MOUSE MODELS OF HYPERLIPIDEMIA AND ATHEROSCLEROSIS

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1. ABSTRACT

The mouse is the most utilized model to study lipids and atherosclerosis. Before the advent of the techniques of genetic manipulation it was well known that different inbred strains had varying degrees of susceptibility to diet-induced atherosclerosis. The C57BL/6 mouse was adopted as the standard even though the arterial lesions induced by even extreme diets were limited in size, complexity, and distribution. This changed with the production of several gene knockout and transgenic mice, which in many cases produced remarkable effects on plasma lipoproteins and arterial lesions even without dietary manipulations. The most typical example remains the apoE deficient model, in which a massive hyperlipidemia is accompanied with the development of severe atherosclerotic plaques at the aortic root and throughout the aortic tree. With the creation of the LDL receptor knockout, and the different knockout and transgenic mice with changes in apoB, apoE, and the HDL system, a solid body of new information has emerged on the mechanisms regulating plasma lipoprotein levels and controlling the initial stages of atherogenesis. This paper presents an overview of the most utilized mouse models and a summary of the results obtained with the technique of bone marrow transplantation, an approach to study macrophages in atherosclerosis.

2. INTRODUCTION

The field of experimental lipid metabolism and atherosclerosis has seen a spectacular expansion in the last eight years, due mostly to the large efforts invested in manipulating the mouse genome to eliminate or add genes relevant to vessel wall biology and health. Although plenty of research work still focuses on animal species spanning from birds to primates (1-3), it is unquestionable that the mouse has become the most widely used model to study lipoproteins and atherogenesis, as well as the standard for the evaluation of strategies affecting arterial plaque development. However, the reader should keep in mind that the transgenic and gene knockout techniques, well established in the mouse system, have been successfully applied to other species. For example, tremendous insights on HDL and LDL metabolism have been obtained from transgenic rabbits overexpressing different apolipoproteins or lipid-modifying enzymes (4), and the only report of metabolically induced coronary death comes from a study of hypertensive rats overexpressing human cholesteryl ester transfer protein (5). This review has the limited objective to summarize and put in perspective the significance and utility of the most common mouse models of dyslipidemia, and to evaluate the impact of these experimental systems on our understanding of atherogenesis.

3. A SIMPLIFIED VIEW OF ATHEROGENESIS

According to the response to injury hypothesis, atherosclerosis is an excessive fibro-proliferative response to an inflammatory insult to the artery wall (6,7). In the setting of even modest hyperlipidemia, plasma lipoproteins enter the artery wall, become modified or oxidized, and lead to the expression of cell surface adhesion molecules by the endothelial cells. Inflammatory cells, principally

Table 1. Atherosclerosis susceptibility among inbred strains of mice after 14 weeks on an atherogenic diet

Susceptibility	Strain	Lesion area/aortic section (μm^2)
Susceptible	C57BL/6, C57BR/cd, C57L, SM	4500-8000
Intermediate	C58, SWR	1670-1690
Low	129, AKR, DBA/2, BALB/c	20-350
Resistant	C3H, NZB, HRS, SJL, CBA, A	No lesions

monocytes and T-cells, enter the subendothelial space, where monocytes differentiate into macrophages and take up modified lipoproteins to form cholesterol ester laden foam cells. The first stage of atherogenesis is the fatty streak, which is characterized by the presence of these macrophage-derived foam cells and small numbers of T-cells. The second stage is characterized by the presence of fibro-proliferative plaques (intermediate lesions) which consist of a central acellular lipid core covered by a fibrous cap containing smooth muscle cells and collagen. The third and final stage of atherogenesis is the formation of complex lesions, characterized by expansion of the central core secondary to cell death, calcification, and capillary hemorrhages. In humans, complex lesions show evidence of thrombus formation with deposition of fibrin and platelets. Plaque rupture and acute thrombotic occlusion of the vessel result in the majority of acute ischemic events. In mice, the thrombotic component of atherosclerosis is less prominent due to differences in the fibrinolytic system (8), and acute vessel obstruction is not commonly observed.

4. PLASMA LIPIDS IN MOUSE AND MAN

Wild-type mice are highly resistant to the development of atherosclerosis. In response to a low-fat, low-cholesterol (normal chow) diet, mice have low plasma levels of cholesterol and most of the cholesterol is in the HDL fraction. A mouse carries more than 85% of its plasma cholesterol in HDL, whereas in humans the major carrier of plasma cholesterol is the LDL (65-85%). Although not well understood, this large difference between species is partly due to several known factors (9), such as: 1. The absence of CETP in mouse plasma (in humans, it transfers cholesteryl esters from HDL to VLDL and triglycerides in the opposite direction); 2. Hepatic lipase, which is membrane-bound in humans and soluble in mice; 3. Reduced synthesis of apoB-100 in mice due to the high degree of editing of the apoB message in the liver; 4. Higher efficiency of murine apoE, compared to its human counterpart, in determining the clearance of remnant lipoproteins.

5. THE C57BL/6 MOUSE MODEL OF DIET-INDUCED HYPERLIPIDEMIA AND ATHEROSCLE-ROSIS

Studies in the late 60's and early 70's first demonstrated that certain inbred strains of mice could develop atherosclerotic lesions in response to diets high in cholesterol and fat, and which contained cholic acid to block cholesterol conversion to bile acids (10-12). Paigen and co-workers fed 10 different in-bred strains of mice a diet consisting of 15% fat, 1.25% cholesterol, and 0.5%

cholic acid, and found that the inbred strains of mice varied in their susceptibility to diet-induced lesion formation (13). Susceptible strains such as C57BL/6 developed foam cell lesions in the subendothelial space near the aortic valve leaflets, whereas other resistant inbred strains did not (Table 1). Paigen and co-workers developed a widely used method for quantifying the extent of atherosclerotic lesions in mice, in which cross-sections obtained from the proximal aorta beginning at the aortic sinus are stained for lipids and the lipid staining area is quantitated (14).

The main effect of this diet on plasma lipids is an increase in the amount of apoB-containing lipoproteins (VLDL, remnants, and LDL) relative to that of HDL. However, the response of HDL to diet was variable among strains and appeared to predict the accelerated atherosclerosis observed in the C57BL/6 mouse, where the post-diet lipoprotein distribution becomes "human-like" with a preponderance of non-HDL cholesterol (15). The lack of complete correlation between susceptibility to atherosclerosis and total plasma cholesterol levels suggested the possibility that other genetic factors may be involved. When crosses between susceptible and resistant strains were used to identify potential genetic susceptibility loci, a new locus, named *Ath-1* was mapped to the short arm of chromosome 1, near but separate from the ApoAII gene (16). However, recent studies by Lusis and co-workers using a statistical genetic analysis called Quantitative Trait Locus Mapping (QTL) failed to detect the *Ath-1* gene locus (17,18).

The diet-induced murine model of atherosclerosis has several limitations: atherosclerotic lesions in C57BL/6 mice are small foam cell plaques present only in the region of the aortic valve leaflets. Unlike the situation in humans, these fatty streak lesions do not progress into fibrous plaques. Furthermore, concerns have been raised that the diet used to induce atherosclerotic lesions in these models is unphysiological because of high concentration of cholesterol and because cholic acid may be pro-inflammatory (19). Lusis and co-workers reported that this diet induces a chronic inflammatory state, characterized by the induction of hepatic NF-kB activation in the expression of acute phase reactants in atherosclerosis susceptible C57BL/6 mice but not in resistant C3H mice (19). Concerns have been raised that this diet might detect genetic differences between inbred strains of mice related more to inflammation than to atherosclerosis susceptibility (19). Despite these concerns, the C57BL/6 diet-induced model of atherosclerosis has proven useful in the examination of the effects of expression of various candidate genes on susceptibility to atherosclerosis (20-22).

Table 2. Features of the most commonly used mouse models of dyslipidemia and atherosclerosis

Mouse Model	Plasma Lipids	Atherosclerosis	Ref. #
ApoE Deficient	Increase in remnant cholesterol and apoB48 lipoproteins; reduction in HDL cholesterol and apoAI	Spontaneous development of lesions in the aortic sinus and throughout the aorta	23, 24, 27, 28
Mutant ApoE Transgenic	Mild increase in cholesterol and triglycerides	No lesions on chow; mild plaque development after high-fat diet	30, 31
LDL-R Deficient	Mild accumulation of LDL on chow; large increase in VLDL and remnants on high-fat diet	No lesions on chow; massive plaque development even on Western-type diet	35, 37, 40
Human ApoB Transgenic	Accumulation of triglyceride-enriched LDL	Lesion development after high-fat diet	42-44, 47
ApoB100-only and ApoB48-only	Single populations of apoB particles	Lesion development on apoE deficient background	54, 55
ApoBEC-1 Deficient/ LDL-R Deficient	Severe hypercholesterolemia due to accumulation of human-like LDL	Spontaneous development of lesions	50
ApoAI Deficient	Drastically reduced levels of HDL cholesterol	No differences from C57BL/6; increased lesion area when human apoB is overexpressed	60, 61, 62
ApoAI Transgenic	Elevated total apoAI and HDL cholesterol levels	Protection from diet-induced lesion development	57, 58
ABC-1 Deficient	Extreme reduction in HDL cholesterol and apoAI	Not reported	69, 70
ACAT-1 Deficient	No changes	Increased atherosclerosis due to free cholesterol toxicity and macrophage death	96, 97

With the advent of the more sophisticated mouse models produced by transgenic or gene targeting techniques, the C57BL/6 mouse is more and more used as control system in the analysis of atherosclerosis attenuation or potentiation by different interventions in engineered mice, rather than as a target to study the biology of atherogenesis. Table 2 shows the salient features of the most commonly used mouse models some of which are described in more detail below.

6. APOE DEFICIENT AND APOE TRANSGENIC MICE

Since two laboratories simultaneously reported the development of recombinant mice deficient in apoE (23,24), the field of experimental atherosclerosis has fully embraced this model and turned it into the gold standard for comparative studies to dissect the relevance of specific influences on atherogenesis. ApoE is the ligand for clearance of remnant lipoproteins by the liver (25). Without apoE, mice develop a phenocopy of human type III hyperlipidemia (26), with severe hypercholesterolemia on a normal chow diet due to accumulations of chylomicron and VLDL remnant lipoproteins, providing evidence that the role of apoE in the mouse system is identical to that in humans. In addition, apoE deficient mice develop spontaneous atherosclerotic lesions at the aortic root and widespread fibrous plaques at the aortic branch points, in a distribution similar to human atherosclerosis (27,28). On a normal chow diet, fatty streak lesions begin to appear after 10 weeks, intermediate lesions containing foam cells and smooth muscle cells appear after 15 weeks, and fibrous plaques appear after 20 weeks. Although extensive fibro-proliferation can narrow the arterial lumen to the point of occlusion, complicated lesions with thrombosis have not been described. Feeding apoE deficient mice a Western type diet (0.15% cholesterol and 21% fat derived from milk fat) results in extreme hypercholesterolemia and accelerates the development and progression of atherosclerotic lesions (27,28).

Although apoE deficient mice are a widespread model of massive hyperlipidemia and spontaneous atherogenesis, absence of apoE is an extreme condition which happens rarely in humans. In fact, the most common cause of type III hyperlipoproteinemia is the presence of a receptor-binding defective form of apoE, such as apoE2 (29). Transgenic mice expressing mutant forms of apoE known as apoE-Cys142 and apoE Leiden develop mild hyperlipidemia on a chow diet, and severe hypercholesterolemia and fatty streak lesions in response to a high-cholesterol, high-fat diet (30,31). Recently, gene-replacement techniques have been used to introduce the common human apoE gene isoforms into the mouse apoE locus (32,33). Exchanging the human apoE2 gene for the mouse apoE gene produces features of type III hyperlipoproteinemia and spontaneous atherosclerosis (32). A limitation of the apoE deficient mice and transgenic mice expressing apoE mutants as models of human atherosclerosis is the fact that type III hyperlipoproteinemia is an extreme and rare condition in humans. However, a vast body of investigations have utilized this model to study the biochemical and cellular events leading to the initiation, progression, arrest, and regression of the arterial plaque. An example of the usefulness of this model will be given below.

7. LDLR DEFICIENT MICE

Elevation of plasma LDL cholesterol is a common risk factor for human coronary artery disease (34). Therefore, efforts have been made to develop murine models of atherosclerosis characterized by elevated levels of LDL cholesterol. To this end, Ishibashi *et al.* described the development of LDL receptor deficient mice through gene-targeting techniques in 1993 (35). LDL receptor deficient mice are a model of familial hypercholesterolemia. In response to a normal chow diet, LDL deficient mice develop moderate hypercholesterolemia with cholesterol levels around 250 mg/dl, due primarily to accumulations in LDL cholesterol. This is in contrast to what happens in humans

lacking the LDL receptor, whose LDL levels in plasma reach up to 1000 mg/dl. The main reason for this difference may be the lower production rate of LDL in the mouse, as a consequence of the high degree of editing of the apoB mRNA in the liver and production of apoB48 particles (36). In addition, LDL receptor deficient mice do not develop significant atherosclerotic lesions on a normal chow diet (35). However, these mice are extremely sensitive to diet-induced hypercholesterolemia. In response to the Western type diet, LDL receptor deficient mice developed severe hypercholesterolemia with cholesterol levels between 1000-1200 mg/dl and robust atherosclerotic lesions throughout the aortic tree (37-39). When fed a diet containing high levels of cholesterol and cholic acid, cholesterol levels raised to >1500 mg/dl and massive xanthomatosis and severe atherosclerotic lesions rapidly developed (40). Although this model is used as a phenocopy of Familial Hypercholesterolemia in humans, the need for a high-cholesterol diet actually produces a dyslipidemia characterized by elevation in remnant and LDL levels, thus diminishing the value of studies addressing atherogenicity of LDL alone.

8. APOB MICE

Elevated levels of plasma apoB100 and LDL cholesterol are well-established risk factors for the development of atherosclerosis (34,41). Therefore, transgenic mice overexpressing human apoB100 have been developed to study the role of apoB100 in lipid metabolism and atherogenesis (42-44). Initial efforts to develop apoB transgenic mice using an apoB100 minigene expression vector made from a hybrid of cDNA and genomic apoB clones yielded very low expression of human apoB100 (44). Transgenic mice expressing high levels of human apoB100 were developed independently by Linton, *et. al.*, and Callow *et. al.*, using a P1 bacteriophage vector containing an 80 kB insert spanning the entire human apoB gene (42,43). Interestingly, these transgenic mice express human apoB only from the liver, as the intestinal enhancer is not present in the transgenic construct (45). The apoB transgenic mice have a plasma lipoprotein profile which more closely parallels that of humans, including a distinct LDL cholesterol peak. On a normal chow diet, the mice have mild hypercholesterolemia and hypertriglyceridemia. The triglyceride elevation is due to enrichment of the LDL cholesterol with triglycerides. The human apoB transgenic mice do not develop spontaneous atherosclerosis on a normal chow diet. However, in response to a high-fat, high-cholesterol diet containing cholic acid, the mice developed severe hypercholesterolemia with cholesterol levels >300 mg/dl due to the presence of a cholesterol ester enriched VLDL and LDL (46). After 18 weeks on a high fat diet, the extent of atherosclerosis in the proximal aorta in the apoB transgenic mice was 11-fold greater than in non-transgenic littermates.

Hobbs and co-workers have bred the human apoB transgene onto the background of LDL receptor deficiency (47). These doubly engineered mice [LDLR$^{-/-}$/TG(apoB+/+)] developed severe hypercholesterolemia with total cholesterol levels of 700-800 mg/dl due almost entirely to accumulation of cholesterol in the apoB100 containing LDL particles, a lipoprotein profile similar to that seen in homozygous familial hypercholesterolemia. Interestingly, the LDLR$^{-/-}$/TG(apoB+/+) mice developed severe aortic atherosclerosis on a chow diet. Furthermore, the addition of the apo(a) transgene to form Lp(a) in these apoB LDL receptor deficient mice did not increase the extent of atherosclerosis (47), suggesting either that the atherogenicity of LDL reaches an unmodifiable maximum at levels above 500 mg/dl, or that Lp(a) is not pro-atherogenic in the mouse system. In contrast, studies by Rubin and collaborators have shown that apo(a) is pro-atherogenic in C57BL/6 mice (48).

As discussed previously, mice deficient for the LDL receptor have only mildly elevated LDL cholesterol levels due to extensive editing of the hepatic apoB mRNA which limits apoB100 synthesis in favor of apoB48 synthesis. The production of apoB48 in the mouse liver is due to apoB mRNA editing which is mediated by apoB mRNA editing catalytic polypeptide-1 (APOBEC-1) (49). Targeted disruption of APOBEC-1 results in mice that produce only apoB100 from the liver (36). By crossing LDLR -/- and APOBEC-1 -/- mice, Davidson and co-workers have generated LDLR-/- mice that cannot edit apoB mRNA and therefore synthesize exclusively apoB100 (50). These mice have markedly elevated cholesterol and apoB100 levels and develop extensive atherosclerosis on a chow diet. Humans with atherosclerosis almost always have high plasma levels of cholesterol-rich apoB100-containing LDL, therefore, the LDLR-/- APOBEC-1-/- mice may provide some advantages over the apoE deficient mice, which show accumulation of apoB48-containing lipoproteins and relatively low levels of apoB100-containing lipoproteins.

Because homozygous elimination of the apoB gene results in embryonic lethality in mid-gestation or birth of pups with exencephalus (51), this engineered mouse could not be established as a model of low cholesterol syndromes. However, several truncated apoB forms have been produced to generate authentic mouse models of apoB deficiency, including the apoB70 (52), apoB39 (53), and apoB83 (45). Finally, Young and coworkers have used targeted mutagenesis of the apoB gene to develop mice that synthesize exclusively apoB48 or apoB100. Both models are viable and fertile (54). In the setting of apoE deficiency lower plasma cholesterol levels are reached in apoB100 mice than in apoB48 mice, indicating that the clearance of apoB48 particles is indeed dependent on apoE-mediated events. Interestingly, atherosclerosis was accelerated in both models in the apoE null background, indicating that the atherogenic potential of apoB48 particles is similar to that of apoB100 particles (55).

9. MICE WITH ENGINEERED HDL

As mentioned above, normal mice have a preponderance of HDL in plasma, which may be the reason for their exceptional resistance to atherosclerosis. Plasma levels of HDL cholesterol and apoAI are inversely related to the risk for coronary artery disease (56). Although apoAI

is the major structural protein of HDL, other proteins, such as apoAII, apoAIV, apoE, and the apoC's, are also abundant among this heterogenous population of lipoprotein particles. Overexpression of human apoAI in transgenic mice resulted in increased plasma levels of HDL cholesterol and decreased atherosclerosis (57). On the basis of this observation, Rubin *et al*. set out to study the contrasting effects of apoE deficiency and apoAI overexpression in the same mouse system, and reported a significant protection from atherosclerosis in these intensely hypercholesterolemic mice by virtue of their increased HDL levels (58). Conversely, expression of transgenic CETP induced lowering of HDL cholesterol and increased aortic lesion formation (59).

Disruption of the mouse apoAI gene through gene-targeting results in hypocholesterolemia due to reduced plasma levels of HDL cholesterol (60). Surprisingly, the apoA-I deficient mice on a mixed genetic background did not display an increased susceptibility to atherosclerosis in response to a high-fat diet, maybe because of the extremely low levels of LDL in these mice (61). However, the lack of apoAI predisposed apoB transgenic mice to accelerated atherosclerosis, proving that low HDL per se is not as damaging as when it is accompanied with hypercholesterolemia (62). In addition, transgenic overexpression of apoAII resulted in increased atherosclerosis despite a significant increase in plasma HDL cholesterol levels (63). Recent evidence indicates that the pro-atherogenic effect of apoAII in mice is by conversion of HDL to proinflammatory particles (64). Also, overexpression of human apoAIV, a minor component of HDL, resulted in reduced atherosclerosis in transgenic mice fed an atherogenic diet compared to controls (65). Finally, overexpression of apoCIII mostly resulted in hypertriglyceridemia due to displacement of apoE and consequent inappropriate processing and clearance of VLDL (39,66).

Taken together, these results combined point to the extreme complexity of the HDL system and suggest that therapeutic manipulations of the reverse cholesterol pathway must go beyond the simple target of raising HDL cholesterol. As an example, the importance of cholesterol efflux for vascular health has become indisputable with the discovery that Tangier disease, a genetic condition characterized by low or absent HDL and early atherosclerosis, is due to a mutation in ATP-binding cassette transporter 1 (ABC1), a member of a superfamily of proteins involved in energy-dependent transport of many substrates across membranes (67,68). Recently, the ABC1 gene has been knocked out in the mouse by two distinct investigative teams. In agreement with the human condition, the ABC1-null mouse shows extremely low levels of HDL as well as the accumulation of lipid-laden macrophages in tissues (69,70).

10. A PRACTICAL APPLICATION OF COMMON MOUSE MODELS: BONE MARROW TRANSPLANTATION AND THE ROLE OF MACROPHAGES IN ATHEROGENESIS

Among the several applications of dyslipidemic mice one can count: 1. The development of therapeutic strategies such as adenoviral or retroviral vector transduction systems, or the use of established or experimental pharmaceuticals; 2. The evaluation of the influences played by other genes, such as cytokines, chemokines, adhesion molecules, platelet factors, components of the coagulation or fibrinolytic cascades, etc, in the modulation of the atherogenic phenotype; 3. The analysis of the physiologic relevance of specific cell types in plaque development in vivo. We and others have developed the approach of bone marrow transplantation as a validated technique to study the biology of macrophage involvement in atherogenesis and to determine whether macrophage-directed gene transfer can represent a viable approach to the treatment of atherosclerosis.

The macrophage expresses a variety of genes that may contribute to atherosclerotic lesion formation. Bone marrow transplantation using gene-targeted mice, both as donors and recipients, provides a useful approach to examine the contribution of macrophage gene expression to the process of atherogenesis (21,38,71-75). Secretion of apoE by the macrophage is seen as a protective process, preventing foam cell formation by stimulating efflux of free cholesterol from the cholesterol loaded macrophage (76) and/or by facilitating reverse cholesterol transport from the artery wall (77). However, an alternative hypothesis suggested that apoE secreted by the macrophage may encourage foam cell formation by associating with the lipoproteins in the extracellular space and promoting increased lipoprotein uptake by the macrophage (78). Studies in transgenic mice have shown decreased atherosclerosis as a consequence of local expression of the human apoE transgene by macrophages (79) or by other cells in the artery wall (80). Bone marrow transplantation studies in apoE deficient mice allowed the question of whether the regulated secretion of native apoE by the macrophage plays a crucial physiologic role in atherogenesis to be addressed.

As a first effort to examine the role of apoE expression by the macrophage in atherosclerosis, apoE (-/-) mice were reconstituted with apoE(+/+) or apoE(-/-) marrow were challenged with the western diet for three months (71). The apoE(+/+)? apoE(-/-) mice showed protection from diet induced hypercholesterolemia relative to controls with mean serum cholesterol levels of 318 mg/dl and 1303 mg/dl, respectively. Analysis of the atherosclerosis by quantitative morphometry revealed that the mean lesion area per mouse was 52 times more in the apoE(-/-)? apoE(-/-) controls compared to the apoE(+/+)? apoE(-/-) mice. Because the reduction of serum cholesterol in the apoE(+/+)? apoE(-/-) mice obscured the contribution of local macrophage apoE expression in the artery wall to the atherosclerotic process, bone marrow transplantation was used to reconstitute C57BL/6 mice with macrophages that were either null or wild-type for the apoE gene, creating in effect a macrophage-specific knock out of apoE (21). The apoE(-/-)? apoE(+/+) retained normal expression of apoE from their hepatocytes, so no changes in serum lipid levels or lipoprotein profiles were detected in comparison to apoE(+/+)? apoE(+/+) controls on either a normal chow diet or an atherogenic diet. After 13 weeks on an atherogenic diet, C57BL/6 mice reconstituted with apoE null marrow developed ten-fold more atherosclerosis than

controls. Thus the lack of apoE expression by the macrophage promotes foam cell formation, supporting a protective role for apoE expression by the macrophage in early atherogenesis (21).

Recently, we have investigated a novel gene therapy approach to atherosclerotic vascular disease by using an apoE-expressing retrovirus to transduce apoE$^{-/-}$ bone marrow, for transplantation into apoE$^{-/-}$ recipient mice (81). Three weeks after bone marrow transplantation, apoE was expressed from arterial macrophages, and was detectable in plasma associated with lipoproteins at 0.5-1% of normal levels, but did not affect plasma cholesterol levels. These studies demonstrated that even very low levels of arterial macrophage-apoE secretion can delay atherogenesis if expressed during foam cell formation, and provide evidence that apoE transgene expression from arterial macrophages may have therapeutic applications. Finally, we performed similar transplant experiments using as recipients mice lacking both apoE and the LDL receptor to determine whether extrahepatic apoE could induce remnant clearance even in the absence of the LDL receptor. Surprisingly, macrophage production of apoE in this system was not accompanied with changes in lipoprotein levels or turnover rates (82), suggesting that the LDL receptor is the primary clearance mechanism for apoB-containing particles and that apoE of hepatic origin may serve a specific role in binding to other receptors, such as the LDL receptor related protein (LRP).

ApoE represents only one of the many potential genes with therapeutic effects on atherosclerosis. Other genes related to lipoprotein metabolism, fibrinolysis, thrombosis, cell proliferation, and oxidation have a potential application for gene therapy of atherosclerosis if expressed from macrophages in the artery wall (81). Lipoprotein receptors expressed on the surface of the macrophage are believed to play an important role in foam cell formation. Macrophages express several receptors capable of taking up native or modified lipoproteins, including the scavenger receptor class A (SR-A) and CD36, which are primarily responsible for the uptake of oxidized or modified arterial lipoproteins (83,84). Targeted disruption of SR-A (85,86) and CD36 (87) protects against atherosclerotic lesion development in mice. Other receptors expressed by the macrophage include the LDL-R and the LRP. Macrophage expression of LDL-R is easily inhibited by excess cholesterol available to the cell (83), suggesting that the physiologic contribution of the LDL-R to lipoprotein uptake by the macrophage may be limited in the presence of elevated levels of LDL cholesterol. However, the macrophage LDL-R has been implicated in the uptake of other atherogenic lipoproteins, such as beta-VLDL and chylomicron remnants (88-90). Transplantation studies in LDLR deficient mice have suggested that the leukocyte LDL-R may not play a major role in lesion development, based on the qualitative observation that both LDL-R(-/-)→LDL-R(-/-) and LDL-R(+/+)→LDL-R(-/-) mice developed extensive atherosclerosis in the aortic valves after 20 weeks on a diet containing 1.25% cholesterol and 0.5% sodium cholate (91,92). Interestingly, macrophages in the aortic lesions of LDL-R(+/+)→LDL-R(-/-) mice stained positively for the LDLR by immunocytochemistry, indicating that LDL-R expression was not completely down regulated. Both of these studies used dietary conditions that induced extreme hypercholesterolemia and the extent of atherosclerosis was examined at a time point when lesions were advanced. Thus, an effect of leukocyte LDL-R expression on foam cell formation might have been obscured under these conditions of extreme hypercholesterolemia and advanced atherosclerosis.

Since LDL-R expression is known to be down-regulated in the presence of high levels of LDL cholesterol, we wanted to examine the contribution of the macrophage LDL-R to foam cell formation under conditions of more moderate hypercholesterolemia. On an atherogenic diet, C57BL/6 mice develop relatively modest hypercholesterolemia due to an accumulation of beta-VLDL, providing an attractive model for testing the hypothesis that the macrophage LDLR influences foam cell formation and atherogenesis in vivo. Therefore, female C57BL/6 mice were transplanted with either LDLR(-/-) marrow or LDLR(+/+) marrow and challenged with an atherogenic diet. As expected, the mice in both groups developed moderate hypercholesterolemia. Although serum cholesterol levels were not significantly different between the two groups at baseline or after 6 weeks on the butter fat diet, after 13 weeks the serum cholesterol levels were 40% higher in the experimental LDL-R(-/-)? C57BL/6 mice, due to accumulation of beta-VLDL. However, quantitative analysis of the extent of atherosclerosis in the proximal aorta revealed that these mice developed a 63% reduction in lesion area compared to the LDL-R(+/+) marrow recipients (38). Our results are compatible with a significant role for the macrophage LDLR in foam cell formation when the atherogenic stimulus is beta-VLDL.

We have recently expanded the approach of murine bone marrow transplantation for studying the role of macrophage gene expression in atherosclerosis by performing fetal liver cell transplantations to evaluate the role of macrophage lipoprotein lipase (LPL) in atherogenesis (20). Expression of lipoprotein lipase (LPL) by the macrophage has been proposed to promote foam cell formation and atherosclerosis, primarily on the basis of *in vitro* studies. Mice homozygous for disruption of the lipoprotein lipase die soon after birth (93,94). Since the fetal liver is the predominant organ of hematopoiesis during mammalian embryogenesis and macrophages are the only leukocytes that express LPL, transplantation of wild type C57BL/6 mice with LPL $^{-/-}$ fetal hematopoietic cells provided an approach for the development of mice with a *de facto* macrophage-specific knockout of LPL expression. These studies indicated that in the setting of an atherogenic diet, macrophage expression of LPL promotes foam cell formation and atherosclerosis *in vivo* (20,95).

Using a similar approach, it was possible to study the effect of the removal of acyl-cholesterol acyltransferase (ACAT) activity in macrophages. Mice lacking macrophage ACAT (96) were used as donors of fetal liver cells, which were then transplanted in irradiated LDL receptor null mice. Although the expectation would be that

the inhibition of cholesterol esterification in the vessel wall reduces foam cell formation, the results from this study clearly showed that excess free cholesterol induces macrophage necrosis and increases lesion area (97). Because of the vast amount of work on the development of ACAT inhibitors to affect cholesterol and atherosclerosis in humans, these data warrant the recommendation that complete blockade of this enzyme be avoided as a criteria for selection of new compounds. This study represents an example of how the mouse system can be used to address questions regarding the biology of the plaque, and to obtain answers that can be translated into clinical considerations.

11. CONCLUSIONS

After almost ten years of continuous progress in engineering the mouse genome to affect lipoprotein metabolism and modulate atherogenesis, the most practical discovery is that the mouse does indeed represent an appropriate model of human dyslipidemia and atherosclerosis. The dissection of several individual genes and cellular pathways has enhanced our understanding of the basic pathophysiology of lipoprotein abnormalities and of the early stages of plaque development. We have a firmer grip on the HDL system and how it relates to atherogenic influences to reduce lesion growth, and we finally realize that the cellular elements of the arterial plaque, most notably the macrophage, can influence the vessel wall directly, and not simply by reaction to changes in plasma lipid concentrations.

Although no animal model can provide direct insights into human atherosclerotic disease, the pace of the technological progress and the critical mass of experimental effort invested in murine systems is such that the benefit for man can easily be foreseen. The challenge for the next ten years will be to utilize the convenient mouse model to identify the best tools and best targets for the prevention and cure of lipid abnormalities and cardiovascular disease in patients.

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