Review Article

A Practical Approach to Using Mice in Atherosclerosis Research

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Abstract

This review discusses the application-side of using mice as an animal model of atherosclerosis, and is directed towards the researcher new to using mice to perform atherosclerosis studies. Although this review will comment on many of the current mouse models that are available, noting their strengths and weaknesses, the majority of this review is relevant to planning experiments involving either apolipoprotein (apo) E deficient or low-density lipoprotein (LDL) receptor deficient mice. Subject matter covered includes a description of the types of lesions expected to form in apoE deficient and LDL receptor deficient mice, the age of the mouse when these various types of lesion are expected to form, and finally, a description of the most popular methods used to perform both biochemical and morphometric analysis of atherosclerotic lesions.

Introduction

Cardiovascular disease resulting from atherosclerosis is the leading cause of morbidity and mortality in Westernised countries. Atherosclerotic lesions form de novo from a focal accumulation of lipoproteins, monocyte-derived macrophages and lymphocytes within the wall of arterial blood vessels. Within this space, lymphocyte signalling promotes the accumulation of cholesterol by intimal-associated macrophages. The source of this cholesterol is lipoprotein particles that pass from the blood into the vessel wall and are subsequently modified by oxidation. Over time, this process culminates in the formation of large structurally unstable lesions that are prone to rupture, thrombus formation, occlusion of the affected vessels and the death of tissue distal to the blockage.

A number of excellent reviews on the use of mice in atherosclerosis research by Allayee et al., Daugherty, Fazio and Linton, Reardon and Getz, and Knowles and Meada have been published in the last two to three years, and each represents a valuable complement to this review.1-5 The most recent cited review by Allayee et al., provides a careful description of how mice offer a practical alternative to humans for studies that focus on determining the genetic bases of atherosclerosis. The reviews by Daugherty and by Fazio and Linton focus on providing a description of the numerous genetically altered (transgenic and gene-targeted-deletion) mouse models currently available to study atherosclerosis.2,3 Finally, the review by Reardon and Getz as well as Knowles and Meada provide a description of how genetically altered mouse models can be used to examine how specific enzymes and receptors involved in lipoprotein metabolism or certain risk factors such as hypertension and diabetes can modulate lesion formation.4,5 Rather than reiterate the content of these five recent reviews, a review dealing with the application-side of using mice in atherosclerosis studies is more appropriate and hence more practical for those researchers new to working with mice in this context. Although this review will comment briefly on many of the current mouse models that are available noting their strengths and weaknesses, the majority of this review is directed towards providing the reader with information relevant to planning experiments involving either apoE deficient (apoE−/−) or LDL receptor deficient (ldlr−/−) mice. Subject matter covered in this review includes a description of the types of lesions expected to form in either apoE−/− or ldlr−/− mice, the age of the mouse when these various types of lesions are expected to form, and finally, a description of the most popular methods used to perform both biochemical and morphometric analysis of atherosclerotic lesions.
Each of the mouse models described briefly in Table 1, and in more detail in the reviews by Daugherty and by Fazio and Linton, will develop atherosclerosis to varying extents, in a time and diet dependent manner. The choice of mouse model should be based on the investigator’s specific needs as it relates to their hypothesis being tested. However, most researchers can do well by using either the apoe-/- or ldlr-/- mouse as their background atherogenic animal model. Not that these two models are far superior to others listed along side Table 1, but rather because these two models have many practical attributes: (i) both the apoe-/- (Strain number, B6.129P2-Apoetm1Unc/J; stock number, 002052) and ldlr-/- (Strain number, B6.129S7-Ldlrtm1Her/J; stock number, 002207) strains are commercially available from the Jackson Laboratory (Bar Harbor, Maine, USA) and are already extensively backcrossed (>10 times) onto the C57BL/6 background (ii) the Jackson Laboratory generally has enough mice available at any one time so there is little down-time as a result of mouse availability (iii) both the apoe-/- and ldlr-/- mice breed well and give birth to reasonable litter sizes (however, anecdotal we find apoe-/- litters to be larger on average (6 to 8 pups) than those from ldlr-/- mice (4 to 6 pups), despite both mouse strains being on the same C57BL/6 background) (iv) finally, the types of lesions formed have been well documented and as provided in Table 2, the researcher can reliably plan to have a specific stage of lesion form within a well-defined time frame. This allows the researcher to better plan his/her experiments based on predicted lesion stages that will form in their control groups.

Mice as an Atherosclerosis Model

Even prior to the advent of transgenics and targeted gene-deletion, the mouse has been viewed for decades as an attractive animal model of atherosclerosis for a number of reasons: (i) the extensive genetic information available on numerous inbred strains eased the task of identifying genetic links to atherosclerosis-susceptibility (ii) the small size and cost of housing allowed for greater experimental numbers to ensure statistical meaning (iii) mice have a short gestation time and can multiply rapidly and (iv) in studies involving the testing of certain pharmacological agents where cost or availability of a particular drug is an issue, mice would require smaller amounts of the drug than identical studies conducted in larger mammals such as swine.

Despite these favorable attributes of the mouse, prior to the 1990’s, which ushered in the technique of creating genetically altered mice, no known inbred mouse strain existed that spontaneously developed atherosclerosis. Extensive literature does exist on the differing susceptibilities of inbred mouse strains to develop atherosclerosis during feeding a modified diet that promotes hyperlipidemia. In the first study of its kind, Paigen et al. found that C57BL/6 mice were the most susceptible to the development of diet-induced atherosclerosis among inbred strains examined. The C3H strain in this study was among the most resistant and BALB/c mice were found to be an example of a strain with intermediate susceptibility. Based on this original finding showing C57BL/6 mice to have an inherent “genetic” susceptibility to atherosclerosis, it has become common practice to perform atherosclerosis studies in this strain, and this includes those studies using genetically modified mice, despite the fact that in many instances this required founder transgenic mice to be tediously backcrossed onto the C57BL/6 strain.

Interestingly, this genetic susceptibility/resistance noted in wild type inbred mice was found to persist when these same strains were made deficient of apoE. In a recent study by Smith et al, apoe-/- mice on six inbred genetic backgrounds were compared for atherosclerotic lesion size in the aortic root in two independent studies. After normalisation to the C57BL/6 strain that was used in both studies, lesion areas were found in the following rank order: DBA/2J>C57BL/6>129/Sv-ter>AKR/J which was almost equal to BALB/cByJ and C3H/HeJ. This type of detailed inbred strain-comparisons highlights the fact that atherosclerosis has a genetic component and when performing an atherosclerosis study using genetically altered mice with a mixed genetic background, one must use paired littersmates when comparing lesion development between control and experimental groups. This same rule of thumb should also be applied when working with transgenic mice on a less than “pure” genetic background achieved by backcrossing founder mice onto a different inbred strain. For example, it would not be appropriate to purchase control C57BL/6 apoe-/- mice (>10 times backcrossed) from the Jackson Laboratories for use in an atherosclerosis study where the experimental apoe-/- mice carrying an additional genetic alteration originated from parents derived in a strain other than C57BL/6 and had not subsequently been backcrossed >10 times to the C57BL/6 strain.

Although no inbred strains of mice are available that spontaneously develop complex atherosclerotic lesions, small “fatty streak-type” lesions can be induced in C57BL/6 mice by feeding them a diet enriched in both cholesterol and fat. Unfortunately, C57BL/6 mice will only develop lesions with features of the earliest stages of the disease, with these lesions usually being confined to the aortic root, associated with the cusps of the aortic valve. Prolonged feeding of a cholesterol and fat-enriched diet has been shown to promote lesion development in C57BL/6 mice that extend.
out into the most proximal portion of the vascular tree (aortic arch), but here too the lesions are very small and are representative of only the earliest stages of atherosclerosis. So, as a truly representative model of atherogenesis, the “wild-type” mouse falls far short of other mammalian models such as non-human primates, and swine, which both develop morphologically similar lesions reflecting all stages of the disease when compared to those found in humans.

### Transgenic Mice

Several transgenic mice have been generated that develop atherosclerotic lesions. In simplest terms, the words transgenic mouse refers to a mouse that has had its genome methodically altered to express more than two copies of a single gene derived from a different strain of mouse, or at least one gene derived from a non-mouse species. In regards
to atherosclerotic lesion development, mice have to become dyslipidaemic before they will develop atherosclerosis. Therefore, overexpressing various genes that are somehow involved in the regulation of lipoprotein metabolism provides the enhanced susceptibility of transgenic mice to develop atherosclerosis in all instances. Provided in Table 1 is a selected list of some of these transgenic mice, and accompanying this list is an indication of the types of atherosclerotic lesions formed; scaling of lesions is based on the American Heart Association-defined stages of human atherosclerosis (stages I to VI).

Mice transgenic for the human apoB100, \(^{23,24}\) the E2 isoform of apoE \(^{25}\) and ApoE3-Leiden, \(^{26}\) the latter a more uncommon form of apoE, have all been used in atherosclerosis studies and each has been shown to develop atherosclerotic lesions to varying degrees (see Table 1).

**Single Gene Replacement Mice**

Unlike transgenic mice, where it is common to express multiple gene copies in various chromosomal locations, the technique of gene replacement converts an endogenous allele to a variant at the same position in the genome. This form of “knock-in” transgenics provides the same tissue-specific expression of the exogenous gene as that of the endogenous gene. This type of gene-replacement strategy has been used to generate mice that express the three most common isoforms of human apoE (E2, E3 and E4). Mouse atherosclerosis studies have been performed in these human apoE “knock-in” mice, \(^{27-29}\) and a summary of the results is also provided in Table 1.

**Single Gene Deleted Mice**

In the early part of the 1990’s the technique of homologous recombination in embryonic stem cells allowed for a specific allele to be deleted and hence permitted the definition of a single protein’s function. Because these mice are genetically engineered to abolish the expression of a specific gene, these mice are commonly referred to as “knock-out” mice. This powerful genetic technique led to the creation of two mouse atherosclerosis models, the apoe-/- \(^{30,31}\) and ldlr-/- \(^{32}\) mice, which develop “spontaneous” or diet-induced atherosclerotic lesions, respectively, that appear morphologically identical \(^{32,33}\) to those found in humans. \(^{19-22}\) Therefore, the introduction of these two “new” animal models of atherosclerosis has truly revolutionised our understanding of the atherogenic process and has allowed the mouse to quickly become the most popular mammalian model of atherosclerosis to date.

**Working Model of Atherosclerosis: Comparison Between Humans and Mice**

The development of an atherosclerotic lesion is a complex process, which in humans begins in early childhood, but may not become clinically relevant until many decades later. As the atherosclerotic lesion develops to a clinically relevant form, the lesion will pass through a set of six predicted stages of development based on morphological criteria. Since this review is intended to highlight the use of mice to study the...
The involvement of the immune system in atherosclerosis has been hypothesised to occur at the very start of the disease. In the arteries of healthy children and teenagers, pre-existing mononuclear cell infiltrations have been identified at regions known to have a greater likelihood to later develop atherosclerosis. These sites, termed “vascular-associated lymphoid tissue” are speculated to function as local immunosurveillance systems that aid in monitoring the bloodstream for potentially harmful endogenous and exogenous antigens. The morphological feature of early stage lesions in both apoe-/- and ldl-r-/- mice are very similar to those found in humans. Like their human
counterpart, these mouse lesions consist of an abnormal accumulation of lipoproteins and a focal “cellular gathering” of mainly T-cells and macrophages, although a small number of B-cells and dendritic cells have also been found.

There is strong evidence that accumulation of plasma-derived lipoproteins in the arterial wall launches specific cell reactions and that this fundamental event initiates the disease. As lipoproteins enter the tunica intima they become trapped by matrix components and then modified by oxidation to a form that has been shown to be chemotactic for monocytes in vitro. In vivo, oxidised lipoproteins may also be chemotactic for other immune cells (T- and B-cells and possibly Natural Killer cells as well), facilitating their recruitment into the vessel wall. Once within the intima, monocytes undergo differentiation to macrophages and begin to clear the modified lipoproteins via scavenger receptors expressed on the macrophage cell surface. This process results in the generation of numerous cholesterol ester-enriched foam cells that come together to form a mass of cells commonly termed a fatty streak. Fatty streak lesions are a common feature in both apo-e-/- and ldl-r-/- mice (see Table 2). Representative images of a stage I, stage II and a stage III lesion from an apo-e-/- mouse are presented in Figure 1. A representative image of a stage III lesion undergoing progression to a stage IV lesion from an apo-e-/- mouse is presented in Figure 2.

Late Lesion Development (stages V to VI)

Stage IV is the first lesion considered advanced by histological criteria because these lesions are the first to possess an accumulation of extracellular lipid, generally known as the lipid core, which occupies an extensive but still well defined region of the tunica intima. The lipid core is thought to develop from an increase and subsequent convergence of the small isolated pools of lipid that begin to collect in stage III lesions. A thickening of the tunica intima is associated with the developing lipid core, which can result in deformity of the arterial wall itself, but generally these lesions do not cause significant narrowing of the vascular lumen. When the intimal area on the lumen side of the lipid core undergoes an increase in fibrous tissue, a “cap” is formed and the lesion is then labelled stage V. Because the upper intimal layer of a stage IV lesion is sometimes indistinguishable from the fibrous cap of a stage V lesion, both stages are generally termed fibrous plaques. A representative image of a stage V lesion from an apo-e-/- mouse is presented in Figure 3. Macrophages, macrophage-derived foam cells, and lymphocytes are generally found densely concentrated along the periphery of the lipid core of both stage IV and V lesions. Lymphocytes and mast cells have also been identified in the regions bordering the shoulder of the lipid core and the fibrous cap. Both stage IV and V lesions are capable of developing fissures, haematomas, and/or thrombi and for this reason both stages are clinically relevant as morbidity and mortality in humans from atherosclerosis is largely due to disruption of stage IV and V lesions. Stage IV or V lesions that contain a haematomata and/or thrombotic deposits are classified as stage VI and may also be referred to as complicated lesions. Although both stage IV and V lesions will form in apo-e-/- mice fed an atherogenic diet for a prolonged period of time (14 to 20 weeks, see Table 2), stage VI lesions have only been reported in the innominate artery of apo-e-/- mice, yet the occurrence of such lesions in this mouse model is under debate, and these lesions have never been documented in the aortic root or along the aortic tree of the apo-e-/- mouse.

Finally, as with apo-e-/- mice, prolonged feeding of ldl-r-/- mice with an atherogenic diet will result in the formation of stage IV lesions consisting of a lipid-filled necrotic core capped with foam cells. However, detailed histological studies have not provided evidence that stage V lesions, containing a true fibrous cap, will form in ldl-r-/- mice.

Next Generation of Mouse Models

In addition to mice designed to carry targeted gene-replacements/deletions or transgenics, alternate techniques of genetic manipulation, such as the Cre- and Flp-recombinase technology to give tissue specific gain or loss of gene expression and the tetracycline-controlled transcriptional regulation system that allows one to turn “on” or “off” a specific transgene upon the administration of doxycycline to the mouse via the animal’s drinking water (See reviews by Nagy, Bockamp et al., Zhu et al., Corbel and Rossi, and Lewandoski) will help ease the growing demand for more sophisticated murine models to conduct biomedical research. The use of viral-mediated tissue-specific gene expression and creation of haematopoietic-driven chimeric mice using bone marrow transplantation has allowed researchers studying atherosclerosis to expand the capabilities of the mouse model and permit one to acquire, in an in vivo setting, a mechanistic insight into the various phases of atherogenesis.

Limitation of the Mouse Model

The small size of the mouse also offers limitations, such as the collection of a sufficient quantity of blood to characterise cellular and chemical entities and the characterisation of lesion samples on an individual mouse basis for both protein and RNA analysis.
Certainly measuring lesion size is required to determine if a particular pharmacological treatment or genetic loss/enhancement of function has a direct affect on atherosclerosis. However, quantification of the extent of atherosclerosis should not be solely a process of measuring the size of an atherosclerotic lesion. Even more information about the process of atherogenesis can be gathered from a detailed examination of morphology as it pertains to the cellular and extracellular composition of the lesion, as this will offer significant mechanistic insight into the atherogenic process itself. Given the complexity of the disease process, the methodology of lesion analysis can be involved and whether the atherosclerotic lesions under investigation are human or experimental animal in origin, investigation of the

Figure 2: Stage IV Lesion. Aortic lesion histology from an apoe-/- mouse fed a Western diet (Dyets Inc. #112286; see legend to Table 2) for 20 weeks from the time the mouse was 8 weeks of age. Panels A-C are images from two serial tissue sections taken from a region of the heart where the aortic sinus becomes the ascending aorta. A segment of heart tissue spanning the aortic sinus and ascending aorta was embedded in OCT medium, snap-frozen, sectioned and stained. Tissue in panel A has been stained with Oil Red O to detect neutral lipids and counter-stained with Hematoxylin. The inset in panel A is the same area represented in both panel B, a higher magnification of panel A, and panel C, a serial tissue section stained with Gomori trichrome to detect collagen. The lesion highlighted in panels B & C is best classified as a stage III lesion undergoing progression to a stage IV lesion. The solid arrows in panels B & C identify a collagen-enriched fibrous-cap covering a developing lipid core (LC). Flanking this developing stage IV lesion are two prominent stage III lesions consisting of lipid enriched foam cells (FC). In panels B & C the tunica media (m) and the leaflet base (lb), which is a raised portion of the aortic wall where the base of the leaflet mergers into the skeleton of the heart, are identified. Magnification: panel A, X40; panels B & C, X200.

Quantification of Atherosclerosis in Mice

Certainly measuring lesion size is required to determine if a particular pharmacological treatment or genetic loss/enhancement of function has a direct affect on atherosclerosis. However, quantification of the extent of atherosclerosis should not be solely a process of measuring the size of an atherosclerotic lesion. Even more information about the process of atherogenesis can be gathered from a detailed examination of morphology as it pertains to the cellular and extracellular composition of the lesion, as this will offer significant mechanistic insight into the atherogenic process itself. Given the complexity of the disease process, the methodology of lesion analysis can be involved and whether the atherosclerotic lesions under investigation are human or experimental animal in origin, investigation of the
disease offers considerable technical challenges due to the range of parameters that may be quantified.

There are a number of methods for the quantification of atherosclerosis in mice and the three vascular beds most commonly analysed are: (i) the aortic root and ascending aorta cut in cross-section (ii) the aortic tree spanning the arch, thoracic and abdominal regions of the aorta opened longitudinally to expose the area of definable lesion covering the luminal en face surface and (iii) serial sectioning of the proximal 1 mm of the brachiocephalic trunk (innominate artery) which is the first major branch off the aorta at the region of the aortic arch. A detailed step-by-step description on how to quantify atherosclerosis in the aortic root, ascending aorta and using the en face preparation of the aortic tree has recently been provided by Daugherty and Whitman.

Figure 3: Stage V Lesion. Aortic lesion histology from an apoe-/- mouse fed a Western diet (Dyets Inc. #112286; see legend to Table 2) for 20 weeks from the time the mouse was 8 weeks of age. Panels A-C are images from two serial tissue sections taken from a region of the heart where the aortic sinus becomes the ascending aorta. A segment of heart tissue spanning the aortic sinus and ascending aorta was embedded in OCT medium, snap-frozen, sectioned and stained. Tissue in panel A has been stained with Oil Red O to detect neutral lipids and counter-stained with Hematoxylin. The inset in panel A is the same area represented in both panel B, a higher magnification of panel A, and panel C, a serial tissue section stained with Gomori trichrome to detect collagen. The lesion highlighted in panels B & C is classified as an advanced, stage V lesion. The solid arrows in panels B & C identify a collagen-enriched fibrous-cap covering a well-developed lipid core (LC). As identified in panel B, a prominent region of lipid enriched foam cells (FC) has formed on top of the fibrous cap. In panels B & C the tunica adventitia (a), and tunica media (m) are identified. Magnification: panel A, X40; panels B & C, X200.
The initial atherosclerosis studies conducted in mice were restricted to the aortic root, as this was the only region where visible atherosclerotic lesion could be reliably found using the C57BL/6 strain of mouse. To date, this same arterial region remains the most commonly used region of the aorta for the quantification and characterisation of atherosclerosis in the mouse model (transgenic and non-transgenic alike). The initial description of this methodology by Paigen et al. involves a 300 μm stretch of the ascending aorta starting at the ostia of the coronary arteries and running distal towards the aortic arch. In serial sectioning of the heart, the coronary ostia are readily identifiable, and for this reason, these ostia represent a perfect “landmark” for the starting point of lesion assessment. The aortic root and ascending aorta are relatively straightforward vascular beds to measure lesion size. However collection of the sections does require considerable technical skill since this methodology relies upon the ability of the investigator to collect all of the sections spanning a 400+ μm region of the ascending aorta. Furthermore, as we have recently described, a more complete histological analysis of the aortic root and ascending regions can be conducted if the entire aortic root and ascending aorta are collected together, which then requires that 90 serial sections be collected spanning a 900 μm stretch of the aortic root.

A second popular technique of lesion analysis is to measure the percentage of lesion area using an en face preparation of the aortic tree. The relative speed and ease of this technique compared to the time-consuming and technically demanding method of aortic root serial sectioning has made en face analysis a popular choice. However, compared to working with aortic tissue cut in serial sections, there are far more limitations to this mode of analysis. The greatest limitation by far is that this type of analysis provides only a one-dimensional representation of the lesion. Thus, when working with an en face preparation, you will only acquire information on lesion size and not complexity, stage of development or compositional make-up. Certainly, if the lesions in the control and experimental groups are at the same stage of development, a difference in percent lesion area is adequate to say that the process of atherogenesis is affected in the experimental population of mice. However, what if the process of atherosclerosis between groups lies within the transitional zone of going from a stage III to IV lesion? Lesion analysis by the en face method would not be able to detect changes in lesion morphology. In this instance, the results of the en face analysis may tell you that there is no significant change in lesion area, and thus you would make the incorrect assumption that the atherogenic process was the same between the control and experimental group(s).

The innominate artery is a small vessel connecting the aortic arch to the right subclavian and right carotid arteries. Rosenfeld et al. and Reardon et al. have both shown a very consistent rate of lesion progression in this arterial region, not only in initial xanthoma formation but also in the development of late-stage lesions showing a narrowed vessel characterised by an atrophic media, perivascular inflammation, loss of continuity of the fibrous cap and in some instances rupture of the plaque at the shoulders of the atherosclerotic lesions and intraplaque haemorrhage. Since these events occur consistently at this arterial site, it has been suggested that this site could be applicable to studying the development of more advanced, stage VI, lesions. However, more work is warranted in order to determine whether these types of lesions form in other mouse models of atherosclerosis.

Only serial sectioning of snap-frozen arterial tissue provides the most flexibility with regards to lesion analysis, since multiple slices of this unfixed tissue allows the investigator to probe the same atherosclerotic lesion for numerous cellular and extra-cellular components using a variety of techniques such as immunocytochemistry and Laser Capture Microdissection (LCM). First, cryosectioned tissue lends itself perfectly to the technique of immunocytochemistry. With the increasing number of commercially available antibodies, immunocytochemistry is a powerful tool for use in defining the compositional make-up of an atherosclerotic lesion. In addition to the technique of immunocytochemistry, one of the newest histological techniques that will revolutionise the way we perceive the process of atherogenesis is the advent of LCM. LCM, which is described in greater detail at the following URL http://dir.nichd.nih.gov/lcm/lcm.htm, is a unique form of microdissection developed in 1996 at the National Institutes of Health. LCM utilises a low-power infrared laser to melt a special thermoplastic film onto cells of interest contained within a sectioned piece of tissue. When this thermoplastic film is lifted, the selected cells remain attached and are captured for further analysis. The technique of LCM allows you to physically isolate a single cell or multi-cellular structures from both paraffin-embedded and frozen tissue. Furthermore, because the LCM technique uses an infrared laser, biological molecules such as RNA, DNA and protein remain undamaged during the microdissection process. Therefore, with tissue isolated using LCM, you are capable of performing a number of downstream molecular assays including mRNA expression analysis using microarrays, two-dimensional gel electrophoresis, Surface Enhanced Laser Desorption/Ionisation (SELDI), and Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight (MALTI-TOF).
given that enough starting material has been collected. Since the LCM technique allows you to extract only atherosclerotic tissue located in the tunica intima and leave behind non-diseased tissue in both the tunica media and adventitia, LCM can enable the researcher to identify differentially expressed genes in a pure atherosclerotic lesion sample, a capability not permitted when standard whole tissue is used. In fact, two groups in the last year have published work that used LCM to extract macrophages from atherosclerotic plaques of both humans and apo-e/- mice and subsequently performed quantitative RT-PCR and Western blotting from the isolated macrophage mRNA and protein, respectively.

**Conclusion**

The advent of genetically altered mice that are rendered susceptible to developing atherosclerosis has provided us with a unique animal model and tool for atherosclerosis research. With the arrival of alternate techniques of genetic manipulations, such as the Cre- and Flp-recombinase technology, tissue-specific gene targeting using virus constructs, and bone marrow transplantation, coupled with state-of-the-art analytical tools such as LCM, the mouse model will move us several steps closer to working out the entire mechanism of atherogenesis. Thus, providing the knowledge to perform the translation research relating to the human condition.

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