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The Mouse as a Model for Human Cardiovascular Disease and Hyperlipidemia

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Abstract
The mouse has been used as an experimental model for atherosclerosis research for only a short time, but the sophisticated genetics of this species has resulted in a number of innovative approaches that are not possible with other models. The availability of inbred, congenic, recombinant inbred, and mutant strains has resulted in the discovery of a number of genes affecting atherosclerosis susceptibility. More importantly, the newer genetic technologies such as quantitative trait loci mapping, transgenic mice, and gene targeted mice are producing important insights into atherosclerosis. This review, focusing on murine models of cardiovascular disease and hyperlipidemia, will be divided into two parts: naturally occurring models and genetically engineered models.

Hyperlipidemia and atherosclerosis in inbred strains of mice
For many years the mouse was not used as an experimental model for atherosclerosis research because of the beliefs that the mouse could not survive on high fat atherogenic diets, that lesions were not reproducible, that most mice did not get lesions, and that lesion pathology did not resemble atherosclerosis in humans [1]. However, the use of lower fat diets solved the survival problem, the use of inbred strains rather than random bred mice solved the reproducibility problem, the use of susceptible strains resulted in most mice getting lesions, and longer experimental times showed that lesions with fibrous caps were produced [2,3]. Recently, lesions that progress beyond the fatty streak stage into complex plaques sharing features with human atherosclerosis have been shown by a number of laboratories [4,5,6].

As with any experimental model, the mouse shows similarities as well as differences when compared to lipoprotein metabolism and atherosclerosis in humans. One difference is that cholic acid must be added to the diet in order to produce lesions. It had been suggested that cholic acid was required for absorption of dietary cholesterol in the mouse, but a recent study demonstrates that cholic acid does not affect absorption but does regulate cholesterol 7-α-hydroxylase, a major pathway for the removal of cholesterol from the body [7•]. Another difference is the distribution of cholesterol among the lipoproteins. When fed standard mouse chow (8% fat), strain C57BL/6 carries 80-90% of the cholesterol in the HDL fraction; however, when these mice are fed a diet of about 30% fat, the quantities of HDL and non-HDL fractions are quite similar to that observed in humans [8]. What is different is the distribution of cholesterol in the non-HDL fraction. In the mouse this is VLDL while in the human it is LDL [9]. The reason for this difference may be that the mouse edits apoB100 to the truncated apoB48 in both the intestine and the liver [10] while the human edits apoB only in the intestine, leaving both apoB48 and apoB100 forms in the plasma. Other important differences

in the lipoprotein system of mice and humans includes the fact that the mouse lacks cholesterol ester transfer protein (CETP) as well as lipoprotein(a) (Lp(a)).

Despite these differences, many findings in the mouse replicate those in humans. Recent studies have shown that dietary n-3 polyunsaturated fatty acids prevent the development of atherosclerotic lesions in mice [11•], that the degree of lesion formation depends on the percent saturation of dietary fat [12•], and that HDL-C levels are inversely proportional to lesion formation [13]. The mouse was used to demonstrate that the atherogenic diet caused greater levels of hepatic conjugated dienes, a measure of oxidation, and also resulted in greater expression of several inflammatory and oxidative stress responsive genes in the susceptible C57BL/6 strain compared to resistant strains [14••]. Additional evidence that oxidation is important in atherosclerosis came from studies of the deposition of lipofuscin, a pigment consisting of terminally oxidized lipids and protein and resulting from oxidative stress. Differences in lipofuscin deposition are associated with differences in susceptibility to lesion formation among mouse strains [15•].

Classical genetic approaches studying atherosclerosis susceptibility in inbred mice

Clearly, the major advantage of adding the mouse to the already extensive repertoire of experimental models for atherosclerosis research is the genetic tools that are available in this species such as inbred, congenic, and recombinant inbred strains. A survey of strains has shown that susceptibility to atherosclerosis is rare among inbred mouse strains but is common in the human population. So far the susceptible inbred mouse strains are SWR, SM, and all those strains that belong to the C57 and C58 family (C57BL/6, C57L, C57BR, C58) [3]. The use of recombinant inbred strains and backcrosses has led to the discovery of several genes affecting atherosclerosis susceptibility such as *Ath-1* [8], *Ath-2* [16], *Ath-3* [17], and *Ath-4* (Nishina and Paigen, personal correspondence), single gene mutants that affect the levels of HDL-cholesterol and atherosclerosis susceptibility. Recombinant inbred strains also were used to map the gene encoding apoJ to mouse chromosome 14 [18••]. ApoJ is a glycoprotein associated with HDL, and it accumulates in the atherosclerotic plaques of both human and mouse. Recombinant inbred strains also were used to suggest that lipoprotein lipase activity in macrophages is higher in atherosclerosis-susceptible strains as compared to resistant strains [19], and that the apoAII gene affects HDL-cholesterol levels in mice fed a low fat diet [20].

The mouse has hundreds of mutant strains that can be used to ask questions concerning the role of various factors in atherosclerosis. An examination of plasma lipids and atherosclerosis in five different mouse mutants, each conferring a phenotype of obesity or diabetes, showed that such mutations are associated with increased HDL-cholesterol and, in four of five mutants, with

resistance to atherosclerosis [21••,22••]. The mouse has many mutants affecting immune function, and these can be used to answer questions concerning the role of individual components of the immune function in atherosclerosis. Such attempts have just begun. Two different laboratories have used a strain of mice carrying a mutant, known as *lpr*, that confers an autoimmune disease. The autoimmune disease is associated with increased vasculitis and lipid deposits in arteries [23,24].

Quantitative trait analysis in mice

Most complex disease traits such as atherosclerosis or hypertension are quantitative rather than qualitative in nature and thought to be determined by many genes, each contributing a small increment to the trait. Recent advances have made possible the mapping of such quantitative traits by providing 3000 polymorphisms easily scored by PCR [25], the theoretical framework for mapping quantitative traits [26], and a computer program, MapMaker, for data analysis [27]. In the area of lipids, the first report using quantitative trait loci (QTL) mapping techniques showed two chromosomal regions that determine cholesterol levels [28••]. The region on chromosome 7 was associated with increased total cholesterol, increased HDL-cholesterol, and increased carcass lipid (overall obesity). The second region on chromosome 6 is associated with increased total cholesterol and increased fat in the subcutaneous fat pad (the outer thigh). The chromosome 7 region contains the locus for the single gene mutation *tubby*, and the chromosome 6 region contains the locus for the mutation *obese* , mutations which cause a phenotype of obesity. Nishina and coworkers showed that the single gene mutants for *tubby* and *obese* are associated with increased total cholesterol, HDL-cholesterol and resistance to atherosclerosis [21••,22••], so these two reports agree in many aspects.

The approach of QTL mapping holds the promise of uncovering new genes involved in lipoprotein metabolism and atherosclerosis. This is an important advance since it has been shown in human studies that variations in the known structural genes for apolipoproteins and lipid metabolizing enzymes can account for only a small part of the variation in lipid levels and heart disease.

Hyperlipidemia and atherosclerosis in genetically engineered mice

The mouse is the easiest mammal in which to overexpress transgenes and presently the only mammal for which embryonic stem cells, essential in the creation of gene knockout animals, are available. Genetic studies have been performed in mice making use of transgenic and gene knockout technology to address hypotheses relevant to lipoprotein metabolism and atherosclerosis. In several of these studies, diet-induced hyperlipidemic C57BL/6 mice have served as a background to study the effects of genes determining HDL composition and concentration. In addition, new diet-induced hyperlipidemic animals have been engineered by over-expression of

transgenes and inactivation of endogenous murine genes involved in lipid transport.

Hyperlipidemic transgenic mice, HDL , and atherosclerosis

As described above, multiple studies have examined the genetics and phenotype of diet-induced atherosclerosis in the C57BL/6 mouse. This animal has been used as the background in which to examine the quantitative effect that overexpression of HDL associated proteins have on atherosclerosis. Overexpression of human apoAI transgenes in the C57BL/6 background raises HDL concentrations and reverses the diet-induced atherosclerosis normally observed in these animals [29]. ApoAI alone is not the entire story. The other apolipoprotein components of HDL affect this lipoprotein's properties in mice. Co-expression of human apoAI and apoAII transgenes in C57BL/6 mice demonstrated that the presence of both human ApoAI and ApoAII on an HDL particles has significantly less antiatherogenic properties than HDL containing exclusively human ApoAI [30••]. Furthermore, overexpression of a mouse apoAII transgene in C57BL/6 x CBA hybrid mice backcrossed for three generations into the susceptible C57BL/6 background develop a hyperlipidemia with a two- to three-fold increase in total plasma cholesterol and the appearance of an alpha-migrating cholesterol ester rich LDL particle [31••]. These animals on a chow diet develop nascent fatty streak lesions. The C57BL/6 hyperlipidemic model has also been useful in demonstrating the atherogenic potential of CETP. C57BL/6 mice overexpressing a simian CETP gene to approximately 15-fold normal human levels develop 50% to 75% elevations in VLDL + LDL cholesterol and a 50% reduction in HDL cholesterol [32••]. When fed the atherogenic diet, the animals overexpressing CETP develop a two-fold increase in the area of lipid staining lesions in the proximal aorta.

Mice have also been created where the murine apoAI gene has been inactivated via homologous recombination in embryonic stem cells [33•]. As expected, these mice have HDL cholesterol levels significantly lower than normal mice. Somewhat surprising is the fact that when these mice were placed on an atherogenic diet they did not appear to have increased susceptibility to atherosclerosis [34•]. These results in the apoAI knockout mice are consistent with studies in humans lacking apoAI, suggesting that low levels of HDL may predispose, but are not inextricably linked, to accelerated atherosclerosis.

Creating the LDL and Lp(a) mouse

Due to the fundamental importance of apoB in determining LDL structure and levels, significant effort has gone into creating mice overexpressing apoB transgenes. Transgenic mice expressing human apoB at significant levels have proven difficult to generate. The apoB cDNA is approximately 14 Kb and the gene is greater than 40 Kb. A variety of minigene constructions have either failed to express apoB or expressed it at low levels when introduced

into the mouse genome [35•]. The difficulty in efficiently expressing apoB cDNAs in transgenic mice suggest that flanking or intronic genomic DNA sequences absent in apoB minigene constructs are necessary for significant expression of this gene in vivo. To circumvent this problem two groups have recently taken advantage of the availability of P1 phagemid libraries containing human genomic inserts, up to 90 Kb in size, to clone the human apoB gene [36••,37••]. A phagemid containing the entire human apoB structural gene plus significant amounts of flanking DNA was isolated and used to create apoB transgenic mice . On comparing multiple lines of human apoB transgenic mice, plasma levels of human apoB in these animals was roughly proportional to the number of copies of the human gene present in the mouse genome.

A surprising finding in these studies of human apoB transgenic mice was the observation that despite greater than 10 Kb of flanking sequences, both 5' and 3' to the human apoB coding sequence, the human apoB transgene expressed in the liver but not the intestine of the transgenic mice. ApoB is expressed in the liver and intestine of both mice and humans and the failure of the human apoB transgene to express in the intestine of the transgenic mice suggests that regulatory elements necessary for intestinal expression of this gene are probably not included within the cloned 90 Kb human apoB genomic fragment. The failure of the human apoB transgene to express in the intestine of mice is reminiscent of studies defining sequences distal to the human apoAI structural gene required for intestinal expression of human apoAI in transgenic mice [38,39]. Murine hepatocytes from the human apoB transgenics edited both human and murine apoB transcripts secreting both apoB100 and apoB48, documenting the cross species capabilities of the murine apoB editing machinery.

Although mice naturally have extremely low concentrations of LDL cholesterol, even when fed a diet high in fat and cholesterol, the mice expressing human apoB did have substantial levels of LDL cholesterol. The cause for the high LDL levels in these animals has yet to be determined. Studies performed in tissue culture have documented inefficient uptake of human LDL by murine LDL receptors [40]. High expressing human apoB transgenic mice have plasma human apoB concentrations greater than 70 mg/dl. Thus possible explanations for the increase in LDL cholesterol in the human apoB transgenic animals include poor LDL receptor mediated clearance of human apoB containing LDL or accelerated production of human apoB100 and marked increases in the synthesis of LDL.

An important finding demonstrated in the studies of the apoB transgenic mice is that, when human apoB transgenic mice were crossed with transgenic mice expressing a human apo(a) transgene, Lp(a) accumulates in the plasma of the doubly transgenic animals. In the study by Callow *et al.* [37••], it was demonstrated that the association between apo(a) and apoB is a covalent one.

In prior studies, it has been shown that, in mice containing the apo(a) transgene alone, apo(a) remains in a lipid-free fraction [35•]. Despite the lipid-free state of apo(a), these animals develop fatty streak lesions when fed an atherogenic diet. Future studies in the apoB/apo(a) mice will address whether lipid association augments the atherogenic potential of apo(a).

Mouse models of primary hypertriglyceridemia

In vitro data over the past fifteen years has suggested that the apoCs may be involved in determining plasma triglyceride levels. These three apolipoproteins have the ability to inhibit lipolysis of triglyceride rich particles [41,42] and inhibit the association of apoE with both the LDL receptor and putative remnant receptor, possibly the LDL receptor-like protein [43]. The possibility that the apoCs are involved in determining plasma triglyceride levels has been tested in transgenic mice. Transgenic mice that overexpress apoCIII develop a severe hypertriglyceridemia with fasting triglyceride levels two- to ten-fold above controls [44]. Mechanistic studies suggest that the defect in the apoCIII transgenic mice is in VLDL clearance [45,46•]. Overexpression of apoCI and apoCII also causes a fasting hypertriglyceridemia. In the apoCI transgenic animals, only limited analyses have been performed [47]. More detailed analysis of the apoCII transgenic mice suggests that, similar to apoCIII transgenic mice, the apoCII transgenic mice have impaired VLDL catabolism [48•].

A fourth protein, apoAIV, has been associated with a hypertriglyceridemic state. Overexpression of human apoAIV in transgenic mice led to a fed hypertriglyceridemia in both low and high expressing mice [49•]. These mice have also proved useful in assessing a putative role for apoAIV in regulating satiety. Several studies in the apoAIV transgenic mice examining the intestinal absorption of fat and fat soluble vitamins failed to demonstrate a role for human apoAIV in mouse intestinal absorption.

ApoE deficiency syndrome and dysbetalipoproteinemia in mice

The first lipoprotein transport gene to be knocked out in mice was the apoE gene [50•,51••,52••]. Since apoE normally acts to remove lipoprotein remnants from the circulation, apoE-deficient mice accumulated VLDL and remnant particles in the plasma. On a chow diet, apoE-deficient mice had total plasma cholesterol levels greater than 500 mg/dl, while on a "Western" diet cholesterol levels can rise to greater than 1500 mg/dl, with the majority of the cholesterol in the VLDL and lipoprotein remnant fractions. These particles were found to be cholesterol ester enriched ß-VLDL, a particle found in humans with dysbetalipoproteinemia and apoE deficiency. ApoE deficiency had little effect on fasting triglyceride levels.

Young apoE-deficient mice, fed a low fat chow diet, developed striking atherosclerosis. The progression of atherosclerosis in these animals is similar to that seen in humans and other atherosclerosis model organisms with

to that seen in humans and other atherosclerosis model organisms with evidence of advanced fibroproliferative atherosclerosis [4,5]. Lesions in these mice are rich in oxidized epitopes, suggesting a role for oxidized lipoproteins in the atherogenesis of apoE-deficient mice.

There are two additional apoE models of hyperlipidemia. Two groups have overexpressed dominant apoE mutants in mice, apoE3-Leiden and apoE3_{142cys} [53•,54•]. Both alleles are associated with a dominant form of type III hyperlipoproteinemia in humans. Overexpression of both proteins in transgenic mice generated a profile similar to that seen in type III hyperlipidemic humans. Both sets of mice developed hypertriglyceridemia and hypercholesterolemia. High plasma levels of the E3_{142cys} mutant protein led to the appearance of ß-VLDL. On a chow diet both sets of *trans*-dominant mutant mice develop nascent fatty streaks (personal communication, Sergio Fazio and Marten Hofker).

The FH mouse

Gene targeting in ES cells has recently been used to create LDL receptor-deficient mice, a model of familial hypercholesterolemia (FH) [55••]. Both heterozygous and homozygous LDL receptor-deficient mice have elevated plasma cholesterol levels. Kinetic studies have demonstrated a significant delay in VLDL and LDL clearance from the plasma of these animals. Although the apoE-deficient mice have a more severe lipoprotein profile than apoE-deficient humans, the LDL receptor-deficient mice have a less severe phenotype. Homozygous familial hypercholesterolemic (FH) humans often develop plasma cholesterol levels of 500 to 1,000 mg/dl. The homozygous FH mice develop plasma cholesterol levels of 250 mg/dl. This difference might reflect an enhanced role for apoE in lipoprotein clearance in mice, an hypothesis supported by the exaggerated hypercholesterolemia in apoE-deficient mice. Alternatively, the lower levels of plasma cholesterol in mice may reflect the low fat chow diet used in these studies. On an atherogenic diet, LDL receptor-deficient mice develop significant elevations in plasma cholesterol to levels over 1,000 mg/dl (personal communication, Joachim Herz). When on the atherogenic diet for an extended time, the LDLR knockout mice develop extensive fibroproliferative atherosclerosis.

Conclusion

Naturally occurring inbred mice and genetically manipulated mice have led to the development of several animal models of human hyperlipidemia. A number of these models have been useful in assessing the role of the many lipoprotein transport genes in normal lipoprotein metabolism. The models have also been instrumental in understanding the pathogenesis of many of these disorders. Several of the engineered mice have been important substrates in which to test the atherogenic potential of these same genes. The various transgenic and knockout mice described above have already provided

studies should continue to systematically assess the role of candidate genes in lipoprotein metabolism and atherosclerosis susceptibility. In addition, through classical mouse genetics, simplified by an expanding genetic and physical map of the mouse genome, anonymous genes may be uncovered that are also involved in lipoprotein metabolism and atherogenesis.

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