Murine models for study of lipoprotein metabolism and atherosclerosis.

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Editorial

Atherosclerosis is a complex disease that is multifactorial in nature (1). While it is clear that alterations in lipoprotein metabolism are etiologically related to atherosclerosis (2), it is equally clear that atherogenesis and its clinical sequalae are determined by many other factors. Even among human subjects homozygous for the same null allele leading to familial hypercholesterolemia, death from cardiovascular causes varies by up to 30 years (3). In an attempt to understand the pathogenesis of this disease, investigators have sought to develop animal models to define the complex events involved and to evaluate therapies designed to ameliorate its consequences. The popularity of individual animal models has waxed and waned over the years. Commonly, rabbits, pigs, pigeons, and subhuman primates have been used, among others. Although rats have been used to study lipoprotein metabolism, only sporadic attempts have been made in the past to use rodents in studies of atherosclerosis. Yet each of these models has been criticized in that they fail to mimic human lipoprotein metabolism or that aortic lesions that develop as a result of a given manipulation were not like that seen in man. While such criticisms may have merit, they nevertheless miss the point that lipoprotein metabolism and atherogenesis are highly complex processes, with many different genetic and environmental events contributing significantly. Dissecting out the individual contributions of all the relevant factors is most difficult, if not impossible, in the human subject. In contrast, a well-thought-out animal model can yield valuable insights into a defined unit process that can then be integrated into an overall understanding of the complex events involved in atherogenesis. In particular, such criticism of lack of relevancy has been levied upon studies of lipoprotein metabolism and atherosclerosis in mice. However, recent advances in murine genetics, as well as advances in molecular biology and the technology that allows us to transfer genes, as well as inactivate them, have brought into prominence the mouse as an animal model in which to study the unit processes involved. The pioneering work of Paigen and her collaborators has defined atherosclerosis susceptible and resistant strains of mice (4, 5) and mapped candidate genes responsible (6, 7). Rubin et al. (8) have shown that overexpression of a human apo AI transgene can inhibit fatty streak formation in susceptible mice. Breslow and colleagues (9) and Maeda and colleagues (10) have used targeted gene disruption to produce lines of "knockout" mice that lack the gene for apo E. These mice have marked hypercholesterolemia on regular chow as well as on high fat diets, and quickly develop both early as well as late, complex lesions typical of advanced atherosclerosis. Granted that the phenotype of these apo E deficient mice differ in many respects from apo E deficient human kindreds, they nevertheless will prove to be of great value in studying individual genetic and environmental influences affecting atherosclerosis susceptibility.

In this issue of *The Journal,* Ishibashi et al. (11) present a brilliant exposition of the power of modern techniques to generate an animal model that teaches us much about lipoprotein metabolism, even though the murine model fails to replicate faithfully, at least in a quantitative manner, what is seen in humans. Using homologous recombination in embryonic stem cells, they produced mice lacking functional LDL receptor genes ($LDLR^{-/-}$). Although plasma levels of IDL/LDL cholesterol were seven- to ninefold greater than wild type littermates, absolute cholesterol levels were still only 228 mg/dl, and increased to only 300–400 mg/dl on a high fat diet. This contrasts sharply with findings in the apo E deficient mice, described above, in which IDL/VLDL cholesterol levels were about 18-fold elevated on chow diet, and 30-fold elevated on a high fat diet and achieved absolute plasma cholesterol levels of 494 mg/dl and 1,821 mg/dl, respectively (9). The phenotypes observed in both types of mice are different than that seen in similarly deficient humans. Homozygous familial hypercholesterolemia subjects have IDL/LDL values fourfold higher than that seen in the $LDLR^{-/-}$ mice, and in general have cholesterol values higher than that seen in apo E–deficient kindreds. By inference, these data suggest important differences in the relative roles of apo B and apo E in mice and man. In man, virtually all the apo B generated by the liver is full length apo B-100. In contrast, ~ 70% of the apo B mRNA in livers of adult mice encode the apo B-48 isoform, an apo B form lacking the LDL receptor–binding domain. These data suggest that VLDL containing apo B-48 may actually be cleared more rapidly than VLDL containing intact apo B-100. Presumably this uptake would be LDL receptor–independent and occur through apo E/apo B-48–mediated mechanisms via hepatic chylomicron receptors. Indeed, future studies may allow testing of this hypothesis as mice with a mutation in the apo B gene making it impossible for the liver to edit B-100 to B-48 will undoubtedly be generated. Upon crossing mice making only VLDL containing apo B-100 with mice lacking the LDL receptor, one might find much more severe hyperlipidemia than was found in the present $LDLR^{-/-}$ mice. With technologies currently available, such studies are feasible and nicely illustrate the great potential of this model.

The paper of Ishibashi et al. indicates yet another powerful way in which such murine models can be most valuable. By using an adenovirus mediated gene delivery vector (12) these authors demonstrate the ability to restore LDL receptor activity to the liver of the $LDLR^{-/-}$ mice and to correct the metabolic abnormality generated by the original gene knockout. While this particular mode of gene replacement therapy may or may not be useful eventually, the availability of $LDLR^{-/-}$ mice should be invaluable in developing gene delivery techniques that one day could be applied successfully in homozygous familial hypercholesterolemia subjects.

Finally, the $LDLR^{-/-}$ mice were generated in the atherosclerosis-susceptible C57B1/6 strain and it can be anticipated that these mice may prove useful in studies of atherosclerosis. Results of such studies will be eagerly awaited. The arterial wall responses in $LDLR^{-/-}$ mice exposed to more modest levels of hypercholesterolemia may differ significantly compared to responses noted in the more dramatic hypercholesterolemia of apo E–deficient mice. By breeding into mice with varying genetic backgrounds and other genetically defined alterations in

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metabolic behavior or vascular wall biology, it can be anticipated that significant insights into the many interacting genes that are involved in the atherogenic process will be elucidated.

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