Animal Model

Mouse Model of Venous Bypass Graft Arteriosclerosis

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Saphenous vein grafts are widely used for treatment of severe atherosclerosis via aortocoronary bypass surgery, a procedure often complicated by later occlusion of the graft vessel. Because the molecular mechanisms of this process remain largely unknown, quantitative models of venous bypass graft arteriosclerosis in transgenic mice could be useful to study this process at the genetic level. We describe herein a new model of vein grafts in the mouse that allows us to take advantage of transgenic, knockout, or mutant animals. Autologous or isogenic vessels of the external jugular or vena cava veins were end-to-end grafted into carotid arteries of C57BL/6J mice. Vessel wall thickening was observed as early as 1 week after surgery and progressed to 4-, 10-, 15-, and 18-fold original thickness in grafted veins at age 2, 4, 8, and 16 weeks, respectively. The lumen of grafted veins was significantly narrowed because of neointima hyperplasia. Histological and immunohistochemical analyses revealed three lesion processes: marked loss of smooth muscle cells in vein segments 1 and 2 weeks after grafting, massive infiltration of mononuclear cells (CD11b/18⁺) in the vessel wall between 2 and 4 weeks, and a significant proliferation of vascular smooth muscle cells (α -actin⁺) to constitute neointimal lesions between 4 and 16 weeks. Similar vein graft lesions were obtained when external jugular veins or vena cava were isografted into carotid arteries of C57BL/6J mice. Moreover, no significant intima hyperplasia in vein-to-vein isografts was found, although there was leukocyte infiltration in the vessel wall. Thus, this model, which reproduces many of the features of human vein graft arteriosclerosis, should prove useful for our understanding of the mechanism of vein graft disease and to evaluate the effects of drugs and gene therapy on vascular diseases. (*Am J Pathol* 1998, 153:1301–1310)

Autologous vein grafts remain the only surgical alternative for many types of vascular reconstruction, but the failure rate of these grafts after 1 year approaches 20%.¹ The development of neointima or arteriosclerosis in the grafted vessel often leads to obliterative stenosis. The hallmarks of neointima lesions are mononuclear cell infiltration, smooth muscle cell proliferation, and extracellular matrix deposition.² The pathogenesis of this disease remains poorly understood, and no successful clinical interventions have been identified.

Several animal models manifesting lesions resembling human vein graft arteriosclerosis have been developed^{3–7} and have helped address specific interventional issues, but they have not helped to clarify the underlying mechanism of the disease. Attracted by the well-defined genetic systems, a number of investigators have begun to use the mouse as an experimental system for atherosclerosis research.^{8–11} Hundreds of inbred lines have been established, the genetic map is relatively well defined, and both congenic strains and recombinant strains are available to facilitate genetic experimentation. In just a few years, murine lipoproteins have been characterized, genetic variants of apolipoproteins have been identified,^{12,13} and genetic variation in susceptibility to atherosclerosis among inbred mouse strains has been demonstrated. The study of vein graft arteriosclerosis in such strains should make it possible to define the specific relations of many genes and cell types to the pathogenesis of this lesion. For example, it has been postulated that hypercholesterolemia is a risk factor for the development of lesions in vein grafts,¹⁴ and it is now possible to study mice that lack apolipoprotein E-containing lipoproteins or low-density lipoprotein receptors.^{13,15} Mice are also available that have deficient macrophage function¹⁶ or lack endothelial adhesion molecules^{17,18} or nitric oxide synthases,^{19,20} which might be important molecules in the development of venous bypass graft arteriosclerosis.

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In the present study, we describe a simple model wherein external jugular or vena cava veins were auto- or isografted into carotid arteries in C57BL/6J mice. We observed intimal lesions within 14 days that progress to marked stenosis in the grafted vessel within 16 weeks. We demonstrated that inflammatory features appeared in the lesions at the early stages followed by smooth muscle cell proliferation and extracellular matrix deposition in the vein graft neointima.

Materials and Methods

Mice

Three month-old male C57BL/6J were purchased from the Charles River Laboratory (Sulzfeld, Germany) and maintained for 1 week on a light/dark (12-hour/12-hour) cycle at 24°C and received food and water *ad libitum* before experimentation. All procedures were performed according to protocols approved by the Institutional Committee for Use and Care of Laboratory Animals. C57BL/6J mice were used as donors and recipients for vein grafts, because these mice are susceptible to atherosclerosis when a cholesterol-enriched diet is administered.⁸⁻¹³ In addition, many mutant or knockout mice are available with this genetic background.

Vein Graft Procedure

Mice were anesthetized with pentobarbital sodium (50 mg/kg body weight, intraperitoneally). Atropine sulfate (1.7 mg/kg body weight) was administered to maintain the respiratory tract in good condition. The operation was performed under a dissecting microscope (Wild M8, Basel, Switzerland). The mouse was fixed in a supine position with its neck extended. A midline incision was made on the ventral side of the neck from the lower mandible to the sternum. The right cleidomastoid muscle was resected.

The vein-grafting procedure is schematically illustrated in Figure 1. The right common carotid artery was mobilized free from the bifurcation in the distal end toward the proximal end as far as possible. The vessel was ligated with an 8-0 silk suture and dissected between the middle ties. The proximal and distal portions of the artery were passed through cuffs made of a polyethylene cannula 0.65 mm in diameter outside and 0.5 mm inside (Portex LTD, London, United Kingdom). The cuff length was 1 mm with a 1-mm handle or extension. The vessel, together with the handle, were fixed by microhemostat clamps (4 mm in length; Martin, Tuttlingen, Germany). The suture at the end of the artery was removed, and a segment of the artery was everted over the cuff body with a stent and fine tweezers and fixed to the cuff with an 8-0 silk suture. Another portion of the artery was similarly prepared (Figure 1, a through d).

Three types of vein grafts, autologous external jugular vein, isogeneic jugular, and vena cava veins, were used in the present study. The right external jugular vein was exposed, and three branches were ligated with an elec-

Figure 1. Schematic representation of vein bypass graft. The right common carotid artery was ligated with an 8-0 silk suture (a) and dissected between the middle ties and passed through the cuffs, respectively (b). 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 was turned inside out with a stent and fine tweezers to cover the cuff body (c), which was fixed to the cuff with an 8-0 silk suture (d). The right external jugular or vena cava vein segment (1 cm) 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 (e). The cuff handle was cut off, and the vascular clamps were removed; pulsations were seen in the grafted vein.

trocoagulator (model SN 54.131; Martin). Both ends were ligated with the suture, and a 1-cm vein segment was removed. For vena cava preparation, a midline abdominal incision was made in the donor animal, and 0.5 ml of saline solution containing 100 units of heparin was injected into the inferior vena cava. After 3 minutes, the anterior thoracic cage was opened from the diaphragm and incised laterally to the internal mammary vessels, and the vena cava (1 cm) was removed. Each vein graft was harvested carefully to avoid mechanical injury during surgical preparation. All grafts were washed with saline solution containing 100 U/ml of heparin.

The vein segment 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 (Figure 1e). The cuff handle was cut off after completion of the anastomoses, the vascular clamps were removed, and evidence of pulsations was sought in both the grafted and native vessels. If there were no pulsations or pulsations diminished within a few minutes of restoration of blood flow, clot formation or occlusion of output was assumed, and the procedure was considered a surgical failure. If there were vigorous pulsations in the grafted vessel, the skin incision was closed with a 6-0 interrupted

suture. One hour was needed to perform the whole operation, and the ischemia time of vein segments was about 15 minutes.

For histological analysis, perfusion was performed as described previously.²¹ Briefly, mice were anesthetized and perfused with 0.9% NaCl solution via cardiac puncture in the left ventricle, and subsequently perfusion fixed with 4% phosphate-buffered formaldehyde (pH 7.2) for 2 and 5 minutes, respectively. The vein grafts were harvested at 1, 2, 4, 8, and 16 weeks postoperatively (six to eight randomly chosen mice at each time point) by cutting the transplanted segments from the native vessels at the cuff end. Samples were fixed with 4% phosphate-buffered formaldehyde at 4°C for 24 hours. For frozen section preparation, mice were sacrificed by cervical dislocation, and vein grafts were harvested, immediately frozen in liquid nitrogen, and stored at -80°C.

Histology and Lesion Quantification

After fixation, the grafts were cut in the middle of the vein segments, dehydrated in graded ethanol baths, cleared in xylol, and embedded in paraffin.²² Histological sectioning began at the center of the graft to avoid the effects of the cuff. Routinely, 7 μ m-thick sections were made throughout the dissected fragments, stained with hematoxylin and eosin (H&E), and examined microscopically (Leitz, Munich, Germany).

Because the venous media is very thin (one or two layered cells or 10 to 20 μ m thick), the thickness of the normal and lesioned vessel walls was simultaneously measured and calculated microscopically. The intima and media were defined as the region between the lumen and the adventitia. The thickness of the vessel wall was determined by measuring four regions of a section along a cross and recorded in micrometers (means $\pm$ SD). Ten cross-sections were obtained by selecting the first of every three sections from each animal. Cell counts in the intima and media were performed on two regions of each section and expressed as the number of nuclei per 100 μ m of the vessel wall.

Immunohistochemical Staining

The procedure used in the present study was similar to that described previously.²³ Briefly, serial 5 μ m-thick frozen sections were cut from cryopreserved tissue blocks, fixed in a cold 1:1 acetone-chloroform mixture for 10 minutes, and washed with phosphate-buffered saline (PBS) for 20 minutes. The sections were subsequently placed in a humidified chamber, where they were overlaid with a rat monoclonal antibody (CD11b/18) against mouse MAC-1 leukocytes (PharMingen, San Diego, CA) and incubated for 1 hour at room temperature. After washing with PBS, sections were incubated with rabbit anti-rat immunoglobulin (Dakopatts, Copenhagen, Denmark) for 1 hour. Sections were washed in PBS three times, incubated with alkaline phosphatase-anti-alkaline phosphatase complex (Dakopatts) for 30 minutes, washed in PBS three times, and developed for 20 min-

utes at room temperature on a shaker using a substrate solution containing 9.8 ml of Tris buffer (0.1 mol/L, pH 8.2), 0.2 ml of dimethylformamide, 8 mg of naphthol AS-MX phosphate, 3 mg of levamisole, and 10 mg of fast red TR salt (Sigma Chemical Co., St. Louis, MO). A counterstaining with hematoxylin was performed at room temperature for 3 minutes. For smooth muscle cell staining, a mouse monoclonal antibody against α -actin (Sigma) labeled with phosphatase was used. The procedure was similar to that described for CD11b/18 labeling except for omission of the second antibody. Semiquantitative evaluation was performed at 10 $\times$ 25 magnification. Positive-stained cells in the intima and media were counted on two regions of each section and expressed as the range of the cell number or the percentage of total nuclei per 100 μ m of the vessel wall.

Statistical Analysis

Statistical analyses were performed on a Macintosh computer with StatView SE+Graphics software using the Mann-Whitney *U* test and analysis of variance, respectively. Results are given as means $\pm$ standard deviations (SD). A *P* value <0.05 was considered significant.

Results

Figure 1 schematically represents the procedure of grafting a vein segment to the carotid artery using the cuff technique. This method is technically simple, easy to learn, and less traumatic than the suturing technique. Using this method, a total of 57 vein grafts in C57BL/6J mice were studied, with a surgical success rate of about 90%. Fifty-four of 57 animals survived until their designated time of harvest, and all vein grafts were patent at the time of harvest. Three vein grafts were nearly occluded because of thrombosis. All control veins and vein grafts were histologically examined, and five per group were immunohistochemically analyzed.

Neointima Formation in Jugular Vein Autografts

Representative histological sections of control external jugular vein and vein grafts are shown in Figure 2. In the control vein, only two layers of cells, presumably a monolayer each of endothelial and smooth muscle cells, respectively, formed the intima and media, whereas the adventitia was composed of connective tissues, including vasa vasorum (Figure 2A). Interestingly, significant cell loss and vessel wall degeneration in the vein graft was observed 1 week after implantation simultaneous to connective tissue deposition and mononuclear cell infiltration in adventitia (Figure 2B). Concordant with these observations is a report that a loss of endothelial and smooth muscle cells in human saphenous vein bypass grafts was demonstrated 1 to 10 days postoperatively,²⁴ suggesting that changes in the early stage of grafts in this mouse model are similar to those in humans. By 2 weeks,

Figure 2. H&E-stained sections of mouse control vein and vein grafts. Mice underwent anesthesia, and the external jugular vein was grafted into the carotid artery. Animals were sacrificed at 0 (A), 1 (B), 2 (C), 4 (D), 8 (E), and 16 (F) weeks after surgery, and the grafted tissue fragments were fixed in 4% phosphate-buffered (pH 7.2) formaldehyde, embedded in paraffin, sectioned, and stained with H&E. Arrowheads indicate the control vessel wall (A) and neointima (B through D). A portion of neointima was shown in E and F; bar = 50 μ m.

mononuclear cells infiltrated into the vessel wall from both lumen and adventitia sides (Figure 2C).

In late vein grafts, 4-, 8-, and 16-week autograft sections (Figure 2, D through F) showed neointima hyperplasia that thickened rapidly. Obviously, three changes can be found: thickening of the vessel wall up to 10 or 20 layers of cells, declining infiltrated mononuclear cells, and increasing matrix protein accumulation in neointima. The 16-week lesion especially showed a lower cell number and an abundance of matrix proteins (Figure 2F). Neointima hyperplasia significantly narrowed the lumen to 20% of the original size by 16 weeks, and no neovascularization in the lesion was observed (Figure 3).

To statistically analyze vein graft remodeling, Figure 4 summarizes data of neointima thickness measured microscopically. Thickening of the vein grafts began as early as 1 week after surgery, although no significant difference was found compared with controls. The thickness of the vessel wall increased significantly in 2-week vein grafts, and neointima hyperplasia progressed throughout all time points. During vein graft remodeling, 4-, 10-, 15-, and 18-fold increases in vessel wall thicknesses were found in 2, 4, 8 and 16 week grafts, respectively (Figure 4B). When cell nuclei in the intima and

media of control veins and neointima of grafted vessels were counted in 100 μ m length, total cell numbers increased significantly in 2-week grafts, were markedly higher in 4- and 8-week grafts, and then began to decline (Figure 5), observations similar to those in other animal models such as rats, rabbits, and pigs.³⁻⁷

Figure 3. H&E-stained section of mouse vein graft 16 weeks after operation. Arrows indicate neointima; bar = 50 μ m.

Figure 4. Neointima thickness in vein autografts. The procedure for animal models and the preparation of H&E-stained sections are the same as described in the legend to Figure 2. Thickness was measured microscopically. Four regions of each section along a cross were measured, and five sections per animal were selected. **A** shows a graph of intima and media or neointima thickness (mean $\pm$ SD) obtained from six to eight animals per time point. **B** shows data (mean) in fold converted from **A**, where the thickness of the control vein intima and media is taken as one. *Significant difference from the control; $P < 0.05$.

Neointima Formation in Vein Isografts

To determine the role of a specific gene or protein in the development of neointima in vein grafts, an animal model of vein isografts is needed. For instance, the effect of low-density lipoprotein receptors of endothelial and smooth muscle cells on vein graft remodeling in normocholesterolemia can be identified by isografting low-density lipoprotein receptor-deficient vein segments into arteries of wild-type littermates. We therefore established several isograft animal models, including vena cava segments isografted to external jugular veins and vena cava or external jugular vein segments isografted to the arteries. Data shown in Figure 6 indicate the process of vein graft remodeling. The structure and diameter (about 0.9 mm) of the vena cava vessel were similar to those of

Figure 5. Neointima hyperplasia in vein autografts. The procedure for animal models and the preparation of H&E-stained sections are the same as those described in the legend to Figure 2. Total H&E-stained nuclei in 100-μm lengths of (neo)intima and media of veins were counted manually. Two opposite areas from each section were counted, and five sections per animal were selected. The graph shows data (mean $\pm$ SD) obtained from six to eight animals per time point. *Significant difference from the control; $P < 0.05$.

external jugular veins (Figure 6, A and B). Both vein segments can be used as graft sources in bypass graft models. In addition, intima thickening in vein-to-vein isografts was much less significant than that of vein-to-artery isografts 4 weeks after surgery (Figure 6C), although there was leukocyte infiltration in the vessel wall.

The results shown in Figure 6, D through F, provide evidence that the formation of neointima in grafted veins was similar in both vena cava isografts and jugular vein autografts. Likewise, mononuclear cell infiltration and matrix protein deposition in the neointima were demonstrated in all three types of grafts. To statistically compare the three vein bypass grafts, the thickness of neointima was measured as described in Figure 4 and as summarized in Figure 7, indicating that neointima hyperplasia occurred in all three grafts. No significant difference in the lesioned areas of either autografts or isografts was seen, suggesting that comparable results can be obtained from both animal models.

Cell Composition in Vein Graft Lesions

There is evidence that lesions of vein graft-induced neointima derived from other animal models and human specimens contain macrophages and smooth muscle cells.^{25,26} To analyze the kinetics of cell components in the development of neointima hyperplasia of vein grafts, immunohistochemical staining using monoclonal antibodies against MAC-1⁺ (CD11b/18) leukocytes and α -actin⁺ smooth muscle cells was performed on frozen sections.

Figure 6. H&E-stained sections of control vein, autografts, and isografts. Mice underwent anesthesia, and the external jugular vein (A) was autografted (D) or isografted (E), or the vena cava (B) was isografted (F) into the carotid artery. C represents the result of vein-to-vein isograft. Animals were sacrificed 4 weeks postoperatively, and the grafted tissue fragments were harvested, embedded in paraffin, sectioned, and stained with H&E. Arrows indicate the vessel wall or neointima; bar = 50 μ m.

The results shown in Figure 8 indicate MAC-1⁺ cell infiltration in vein graft lesions. It is known that MAC-1⁺ cells are monocytes/macrophages, natural killer cells, and granulocytes. We found that the majority of infiltrated cells in neointima were mononuclear cells, ie, monocytes/macrophages. In control veins, MAC-1⁺ monocytes/macrophages were rarely seen in the intima and media, whereas abundant infiltration of these positive cells were found in intima and/or adventitia of 1- and 2-week vein grafts (Figure 8, B and C). MAC-1⁺ monocytes/macrophages were detected at the luminal surface at 1 and 2 weeks postengraftment and were seen transmurally by 4 and 8 weeks (Figure 8, D and E). MAC-1⁺ cells were predominant in the neointima of 2- and 4-week grafts (Table 1), were distributed in both luminal and adventitial sites in 8-week vein grafts, and were found occasionally in the lesions of 16-week vein grafts (Figure 8, D through F; Table 1). Thus, the numbers of MAC-1⁺ monocyte/macrophage infiltration increased during the first 4 weeks and decreased thereafter.

Smooth muscle cell proliferation is a key event in the process of vascular remodeling and in the development of atherosclerosis.²⁷⁻³⁰ It would be interesting to see whether such cell proliferation also occurs in the neointima hyperplasia of mouse vein graft models. α -Actin is widely used as a marker of vascular smooth muscle cells, although not all smooth muscle cells positively stain with

antibodies against α -actin. Figure 9 and Table 1 demonstrate the presence of abundant smooth muscle cells in venous bypass graft lesions 4 to 16 weeks after surgery. Initially, a monolayer of smooth muscle cells in normal vein walls stained strongly positive (Figure 9A), and very weak staining, if any, was observed at 1 and 2 weeks. No staining was seen in vein segments stained with normal rat serum as a negative control (data not shown). Importantly, α -actin⁺ smooth muscle cells became abundant in neointima of 4-week vein graft neointima (Figure 9B; Table 1) and constituted a majority of cell components in 8- and 16-week lesions (Figure 9, C and D; Table 1). Our results indicate that smooth muscle cell proliferation is a main cause of lesion formation in venous bypass grafts in the mouse model, similar to human vein graft remodeling.

Discussion

Advantages of the Model

Recently, many investigators have developed murine genetic models in which genes are either overexpressed, deleted, or mutated. Such mouse models have considerable advantages over other animal systems in that they overcome the need to administer factors or their inhibitors, which can be problematic and often difficult to quan-

Figure 7. Comparison of neointima thickness between venous bypass auto- and isografts. The procedure for animal models and the preparation of H&E-stained sections are the same as those described in the legend to Figure 6. The thickness was measured microscopically. The graph shows data of neointima thickness (mean $\pm$ SD) obtained from six to eight animals per group. There are no significant differences among groups.

tify. In the present report, we establish and characterize a new model for the study of neointima formation of venous bypass grafts in mice. When used with vein autografts in mice subjected to targeted gene deletion, the model could provide a powerful tool for dissecting the relative contributions of such genes, including low-density lipoprotein receptors, endothelial adhesion molecules, nitric oxide synthase, and growth factors, in the development of neointima hyperplasia. When used with vein isografts that can be treated *ex vivo* with drugs or gene transfer, the thinner vessel wall allows easy penetration by small molecules and plasmids from the adventitia side. By using this model, we believe that significant progress in understanding the pathogenesis and treatment of vein graft disease may be seen in the near future.

In many respects, the morphological features of this murine vascular graft model resemble those of human venous bypass graft disease.^{25,26} First, a marked loss of smooth muscle cells has been observed in lesions in the early stage of vein grafts of humans and mice (Ref. 24; Figure 2B). Secondly, the human lesions have an inflammatory nature characterized by mononuclear cell infiltration in the early stage of vein bypass grafts, and the lesions seen in our mouse models contain abundant MAC-1⁺ monocytes/macrophages (Ref. 31; Figure 8). Finally, both the human and mouse lesions are characterized by concentric intimal proliferation with a domi-

nance of smooth muscle cells in late specimens (Ref. 26; Figures 3 and 9). Thus, this model could be useful in the study of human venous bypass graft arteriosclerosis.

Because differences exist between all animal models and human diseases, we should point out where the mouse model is not consistent with the human condition. For example, it appears that there is more inflammation in the adventitia in the mouse model than is seen in the human condition, although the mechanism resulting in this difference is not clear. Atherosclerotic lesions, including foam cell accumulation and necrotic core formation in the intima, can be found in human vein grafts beyond the 1st year after bypass surgery.^{26,32} It would be difficult to study late-stage atherosclerosis using the mouse model because of the shorter life span and lack of spontaneous atherosclerosis in mice. In addition, mural microthrombi, fibrin deposition, and acute inflammation leading to thrombosis are often observed in human venous bypass grafts.^{26,32} In the mouse model, the rate of thrombus formation is very low, possibly because of the shorter time of ischemia and lower degree of mechanical injury to the mouse graft.

Possible Mechanism of Neointima Hyperplasia

Understanding the pathogenesis of vein graft arteriosclerosis is often extrapolated from studies on (spontaneous) atherosclerosis in arteries,³³ but the features of lesions and pathogenic processes of graft-induced arteriosclerosis differ from those of spontaneous atherosclerosis. For instance, the development of arteriosclerosis in vein grafts is rather rapid compared with that in the arteries, which begins in childhood. In the present study, we demonstrate that early changes of grafted vein segments included leukocyte infiltration followed by smooth muscle cell proliferation. Surgical or traumatic and ischemic injury to the vein segments may be partially responsible for the lesion formation at the early stage in the vein grafts. However, accumulating evidence indicates that mechanical stress plays a crucial role in the neointima formation via enhancing gene expression of adhesion molecules, growth factors, cytokines, and matrix proteins.³⁴ It has been demonstrated that exposure of endothelial cells to shear (mechanical) stress results in increased expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and monocyte chemoattractant protein-1 via activation of transcription factor nuclear factor- κ B and activator protein-1.^{35–39} These molecules are essential for leukocyte-endothelial cell interaction and subsequently cell infiltration, which is characteristic for the early lesions of vein grafts that undergo elevated blood pressure. Thus, our observations, together with those of others, suggest that mechanical stress is one of the most important factors in initiating inflammation of vein graft arteriosclerotic lesions.

Following vein graft inflammation is smooth muscle cell proliferation, a hallmark of late-stage lesions. Although the precise mechanism initiating such cell proliferation remains to be elucidated, evidence indicates a role for mechanical stress.⁴⁰ In hypertension, mechanical force

Figure 8. Immunohistochemical staining demonstrates the presence of MAC-1⁺ mononuclear cell infiltration in vein grafts. Sections derived from vein grafts at 1 (A and B), 2 (C), 4 (D), 8 (E), and 16 (F) weeks were labeled with normal rat serum (A) or a rat monoclonal antibody (CD11b/18; B through F) against MAC-1⁺ leukocytes and developed with alkaline phosphatase-anti-alkaline phosphatase techniques. A counterstaining (blue) with hematoxylin was performed. Filled arrows indicate neointima, and open arrows indicate examples of typical MAC-1-positive cells (red). D through F show a portion of neointima; bar = 20 μ m.

on the arterial wall increases up to 30%, resulting in marked alterations in signal transduction and gene expression in vascular smooth muscle cells, which contribute to cell differentiation, proliferation, and matrix protein synthesis.^{40–43} In grafted veins, mechanical force on the vessel segment suddenly increases more than 10-fold (arterial *versus* venous blood pressure), which provides a strong stimulus to smooth muscle cells. How the mechanical stimuli are converted into a biological signal in cells *in vivo* remains to be studied. We previously demonstrated that acutely elevated blood pressure, mechanical stress, or balloon injury to the carotid artery induce activation of mitogen-activated protein kinases, an essential signal transducer for cell growth.^{44,45} Recently, we observed that physical forces rapidly induced phosphorylation of

platelet-derived growth factor receptor α , supporting the mechanical stress-stimulated activation of platelet-derived growth factor receptor α .⁴⁶ Thus, mechanical stresses may directly perturb the cell surface or alter receptor conformation, thereby initiating signaling pathways normally used by growth factors.

In summary, we have established a new model of vein graft arteriosclerosis in mice. The lesion was characterized by mononuclear cell infiltration followed by smooth muscle cell proliferation and matrix protein deposition. Although the pathogenetic mechanism remains unknown, we hypothesize that increased mechanical force is an initial signal that stimulates gene expression of adhesion molecules, including intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and

Table 1. MAC-1⁺ and α -Actin⁺ Cells in Media and Neointima

Graft age (week)	The number or %/100 μ m		
	MAC-1 ⁺ cells	α -actin ⁺ cells	Total nuclei
0	0–2 (no.)	5–9 (no.)	11 $\pm$ 5
1	3–8 (no.)	0–3 (no.)	13 $\pm$ 6
2	8–14 (no.)	0–1 (no.)	29 $\pm$ 11
4	50 $\pm$ 20 (%)	10 $\pm$ 8 (%)	123 $\pm$ 44
8	25 $\pm$ 19 (%)	58 $\pm$ 21 (%)	121 $\pm$ 29
16	3 $\pm$ 5 (%)	69 $\pm$ 23 (%)	94 $\pm$ 21

The positive-stained cells (red) and total number of nuclei (blue) were counted. In each section, two regions (100 μ m/region) were evaluated. Data from groups 0, 1, and 2 weeks represent ranges of the number (no.) of positive cells since total nuclei per 100 μ m were less than 30. The percentage of positive cells in groups 4, 8, and 16 weeks is shown. The total number of nuclei/100 μ m is shown in the last column. The values are ranges of the number or means $\pm$ SD from two to four sections per animal ($n = 5$).

Figure 9. Immunohistochemical staining demonstrates the presence of α -actin⁺ smooth muscle cells in vein grafts. Sections derived from vein grafts at 0 (A), 4 (B), 8 (C), and 16 (D) weeks were labeled with a mouse monoclonal antibody against α -actin conjugated with an alkaline phosphatase and developed with the substrate with counterstaining. **Filled arrows** indicate normal intima/media (A) or neointima (B through D), and **open arrows** indicate examples of typical α -actin-positive cells (red). B through D show a portion of neointima; bar = 50 μ m.

monocyte chemotactic protein-1, which evokes an inflammation process in the grafted veins. Mechanical force may also be responsible for initiating the growth factor-mitogen-activated protein kinase signal pathways essential for cell growth. Further studies using this animal model could significantly enhance our understanding of the mechanism of vein graft arteriosclerosis and provide valuable information for therapeutic intervention in vascular diseases.

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