ANIMAL MODELS OF ATHEROSCLEROSIS

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I. ABSTRACT

Cardiovascular disease is currently the predominant cause of mortality worldwide and its incidence is expected to increase significantly during the next decades owing to the unhealthy effects of modern life-style habits (e.g., obesity and lack of physical exercise). Cardiovascular death is frequently associated with acute myocardial infarction or stroke, which are generally the ultimate consequence of an underlying atherosclerotic process. Small and big animal models are valuable tools to understand the molecular mechanisms underlying atherosclerotic plaque formation and progression, as well as the occurrence of associated ischemic events. Moreover, animal models of atherosclerosis are pivotal for testing mechanistic hypothesis and for translational research, including the assessment of dietary and/or pharmacological interventions and the development of imaging technologies and interventional devices. In this chapter, we will describe the most widely-used animal models that have permitted major advances in atherosclerosis research and significant improvements in the treatment and diagnosis of atherosclerotic disease.

KEYWORDS

Cardiovascular disease, atherosclerosis, animal models, rabbit, swine, mouse, rat, primate

II. ATHEROSCLEROSIS DEVELOPMENT: BASIC CONCEPTS

Cardiovascular disease (CVD) is currently the predominant cause of mortality worldwide with more than 17 million annual deaths, and global cardiovascular deaths are predicted to increase to more than 23 million by 2030 (1). In most cases, cardiovascular death is provoked by acute myocardial infarction or stroke, which are generally the ultimate consequence of an underlying atherothrombotic process. Atherosclerosis is a complex inflammatory disease characterized by the progressive hardening and thickening of the vessel wall owing to the development of complex lesions that narrow the arterial lumen (atherosclerotic plaque, atheroma or neointimal lesion) **(Figure 1).** Atherosclerotic plaque formation is triggered and sustained by the dysfunction of the endothelium associated with the chronic exposure to cardiovascular risk factors (e. g., hyperlipidemia, hypertension, diabetes, smoking, etc). A major cardiovascular risk factor is the existence in the blood of high levels of low density lipoproteins (LDL) that accumulate in the subendothelial space of the arterial wall and undergo oxidative modifications that trigger an inflammatory response (2, 3). Locally produced oxidized LDL (oxLDL) induce the expression in the endothelial cells of chemotactic proteins, such as monocyte chemoattractant protein-1 (MCP-1) (4, 5), and adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), E-selectin and P-selectin (6, 7). This leads to the recruitment of blood-borne monocytes in the injured arterial wall, which subsequently differentiate into macrophages of different subtypes (macrophage polarization) (8). Neointimal macrophages avidly internalize oxLDL and become foam cells that critically contribute to plaque development by secreting a plethora of mediators that perpetuate the inflammatory process in the vessel wall (9). This maladaptive, non-resolving inflammatory response is the driving force of atherosclerotic plaque development. It further promotes the recruitment of circulating monocytes and T-cells that boost inflammation in the arterial wall, and also stimulates the migration of vascular smooth muscle cells (VSMCs) from the tunica media into the subendothelial space, where they exhibit abnormally high proliferation and secrete extracellular matrix proteins that also contribute to atheroma growth (10-13). Moreover, the excessive accumulation of oxidized lipids leads to

endoplasmic-reticulum stress-associated apoptosis of macrophages, generating apoptotic bodies that cannot be properly disposed and therefore enhance plaque inflammation and instability (14). At advanced stages of the disease, rupture of high-risk vulnerable plaques exposes their thrombogenic compounds to the circulating blood, leading to luminal thrombosis and eventually to life-threatening acute ischemic events (e.g. myocardial infarction, stroke) (15).

III. ANIMAL MODELS OF ATHEROSCLEROSIS

The first evidence that atherosclerosis can be induced in laboratory animals was provided in 1908 by Ignatowski, who demonstrated the formation of lesions in the aortic wall of rabbits that were fed a diet enriched in animal proteins (mainly from meat, milk and egg yolks) (16). Since then, various animal species have been used as experimental models of atherosclerosis, including rabbits, mice, rats, guinea pigs, hamsters, birds, swines, dogs and non-human primates. A thorough examination of these animal models —especially rodents, swines and rabbits— has greatly increased our understanding of the pathophysiological mechanisms that lead to the formation and development of the atherosclerotic plaque. In spite of the many differences existing between these models, all of them share the requirement of high levels of blood plasma cholesterol for atherosclerotic plaque to develop. The initial observations of this remarkable characteristic of every animal model of atherosclerosis were crucial for the discovery of the important role of cholesterol in atherosclerosis development. The next sections discuss the main advantages and disadvantages of the most commonly utilized animal models of atherosclerosis, which are summarized in **Tables 1-4**. Other available animal models of atherosclerosis that are used less frequently are discussed elsewhere (17, 18).

IIIA. Rabbit models of atherosclerosis

The rabbit was the first animal model used in atherosclerosis research (16) and was pivotal in the initial experiments that led to the discovery of the role of elevated plasma cholesterol as a critical factor in the initiation of atherosclerosis (19, 20). Although currently less used than mice, the rabbit is still one of the most frequently employed animal models for atherosclerosis research due to its easy handling as well as its inexpensive maintenance and high availability.

The most commonly rabbit strain that is used in atherosclerosis research is the New Zealand White (NZW), which is not inherently prone to atherosclerosis due to its low levels of plasma cholesterol when maintained under standard chow (around 50 mg/dl) (20). Induction of vascular lesions in NZW rabbits generally requires feeding a high-cholesterol diet (ranging from 0.2 to 2% cholesterol), which rapidly increases plasma cholesterol levels by up to 8 times (21) and leads to the formation of foam cells-enriched fatty streaks in several vascular regions (especially the aortic arch and the thoracic aorta) (22). However, some studies have raised doubts on the mechanisms underlying the formation of atherosclerotic plaques in this herbivorous animal model, especially because of the highly abnormal diet that is required to induce vascular lesions. Indeed, other dietary manipulations, such as the substitution of casein for soy protein in the diet, are atherogenic in the rabbit in the absence of a high cholesterol diet (23). In addition, the development of advanced and complex atherosclerotic plaques containing a lipid core surrounded by VSMCs generally requires long periods of cholesterol-feeding in NZW rabbits (from 6 months to several years) (24-27). However, long-term fat-feeding of rabbits is discouraging, because it is frequently accompanied by noxious side-effects and increased mortality owing to hepatic toxicity. Moreover, it induces a massive inflammatory response that does not resemble the chronic low-grade inflammatory response associated to human atherosclerosis. Some studies support the notion that the formation of advanced lesions in the rabbit depends on the age of the animals that are challenged with a cholesterol-rich diet. Old rabbits (3-4.5 years old) frequently develop fibrous plaques after being fed a high cholesterol

diet, while young rabbits (4-month-old) do not exhibit such advanced lesions after the same period of cholesterol feeding (28). However, it must be noted that rabbits show high biological variability with respect to individual responsiveness to dietary cholesterol, and that lesion morphology varies significantly depending on the cholesterol content of the diet. Short-term feeding with diets containing high amounts of cholesterol (1-2 %) mostly induces macrophage-rich fatty streaks, while long-term diets with lower amounts of cholesterol (0.2-0.75%) generate more complex lesions, with VSMC infiltration and cholesterol deposits (20, 24-27, 29). In addition, it has been shown that complex atherosclerotic lesions can be induced in NZW rabbits by intermittent cycles of fat feeding with periods of normal diet (27, 30). A widely-used alternative method to accelerate the development of advanced lesions in rabbits combines a high-fat/high-cholesterol diet with angioplasty-induced aortic denudation of the aorta, generally from the aortic arch to the iliac arteries (31-38). This combined protocol greatly accelerates the formation of atherosclerotic lesions and, importantly, produces plaques that exhibit a lipid core surrounded by a fibrous cap due to increased proliferation of VSMCs, thus resembling more closely human advanced plaques than those produced by feeding rabbits with a high-cholesterol diet alone.

Some rabbit strains carry genetic mutations that lead to hyperlipidemia and atherosclerosis. This is the case of Watanabe, St Thomas, and Houston RT strains, which present genetic abnormalities in lipid metabolism (39-42). The most widely-used hyperlipidemic rabbit strain in atherosclerosis research is the Watanabe heritable hyperlipidemic (WHHL) rabbit (39), which has been particularly important for the development of several cholesterol lowering antiatherosclerotic drugs (43). WHHL rabbits carry an inactivating mutation in the gene encoding the LDL receptor (LDLR), a major mediator of the hepatic clearance of circulating lipoproteins (44, 45). Consistently, WHHL rabbits spontaneously exhibit hypercholesterolemia, with increased plasma levels of atherogenic very low density lipoproteins (VLDL) and reduced levels of atheroprotective high density lipoproteins (HDL), together with visceral fat accumulation and hyperinsulinemia. Furthermore, WHHL rabbits develop aortic atherosclerotic

plaques, ranging from fatty streaks to more advanced, fibrous lesions (46), and selective breeding has allowed the generation of WHHL rabbits that exhibit severe coronary atherosclerosis (47) and develop spontaneous myocardial infarctions (48).

The generation of transgenic rabbits with altered expression of specific genes involved in cholesterol and lipoprotein metabolism has provided notable insight into the important role of cholesterol trafficking in atherosclerosis development. For instance, NZW rabbits with liver-specific expression of human apolipoprotein A-I (the major component of plasma HDL) exhibit significantly smaller atherosclerotic lesions than non-transgenic controls (49, 50). Similarly, transgenic rabbits that overexpress human lecithin-cholesterol acyl transferase, an enzyme that catalyzes the conversion of free cholesterol into cholesteryl esters, have markedly reduced atherosclerosis compared to control rabbits when fed a high cholesterol diet (51). WHHL rabbits have also been subjected to genetic manipulation. For example, WHHL rabbits that express human apolipoprotein(a), which assembles into atherogenic lipoprotein(a) particles in plasma, exhibit accelerated atherosclerosis development and more complex lesions than non-transgenic controls (52). Likewise, human lipoprotein lipase overexpression in this rabbit strain leads to enhanced aortic atherosclerosis (53).

IIIB. Swine models of atherosclerosis

The development, morphology, and function of the cardiovascular system in swine as well as their lipoprotein profiles and metabolism closely resemble that of humans. Gottlieb and Lalich reported the first large quantitative data upon the spontaneous occurrence of atherosclerosis in the porcine aorta by analyzing more than 2,000 specimens obtained at slaughterhouses (54). Several strains of large farm pigs that bear mutant alleles for the LDLR or apolipoprotein B loci have been available for more than two decades (55-58). These strains spontaneously develop humanoid atherosclerosis in most arterial beds, including the coronary arteries, and disease

progresses in time periods inversely proportional to cholesterol levels. Like in humans, early lesions in swine models of familial hypercholesterolemia consist of fatty streaks rich in lipid-laden macrophages, which progress to complicated lesions with calcification and features of vulnerable plaques, including abundant neovascularization, necrotic cores, a thin fibrous-cap, and intraplaque hemorrhage. Although these characteristics make familial hypercholesterolemic pigs a suitable preclinical model for testing new anti-atherogenic and anti-thrombotic drugs and for developing imaging technologies and interventional devices (59), their use is limited due to the long periods of time that are required to develop complex atherosclerotic lesions even when challenged with atherogenic diets (2-3 years) and the large size and weight they reach (>200 kg). These difficulties in care and high maintenance cost are reduced with the use of smaller swine strains, such as the Yucatan miniature pig and several sublines that have been derived from the primary population, which also develop humanoid complicated lesions with abundant necrosis and cholesterol deposits and extensive calcification (60-65). Very recently, Thim et al. (66) reported the generation of a downsized hypercholesterolemic pig strain named FBM that was produced by crossing the Rapacz familial hypercholesterolemic pig bearing the R84C LDLR mutation with a smaller pig (Chinese Meishan) and then crossing the offspring with an even smaller minipig from Brentocelles, France. FBM pigs breed like normal pigs, develop atherosclerotic lesions on standard diet and disease progression is aggravated by atherogenic diet feeding, whereby plasma total cholesterol rose to >20 mmol/l (>800 mg/dl) and plaques mirrored human-like features, including a large necrotic core covered by a thin and inflamed fibrous cap, neovascularization, intraplaque hemorrhage, and expansive remodeling (66).

Like in other animal models, atherosclerotic plaque development in swine can be accelerated by combining high-cholesterol diet with locally-produced vascular injury inflicted by different means, including guide-wire-induced injury (67), endovascular balloon inflation with or without stent deployment (59, 62, 66), partial vessel ligation (68), and balloon angioplasty followed two weeks later by percutaneous intramural injection of a mixture of cholesteryl esters and human oxLDL (69, 70). Not only atherogenic diets plus vascular injury protocols reduce the

difficulties in care and high maintenance cost associated with the use of swine models by reducing the duration of the study, but are also highly relevant models for translational research in the field of percutaneous interventions and cardiovascular imaging.

Diabetes and hypercholesterolemia frequently co-exist in patients with metabolic syndrome, who are at significantly higher risk for atherosclerosis and its complications than the general population. The incidence of diabetes, particularly type 2-diabetes, is expected to increase significantly during the next decades owing to the unhealthy effects of modern life-style habits (e.g., obesity and lack of physical exercise). However, studies examining mechanisms underlying diabetes-accelerated atherosclerosis are scant in part due to the lack of suitable humanoid pre-clinical models. The combination of hypercholesterolemia and diabetes (induced by intravenous administration of streptozotocin, which destroys over 80% of pancreatic beta cells) accelerates atherosclerotic lesions in the aorta and coronary and femoral arteries of Yorkshire pigs (71, 72). In addition to reducing the duration of experiments, the combination of diabetes and hypercholesterolemia in the pig makes animal handling easier (diabetic pigs gain weight slower than non-diabetic controls) and leads to the formation of advanced human-like atherosclerotic lesions. However, treatment of complications associated with diabetes (eg, hypoglycemia, hyperglycemia, gastroparesis, and infections) is required when working with swine models of diabetes-induced atherosclerosis. The combination of diabetes and hypercholesterolemia has proven relevant in several settings, including the demonstration that selective inhibition of lipoprotein-associated phospholipase A(2) (Lp-PLA(2)) with darapladib substantially reduces inflammation and the development of advanced coronary atherosclerosis (73).

IIIC. Non-human primate models of atherosclerosis

Non-human primates are attractive for atherosclerosis research because their general anatomical resemblance and their phylogenetic proximity to humans, thus making likely that

data derived from these animals are more directly applicable to the clinical scenario. However, New World monkeys have been of limited value in atherosclerosis research primarily because of the complications that are associated with their chronic renal disease, which affects both their lipoprotein metabolism and atherosclerotic plaque development (74-76). In contrast, extensive research has been conducted in Rhesus monkeys, which have been shown to develop spontaneous atherosclerosis in several vessels, including the coronary arteries. Moreover, feeding a high cholesterol diet greatly accelerates the development of atherosclerosis and frequently induces fatal myocardial infarction in these primates (77, 78). Atherosclerosis regression upon low-fat feeding has also been demonstrated in this model (79, 80). Similarly, cynomolgus and Cebus monkeys develop atherosclerosis and have been used as experimental models of this disease (81-83). Like in humans, age has a striking effect on the susceptibility to atherosclerosis in non-human primates; young animals are reasonably resistant to the development of atherosclerotic lesions, but become much more susceptible as they age (83, 84). In addition, the same gender differences that exist in human atherosclerosis are evident in non-human primates, with males being more affected than females (84). Unfortunately, in spite of these striking similarities between human and primate atherosclerosis, the difficult handling of these animals, their high economical cost, and the important ethical concerns strongly limit their utility in research.

IIID. Rodent models of atherosclerosis

Pioneer studies of experimental atherosclerosis have been mainly performed in rabbits, pigs and non-human primates. Although research with these species certainly provided valuable insight into the pathophysiology of atherosclerosis, it also encountered a number of important obstacles that limited their value as experimental models, as discussed above. In contrast, rodents, in particular mice, have many advantages for experimental studies and are currently the most frequently used laboratory animals in atherosclerosis research.

Mice. Due to their small size, mice can be handled and maintained easily. Moreover, mice can live up to 3 years, and exhibit a quick generation time (3 weeks for gestation and 6-8 weeks to reach sexual maturity), allowing the breeding of large cohorts in a short time frame. Moreover, the mouse is the most commonly used mammal for genetic manipulation and a vast diversity of transgenic and knock-out mice is readily available to conduct atherosclerosis research. However, the use of mice as an experimental model of atherosclerosis faces one major obstacle: this species is extremely resistant to atherosclerosis. The reasons for this may be various. Cholesterol metabolism is very different in mice and humans, and plasma cholesterol and lipoprotein patterns are particularly dissimilar in both species. Plasma cholesterol levels are generally low in mice fed standard chow, ranging from 60 to 100 mg/dl depending on the background strain. Moreover, while the major circulating lipoprotein in humans is pro-atherogenic LDL, plasma LDL levels in wild-type mice are extremely low and more than 85% of cholesterol is carried on atheroprotective HDL (85). This remarkable difference is probably mostly due to the absence in mice of plasma cholesterol ester transfer protein (CETP), an enzyme that catalyzes the transfer of cholesteryl esters and triglycerides between different lipoproteins (86). In addition, mice and men have several differences in cardiovascular physiology and anatomy. The human heart rate is normally 60 to 90 beats per minute, whilst in the mouse is around 300 beats per minute. Furthermore, the postural differences between mice and men affect hemodynamics, an important determinant of atherosclerosis susceptibility (87-89). In spite of the natural resistance of mice to atherosclerosis, the combination of dietary challenges and genetic manipulation has allowed the generation of several murine models of atherosclerosis, which are discussed below.

The first mouse model of atherosclerosis was initially characterized during 1960s by Wissler and coworkers, who used an experimental diet that contained very high amounts of fat (30%) and cholesterol (5%), and 2% cholic acid, which promotes hypercholesterolemia by blocking

cholesterol conversion to bile acids (90). Although this extreme diet induced the formation of fatty streaks in different vascular regions of the mouse, it was also pro-inflammatory and highly toxic, leading frequently to weight loss and high susceptibility to infections (90). Using a less toxic diet that contained 15% fat, 1.25% cholesterol and 0.5% cholic acid, Paigen and collaborators found high variability in diet-induced atherosclerosis when comparing 10 different inbred strains of mice (91). The most susceptible strain was C57BL/6, which developed mild hypercholesterolemia — around 200 mg/ml — and fatty streak lesions in the aortic root after 3 to 9 months of fat-feeding, whereas other resistant inbred strains, such as the C3H, did not exhibit atherosclerotic lesions under the same dietary regimen (91). Of note, lesions in the aorta of fat-fed C57BL/6 mice were small, consisted almost exclusively of macrophages and did not progress to fibrous plaques, unlike the situation in humans. This shortcoming, together with the toxicity and the pro-inflammatory actions of cholic acid, challenged the utility of wild-type mice for atherosclerosis research. Nevertheless, wild-type mice have proven useful to identify several genes involved in the etiopathogenesis of atherosclerosis (92-94).

A major breakthrough in atherosclerosis research occurred in 1992 with the generation of mouse strains with genetic disruption of apolipoprotein E (apoE) (95, 96). apoE is a structural component of all lipoproteins other than LDL and is a critical ligand for the hepatic clearance of plasma lipoproteins mediated by LDLR and LDLR-related proteins (97-99). Consistently, even on standard low-fat chow, apoE-deficient mice exhibit marked hypercholesterolemia (around 400 mg/dl, which represents a ~5-fold increase in plasma cholesterol levels compared to wild-type mice), and a dramatic shift in the plasma lipoprotein profile, with pro-atherogenic VLDL as the most abundant circulating particles, similar to the situation in human type III hyperlipidemia (100). In addition, apoE-deficient mice spontaneously develop atherosclerotic plaques in several vascular beds, predominantly in the aortic root, the aortic arch and the different branch points along the aorta. Atherosclerotic lesions in apoE-null mice are heterogeneous, ranging from fatty streaks rich in foam cells to complex lesions that exhibit macrophage-enriched shoulders and necrotic cores with a well-formed fibrous cap that includes VSMCs and extracellular matrix

(**Fig.1**). Moreover, atherosclerosis is greatly accelerated in apoE-deficient mice challenged with high-cholesterol high-fat diets that are not supplemented with cholic acid (101, 102). The most frequently-used atherogenic diet in mice is the Western-type diet, which contains 0.15% cholesterol and 21% fat derived from milk fat. When fed this diet, apoE-deficient mice exhibit over a three-fold elevation in plasma cholesterol levels compared to animals fed regular chow, and develop complex fibrous plaques in the aortic sinus after 10-14 weeks of fat-feeding. This rapidity of lesion progression is an important advantage in the experimental setting in comparison with other animal models of atherosclerosis. Although apoE-deficient mice have turned into the gold standard for experimental atherosclerosis studies, this model has also some important limitations. A major drawback is that most plasma cholesterol in apoE-deficient mice is mostly confined to VLDL particles and not to LDL particles, as it occurs in humans. Furthermore, there is increasing evidence that apoE has extra atheroprotective properties in addition to mediating lipoprotein clearance, including antioxidant, antiproliferative and anti-inflammatory actions. Therefore, because atherosclerosis is an inflammatory disease, the potential immunomodulatory actions of apoE must be considered when analyzing data obtained in this animal model.

In addition to apoE-deficient mice, other genetically-manipulated mouse strains have been generated that are prone to atherosclerosis and show particular advantages in certain experimental settings. A widely-used model is the LDLR-deficient mouse, which has a milder lipoprotein alteration than apoE-deficient mice when fed standard low-fat chow, with plasma cholesterol levels around 250 mg/dl due mainly to the accumulation of LDL (103, 104). Although LDLR-deficient mice do not develop significant atherosclerosis on a normal chow diet, high-fat feeding leads to severe hypercholesterolemia (~900 mg/dl) with accumulation of VLDL and LDL and extensive atherosclerosis (104). A major disadvantage of this murine model is that the development of human-like complex fibrous lesions typically requires longer periods of fat-feeding than in apoE-deficient mice. However, LDLR-deficient mice are advantageous compared to apoE-null mice when performing bone marrow transplantation studies. apoE is

locally produced by macrophages in the atheroma (105), therefore bone marrow transplantation studies with apoE-deficient mice require that the donor also carries apoE deficiency to avoid the atheroprotective action of apoE secreted by bone-marrow derived macrophages. In contrast, LDLR expression in hematopoietic cells is minimal and irrelevant in the process of atherogenesis, and this allows the utilization of wild-type mice as bone marrow donors in experiments using LDLR-null mice as recipients. Another important difference is that LDLR-deficient mice are more prone to the development of obesity and insulin resistance than apoE-deficient mice (106), a particularly relevant issue when analyzing the role of genes involved in metabolic control and atherosclerosis development. In contrast, apoE-deficient mice are more susceptible to injury-induced neointimal formation (107, 108), and therefore may represent a better experimental model for the investigation of the molecular mechanisms underlying restenosis post-angioplasty. Although the pros and cons of each model are frequently debated, results with apoE-deficient and LDLR-deficient mice are generally comparable. Another genetically-modified mouse that has been used as an experimental model of atherosclerosis — although less frequently than apoE-null and LDLR-null — is the apoE*3Leiden (apoE3L) transgenic mouse, which carries a mutated form of the human *APOE3* gene. Although apoE3L mice express the endogenous murine wild-type apoE, forced expression of the human *APOE3* mutant gene highly impairs the hepatic clearance of lipoproteins and this leads to hypercholesterolemia and atherosclerosis upon cholesterol feeding (109).

Even though genetically-engineered mice are currently the most widely used animal models of atherosclerosis, they have been classically considered to lack the most pathologically-relevant feature of human atherosclerosis, i.e., plaque rupture, which is the leading cause of life-threatening acute ischemic events (eg, myocardial infarction and stroke). However, while this appears to be generally true in the case of atherosclerotic plaques in the aortic sinus — the arterial region most typically analyzed in murine atherosclerosis studies — it might not be the case in other vascular regions in the mouse, because a number of studies have reported that plaque rupture spontaneously occurs in the brachiocephalic artery (also known as the

innominate artery) of apoE-deficient mice (110, 111). However, this conclusion often relies exclusively on some particular histopathological markers of plaque rupture or on a definition of plaque rupture that differs from the concept in humans in the sense that it does not include neither luminal thrombosis nor intraplaque hemorrhage (112). Certainly, high controversy still exists about the definition and occurrence of spontaneous plaque rupture in murine models of atherosclerosis and further studies are required to clarify this important aspect. However, a number of models of artificially-induced atherosclerotic plaque rupture and/or atherothrombosis in hypercholesterolemic mice have been proposed, and some of them have provided important insight into the molecular mechanisms that determine plaque vulnerability. One such approach is to mechanically provoke rupture of the atherosclerotic plaque. For example, platelet- and fibrin-rich thrombi can be induced within atheromata of apoE-deficient mice by compressing the aorta between forceps (113). Similarly, carotid artery ligation followed by the application of a polyethylene cuff has been shown to induce intraplaque hemorrhage and luminal thrombus formation in apoE-deficient mice (114). Photochemical injury has also been used to induce atherothrombosis in this murine model (115). In addition, a number of genetic manipulations have been shown to promote plaque instability and rupture, including macrophage-specific expression of active metalloprotease-9 (MMP-9), which induces fibrin deposition and intraplaque hemorrhage in apoE-deficient mice (116), as well as adenovirus-mediated overexpression of p53 in combination with treatment with the vasopressor compound phenylephrine (117).

Rats. The rat has been pivotal for physiological and metabolic research since the development of the first defined rat strain at the Wistar Institute in the 1920s (118). Like wild-type mice, rats lack plasma CETP and carry most plasma cholesterol in HDL particles. Consistently, the rat is highly resistant to atherosclerosis, with the exception of some particular strains that develop arterial fatty lesions that however do not resemble human atherosclerotic plaques (119-121). In

addition, rats are poorly responsive to fat-feeding, and hyperlipidemia and atherosclerosis can only be induced using diets that contain an extremely high content of cholesterol and fat, together with cholic acid and thiouracil, which induces hypotiroidism and thereby lowers hepatic clearance of lipoproteins (122, 123). Therefore, wild-type rats are not generally considered a suitable model of experimental atherosclerosis. However, some rat strains that carry mutations which cause hyperlipidemia have been reported to develop atherosclerotic plaques in different vascular beds (121, 124-126). The JCR:LA-corpulent strain is of particular interest because it develops extensive atherosclerosis and ischemic lesions of the heart, as well as insulin resistance and other metabolic dysfunctions (127-133). In addition, the recent development of molecular techniques for the manipulation of the rat genome (134) is expected to hasten the generation of transgenic and knock-out rats of interest for atherosclerosis research. Of note in this regard, rats that are genetically-engineered to overexpress human CETP exhibit dyslipidemia and coronary artery lesions with a strong male-to-female bias, like in humans (135, 136). In spite of this, the mouse remains the most widely-used rodent model of atherosclerosis, mostly because of the easier breeding and genetic manipulation of this species.

Other rodents. Hamsters and guinea pigs have been used as experimental models of atherosclerosis. Unlike mice and rats, hamsters express plasma CETP and carry a significant fraction of plasma cholesterol in LDL particles and thus are metabolically closer to humans than other rodents (137, 138). Several studies have demonstrated the development of hypercholesterolemia and atherosclerotic plaques in the aorta of Golden Syrian hamsters, ranging from fatty streaks to more complex lesions (139-141). However, more recent work has not consistently replicated the extension and/or morphology of atherosclerotic lesions in hamsters (142), and therefore this model is not frequently used in atherosclerosis research. Guinea pigs also express CETP and transport the majority of circulating cholesterol in LDL particles (137), and high cholesterol diets induce the development of early atherosclerotic

plaques in the arterial wall of these animals (143). However, advanced atherosclerotic lesions are not generally observed in guinea pigs, thus limiting the usefulness of this model in atherosclerosis research.

IV. CONCLUDING REMARKS. Since the initial evidence that atherosclerosis development can be induced in laboratory animals was provided by Ignatowski in 1908, various animal species have been used as experimental models of this disease. While most animal models are still being used to a greater or lesser extent, none of them can be considered an ideal model of the human pathology. The choice of the most appropriate animal model depends on the nature of the research that is to be performed. Atherosclerosis in pigs and non-human primates closely recapitulates the main morphological and biochemical characteristics of human atherosclerosis, therefore the results obtained in large animal models are more easily extrapolated to humans in translational research studies, such as the development of imaging techniques and interventional devices and the assessment of novel therapeutic strategies. However, research with large animal species encounters a number of important obstacles, such as difficulties in handling, the high economical cost of maintenance, and important ethical considerations in the case of studies with non-human primates. In contrast, small animals, such as mice, rats and rabbits, have many advantages for experimental studies (e.g. easy handling, low cost), but do not typically develop the advanced vulnerable atherosclerotic plaques that are characteristic of human individuals suffering severe CVD. Genetically-engineered mouse models have been crucial to elucidate the molecular mechanisms underlying atherosclerotic plaque initiation and progression, and the recent advent of strategies to tissue-specifically and/or temporally control the genetic manipulation offers added value to murine models. The used of all animal models available nowadays will undoubtedly continue to permit major advances in atherosclerosis research that should translate into improved treatment, prevention and diagnosis of atherosclerosis and associated ischemic diseases.

V. ACKNOWLEDGMENTS

Work in the author's laboratories is supported by the Spanish Ministry of Science and Innovation (MICINN) (grants SAF2010-16044 and SAF2008-04629) and Fondo de Investigación Sanitaria - Instituto de Salud Carlos III (RECAVA: grant RD06/0014/0021, and PI10/02268). The CNIC is supported by the MICINN and the Pro-CNIC Foundation.

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FIGURE LEGEND

Figure 1. Atherosclerotic plaque development. The scheme shows the main events that occur in the vessel wall during atherosclerosis development, starting with a 'healthy' artery wall (left) and progression to an advanced atherosclerotic lesion (right). Not shown in the scheme is plaque rupture and thrombus formation at advanced disease stages, which can provoke life-threatening acute ischemic events (eg, myocardial infarction and stroke). The photomicrographs in **A-G** show representative imunohistological staining in the aortic sinus and the ascending aorta of apoE-deficient mice, the most widely-used mouse model of atherosclerosis. **(A)** Cell accumulation within the subendothelial space in an early atheroma. Red: nuclei; green: endothelial cells detected by von Willebrand immunofluorescent staining. Elastic laminae in the tunica media exhibit green autofluorescence. **(B)** Macrophage infiltration into the subendothelial space of the arterial wall. Blue: nuclei; green: neointimal macrophages detected by F4/80 immunofluorescent staining; red: medial VSMCs detected by smooth-muscle-α-actin immunofluorescent staining. **(C)** Cell proliferation in a fatty streak. Blue: nuclei; red: medial VSMCs detected by smooth-muscle-α-actin immunofluorescent staining; green: neointimal macrophages detected by F4/80 immunofluorescent staining; white: nuclei in proliferating celles detected by Ki67 immunofluorescent staining. Arrows mark proliferating macrophages in the atheroma. **(D)** Intermediate vascular lesion with a thin fibrous cap. Blue: nuclei; red: VSMCs detected by smooth-muscle-α-actin immunofluorescent staining; green: macrophages detected by F4/80 immunofluorescent staining. **(E)** Electron microscopy image of an intermediate atherosclerotic lesion with a fibrous cap and lipid core (asterisk). **(F)** Advanced atherosclerotic plaque with fibrous cap rich in VSMCs (detected by smooth-muscle-α-actin immunohistochemical red staining). **(G)** Advanced atherosclerotic plaque with necrotic core (asterisk). Macrophages detected by Mac3 immunohistochemical staining are shown in brown. Nuclei are counterstained with hematoxylin.

TABLE 1: Advantages and disadvantages of rabbit models of atherosclerosis

Advantages	Easy to maintain and handle, no special requirements
	Low economical cost
	High availability
	Lipoprotein metabolism relatively similar to humans (except for hepatic lipase deficiency in rabbits)
	Good response to dietary cholesterol
	Availability of hyperlipidemic mutant strains
Disadvantages	Highly abnormal diet required for the development of hypercholesterolemia and atherosclerosis
	Long-term high cholesterol feeding induces massive inflammation and hepatic toxicity

TABLE 2: Advantages and disadvantages of swine models of atherosclerosis

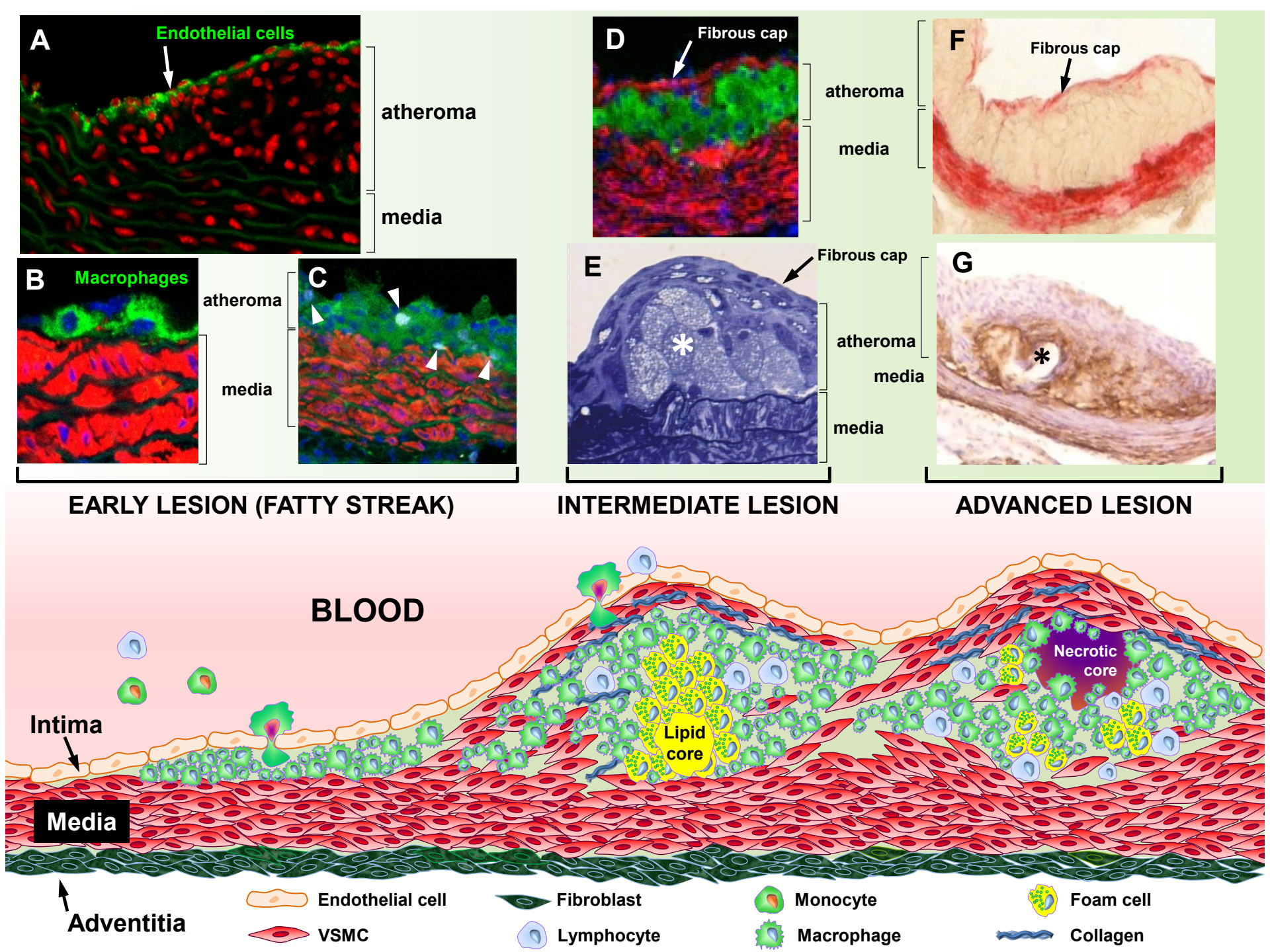
Advantages	Cardiovascular anatomy very similar to humans
	Spontaneous formation of atherosclerosis lesions, even in low-fat standard chow
	Morphology of vascular lesions similar to humans
	Lesion distribution similar to humans, predominantly in aorta, coronary and carotid arteries
	Lipoprotein metabolism similar to humans (except for apolipoprotein II deficiency in swines)
Disadvantages	High cost of purchase and maintenance
	Difficulty in handling (except for mini-pig strains)
	Atheroma formation requires longer time than in other species

TABLE 3: Advantages and disadvantages of non-human primate models of atherosclerosis

Advantages	Phylogenetically close to humans
	Spontaneous formation of atherosclerosis lesions (in some strains, even in low-fat standard chow)
	Vascular lesions similar to humans, including vulnerability features and thrombosis
Disadvantages	High cost of purchase and maintenance
	Limited availability
	Requirement of special animal facilities
	Ethical concerns

TABLE 4: Advantages and disadvantages of mouse models of atherosclerosis

Advantages	Easy breeding and handling
	Short generation time
	Well-defined genetics and availability of inbred strains
	Well established protocols for directed genetic manipulation
Disadvantages	High resistance to atherosclerosis development in wild-type mice. Requirement of genetically-modified mice (e.g. apoE-deficient, LRLD-deficient)
	Plasma lipid profile markedly different to humans
	Differences in the morphology of the arterial wall due to the small size of murine vessels (e.g. reduced thickness of the medial layer, lack of vasa vasorum)
	Absence of plaque rupture and luminal thrombosis in most vessels


A
Endothelial cells
atheroma
media
B
Macrophages
atheroma
media
C
D
Fibrous cap
atheroma
media
E
Fibrous cap
atheroma
media
F
Fibrous cap
G
EARLY LESION (FATTY STREAK)
INTERMEDIATE LESION
ADVANCED LESION
BLOOD
Intima
Media
Adventitia
Lipid core
Necrotic core
Endothelial cell
VSMC
Fibroblast
Lymphocyte
Monocyte
Macrophage
Foam cell
Collagen