely yield new candidates for human susceptibility, and *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ mutations should accelerate their identification. Nevertheless, testing each gene effect on multiple genetic backgrounds by breeding is of borderline practicality. Regenerating interesting mutations such as *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ by using embryonic stem cells from inbred strains other than 129 (such as atherosclerosis-susceptible C57BL/6 mice or the less-susceptible strains DBA/1 or BALB/c) may prove

easier than the time-consuming, costly, and labor-intensive task of backcrossing to congenicity.

Further uses of gene targeting and transgenic mice assure that additional models of atherosclerosis will be developed. The development of mouse models in which plaque rupture occurs is particularly important, as discussed above. Also, the application of Cre-lox technology to develop tissue-specific and/or time-dependent knockouts109 will prove valuable for dissecting the mechanism of atherosclerosis, although such animals do not strictly represent inherited genetic variations, which are of course lifelong and affect multiple organ systems.

Finally, we emphasize that mouse knockout experiments are the genetic equivalent of recessively inherited conditions in humans due to the loss of gene function. This type of condition makes only a small contribution to the total human burden of atherosclerosis. The pattern of inheritance of atherosclerosis in humans is more compatible with susceptibility being due to combinations of small, *quantitative* changes in gene function, analogous to the situation that occurs in mice that are heterozygous for a gene modification. Thus, it is extremely important not only to study whether a complete absence of gene function affects atherosclerosis but also to determine whether *quantitative* changes in the expression of genes affect the condition. Genes that affect atherosclerosis in mice in the heterozygous state are probably the best candidate genes for being related to atherosclerosis susceptibility in humans. Thus, the study of heterozygotes is extremely important (for more on the importance of quantitative changes, see Reference 95).

Conclusions: “Mice to Humans” and “Humans to Mice”

Genes implicated in processes such as inflammation, hyperglycemia, HTN, or coagulation/fibrinolysis that have also been shown to affect the development of atherosclerosis in mice need to be studied in human populations to look for alleles that may account for susceptibility. Experiments to identify such modifiers benefit greatly by being carried out in parallel in mice and humans. For example, a recent epidemiological study has shown that a polymorphism in the *eNOS* gene is a risk factor for coronary artery disease and MI,110 a finding that is supported by the relationship that we have found between eNOS deficiency and atherogenesis.84

Human polymorphisms shown to be *associated* with atherosclerosis should be tested for *causation* in mice. One way to carry out such tests is to “humanize” the mouse by generating animals that have the same allelic differences, such as single-nucleotide polymorphisms (SNPs), that occur in human populations. For example, the effects of a pair of SNP differences in the human *APOE* gene were evaluated in mice. The 3 common *APOE* alleles in the human population, *APOE*2*, *APOE*3*, and *APOE*4*, differ only in 2 coding nucleotides resulting in amino acid changes. Various population-based studies have suggested that these small differences influence lipid metabolism in humans.111 Recently, our laboratory used mice with a targeted replacement of the endogenous mouse allele with the different human alleles to show that protein structures coded by these alleles are responsible for plasma retention of lipoproteins and atherosclerosis susceptibility.112,113

The effect of any single SNP is likely to be small. However, eventually it will be possible to combine many of these small variations in a single mouse. Comparing various "combinations" should yield important information about which combinations are particularly deleterious and which are protective. It is certainly not easy to breed these "compound" mutants. However, once "prototype" animals have been obtained, cloning via nuclear transfer is a potential future way to facilitate the generation of sufficient numbers of these animals to carry out meaningful studies.114,115

The ability to go back and forth between humans and mice, made possible by targeted mouse models, has and will continue to play an integral part in the study of atherosclerosis. The pathogenesis of atherosclerosis is complex, polygenic, and multifactorial. Knowledge of the genetic determinants of atherosclerosis, aided by mouse studies, will allow us to identify disease-prone persons and design specific preventive measures for them and treatments tailored for those for whom prevention is no longer an option.

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TABLE 1

Genetic Modifiers of Atherosclerosis Related to Inflammation

Inflammation	Gene Altered	Atherosclerosis Model	Genetic Background	Diet	Fold Change			Reference No.
					Increase (↑)	No Change (↔)	Decrease (↓)	
Cytokines and receptors								
1	MCP-1–/–	*LDLR* $^{-/-}$	129/B6	High fat			5↓	30
2	CCR-2–/–	*Apoe* $^{-/-}$	129/B6	Normal chow			3↓	32
	+/–						1.4↓	
3	CCR-2–/–	*Apoe* $^{-/-}$	129/B6	High fat			1.4↓	31
	+/–					↔		
4	Bone marrow replacement with MCP-1 tg cells	*Apoe* $^{-/-}$	FBVX/B6	Normal chow	1.6↑			33
5	CD154–/–	*Apoe* $^{-/-}$	B6	Normal chow			5↓	38
6	IFN-γR–/–	*Apoe* $^{-/-}$	129/B6	High fat			1.6↓	39
7	Bone marrow replacement with CXCR-2–/– cells	*LDLR* $^{-/-}$	B6	High fat			2.5↓	45
Inflammatory mediators								
8	iNOS–/–	*Apoe* $^{-/-}$	B6	Normal chow		↔		84
	+/–					↔		
9	12/15-Lipoxygenase–/–	*Apoe* $^{-/-}$	B6	Normal chow			2.5↓	42
	+/–					↔		
Adhesion molecules								
10	P-selectin–/–	*Apoe* $^{-/-}$	B6	Normal chow			2↓	25
		LDLR $^{-/-}$	129/B6	High fat			1.25↓	26, 27
11	E-selectin–/–	*Apoe* $^{-/-}$	B6	Normal chow			1.3↓	25
12	P,E-selectin–/–	*LDLR* $^{-/-}$	129/B6	High fat			2↓	27
13	ICAM–/–	*Apoe* $^{-/-}$	B6	Normal chow			1.4↓	25
Immunodeficient models								
14	MCSF–/–	*Apoe* $^{-/-}$	129/B6/C3H	Normal chow			5↓	23
	+/–						2↓	
15	RAG1–/–	*Apoe* $^{-/-}$	129/B6	Normal chow			1.7↓	35

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Inflammation	Gene Altered	Atherosclerosis Model	Genetic Background	Diet	Fold Change			Reference No.
					Increase (↑)	No Change (↔)	Decrease (↓)	
		Apoe $^{-/-}$	129/B6	High fat		↔		
16	RAG2–/–	*Apoe* $^{-/-}$	129/B6	High fat		↔		36

tg indicates transgenic; IFNR, interferon receptor; ICAM, intercellular adhesion molecule.

TABLE 2

Hyperglycemia and Atherosclerosis

Hyperglycemia	Treatment	Atherosclerosis Model	Genetic Background	Diet	Fold Change			Reference No.
					Increase (↑)	No Change (↔)	Decrease (↓)	
1	Streptozocin	*Apoe* $^{-/-}$	B6	Normal chow	2↑			54
2	Streptozocin	*Apoe* $^{-/-}$	B6	Normal chow	5↑			55
3	Streptozocin+sRAGE treatment	*Apoe* $^{-/-}$	B6	Normal chow		↔		55
4	Streptozocin	*LDLR* $^{-/-}$	129/B6	High fat		↔		53

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TABLE 3

Genetic Modifiers of Atherosclerosis Related to Hypertension and Coagulation

	Gene Altered	Atherosclerosis Model	Genetic Background	Diet	Fold Change			Reference No.
					Increase (↑)	No Change (↔)	Decrease (↓)	
Hypertension								
1	eNOS–/–	*Apoe* $^{-/-}$	B6	Normal chow	1.8↑			84
	+/–				1.2↑			and unpublished data
2	eNOS–/–	*Apoe* $^{-/-}$	B6	High fat	1.8↑			86
	+/–				↔			
3	ACE+/–	*Apoe* $^{+/-}$	F_1 (129/B6)	High fat		↔		94
Coagulation								
4	fibrinogen–/–	*Apoe* $^{-/-}$	129/CF1/B6			↔		97
5	Plasminogen–/–	*Apoe* $^{-/-}$	129/Black Swiss/B6		7↑			103
6	PAI-1–/–	*Apoe* $^{-/-}$	B6			↔		102
		LDLR $^{-/-}$	B6	High fat		↔		
7	PAI-1 tg	*Apoe* $^{-/-}$	B6			↔		102
		LDLR $^{-/-}$	B6	High fat		↔		

tg indicates transgenic.