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MINIREVIEW

Advances in Experimental Dyslipidemia and Atherosclerosis

Mohammed H. Moghadasian, Jiri J. Frohlich, and Bruce M. McManus

Healthy Heart Program, The iCAPTUR[4]E Centre/UBC McDonald Research Laboratories, and Department of Pathology and Laboratory Medicine, St. Paul's Hospital and the University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY: Among the models of dyslipidemia and atherosclerosis, a number of wild-type, naturally defective, and genetically modified animals (rabbits, mice, pigeons, dogs, pigs, and monkeys) have been characterized. In particular, their similarities to and differences from humans in respect to relevant biochemical, physiologic, and pathologic conditions have been evaluated. Features of atherosclerotic lesions and their specific relationship to plasma lipoprotein particles have been critically reviewed and summarized. All animal models studied have limitations: the most significant advantages and disadvantages of using a specific animal species are outlined here. New insights in lipid metabolism and genetic background with regard to variations in pathogenesis of dyslipidemia-associated atherogenesis have also been reviewed. Evidence suggests that among wild-type species, strains of White Carneau pigeons and Watanabe Heritable Hyperlipidemic and St. Thomas's Hospital rabbits are preferable to the cholesterol-fed wild-type animal species in dyslipidemia and atherosclerosis research. Evidence for the usefulness of both wild-type and transgenic animals in studying the involvement of inflammatory pathways and *Chlamydia pneumoniae* infection in pathogenesis of atherosclerosis has also been summarized. Transgenic mice and rabbits are excellent tools for studying specific gene-related disorders. However, despite these significant achievements in animal experimentation, there are no suitable animal models for several rare types of fatal dyslipidemia-associated disorders such as phytosterolemia and cerebrotendinous xanthomatosis. An excellent model of diabetic atherosclerosis is unavailable. The question of reversibility of atherosclerosis still remains unanswered. Further work is needed to overcome these deficiencies. (*Lab Invest 2001, 81:1173–1183*).

Atherosclerosis is a multifactorial disease; a number of genetic and environmental factors contribute to its development. Approximately 100 years ago Ignatowski (1908) reported experimental atherosclerosis in rabbits. Since then, a strong association between certain types of dyslipidemia (such as hypercholesterolemia, hypertriglyceridemia, combined hyperlipidemia, phytosterolemia, etc.) and development of atherosclerotic lesions has been documented by a number of clinical trials and epidemiologic and experimental studies. To better understand the relationship between disorders of lipid metabolism and atherogenesis, a number of animal models have been used. In this regard, until recently, dietary lipid manipulation and use of naturally defective animals, such as Watanabe heritable hyperlipidemia (WHHL) rabbits, have been the focus of most experimental settings. Nowadays, gene deletion technology has allowed researchers to produce a variety of transgenic animal models closely resembling particular human lipoprotein disorders.

In general, the ideal animal model for human dyslipidemias and atherosclerosis should fulfill several requirements. These requirements include low cost, ease of housing, size, speed of breeding, and a well-defined genetic background. Moreover, the animal models should share the pathophysiology of the disease with humans. Atherosclerotic plaque complications such as calcification, erosion, ulceration, hemorrhage, plaque rupture, thrombosis, and stenosis and the formation of aneurysms should be considered important features. Table 1 summarizes the advantages and disadvantages of most commonly used laboratory animal species in the field of experimental dyslipidemia and atherosclerosis.

It seems unlikely that any single animal model will meet all requirements. Thus, investigators have to choose the most appropriate model for testing a particular hypothesis. Despite many advances, there are still no suitable animal models for rare types of dyslipidemia associated with atherosclerosis, such as phytosterolemia and cerebrotendinous xanthomatosis. However, with advances in gene deletion/alter-

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Address reprint requests to: Dr. Mohammed H. Moghadasian, Healthy Heart Program and Department of Pathology and Laboratory Medicine, St. Paul's Hospital and the University of British Columbia, 180–1081 Burrard Street, Vancouver, BC, Canada V6Z 1Y6. E-mail: mhmoghad@interchange.ubc.ca

Table 1. Advantages and Disadvantages of Certain Animal Species in Human Atherosclerosis Investigation

Animals	Advantages	Disadvantages
Mice	Well-defined genetic; easy breeding; short generation time; inbred availability; easy handling and housing; availability of several transgenic lines	Highly resistant to atherogenesis; high HDL; no CETP; difficulties in frequent blood sampling and dissection of medium/small-size vessels
Rabbits	Naturally LDL-receptor deficient strain; naturally hypertriglyceridemic strain; good size; easy to keep and handle; known to many investigators; good response to dietary cholesterol; availability of transgenic lines	Lesion locations less similar to those in man; very high plasma cholesterol needed to induce atherosclerosis; hepatic lipase deficient; no spontaneous atherosclerosis; cholesterol storage syndrome on cholesterol feeding
Pigeons	Atherosclerosis susceptible strains; location, histology, and progress of lesions similar to those in humans; low cost and easy handling; sufficient size; good response to dietary cholesterol; short generation time; relatively long life span	Nonmammalian; lack of apo E, B_{48}, and chylomicron formation; viral infection seen associated with atherosclerosis; considerable changes in lipoprotein metabolism during egg-laying
Nonhuman primates	The closest species to humans; some strains respond well to dietary cholesterol; spontaneous early stage atherosclerosis in some strains	Variations in site of lesions; expensive and difficult to house and handle; limitation in availability; ethical concerns
Swine	Some physiological/anatomical similarities to humans; spontaneous atherosclerosis particularly in abdominal aorta; availability of miniature pigs; natural lipoprotein mutant strains	High cholesterol diet (4–5% w/w); less-known to investigators; very low baseline cholesterol level; difficulties in care and high maintenance cost
Dogs	Some physiological/anatomical similarities to humans; well-characterized lipoprotein profile	Atherosclerosis-resistant species; high HDL; expensive; poor response to dietary cholesterol; ethical concerns

LDL, low-density lipoprotein; HDL, high-density lipoprotein; CETP, cholesteryl ester transfer protein; apo, apolipoprotein.

ation technology, these models will be created. This technology will increase the variety of suitable animal models for studies of the pathogenesis of human diseases.

The aim of this review is to summarize the important characteristics of various laboratory animal models commonly used in the studies of disorders of lipid metabolism associated with atherosclerosis.

Rodents

Mice

Unlike humans and several other animals, mice do not possess plasma cholesteryl ester transfer protein (CETP) and, therefore, about 70% of the plasma total cholesterol is found in high-density lipoprotein (HDL) particles. This may be the major contributing factor to its resistance to atherogenesis. Poor response to dietary cholesterol supplementation may be an additional limitation in using mice in experimental atherogenesis.

Unlike their wild-type counterparts, transgenic mice provide an excellent opportunity to study the interactions of gene(s) and environment in atherogenesis. Mouse apolipoprotein (apo) E gene was the first successfully deleted gene (Zhang et al, 1992). Several other relevant transgenic murine models, such as low-density lipoprotein (LDL) receptor-knockout (KO) (Veniant et al, 1998), hepatic lipase-KO (Mezdour et al, 1997), human apo B_{100} expression (Purcell-Huynh et al, 1995), and human CETP expression (Marotti et al, 1993), have also been developed. Among them, apo E-KO, LDL receptor-KO, and human apo B_{100} transgenic mice have shown marked atherogenesis throughout their arterial tree. The atherosclerotic lesions have several characteristic features of human atheromas. Several stages in the development of atherosclerotic lesions in apo E-KO mice, as one of the most extensively used models in atherosclerosis studies, are illustrated in Figure 1.

In particular, apo E-KO mice develop severe hypercholesterolemia associated with advanced atherogenesis even on low-fat chow (Zhang et al, 1992). The degree of hypercholesterolemia and the rate of lesion development can be accelerated by a high-fat/high-cholesterol diet (Moghadasian et al, 1997, 1999b). This accelerated atherogenesis can be prevented by low doses of apo E with or without significant decreases in plasma cholesterol levels (Mitchell et al, 2000; Thorngate et al, 2000).

Severe hypercholesterolemia and atherogenesis in LDL receptor-KO mice (a model of human familial hypercholesterolemia) can be induced only by atherogenic diets (Veniant et al, 1998). Similar to LDL recep-

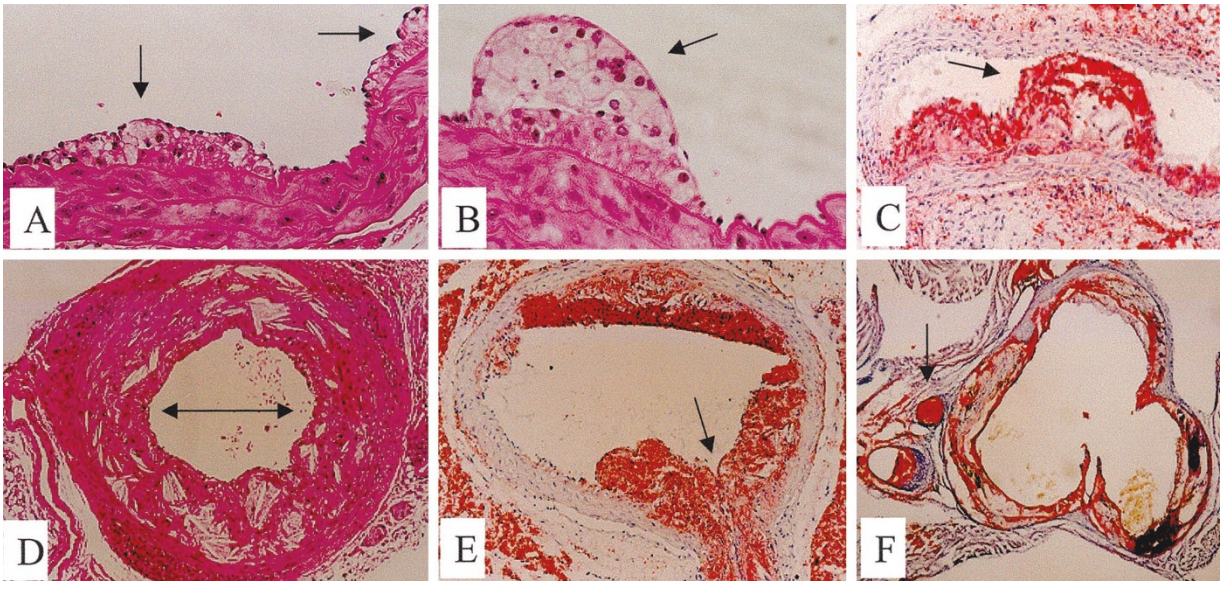

Figure 1.
Various stages in the development of atherosclerotic lesions in both the aortae and coronary arteries of apolipoprotein E-deficient mice: from fatty streaks to complete stenosis. *Arrows* show: A, fatty streaks, hematoxylin and eosin (H&E); B, foam cell accumulation, H&E; C and D, narrowing of the vessel, oil red O (ORO) and H&E, respectively; E and F, complete obstruction at branch point and stenosis, ORO. Original magnification, ×25.

tor–deficient mice, human apo B_{100} transgenic mice do not develop atherosclerotic lesions when fed with low-fat chow (Purcell-Huynh et al, 1995); however, a high-fat atherogenic diet results in hypercholesterolemia and severe atherogenesis in 18 weeks (Purcell-Huynh et al, 1995). These lesions progress over time; advanced lesions (similar to those seen in apo E-KO mice) with a necrotic core, abundant cholesterol clefts, extracellular fat, and fibrous cap develop over 6 months of feeding with atherogenic diets (Purcell-Huynh et al, 1995). Thus, if using mouse chow is preferable for the investigation of atherogenesis, apo E-KO mice are preferable to either human apo B_{100} transgenic or LDL receptor–deficient mice.

Apo E-Leiden transgenic mice also develop hypercholesterolemia and atherosclerosis on high-fat/high-cholesterol diets (Groot et al, 1996). It has been documented that high dietary fat/cholesterol consumption is positively associated with an increase in the severity of atherosclerotic lesions and with a craniocaudal progression of lesion development (Lutgens et al, 1999). In this animal model, enhanced DNA synthesis and apoptosis were found in early lesions and in advanced plaques, respectively. Replacement of mouse apo E gene with human apo E_2 gene resulted in human type III hyperlipoproteinemia with spontaneous atherogenesis (Huang et al, 1996).

Cross breeding of human apo B_{100} with LDL receptor–deficient mice produces a highly susceptible strain ($HuBTg^{+/+}Ldlr^{-/-}$) which develops severe hypercholesterolemia and atherosclerosis when fed low-fat chow (Sanan et al, 1998). Although hypercholesterolemia and atherosclerosis in $HuBTg^{+/+}Ldlr^{-/-}$ animals resemble those seen in apo E-KO mice, they differ from apo E-KO mice in that LDL and not β-VLDL (as in apo E-KO mice) contains most of their plasma cholesterol (Zhang et al, 1992). Because the metabolism of LDL is different from that of β-VLDL, there are likely two different pathophysiologic pathways for atherogenesis in those two lines of transgenic mice.

Foger et al (1999) observed an interesting phenomenon when they cross-bred human lecithin cholesterol acyl transferase (LCAT) transgenic mice with CETP transgenic mice. Unlike their parents, the offspring had lower total cholesterol with a reduced atherosclerosis burden. Further investigation showed that expression of CETP in LCAT mice normalized both plasma clearance of [^{3}H]-labeled cholesteryl ester from HDL particles and its uptake by hepatocytes. These observations led to the conclusion that the antiatherogenic property of CETP in LCAT mice is most likely due to restoring the functional properties of HDL particles and increasing their uptake by the liver (Foger et al, 1999). Acyl coenzyme A:cholesterol acyl transferase (ACAT) may play a role in pathogenesis of atherosclerosis by catalyzing the formation of cholesteryl esters in macrophages during the process of foam cell formation. However, LDL receptor-KO mice reconstituted with ACAT1-deficient macrophages unexpectedly developed severe atherosclerotic lesions rich in free cholesterol (Fazio et al, 2001).

Overexpression of apo C-III in mice is associated with hypertriglyceridemia and atherosclerosis, whereas overexpression of apo A-IV or apo A-I reduces atherosclerosis and increases HDL-C levels. A line of transgenic mice with apo E-KO background was recently created to study the effects of the combination of all three of the above-mentioned human apolipoproteins on atherosclerosis (Vergnes et al, 2000). Overexpression of apo A-I/C-III/A-IV gene cluster resulted in an appreciable reduction in atherosclerosis and increased plasma triglyceride levels. Thus, it can be suggested that whereas apo C-III overexpression increases plasma triglyceride levels, expression

of apo A-I and A-IV protects animals against atherosclerosis. Overexpression of human apo A-I in apo E-KO mice resulted in a significant reduction in macrophage accumulation in their aortic roots. This was associated with reduced β-VLDL oxidation, downregulated ICAM-1 and vascular cell adhesion molecule (VCAM)-1 expression, diminished ex vivo leukocyte adhesion, and reduced endothelial cytosolic Ca^{2+} signaling through PAF-like bioactivity (Theilmeier et al, 2000). Another recent study showed that overexpression of human apo A-II in apo E-KO mice results in a marked elevation (up to 24-fold) in plasma triglyceride levels accompanied by decreased HDL-C levels and increased atherosclerosis (Escola-Gil et al, 2000). Overall, this line of transgenic mice features several characteristics of human combined hyperlipidemia and therefore allows us to study how apo A-II may regulate VLDL metabolism.

Induction of hyperhomocystinemia by a diet low in folate and vitamins B_6 and B_{12} and high in methionine in apo E-KO mice resulted in enhanced atherogenesis accompanied by increased expression of VCAM-1, tissue factor, and matrix metalloproteinase-9. Dietary supplementation with folate and vitamins B_6 and B_{12} significantly suppressed homocysteine-mediated changes (Hofmann et al, 2001). As vitamin E reduces atherogenesis in apo E-KO mice (Pratico et al, 1998), vitamin E deficiency caused by disruption of the α-tocopherol transfer protein gene (Ttpa) increased atherosclerosis in these mice (Terasawa et al, 2000). On the other hand, antioxidant probucol significantly promotes atherogenesis in apo E-KO mice (Moghadasian et al, 1999b).

It should be noted that the animals' genetic background may play a significant role in their susceptibility to atherogenesis. A recent study has demonstrated that C57BL/6J (more atherosclerosis susceptible strain) apo E-KO mice are more prone to atherosclerosis than FVB/NJ apo E-KO mice (Dansky et al, 1999).

Rabbits

Rabbits share with humans several aspects of lipoprotein metabolism, such as similarities in composition of apolipoprotein B containing lipoproteins (Chapman, 1980), production of apo B_{100}-containing VLDL by the liver (Greeve et al, 1993), plasma CETP activity (Nagashima et al, 1988), and high absorption rate of dietary cholesterol (Yang et al, 1998). Unlike humans, rabbits are hepatic lipase–deficient and do not have an analog of human apo A-II (Chapman, 1980; Warren et al, 1991). WHHL and St. Thomas's Hospital (STH) rabbits are naturally defective; WHHL rabbits resemble human familial hypercholesterolemia (Aliev and Burnstock, 1998) and STH rabbits resemble human hypertriglyceridemia and combined hyperlipidemia (Beaty et al, 1992; Nordestgaard and Lewis, 1991). Atherogenic diets are usually associated with hypercholesterolemia and the development of atherosclerotic lesions in the aortic arch and thoracic aorta rather than in the abdominal aorta that is almost always affected in humans (Yang et al, 1998).

Transgenic rabbits are now available; human apo B_{100} transgenic rabbits with New Zealand White (NZW) background have increased levels (up to 2- to 3-fold) of both plasma total cholesterol and triglycerides and have low HDL cholesterol (Fan et al, 1995). Overexpression of human apo A-I or human LCAT in NZW or in WHHL rabbits was associated with an increase in HDL cholesterol and a decrease in the extent of atherosclerosis compared with controls (Duverger et al, 1996; Hoeg et al, 1993, 1996). To understand the interaction of LCAT with LDL receptors in atherogenesis processes, human LCAT gene was introduced into LDL-receptor deficient (WHHL) rabbits (Brousseau et al, 2000). The transgenic rabbits had a significantly elevated HDL-C with no significant change in LDL-C. Further metabolic studies showed that the fractional catabolic rate of apo B_{100} also was not affected by expression of human LCAT in WHHL rabbits. Thus, it was concluded that the LCAT antiatherogenic effect requires LDL receptor activity.

Other lines of either NZW or WHHL transgenic rabbits, such as lipoprotein (a) (Rouy et al, 1998), apo E_2 (Huang et al, 1997), apo E_3 (Fan et al, 1998), and hepatic lipase (Fan et al, 1994), are useful tools for understanding the pathogenesis of atherosclerosis and dyslipidemia. For example, male apo E_2 transgenic rabbits develop type III hyperlipoproteinemic phenotype associated with severe aortic atherogenesis; these changes are less severe in females (Huang et al, 1997). Overexpression of LCAT in transgenic rabbits attenuates the effects of atherogenic diets on both plasma lipid levels and atherogenesis (Hoeg et al, 1996). Similarly, protection against diet-induced hypercholesterolemia and atherosclerosis has been observed in human apo A-I transgenic rabbits (Duverger et al, 1996). On the other hand, expression of human apo (a) resulted in coronary atherosclerosis and deposition of human apo (a) in the vessel wall in cholesterol-fed rabbits (Fan et al, 2001).

Avian

Pigeons

Pigeons have relatively high levels of plasma cholesterol, most of it in HDL particles (St. Clair, 1983), thus to some extent they are naturally hypercholesterolemic. Consumption of a high-cholesterol diet results in even more augmented hypercholesterolemia. Certain strains of pigeons, such as White Carneau (WC), develop atherosclerosis while consuming a commonly used grain diet (Jerome and Lewis, 1985; St. Clair, 1983). On the other hand, Show Racer (SR) pigeons are resistant to atherogenesis even when fed a high-cholesterol diet (St. Clair, 1983). An atherogenic diet (0.5% cholesterol and 10% lard w/w) increased plasma total cholesterol levels up to 2,000 mg/dl (>6 times compared with controls) (Barakat and St. Clair, 1985). On a cholesterol-free diet about 70% of plasma cholesterol is carried by HDL, whereas in cholesterol-

fed pigeons, β-VLDL and LDL are the predominant lipoproteins (Barakat and St. Clair, 1985). Unlike humans, pigeons do not have apo E and apo B_{48} (Barakat and St. Clair, 1985). The atherosclerotic lesions in WC pigeons are usually found in the thoracic aorta and abdominal aorta, and brachiocephalic, iliac, carotid, renal, and coronary arteries. The lesions contain foam cells, cholesterol clefts, and an increased amount of extracellular matrix; advanced plaques may have foci of calcification, hemosiderin accumulation, and neovascularization and may eventually end up with ulceration, hemorrhage, thrombus formation, and myocardial infarction (Prichard et al, 1964). Moreover, several strains of pigeons with various morphologic features of atherosclerosis have been produced through a number of genetic studies (Wagner et al, 1973).

Sterol balance studies revealed that the WC breed excretes less natural sterols than the SR breed (Siekert et al, 1975); this may contribute to the differences in diet-induced hypercholesterolemia and atherosclerosis observed in these two strains. Intestinal bypass of the distal one-third of the bowel may cause regression of the early lesions in the CW breed as determined by a 50% reduction in lesion cholesteryl esters (Kottke et al, 1974). The bypass did not result in an increase in intestinal cholesterol or bile acid excretion (Flynn et al, 1976). It should be noted that unlike in humans, in pigeons bile acids are absorbed in the proximal intestine rather than the distal intestine (Spittle et al, 1976). In addition to lipid metabolism and lesion development, pigeons show similarity to humans in other features of atherosclerosis, including increased platelet adherence, thrombosis, and impaired endothelial and vascular smooth cell function (Lewis and Kottke, 1977; Randolph and St. Clair, 1984). Thus, it is reasonable to suggest that CW pigeons are one of the best models for studying human atherosclerosis.

Swine

Severe coronary and aortic atheroma can be induced in swine (Griggs et al, 1986; Holvoet et al, 1998; Ratcliffe and Luginbuhl, 1971; Stout, 1982). In general, left coronary arteries are more prone to atherogenesis than right coronary arteries.

Advanced atherosclerotic lesions are induced in coronary arteries of miniature pigs by high-cholesterol (4% w/w) diets. These lesions contain oxidized LDL; the level of the lesions' oxidized LDL is significantly correlated with intimal areas, but not with plasma LDL cholesterol (Holvoet et al, 1998).

A strain of pigs has been reported to have three lipoprotein-associated mutations (designated Lpb5, Lpr1, and Lpu1) with concurrent marked hypercholesterolemia and development of atherosclerosis on a low-fat, cholesterol-free diet (Prescott et al, 1991; Rapacz et al, 1986). Atherosclerotic lesions in coronary arteries of these mutant pigs have now been characterized: fatty streaks are observed by the age of 7 months and the lesions further advance with time. Advanced lesions in a 2-year-old animal were composed of extracellular lipids in the form of crystals, foam cells, necrotic core, moderate medial hyperplasia, and had a thin fibrous cap (Rapacz et al, 1986). These animals develop atherosclerotic lesions in several arteries including coronary, iliac, and femoral; advanced and complicated lesions similar to those seen in humans are found by two years of age (Prescott et al, 1991). It is of interest that these animals, unlike humans with familial hypercholesterolemia or WHHL rabbits, have normal LDL receptor activity (Rapacz et al, 1986).

Carnivores

Dogs

Spontaneous atherosclerosis is rare in dogs; dogs are even resistant to mild-hyperlipidemia induced by cholesterol-supplemented diets (Mahley et al, 1974). However, a high-fat/high-cholesterol diet deficient in essential fatty acids can make dogs severely hyperlipidemic and prone to atherogenesis. Several studies have used diets supplemented with 5% (w/w) cholesterol and 16% (w/w) hydrogenated coconut oil to produce canine atherogenesis (Butkus et al, 1976; McCullagh et al, 1976). Consumption of this diet deficient in essential fatty acids for a 1-year period was accompanied by an approximately 8-fold increase in plasma total cholesterol levels and development of severe atherosclerotic lesions in the abdominal aorta and coronary, celiac, and iliac arteries compared with controls (Butkus et al, 1976). Cholesteryl oleate was the major component of lipid fractions in both plasma and in the atherosclerotic plaques. Interestingly, replacement of 25% of hydrogenated coconut oil with safflower oil (high in linoleic acid) markedly reduced the extent of hyperlipidemia and atherogenesis (Butkus et al, 1976). This was associated with a shift in the predominant cholesteryl esters from cholesteryl oleate to cholesteryl linoleate in both plasma and arterial wall of the dogs.

Mahley et al (1974) characterized plasma lipoprotein particles and development of atherosclerotic lesions using high-fat/high-cholesterol diets in hypothyroid dogs. Under these conditions the dogs were characterized as either "hyperresponders" or "hyporesponders." Hyporesponders did not develop atherosclerosis, despite a more than 4-fold increase in their plasma total cholesterol. On the other hand, hyperresponder dogs developed advanced atherosclerotic lesions with a more than 12-fold increase in their plasma total cholesterol concentrations. Both groups of dogs had increased levels of cholesterol in lipoprotein fractions with a density of <1.006 to 1.040 g/ml compared with controls. However, lipoprotein fractions with a density of 1.040–1.080 g/ml increased in hyporesponders and decreased in hyperresponders. Hyperresponder dogs had a more than 90% reduction in their lipoproteins with density of 1.08–1.21 g/ml compared with controls; this decrease was only 30% in hyporesponder dogs.

Nonhuman Primates

Several nonhuman primates, such as squirrel monkeys, baboons, and wooly and spider monkeys, may develop spontaneous early stage (fatty streaks) atherosclerosis (Carey, 1978). Monkeys can be divided into hyperresponders and hyporesponders. This also applies to the severity and degree of atherosclerotic lesions (Bullock et al, 1975; Carey, 1978; Clarkson et al, 1971; Pronczuk et al, 1991). In addition, there is no consistency in the location of lesions in several strains of monkeys studied. For instance, male rhesus monkeys develop lesions at the bifurcation of the anterior descending and circumflex branches of the left coronary artery (Stary, 1976), whereas the carotid bifurcation and coronary arteries are the major sites of lesions in cebus monkeys (Bullock et al, 1969). The cynomolgus monkeys usually develop lesions in their coronary arteries but not in the aorta (Kramsch and Hollander, 1968), whereas the extent of lesions in abdominal aorta is much greater than in thoracic aorta in African green monkeys (Bullock et al, 1975). Three cases of LDL receptor deficiency in a rhesus monkey family associated with increased levels of LDL cholesterol, lipoprotein (a), and advanced atherosclerotic lesions in the aorta, and to a lesser extent in coronary arteries, were reported (Kusumi et al, 1993; Scanu et al, 1988).

The variability in lesion development, high cost, limited animal availability, and possible hazard and difficulties in handling them, together with ethical questions are major limitations in the use of these animals in studying lipid metabolism and atherosclerosis.

Experimental Evidence of Regression of Atherosclerotic Lesions

The question whether atherosclerosis is reversible is still one of the major research interests. One of the earliest successful regression studies was carried out by Horlick and Katz (1949). These investigators documented that cessation of cholesterol feeding led to a rapid fall in plasma cholesterol levels, along with definite regression and healing of induced atherosclerotic lesions in both thoracic and abdominal aortas of chicks. However, dietary means did not result in a significant regression in experimentally induced atherosclerotic lesions in rabbits (Vesselinovitch et al, 1974). Similarly, lovastatin treatment (10 mg/day or 20 mg/day) was not associated with regression of aortic atheromatous lesions in New Zealand male rabbits (Zhu et al, 1992).

Attempts to induce regression of diet-induced atheromas in rhesus monkeys also failed to show the efficacy of low-fat diets (Strong et al, 1994). Dietary intervention (either basal diet or basal diet supplemented with both dipyridamole [10 mg/kg] and aspirin [50 mg/kg] for 1 year) did not significantly reduce atherosclerotic narrowing induced by a high-fat diet in cynomolgus monkeys (Hollander et al, 1979). Whereas Clarkson et al (1981) found no evidence for reversibility of atherosclerosis in monkeys with plasma cholesterol levels of about 300 mg/dl, they reported that regression of coronary atherosclerosis in *Macaca mulatta* can be achieved over a period of 4 years, if their plasma cholesterol levels remain about 200 mg/dl (Clarkson et al, 1984). These observations suggest that regression of atherosclerosis in monkeys depends on the degree of their response to dietary cholesterol; hyporesponders showed evidence for regression, whereas hyperresponders did not (Clarkson et al, 1984). Dietary intervention induced regression of early atherosclerotic lesions, but not of advanced lesions, in the abdominal aorta of swine (Daoud et al, 1981). Similar to above observations, neither phytosterol treatment nor low-fat diets caused regression of advanced atherosclerotic lesions in the aortic roots of apo E-KO mice (Moghadasian et al, 1999a). However, liver-directed gene transfer of human apo E_3 induced regression of atherosclerotic lesions in apo E-KO mice. These changes were accompanied by reduced plasma cholesterol levels, accumulation of apo E, decreased foam cells, increased smooth muscle cells, and increased matrix content in the atherosclerotic lesions (Desurmont et al, 2000). Similarly, liver-directed human apo A-I gene transfer resulted in significantly reduced total lesion area, along with a significant increase in HDL cholesterol levels, in apo E-KO mice (Tangirala et al, 1999). Expression of human apo E_3 in cholesterol-fed LDL receptor-KO mice resulted in an increase in plasma apo E levels with no significant changes in plasma lipoprotein profile. These changes were associated with a significant regression in atherosclerotic lesions accompanied by decreased foam cells and slightly increased extracellular matrix components (Tangirala et al, 2001).

Experimental Evidence of Inflammatory Elements in Atherosclerosis

Interactions between oxidized LDL and inflammatory cells such lymphocyte-derived macrophages play a crucial role in the pathogenesis of atherosclerosis. In fact, the fatty streaks are composed of macrophages and T lymphocytes, typical inflammatory cells. Extracellular deposition of amorphous and membranous lipids accelerates the influx of these inflammatory cells, leading to further stages of atherosclerotic lesion development. Continuation of these inflammatory processes results in increasing numbers of these cells in the lesion and release of hydrolytic enzymes, cytokines, chemokines, and other inflammatory mediators causing focal necrosis. Moreover, the inflammatory mediators promote lipoprotein flux into the artery wall and thus further enhance the atherosclerosis processes. The entrapment of lipoproteins may initiate a vicious cycle of inflammation, modification of lipoproteins, and further inflammation in the artery wall. The concept of association of inflammatory pathways (as mentioned above) with atherosclerosis has been recently reviewed in detail by Ross (1999).

Apo E-KO mice have been used to study the role of certain inflammatory mediators in atherogenesis. For example, Nakashima et al (1998) reported that VCAM-1 is localized over the surface of groups of

endothelial cells in lesion-prone sites in apo E-KO mice; the expression of VCAM-1 was correlated with the extent of exposure to plasma cholesterol. In agreement with this report, Pasceri et al (2000) showed that troglitazone treatment was associated with a significant inhibition of VCAM-1 expression and reduction in monocyte/macrophage homing to atherosclerotic plaques in apo E-KO mice. Immunization of apo E-KO mice with either homologous plaque homogenates or homologous malondialdehyde-LDL was associated with a significant reduction in atherosclerotic lesion development, most likely through a T-cell–dependent antibody pathway. Elevated titers of IgG antibodies correlated with decreased lesion development (Zhou et al, 2001). Similarly, elevated IgG and IgM autoantibody titers to malondialdehyde-LDL and Cu-oxidized LDL in cholesterol-fed LDL receptor-KO mice significantly correlated with lesion oxidized LDL content, atherosclerotic surface area, and aortic weight (Tsimikas et al, 2001). Similarly, autoantibodies to oxidized phospholipids significantly correlated with the extent of aortic atherosclerosis in apo E-KO mice (Pratico et al, 2001).

Moreover, antioxidant treatments (probucol or vitamin E) was associated with a significant decrease in atherosclerosis and VCAM-1 mRNA in cholesterol-fed New Zealand rabbits (Fruebis et al, 1999). Similarly, treatment with antibody against mouse CD40L significantly reduced the size of atherosclerotic lesions by 59% and their lipid content by 79% in LDL receptor-KO mice fed a high-cholesterol diet (Mach et al, 1998). These changes were accompanied by a marked decrease in the number of macrophages (−64%) and T lymphocytes (−70%) with decreased expression of VCAM-1 in the atherosclerotic lesions. These data support the involvement of inflammatory pathways in pathogenesis of atherosclerosis in hyperlipidemic mice.

Repeated inoculation with *Chlamydia pneumoniae* resulted in inflammatory response in both C57BL/6J and apo E-KO mice (Campbell et al, 2000). Inflammation was associated with a statistically significant increase in atherosclerotic lesion size in apo E-KO mice; however, there was no evidence of atherosclerosis in control (C57BL/6J) mice (Campbell et al, 2000). Inoculation of apo E-KO mice with either murine Cytomegalovirus or *Chlamydia pneumoniae* or a combination of these two pathogens resulted in an increase in atherosclerotic lesion size by 84%, 70%, and 45%, respectively. Increased levels of circulating interferon-γ might play a role in higher atherogenicity activity of murine Cytomegalovirus (Burnett et al, 2001). Early treatment of *Chlamydia pneumoniae* infection with azithromycin prevented exacerbation of atherogenesis in apo E-KO mice, whereas late treatment did not affect the outcome (Rothstein, 2001).

Experimentally induced *Chlamydia pneumoniae* infection resulted in a significant increase in arterial intimal thickening in mildly hyperlipidemic rabbits; azithromycin treatment prevented this process (Muhlestein, 2000). Similarly, inoculation of New Zealand rabbits with bovine herpesvirus type-4 was associated with accelerated atherosclerosis (Lin et al, 2000).

Discussion

Before the gene technology era, dietary cholesterol supplementation was the focus of experimental atherosclerosis. Accumulation of cholesterol in other tissues due to dietary cholesterol supplementation has led to criticism of the use of cholesterol-fed animal models in atherosclerosis research (Stehbens, 1986).

During the past decade, gene technology has been used to create transgenic animals. This has remarkably increased our understanding of the interaction between genetic and environmental factors in the development, prevention, and treatment of many human disorders, including dyslipidemias and atherosclerosis. However, we still do not have models for disorders such as fatal atherosclerosis associated with phytosterolemia or cerebrotendinous xanthomatosis.

Of wild-type animals, various strains of WHHL and STH rabbits and WC pigeons have features similar to human atherosclerosis. These animals are relatively inexpensive and can be easily obtained and handled. Thus they may continue to serve us in studying the pathogenesis of the disease and its dietary and/or drug management.

Transgenic rabbits and mice are an excellent addition to wild-type laboratory animal models. They have enabled us to research a particular aspect of genetic factor contributing to the pathogenesis of human disease. For example, lipoprotein transfer gene expression revealed the importance of regions controlling tissue-specific expression of various apoproteins such as apo A, apo B, apo E, and apo C. Overexpression of human proteins in animals lacking that protein is another important accomplishment; overexpression of CETP in mice and human hepatic lipase in rabbits are excellent examples.

Recently, considerable attention has been paid to the association of inflammatory responses to *Chlamydia pneumoniae* infection with atherosclerosis. In this regard, both wild-type and transgenic hyperlipidemic animals have been used. Although evidence for a role for *Chlamydia pneumoniae* seems to be quite strong, more data are required to understand to what extent it plays a causal role in the development of human atherosclerotic lesions.

We now have an understanding of the process of induction of atherosclerotic lesions in experimental animals, but our understanding of the process of regression of atherosclerotic lesions is still incomplete. The focus of most of the research on regression of atherosclerosis has been on the evaluation of plaque features such as size, lipid content, and cellular and extracellular components. Each of these components may have a distinct response to dietary or other regression interventions. One of the major limitation of regression of lesions induced by a high-cholesterol diet is probably the massive accumulation of cholesterol in various tissues. This may not allow us to observe regression of the lesions by mild dietary

approaches over a relatively short period. On the other hand, the rapid fall in plasma total or the increase in HDL cholesterol levels in KO mice by gene transfer technology results in rapid regression of atherosclerotic lesions.

All of the animal models have significant limitations. Data banks and new technologies will help in choosing the best available model. Despite the many achievements over the past decade, we still do not completely understand the mechanisms of relationship between certain dyslipidemias and atherosclerosis and how to regress the established atherosclerotic lesions. Further investigation is needed either to develop more transgenic animals or to discover a particular animal model for studying a particular aspect of the disease. Moreover, we need better-defined laboratory models to develop safer, more effective, and less invasive therapeutic approaches for the disease.

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