American Journal of Pathology, Vol. 165, No. 1, July 2004
Copyright © American Society for Investigative Pathology

Review

Mouse Models of Arteriosclerosis

From Arterial Injuries to Vascular Grafts

Qingbo Xu

From the Department of Cardiac and Vascular Sciences, St. George's Hospital Medical School, London, United Kingdom

Animal models are designed to be preliminary tools for better understanding of the pathogenesis, improvement in diagnosis, prevention, and therapy of arteriosclerosis in humans. Attracted by the well-defined genetic systems, a number of investigators have begun to use the mouse as an experimental system for arteriosclerosis research. Hundreds of inbred lines have been established, and the genetic map is relatively well defined, and both congenic strains and recombinant strains are available to facilitate genetic experimentation. Because arteriosclerosis is a complicated disease, which includes spontaneous (native) atherosclerosis, transplant arteriosclerosis, vein graft atherosclerosis, and angioplasty-induced restenosis, several mouse models for studying all types of arteriosclerosis have recently been established. Using these mouse models, much knowledge concerning the pathogenesis of the disease and therapeutic intervention has been gained, eg, origins of endothelial and smooth muscle cells in lesions of transplant and vein graft atherosclerosis. This review will not attempt to cover all aspects of mouse models, rather focus on models of arterial injuries, vein grafts, and transplant arteriosclerosis, by which the major progress in understanding the mechanisms of the disease has been made. This article will also point out (dis)advantages of a variety of models, and how the models can be appropriately chosen for different purposes of study. ***(Am J Pathol 2004, 165:1–10)***

Arteriosclerosis is characterized by smooth muscle cell hyperplasia or hypertrophy and matrix protein accumulation in the intima and/or media with or without lipid deposition, resulting in thickening and stiffness of the arterial wall.[1] Arteriosclerosis includes (spontaneous) atherosclerosis, accelerated arteriosclerosis (namely, transplant arteriosclerosis), restenosis after percutaneous transluminal coronary angioplasty, and vein graft atherosclerosis.[2] Atherosclerosis research with animal models, as known today, is nearly 100 years old.[3] Knowledge of the pathogenesis and therapy of atherosclerotic disease and the use of animal models in arteriosclerosis research have evolved almost simultaneously. The use of animal models in the study of arteriosclerosis is essential to answer many questions. For instance, evaluation of a risk factor as a single independent variable, with almost complete exclusion of other factors, can best be performed in animals free of intercurrent diseases or abnormalities and with well known genetic characteristics.[4] On the other hand, the role of vascular injury because of angioplasty, alloimmune responses, or vein grafts can be investigated alone or in combination with other factors that either aggravate or have beneficial effects. Furthermore, experiments using animals are the only way to develop and test new diagnostic, preventive, and therapeutic procedures for both ethical and practical reasons. The investigator can choose the species, time, and method, as well as obtain tissue, and serum samples as well as other material needed for measurements under optimal conditions, selective circumstances that are difficult, if not impossible, in studies with human patients.

Attracted by the availability of well-defined genetic systems of transgenic and knockout mice, a number of investigators have begun to use the mouse as an experimental system for arteriosclerosis research.[4] Hundreds of inbred lines have been established, and the genetic map is relatively well defined, and both congenic strains and recombinant strains are available to facilitate genetic experimentation. The animal model of hyperlipidemia and atherosclerosis in apoE- and low-density lipoprotein receptor-deficient mice has been widely used.[4–6] Such mouse models have considerable advantages over other animal systems in that they overcome the need to admin-

Supported by grants from British Heart Foundation (PG/01/170) and the Oak Foundation.

Accepted for publication February 27, 2004.

Address reprint requests to Qingbo Xu, M.D., Ph.D., Department of Cardiac and Vascular Sciences, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK. E-mail: q.xu@sghms.ac.uk.

Table 1. Comparison of Vascular Injury Models

	Wire injury	Electronic injury	Ligation	Collar
EC integrity	No	No	Yes	Yes
Medial injury	Yes	Yes	No	No
Blood flow	Unchanged	Unchanged	Changed	Unchanged
Thrombosis	Slightly	Heavily	Possibly	No
Neointima	++	++++	+++	++
Surgery difficulties	+++	+	+	++

EC, endothelial cell.

ister a cholesterol diet. Since several reviews summarizing the effects of a variety of genes on native atherosclerosis have been published,[5,7] the present subtitle will not cover the animal models of spontaneous atherosclerosis, ie, hyperlipidemia-induced atherosclerosis, rather focus on other types of mouse models for arteriosclerosis, including arterial injuries, vein graft, and vessel transplantation.

Arterial Injuries

Angioplasty is very often used to treat patients with severe coronary artery disease. The coronary blood flow in the majority of patients is recovered after the treatment. The problem is restenosis of the vessel because of the formation of neointimal lesions.[8] The hallmarks of neointima lesions are smooth muscle cell proliferation and extracellular matrix deposition.[9] The pathogenesis of this disease remains poorly understood. Most knowledge concerning the mechanisms of restenosis formation was derived from studies of animal models. In the late 1970s, Clowes and colleagues[10] established the rat arterial injury model, by which a great number of articles describing the process of restenosis were published. In 1993, Lindner and colleagues[11] developed the first mouse model of arterial injury using a flexible wire. Subsequently, ligation and electronic injury models were also established.[12,13] These models are being widely used by many laboratories and have generated a large number of publications during the last 5 years.[14–19] Because it is difficult to include all of the data derived from the use of these models, a brief summary on several of the models follows (Table 1).

Wire-Injury Model

Technically, this model is similar to that of rat carotid arterial injury. Briefly, the bifurcation of the carotid artery was exposed and two ligations were placed around the external carotid artery, which was then tied off with the distal ligature. An incision hole was made between the two ligatures, where a flexible wire was introduced into the common carotid artery. After passing the vessel three times, Lindner and colleagues[11] observed that complete removal of the endothelium was achieved with a flexible wire. A platelet monolayer covered the denuded surface, and damage to underlying medial smooth muscle cells was detected. Injection of [^{3}H]thymidine displayed replication of medial smooth muscle cells, which was found to be 1.6% at 2 days after denudation and 9.8% at 5 days. Smooth muscle cells were observed in the intima by day 8, and by 2 weeks the intimal lesion had a similar cell content as the media. In most animals, repair of the endothelial lining was complete 3 weeks after injury. Using an outbred strain, they found that within 2 weeks after injury, intimal lesions would form in areas that were still denuded. These intimal lesions, however, were not very extensive and usually did not exceed two or three cell layers in thickness.[11,12]

Carotid Artery Ligation

An alteration in blood flow has been shown to increase intimal lesion formation in vascular grafts and balloon-injured vessels,[20] thus indicating that alterations in biomechanical stress will affect the proliferative response of smooth muscle cells.[21,22] Furthermore, a number of studies have demonstrated that vessels adapt to chronic changes in blood flow by undergoing compensatory adjustments in their lumen size.[23,24] Based on this knowledge, Kumer and Lindner[12] developed a murine model of carotid artery ligation, in which blood flow in the common carotid artery was disrupted by ligating the vessel near the distal bifurcation. The surgery procedure for this model is relatively simple, ie, ligating the common carotid artery near the bifurcation. Neointimal lesions in the common carotid artery can be seen 2 to 4 weeks after ligation. This model differs from others in that they do not require mechanical trauma and widespread endothelial denudation to induce smooth muscle cell proliferation. It should be noted that the model might not mimic a physiological or pathological situation. However, vascular lesions in humans often develop at sites of altered hemodynamics associated with low shear stress.[25] Therefore, it is conceivable that the factors responsible for intimal lesion formation at these sites might differ from those involved in intimal hyperplasia after arterial injury associated with endothelial denudation. Nevertheless, this model is often used to test the functions of genes in the neointimal formation in knockout mice because of its simplicity.

Other Types of Arterial Injury

Carmeliet and colleagues[13] described a mouse model of carotid artery injury induced by perivascular electric stimulation, in which femoral arteries in mice were injured perivascularly via a single delivery of an electric current. They found that electric injury destroyed all medial

smooth muscle cells, denuded the injured segment of intact endothelium, and transiently induced platelet-rich mural thrombosis. A vascular wound-healing response resulted that was characterized by degradation of the mural thrombus, transient infiltration of the vessel wall by inflammatory cells, and progressive removal of the necrotic debris. Topographic analysis revealed repopulation of the media and accumulation in the neointima of smooth muscle cells originating from the uninjured borders and progressing into the necrotic center. Thus, electric injury of arteries provides a model of vascular wound healing with arterial neointima formation and re-endothelialization that may be useful for the genetic analysis of its molecular mechanisms in transgenic mice.

In 1989, Booth and colleagues[26] established the rabbit model by placing a perivascular collar resulting in the neointimal formation in the carotid artery. Moroi and colleagues[27] adapted this model to mice, in which the left femoral artery was isolated and loosely sheathed with a 2.0-mm polyethylene tube made of PE-50 tubing (inner diameter, 0.56 mm; outer diameter, 0.965 mm) and tied in place with an 8-0 suture. The tube is larger than the vessel, and does not obstruct blood flow. It results in predictable formation of neointima in mice throughout a 14-day period. In this tube model, the endothelial cells are not directly manipulated or removed, allowing study of the effect of individual endothelial factors, including endothelium-derived nitric oxide (NO). In this model, the primary endpoint is neointimal formation, and is complementary to other models. This model has been proven to be reproducible, easily quantitated, and lends itself well to analysis of individual gene products that can be manipulated by transgenic approaches and targeted gene disruption, albeit the mechanism of intimal formation is unknown.

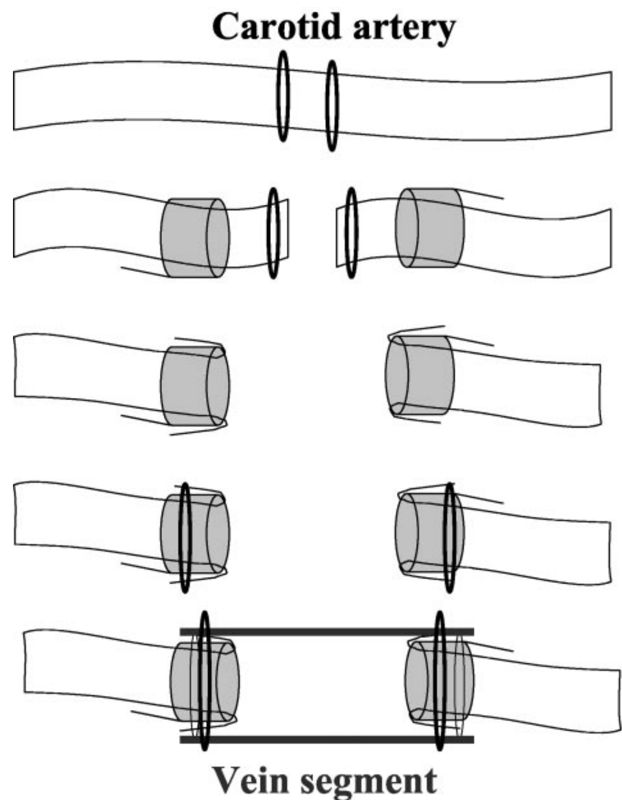

Figure 1. Schematic representation of vein bypass graft (adapted from Zou et al[30]). The right common carotid artery was ligated with 8-0 silk suture, dissected between the middle ties, and passed through cuffs, respectively. The vessel, together with the cuff handle, was fixed with microhemostat clamps, the suture at the end of the artery was removed and a segment of the artery turned inside out to cover the cuff body. The vena cava vein segment was harvested and grafted between the two ends of the carotid artery by sleeving the ends of the vein over the artery cuff and suturing them together with an 8-0 suture ligation.

Vein Graft Atherosclerosis

Vascular grafts are widely used in aortocoronary bypass graft surgery.[28] The small caliber autogenous saphenous vein is usually used as a graft, but occlusion of the graft vein often occurs after bypass operations. A small fraction (perhaps 5 to 10%) of saphenous vein grafts used as aortocoronary bypass vessels occlude within 1 month, ~10 to 20% occlude within 1 year, and by 10 to 12 years postoperatively, only ~50 to 60% remain patent. Three pathological processes are primarily responsible for graft occlusion: thrombosis (early closure), intimal hyperplasia (a few months to a few years), and atherosclerosis (usually after 1 year).[29] Understanding the pathogenesis of vein graft atherosclerosis is often extrapolated from studies on (spontaneous) atherosclerosis in arteries, but the features of the lesions and the pathogenic processes of graft-induced atherosclerosis differ from spontaneous atherosclerosis. Therefore, appropriate mouse models for vein grafts would be needed for studying this disease.

Technical Feasibility of the Mouse Model

Before the first mouse model of vein graft arteriosclerosis was successfully established, we tested several methods to anastomose the ends of the vein segment and arteries, including using a suture, a nylon membrane, a plastic tube, and a cuff. Finally, we found that only a cuff technique is feasible to obtain a reproducible result[30] (Figure 1). Because the vein graft procedure was previously described in detail,[30] here I just briefly describe the method and include a 3-minute movie (supplement), which would be helpful for the investigators who wish to use this model. When you watch the movie, you see that the right common carotid artery was mobilized free from the bifurcation at the distal end toward the proximal, cut in the middle, and a cuff placed at the end. The cuff was made of an autoclavable nylon tube 0.63 mm in diameter outside and 0.5 mm inside. The artery was turned inside out over the cuff and ligated. The vessel segment of either vena cava or jugular vein from donor was grafted between the two ends of the carotid artery by sleeving the ends of the vein over the artery cuff and ligating them together with the 8-0 suture. As displayed in the movie, pulsation can be seen after removing the clamps. The complete grafting procedure required 30 to 40 minutes.

This simplified mouse model of vein grafts has several advantages: first, the operation procedure is simple and easy to learn. The vast majority of investigators could perform the surgery after a short period of training. Sec-

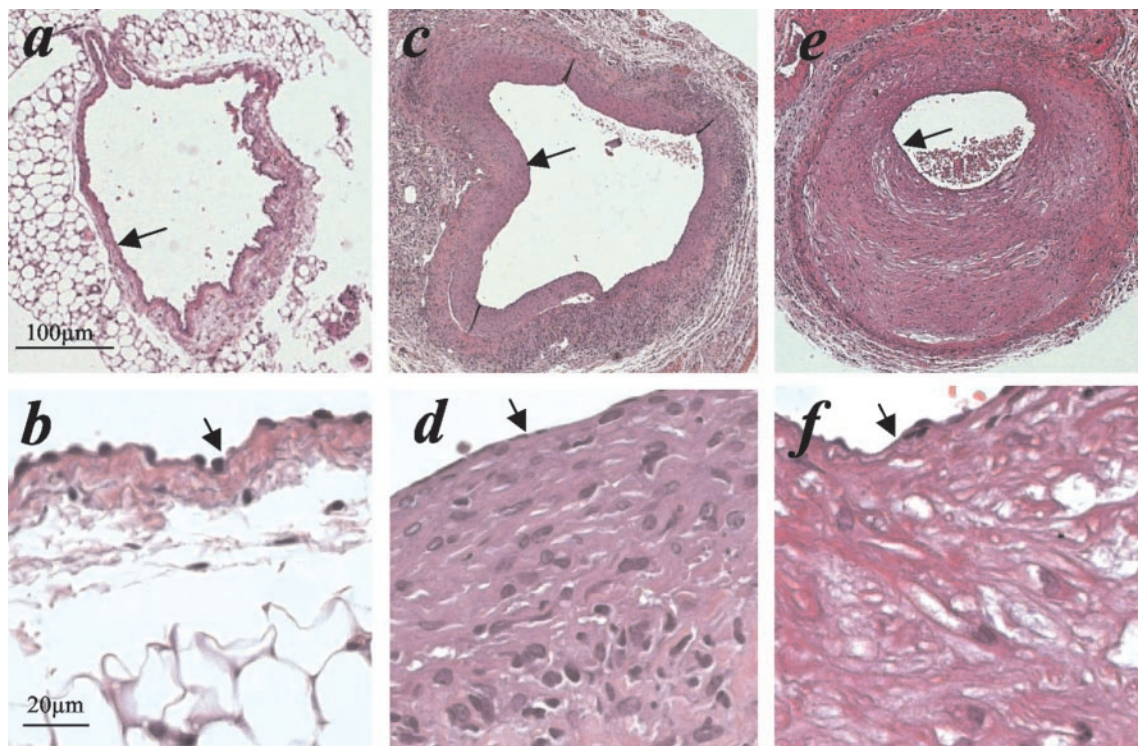

Figure 2. Morphology of neointimal and atherosclerotic lesions of vein grafts in mice. Vena cava (**a**, **b**) of the mouse was grafted into carotid arteries of wild-type (**c**, **d**) or apoE−/− (**e**, **f**) mice. Animals were sacrificed 8 weeks after surgery, and the grafted tissue fragments fixed, embedded in paraffin, sectioned, and stained with H&E. **Arrows** indicate the surface of vessel intima. Note that a proportion of lesions is shown in **d** and **f**.

ond, the traumatic and ischemic injuries to the grafts are minimal. Half an hour is needed to perform the whole operation by our trained surgeon, and the ischemia time of artery segments is between 5 to 10 minutes. Third, the successful rate of surgery is higher because the operation is performed in the neck region and takes a shorter time. Finally, two types of donor organs, jugular vein and vena cava, have been grafted into carotid arteries in the present experiments. Although accelerated arteriosclerosis develops in both vein grafts, the following differences exist. Vena cava is relatively easy to be sleeved over the cuff, which can be used for grafting the vessel donated by another mouse in a same strain or littermate. Because it is an isograft, the role of the specific gene tested in the disease development could be distinguished between the vessel wall and the host, eg, *apoE−/−* vena cava grafted to an *apoE+/+* recipient. Therefore, both donor organs can be used for vein grafts and the technique is feasible for the investigators.

The Pathogenesis of Vein Graft Atherosclerosis

With the help of the mouse model, it is possible to understand the cellular and molecular mechanisms of vein graft atherosclerosis. It was demonstrated that one of the earliest cellular events in neointima formation in arteriosclerosis is cell death, in which biomechanical stress is a critical initiator.[31] After cell death, massive mononuclear cell infiltration into the vessel wall occurs. The mechanism by which monocytes/macrophages are continuously recruited to the neointima of the vessel wall may involve two factors: Biomechanical stress directly stimulating endothelial cell and smooth muscle cell expression of adhesion molecules and chemokines; secondly dead cells may be an additional force for the induction of inflammatory responses in the vessel wall.[21] Eventually, cell differentiation, proliferation, and accumulation in the intima progress. In this process, biomechanical stress activates platelet-derived growth factor receptor-MAPK pathways, leading to cell proliferation.[22] Additional factors stimulating cell growth could be cytokines and growth factors released from inflammatory cells. Finally, vein graft atheroma could be observed in vein grafts performed in apoE−/−[32] or apoE*3-Leiden[33] transgenic mice, which are morphologically similar to the lesion in humans (Figure 2).

Based on the knowledge of the cellular processes obtained using the mouse model, we could then test the role of specific genes in the pathogenesis of atherosclerosis. For instance, mice lacking transcription factor p53, which is crucial in cell apoptosis, developed neointimal lesions in vein grafts with a twofold increase compared to wild-type controls.[34] Importantly, vascular cell apoptosis was significantly reduced in *p53*$^{-}$/$^{-}$ vein grafts, which coincided with oxidative DNA damage in vein grafts. In cultured smooth muscle cells from *p53*$^{-}$/$^{-}$ mice, apoptosis was increased in response to the death receptor ligand tumor necrosis factor-α, but decreased in response to the NO donor sodium nitroprusside, suggesting that different signaling pathways are involved in tumor necrosis factor-α- and NO-induced apoptosis, respectively.[35] Recent observations have shown that upstream

activators of p53 were involving p38 MAPK-integrin pathways in mechanical stress-induced apoptosis.[36,37]

Mechanical stretch has a critical role in lesion formation and so Lardenoye et al[38] investigated the effect of using an external stent to reduce wall stress. The result was more than 80% reduction in vein graft atherosclerosis and less vascular cell apoptosis. Cell death in vein grafts is followed by an inflammatory response during which expression of endothelial molecules, eg, ICAM-1. When vein grafts were performed in ICAM-1 knockout mice, a 50% reduction of arteriosclerotic lesions was seen. Similarly, interruption of cell migration and proliferation by locally applied drugs, eg, suramin,[39] and gene transfer, eg, tissue inhibitor of MMP2,[40] resulted in either reduced lesions or alterations in the process of graft remodeling.

Cell Origins of Vein Graft Lesions

Another significant progression made by using the mouse model is to answer the question of where lesional cells are derived from. After cell death in the early stage of vein grafts, endothelial regeneration and smooth muscle accumulation in the intima occur. Traditionally, it was believed that denuded endothelium can be replaced by the remaining cells in the vicinity and smooth muscle cells migrate from the media.[41] However, data of vein grafts in mice do not support this hypothesis and rather suggest a role of stem cells in repairing the damaged vessels. Using transgenic mice expressing LacZ gene only in endothelial cells, we provided the first evidence that the regenerated endothelial cells of vein grafts originated from recipient circulating blood, and not the remaining endothelial cells of donor vessels.[42] We also demonstrate that approximately one third of endothelial cells of vein grafts are derived from bone marrow stem cells. These data establish that circulating progenitor endothelial cells adhere to the graft and subsequently cover the surface of neointimal and atherosclerotic lesions of vein grafts.

Similarly, we provide solid evidence that neointimal and atherosclerotic smooth muscle cells of vein grafts originate from recipients and donor vessels, as identified directly by smooth muscle SM22-driven β-gal expression.[43] We observed that $\sim$22% of smooth muscle cells in atherosclerotic lesions were derived from recipients and 69% from grafted vessels.[43] These data establish the heterogeneous origins of smooth muscle cells in both neointimal and atherosclerotic lesions of vein grafts. Thus, these findings are crucial for understanding the pathogenesis of vein graft atherosclerosis, and for establishing a new strategy of therapeutic intervention for the disease.

Transplant Arteriosclerosis

Allograft-accelerated transplant arteriosclerosis is the main limitation of long-term survival of patients with solid organ transplantation. Characteristics of the lesions include endothelial damage, mononuclear cell infiltration, smooth muscle cell proliferation, and matrix protein deposition in the intima of the vessel wall.[44–46] The lesions eventually culminate in vascular stenosis and ischemic graft failure. In comparison to other types of arteriosclerosis, the initiating cause of the disease is relatively clear, ie, alloimmune reactions to the vessel wall. However, the pathogenesis of transplant arteriosclerosis is not fully understood, and mouse models would be helpful for clarifying the molecular mechanisms of the disease. So far, three types of mouse models have been established, including side-to-end grafting to carotid artery,[47] end-to-end to infrarenal aorta,[48] and end-to-end to carotid artery using a cuff technique.[49]

Shi and colleagues[47] have established the first mouse model of transplant arteriosclerosis by suturing end-to-side of vessel segments to carotid arteries, and Koulack and colleagues[48] developed an aortic transplantation to infrarenal aorta by end-to-end anastomosis. These mouse models have been proven to be useful tools in studying the pathogenesis of transplant arteriosclerosis.[50–54] In 2000, we described a simplified mouse model of transplant arteriosclerosis,[49] which was performed by end-to-end anastomosis to carotid artery using a cuff technique (Figure 1) similar to the method described for vein bypass grafts.[30] In our experience, this model has some advantages, eg, a simple operation procedure, less traumatic and ischemic injuries to the grafts, and a high surgical success rate. Neointimal lesion development in transplanted vessel segments of both carotid and aortic arteries is comparable to that of transplanted arteries by other methods. For instance, Shi and colleagues[54] demonstrated that neointimal lesions were reduced 52% in allograft arteries donated by *ICAM-1−/−* mice, whereas our observations indicate a 60% reduction in neointimal lesions of *ICAM-1−/−* arteries.[49]

Usage of Knockout Mice

Accumulating data indicates that knockout mice are a powerful tool for studying the genes or molecules contributing to transplant arteriosclerosis. Many reports have shown the role of cytokines in the development of transplant arteriosclerosis, eg, IL-10, tumor necrosis factor-α, and interferon (IFN)-γ.[55–57] Others have demonstrated the effects of genes related to cell proliferation, lipid metabolism, and NO production on disease progress.[58] In this review I will not cover all knockout mice studied in transplant arteriosclerosis, but give some examples to emphasize the role of the murine model.

IFN-γ regulates the proliferation and function of activated T lymphocytes, and plays a pivotal role in rejection by activating macrophages.[59] Two groups studied the effects of IFN-γ on transplant arteriosclerosis using *IFN-γ-deficient* mice.[56,57] They found that T lymphocytes and macrophages infiltrated coronary arteries of allografts in the wild-type recipients. In contrast, *IFN-γ*-deficient recipients did not develop thickening of the arterial intima, despite a T lymphocyte and macrophage infiltrate in the parenchyma. IFN-γ powerfully stimulates the increased

synthesis and cell surface expression of both class I and II major histocompatibility complex antigens *in vivo*, including bone marrow-derived immunocompetent cells, as well as cells of vessel wall. It can directly activate macrophages, and enhance expression of major histocompatibility complex products, other components of the antigen presentation pathway, and adhesion molecules.[60] This result was also confirmed by treatment with a neutralizing antibody against IFN-γ.

A second example is that the data obtained from the use of NO synthase (NOS) knockout mice. Inducible nitric oxide synthase (iNOS) is up-regulated in rejecting allografts and is protective against allograft arteriosclerosis; it suppresses neointimal smooth muscle cell accumulation and inhibits adhesion of platelets and leukocytes to the endothelium.[61] Lee and colleagues[62] examined the effects of selective eNOS deficiency in aortic allografts in a murine chronic rejection model using grafts from eNOS knockout mice (C57BL/6) and normal C3H as recipients. eNOS-deficient grafts showed significantly increased intima/media ratios at day 30 compared to controls. Immunostaining demonstrated that in eNOS KO grafts, eNOS was not detectable whereas iNOS was expressed prominently in infiltrating recipient mononuclear cells. They further demonstrated that early overexpression of iNOS by *ex vivo* gene transfer completely prevented the development of arteriosclerosis associated with eNOS deficiency. Therefore, eNOS plays a protective role in allografts, and in eNOS-deficient allografts, early overexpression of iNOS is capable of preventing the development of allograft arteriosclerosis. These data support the notion of the usefulness of knockout mice in investigating transplant arteriosclerosis.

Cell Origins in Transplant Arteriosclerosis

Another important contribution to our understanding the pathogenesis of transplant arteriosclerosis by using mouse models is clarification of cell origins in arteriosclerotic lesions of allografts. For a considerable time, it was believed that donor vessel cells contribute to the formation of arteriosclerotic lesions in allografts.[63] However, the results of mouse models from several laboratories have established that smooth muscle cells within the intimal lesions are completely derived from the recipients.[64–67]

Concerning the source of recipient-derived smooth muscle cells, Shimizu and colleagues[64] and Sata and colleagues[65] demonstrated that ~10 to 20% of α-actin+ cells in the neointimal lesions of allografts were co-localized with β-galactosidase+ (gal) cells in a chimeric mouse expressing β-gal in bone marrow cells. They concluded that host bone-marrow cells are, at least in part, a source of smooth muscle-like cells in transplant neointimal lesions. In fact, a large number of leukocytes infiltrate the vessel wall of allografts in the development of the disease, and they are in close contact with smooth muscle cells in the lesion. This would make it difficult to distinguish whether the α-actin/β-gal double-stained cell(s) in tissue sections are from one cell or two adjacent

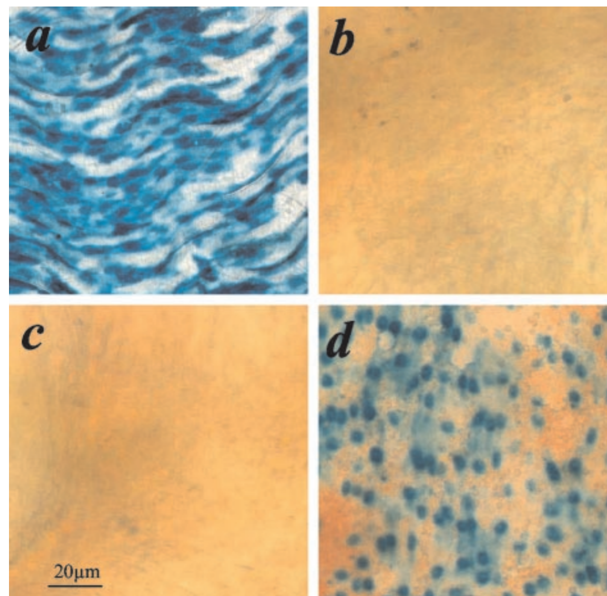

Figure 3. *En face* staining of aortic endothelial cells demonstrating the recipient origin. Freshly harvested aorta segments (**a**) from TIE2-LacZ mice were allografted into the carotid arteries of BALB/c mice (**b**). Freshly harvested aortic segments (**c**) from a BALB/c mouse were allografted into the carotid arteries of TIE2-LacZ mice (**d**). The grafts were harvested 4 weeks postoperatively and incubated with a substrate X-gal. After processing, *en face* photographs were taken. Blue color indicates β-gal+ cells.

cells. For better identification of smooth muscle cell origins, we designed experiments with aortic allografts in three types of transgenic mice expressing β-gal, ie, 1) all tissues (ROSA26), 2) only smooth muscle cells (SM-LacZ[68]), and 3) apoE knockout mice carrying LacZ genes in smooth muscle cells (SM-LacZ/apoE−/−[69]). We confirmed the recipient origin of smooth muscle cells in both neointimal and atherosclerotic lesions of allografts, but no β-gal-positive cells were found in the lesions of vessels grafted to mice with SM-LacZ bone marrow.[67] Therefore, bone marrow is unlikely to be a source of neointimal smooth muscle cells in transplant atherosclerosis.

Mouse models also simplify the identification of the origin of endothelial cells in transplant vessels. Very recently, we demonstrated that the regenerated endothelial cells of arterial allografts are originated from recipient circulating blood, rather than from remaining endothelial cells of donor vessels.[70] These data establish that circulating progenitor cells are sources of cells that contribute to neointimal lesions of allografts (Figure 3). This means that all cells of lesions are derived from the recipients, rather than donor vessel wall per se. Interestingly, this result was confirmed to be true for transplant arteriosclerosis in humans, ie, recipient origins of smooth muscle cells,[71] indicating a possibility of direct data translation from the mouse model to the human disease.

Choosing the Model

Although the mouse model is widely used by many laboratories, some problems might occasionally appear because of insufficient knowledge of the specific animal

models. Investigators, who are not experienced with the mouse model, would need to consider several issues before starting their experiments. Because the author's laboratory has experience with the generation of double-knockout mice in *apoE−/−* background, developed the model of vein graft arteriosclerosis, adapted a transplant model, and tested other models as well,[30,32,33,43,49,67,72,73] in this review, an attempt is made to provide some updated guidelines for the selection of animals that are most appropriate for the study of different aspects of arteriosclerosis.

Aim of the Research

Obviously, each study has different aims and requires different animal models. Some of them are clear and easy to determine. For instance, allograft transplantation would be needed to test the effects of genes or drugs on allo-immune responses in the development of transplant arteriosclerosis. If functions of genes related to endothelial cells are studied, endothelial injury models may be not suitable, which is also less suitable for studying inflammatory process, because of less inflammation in wire-injured models compared to vessel grafts. Thus, the researcher should carefully choose the model according to the nature of a variety of models described above. It is necessary to compromise and to select animals with characteristics that most satisfactorily fit the problem under study, ie, meet the aim of your study.

Genetic Background

It is well known that the genetic background of mice significantly influences the formation of atherosclerotic lesions in hyperlipidemic models.[7] Mice commonly used for studying hyperlipidemia-induced atherosclerosis are C57BL/6J strain, whereas C3H/HeJ, BALB/cJ and A/J are not sensitive to a cholesterol diet. Concerning the injury models, data indicate that neointimal lesions vary between different stains, but differ from diet-induced atherosclerosis, suggesting that injury-induced neointimal hyperplasia and diet-induced atherosclerosis are controlled by distinct sets of genes; the former appeared to be determined by recessive genes at ≥2 loci.[74] We compared neointimal lesions in vein isografts between C57BL/6J and BALB/c strains and did not find a significant difference in either inflammatory responses or the thickness of lesions, suggesting there is less effect of genetics on vein graft models (unpublished data). For transplant arteriosclerosis, different major histocompatibility complex class II antigens between donors and recipients are needed, eg, between C57BL/6J and BALB/c mice. Thus, careful selection of the model with different genetic background for your experiments is essential for the successful performance of the study.

Surgical Scale

Because of the small size, microsurgery is needed for all types of mouse models for studying arteriosclerosis. Before the model is selected, the investigator should consider the technical issues in establishing animal models. For instance, the quantification of atherosclerotic lesions in the root regions of the aorta and on aortic *en face* would be needed for hyperlipidemia-induced atherosclerosis. The technique for preparing sections and integrated aortas is relatively easier, but appropriate training is still required. Technically, the most difficult models would be vein bypass grafts and vessel transplant, although the cuff method makes the surgery much simplified.[30] However, it is possible for a trainee to get the first mouse living after surgery within 1 week. The supplemented movie for vein bypass grafts in mice could be helpful for researchers who are interested in the model (supplement). The level of surgical difficulties in establishing mouse models of vessel injury is much lower than that of vessel grafts. Especially, the model of carotid artery ligation could be managed by staff with minimal training in the laboratory. Therefore, it is fortunate that there are several models from which to choose to gain maximum applicability of the results to a greater understanding of arteriosclerosis.

Economic and Ethic Concerns

Choosing the most appropriate animal model for the study of a specific problem almost always presents a dilemma. In the consideration and fulfillment of primary requirements, which are the suitable and pertinent characteristics for the problems under investigation, one cannot ignore the availability and the expense of the required mice. Thus, quite often, compromises have to be made if an investigator is to fulfill the essential needs of a well-planned study. However, if one is to venture in to giving advice regarding a choice of the most satisfactory animal models for selected studies, there are a few examples. These include generation of double-knockout mice and establishment of plaque rupture models. Both models are time consuming and expensive. For example, if you have a knockout mouse with 129/B6 genetic background and want to cross to *apoE−/−* mice (C57BL/6J), it will take at least 2 years to obtain double-knockout mice in C57BL/6J background. The cost for the personnel and animal hospitalization for 2 years will be a great amount. In addition, because of ethic concerns, both project and personal licenses are usually required for all animal experiments. It takes 6 to 8 months in some countries from the application to obtain both licenses. Therefore, the investigators have to take account of the time schedule before planning their animal experiments.

Limitation

Although the mouse offers an incredibly valuable tool for the study of arteriosclerosis in the laboratory, it is essential for the investigator to be aware of similarities and differences that exist between animal models and human disease, and between various strains. This knowledge will prevent the likelihood of drawing false conclusions, and will allow an accurate evaluation of results.

The Difference between the Mouse and Human

During the last decade, a large proportion of our knowledge concerning the molecular and cellular mechanisms of arteriosclerosis comes from the use of mouse models. However, we must bear in mind that results obtained in the experimental animals may not translate directly to humans. Several crucial issues should be addressed when the data from mice are interpreted to the equivalent situation in humans. First, mice do not develop spontaneous atherosclerosis without genetic manipulation. Atherogenesis in humans usually occurs at lower lipid levels and throughout a much more prolonged time scale than generally used in experiments on mice genetically altered to enhance their susceptibility to atherosclerosis. Second, mice weigh ~25 g, some 3000 times less than the average man. Anatomical and physiological conditions vary between the mouse and human. For instance, a section of coronary artery in the mouse contains ~3000 times fewer cells than an equivalent section of human coronary artery. This is reflected in the histology of mouse arteries, in which the endothelial layer lies directly on the internal elastic lamina and the media consists of only two or three layers of smooth muscle cells. Finally, some models of mice, eg, vessel ligation, electric injury, and collar placement in the artery, do not mimic a physiological or pathological situations in humans. Therefore, caution must be advised when extrapolating from mouse model data to supposed human equivalents.

Genetic Background

As mentioned above, genetic background significantly influences the results of atherosclerosis, especially in hyperlipidemia animal models. For example, the extent of atherosclerosis among apoE knockout mice on a standard atherosclerosis-prone C57BL/6 background was found to be seven times greater than apoE knockout mice with an atherosclerosis-resistant FVB genetic background.[5] The ideal solution to this problem is to use inbred isogenic strains in which the experimental and control mice differ only at the target locus. For other models, genetic background of mice is also important. Therefore, a detailed description of the genetic background of all mouse models used in transgenic experiments should be given, and that the genetic background should always be taken into account when assessing experimental results.

Lesion Quantification

One of major impacts of the mouse on our understanding of arteriosclerosis has involved its genetic manipulation (ie, transgenic and knockout models) and treatment to examine the difference in lesion size in aortic roots or grafted/injured vessels. Most investigators are using the methods described by Paigen and colleagues[75] in 1987 to quantify the lesion areas on sections of aortic sinus in mouse models of hyperlipidemia-induced atherosclerosis. Two factors can markedly influence the results of lesion quantification. One is the angle of the heart that is fixed to the head of cryocut machine, which can lead to a variable size of lesions. Another is that the lesion size at the different levels of sections derived from aortic sinus (~300 μm) of different animals cannot be compared between two groups. This means that false results could be obtained if a different angle or levels of sections are compared between two groups. Similarly, injured vessels and vessel grafts from different groups can only be compared if the sections are derived from similar angle and location when sections are prepared from the vessel.

Perspectives

Studies aimed at testing and documenting either mechanistic or therapeutic interactions in mouse models have significantly improved our understanding of atherogenesis. For instance, smooth muscle cells in lesions of transplant arteriosclerosis in mice were derived from donor stem cells, which was verified to be similar to the cell origin of lesions in humans.[71] Therefore, because of results from animal studies, combined therapy, using dietary intervention and one or more drugs, eg, statin, is used in a variety of preventive and therapeutic approaches in humans. With the use of existing and newly developed mouse models to study atherosclerosis, one can anticipate that, in addition to already acquired knowledge concerning interrelationships between inflammation, lipid metabolism, the role of the artery wall, and genetic influences, there will be more opportunities to increase our comprehension of the lesion at the cellular and molecular levels. This knowledge should help us to acquire the scientific means to control the untoward disease process of arteriosclerosis in humans.

Acknowledgments

I thank all collaborators who contributed to the work summarized in the review and Dr. E. Torsney for the critical reading of the manuscript.

References

1. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull Jr W, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW: A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1994, 89:2462–2478
2. Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB, Chesebro JH: Syndromes of accelerated atherosclerosis: role of vascular injury and smooth muscle cell proliferation. J Am Coll Cardiol 1990, 15:1667–1687
3. Ignatowski AC: Influence of animal food on the organism of rabbits. Izvest Imper Voennomed Akad St Petersburg 1908, 16:154–173
4. Breslow JL: Transgenic mouse models of lipoprotein metabolism and atherosclerosis. Proc Natl Acad Sci USA 1993, 90:8314–8318
5. Knowles JW, Maeda N: Genetic modifiers of atherosclerosis in mice. Arterioscler Thromb Vasc Biol 2000, 20:2336–2345
6. de Winther MP, Hofker MH: New mouse models for lipoprotein metabolism and atherosclerosis. Curr Opin Lipidol 2002, 13:191–197
7. Allayee H, Ghazalpour A, Lusis AJ: Using mice to dissect genetic

factors in atherosclerosis. Arterioscler Thromb Vasc Biol 2003, 23:1501–1509
8. Levine GN, Chodos AP, Loscalzo J: Restenosis following coronary angioplasty: clinical presentations and therapeutic options. Clin Cardiol 1995, 18:693–703
9. Schwartz SM, deBlois D, O'Brien ER: The intima. Soil for atherosclerosis and restenosis. Circ Res 1995, 77:445–465
10. Clowes AW, Breslow JL, Karnovsky MJ: Regression of myointimal thickening following carotid endothelial injury and development of aortic foam cell lesions in long term hypercholesterolemic rats. Lab Invest 1977, 36:73–81
11. Lindner V, Fingerle J, Reidy MA: Mouse model of arterial injury. Circ Res 1993, 73:792–796
12. Kumar A, Lindner V: Remodeling with neointima formation in the mouse carotid artery after cessation of blood flow. Arterioscler Thromb Vasc Biol 1997, 17:2238–2244
13. Carmeliet P, Moons L, Stassen JM, De Mol M, Bouche A, van den Oord JJ, Kockx M, Collen D: Vascular wound healing and neointima formation induced by perivascular electric injury in mice. Am J Pathol 1997, 150:761–776
14. Leidenfrost JE, Khan MF, Boc KP, Villa BR, Collins ET, Parks WC, Abendschein DR, Choi ET: A model of primary atherosclerosis and post-angioplasty restenosis in mice. Am J Pathol 2003, 163:773–778
15. Wessely R, Hengst L, Jaschke B, Wegener F, Richter T, Lupetti R, Paschalidis M, Schomig A, Brandl R, Neumann FJ: A central role of interferon regulatory factor-1 for the limitation of neointimal hyperplasia. Hum Mol Genet 2003, 12:177–187
16. Galis ZS, Johnson C, Godin D, Magid R, Shipley JM, Senior RM, Ivan E: Targeted disruption of the matrix metalloproteinase-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling. Circ Res 2002, 91:852–859
17. Manka D, Collins RG, Ley K, Beaudet AL, Sarembock IJ: Absence of p-selectin, but not intercellular adhesion molecule-1, attenuates neointimal growth after arterial injury in apolipoprotein E-deficient mice. Circulation 2001, 103:1000–1005
18. Schober A, Manka D, von Hundelshausen P, Huo Y, Hanrath P, Sarembock IJ, Ley K, Weber C: Deposition of platelet RANTES triggering monocyte recruitment requires P-selectin and is involved in neointima formation after arterial injury. Circulation 2002, 106:1523–1529
19. Zimmerman MA, Selzman CH, Reznikov LL, Miller SA, Raeburn CD, Emmick J, Meng X, Harken AH: Lack of TNF-alpha attenuates intimal hyperplasia after mouse carotid artery injury. Am J Physiol 2002, 283:R505–R512
20. Gimbrone Jr MA, Resnick N, Nagel T, Khachigian LM, Collins T, Topper JN: Hemodynamics, endothelial gene expression, and atherogenesis. Ann NY Acad Sci 1997, 811:1–11
21. Xu Q: Biomechanical-stress-induced signaling and gene expression in the development of arteriosclerosis. Trends Cardiovasc Med 2000, 10:35–41
22. Li C, Xu Q: Mechanical stress-initiated signal transductions in vascular smooth muscle cells. Cell Signal 2000, 12:435–445
23. Guyton JR, Hartley CJ: Flow restriction of one carotid artery in juvenile rats inhibits growth of arterial diameter. Am J Physiol 1985, 248:H540–H546
24. Langille BL, O'Donnell F: Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. Science 1986, 231:405–407
25. Ku DN, Giddens DP, Zarins CK, Glagov S: Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. Arteriosclerosis 1985, 5:293–302
26. Booth RF, Martin JF, Honey AC, Hassall DG, Beesley JE, Moncada S: Rapid development of atherosclerotic lesions in the rabbit carotid artery induced by perivascular manipulation. Atherosclerosis 1989, 76:257–268
27. Moroi M, Zhang L, Yasuda T, Virmani R, Gold HK, Fishman MC, Huang PL: Interaction of genetic deficiency of endothelial nitric oxide, gender, and pregnancy in vascular response to injury in mice. J Clin Invest 1998, 101:1225–1232
28. Davies MG, Hagen PO: Pathobiology of intimal hyperplasia. Br J Surg 1994, 81:1254–1269
29. Mehta D, Izzat MB, Bryan AJ, Angelini GD: Towards the prevention of vein graft failure. Int J Cardiol 1997, 62(Suppl 1):S55–S63
30. Zou Y, Dietrich H, Hu Y, Metzler B, Wick G, Xu Q: Mouse model of venous bypass graft arteriosclerosis. Am J Pathol 1998, 153:1301–1310
31. Mayr M, Li C, Zou Y, Huemer U, Hu Y, Xu Q: Biomechanical stress-induced apoptosis in vein grafts involves p38 mitogen-activated protein kinases. FASEB J 2000, 14:261–270
32. Dietrich H, Hu Y, Zou Y, Huemer U, Metzler B, Li C, Mayr M, Xu Q: Rapid development of vein graft atheroma in ApoE-deficient mice. Am J Pathol 2000, 157:659–669
33. Lardenoye JH, de Vries MR, Lowik CW, Xu Q, Dhore CR, Cleutjens JP, van Hinsbergh VW, van Bockel JH, Quax PH: Accelerated atherosclerosis and calcification in vein grafts: a study in APOE*3 Leiden transgenic mice. Circ Res 2002, 91:577–584
34. Mayr U, Mayr M, Li C, Wernig F, Dietrich H, Hu Y, Xu Q: Loss of p53 accelerates neointimal lesions of vein bypass grafts in mice. Circ Res 2002, 90:197–204
35. Mayr M, Hu Y, Hainaut H, Xu Q: Mechanical stress-induced DNA damage and rac-p38MAPK signal pathways mediate p53-dependent apoptosis in vascular smooth muscle cells. FASEB J 2002, 16:1423–1425
36. Wernig F, Xu Q: Mechanical stress-induced apoptosis in cardiovascular system. Prog Biophys Mol Biol 2002, 78:105–137
37. Wernig F, Mayr M, Xu Q: Mechanical stretch-induced apoptosis in smooth muscle cells is mediated by beta1-integrin signaling pathways. Hypertension 2003, 41:903–911
38. Lardenoye JH, De Vries MR, Grimbergen JM, Havekes LM, Knaapen MW, Kockx MM, van Hinsbergh VW, van Bockel JH, Quax PH: Inhibition of accelerated atherosclerosis in vein grafts by placement of external stent in apoE*3-Leiden transgenic mice. Arterioscler Thromb Vasc Biol 2002, 22:1433–1438
39. Hu Y, Zou Y, Dietrich H, Wick G, Xu Q: Inhibition of neointima hyperplasia of mouse vein grafts by locally applied suramin. Circulation 1999, 100:861–868
40. Hu Y, Baker AH, Zou Y, Newby AC, Xu Q: Local gene transfer of tissue inhibitor of metalloproteinase-2 influences vein graft remodeling in a mouse model. Arterioscler Thromb Vasc Biol 2001, 21:1275–1280
41. Ross R: The pathogenesis of atherosclerosis—an update. N Engl J Med 1986, 314:488–500
42. Xu Q, Zhang Z, Davison F, Hu Y: Circulating progenitor cells regenerate endothelium of vein graft atherosclerosis, which is diminished in apoE-deficient mice. Circ Res 2003, 93:e76–e86
43. Hu Y, Mayr M, Metzler B, Erdel M, Davison F, Xu Q: Both donor and recipient origins of smooth muscle cells in vein graft atherosclerotic lesions. Circ Res 2002, 91:e13–e20
44. Uretsky BF, Murali S, Reddy PS, Rabin B, Lee A, Griffith BP, Hardesty RL, Trento A, Bahnson HT: Development of coronary artery disease in cardiac transplant patients receiving immunosuppressive therapy with cyclosporine and prednisone. Circulation 1987, 76:827–834
45. Weis M, von Scheidt W: Cardiac allograft vasculopathy: a review. Circulation 1997, 96:2069–2077
46. Libby P, Pober JS: Chronic rejection. Immunity 2001, 14:387–397
47. Shi C, Russell ME, Bianchi C, Newell JB, Haber E: Murine model of accelerated transplant arteriosclerosis. Circ Res 1994, 75:199–207
48. Koulack J, McAlister VC, Giacomantonio CA, Bitter-Suermann H, MacDonald AS, Lee TD: Development of a mouse aortic transplant model of chronic rejection. Microsurgery 1995, 16:110–113
49. Dietrich H, Hu Y, Zou Y, Dirnhofer S, Kleindienst R, Wick G, Xu Q: Mouse model of transplant arteriosclerosis: role of intercellular adhesion molecule-1. Arterioscler Thromb Vasc Biol 2000, 20:343–352
50. Koulack J, McAlister VC, MacAulay MA, Bitter-Suermann H, MacDonald AS, Lee TD: Importance of minor histocompatibility antigens in the development of allograft arteriosclerosis. Clin Immunol Immunopathol 1996, 80:273–277
51. Shi C, Lee WS, He Q, Zhang D, Fletcher Jr DL, Newell JB, Haber E: Immunologic basis of transplant-associated arteriosclerosis. Proc Natl Acad Sci USA 1996, 93:4051–4056
52. Sun H, Subbotin V, Chen C, Aitouche A, Valdivia LA, Sayegh MH, Linsley PS, Fung JJ, Starzl TE, Rao AS: Prevention of chronic rejection in mouse aortic allografts by combined treatment with CTLA4-Ig and anti-CD40 ligand monoclonal antibody. Transplantation 1997, 64:1838–1843
53. Moons L, Shi C, Ploplis V, Plow E, Haber E, Collen D, Carmeliet P: Reduced transplant arteriosclerosis in plasminogen-deficient mice. J Clin Invest 1998, 102:1788–1797

54. Shi C, Feinberg MW, Zhang D, Patel A, Sim CU, Dong ZM, Chapman SM, Gutierrez-Ramos JC, Wagner DD, Sibinga NE, Haber E: Donor MHC and adhesion molecules in transplant arteriosclerosis. J Clin Invest 1999, 103:469–474
55. Furukawa Y, Becker G, Stinn JL, Shimizu K, Libby P, Mitchell RN: Interleukin-10 (IL-10) augments allograft arterial disease: paradoxical effects of IL-10 in vivo. Am J Pathol 1999, 155:1929–1939
56. Nagano H, Mitchell RN, Taylor MK, Hasegawa S, Tilney NL, Libby P: Interferon-gamma deficiency prevents coronary arteriosclerosis but not myocardial rejection in transplanted mouse hearts. J Clin Invest 1997, 100:550–557
57. Tellides G, Tereb DA, Kirkiles-Smith NC, Kim RW, Wilson JH, Schechner JS, Lorber MI, Pober JS: Interferon-gamma elicits arteriosclerosis in the absence of leukocytes. Nature 2000, 403:207–211
58. Lee JK, Borhani M, Ennis TL, Upchurch Jr GR, Thompson RW: Experimental abdominal aortic aneurysms in mice lacking expression of inducible nitric oxide synthase. Arterioscler Thromb Vasc Biol 2001, 21:1393–1401
59. Nathan CF, Murray HW, Wiebe ME, Rubin BY: Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J Exp Med 1983, 158:670–689
60. Skoskiewicz MJ, Colvin RB, Schneeberger EE, Russell PS: Widespread and selective induction of major histocompatibility complex-determined antigens in vivo by gamma interferon. J Exp Med 1985, 162:1645–1664
61. Shears LL, Kawaharada N, Tzeng E, Billiar TR, Watkins SC, Kovesdi I, Lizonova A, Pham SM: Inducible nitric oxide synthase suppresses the development of allograft arteriosclerosis. J Clin Invest 1997, 100: 2035–2042
62. Lee PC, Wang ZL, Qian S, Watkins SC, Lizonova A, Kovesdi I, Tzeng E, Simmons RL, Billiar TR, Shears II LL: Endothelial nitric oxide synthase protects aortic allografts from the development of transplant arteriosclerosis. Transplantation 2000, 69:1186–1192
63. Kennedy Jr LJ, Weissman IL: Dual origin of intimal cells in cardiac-allograft arteriosclerosis. N Engl J Med 1971, 285:884–887
64. Shimizu K, Sugiyama S, Aikawa M, Fukumoto Y, Rabkin E, Libby P, Mitchell RN: Host bone-marrow cells are a source of donor intimal smooth-muscle-like cells in murine aortic transplant arteriopathy. Nat Med 2001, 7:738–741
65. Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, Hirai H, Makuuchi M, Hirata Y, Nagai R: Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat Med 2002, 8:403–409
66. Li J, Han X, Jiang J, Zhong R, Williams GM, Pickering JG, Chow LH: Vascular smooth muscle cells of recipient origin mediate intimal expansion after aortic allotransplantation in mice. Am J Pathol 2001, 158:1943–1947
67. Hu Y, Davison F, Ludewig B, Erdel M, Mayr M, Url M, Dietrich H, Xu Q: Smooth muscle cells in transplant atherosclerotic lesions are originated from recipients, but not bone marrow progenitor cells. Circulation 2002, 106:1834–1839
68. Moessler H, Mericskay M, Li Z, Nagl S, Paulin D, Small JV: The SM 22 promoter directs tissue-specific expression in arterial but not in venous or visceral smooth muscle cells in transgenic mice. Development 1996, 122:2415–2425
69. Ludewig B, Freigang S, Jaggi M, Kurrer MO, Pei YC, Vlk L, Odermatt B, Zinkernagel RM, Hengartner H: Linking immune-mediated arterial inflammation and cholesterol-induced atherosclerosis in a transgenic mouse model. Proc Natl Acad Sci USA 2000, 97:12752–12757
70. Hu Y, Davison F, Zhang ZG, Xu Q: Endothelial replacement and angiogenesis in arteriosclerotic lesions of allografts are contributed by circulating progenitor cells. Circulation 2003, 108:3122–3127
71. Glaser R, Lu MM, Narula N, Epstein JA: Smooth muscle cells, but not myocytes, of host origin in transplanted human hearts. Circulation 2002, 106:17–19
72. Metzler B, Mair J, Lercher A, Schaber C, Hintringer F, Pachinger O, Xu Q: Mouse model of myocardial remodelling after ischemia: role of intercellular adhesion molecule-1. Cardiovasc Res 2001, 49:399–407
73. Zou Y, Hu Y, Mayr M, Dietrich H, Wick G, Xu Q: Reduced neointima hyperplasia of vein bypass grafts in intercellular adhesion molecule-1-deficient mice. Circ Res 2000, 86:434–440
74. Kuhel DG, Zhu B, Witte DP, Hui DY: Distinction in genetic determinants for injury-induced neointimal hyperplasia and diet-induced atherosclerosis in inbred mice. Arterioscler Thromb Vasc Biol 2002, 22:955–960
75. Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA: Quantitative assessment of atherosclerotic lesions in mice. Atherosclerosis 1987, 68:231–240