

MECHANISMS AND PREVENTION OF INTIMAL THICKENING OF THE AUTOGENOUS VEIN GRAFTS — POSSIBLE INVOLVEMENT OF NITRIC OXIDE —

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ABSTRACT

Platelet thrombosis, intimal hyperplasia, and the progression of atherosclerosis are the most important factors determining the patency of vein grafts for arterial occlusive disease. Interactions between aggregating platelets and the vessel wall play an important role in all of these processes. The endothelium modulates the underlying vascular smooth muscle by releasing nitric oxide (NO), a potent vasodilator and anti-aggregating substance. This review focuses on vascular modulation by NO in vein grafts.

Key Words: Vein graft, Intimal thickening, Endothelium, Nitric oxide, Gene therapy

INTRODUCTION

Although grafting with an autologous vein as a vascular substitute in the treatment of peripheral arterial occlusive disease is the surgical procedure that yields the best long-term results¹⁻³⁾, late vein-graft failure remains a significant problem for vascular surgeons. Platelet thrombosis, intimal hyperplasia, and the progression of atherosclerosis are the most important factors determining the patency of vein grafts for arterial occlusive disease. Interactions between aggregating platelets and the vessel wall play an important role in all of these processes.

The significant role of the vascular endothelium in vasomotor regulation has been demonstrated.^{4,5)} The endothelium produces potent vasodilators such as prostacyclin⁵⁾ and nitric oxide (NO)⁴⁻⁶⁾. This review focuses on vascular modulation by NO in vein grafts. In addition, it describes the current status of experimental studies concerning the differences in the biological behavior between the vein and arterial grafts.

CLINICAL OUTCOME OF HUMAN BYPASS GRAFTS

The saphenous vein remains the most durable conduit for small-vessel arterial bypass. Despite the excellent results achieved with saphenous veins for peripheral bypass, approximately 30% of these autogenous vein grafts occlude within 5 years.¹⁻³⁾ In cardiac surgery, the internal

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mammary artery (IMA) and saphenous vein were used for aortocoronary bypass. In one series, IMA grafts and saphenous vein grafts had patency rates of 95 and 93% respectively, at 1 year, but at 5 and 10 years the patency rates of the IMA grafts were 88 and 83% which were superior to the 74 and 41% rates for saphenous vein grafts^{7,8}. Trauma to the saphenous vein during surgical preparation can denude the endothelium, impair the intrinsic vein's fibrinolytic activity, and damage the vessel wall, thereby predisposing the vein to develop early thrombosis⁹. Careful harvesting of the graft, with particular attention paid to the use of modified storage solutions, avoiding overdilatation has been shown to improve the patency and preserve the ingrate of the graft in both animal models and the clinical settings.

Thus far, the biological advantages of arterial grafts have been reported in terms of aortocoronary bypass. The proliferation of vascular smooth muscle cells from the venous system in response to pulsatile stretch appears to be markedly greater than that of arterial vascular smooth muscle cells¹⁰. Comparative morphological and angiographic studies of IMA and saphenous vein bypass grafts that have been implanted long-term show that accelerated atherosclerosis commonly occurs in saphenous vein grafts but tends to be extremely rare in IMA grafts. Several potential explanations may be offered for the superiority of the IMA graft¹¹. The media of the artery may derive nourishment from the lumen as well as from the vasa vasorum, and the internal elastic lamina of the IMA is uniform. Moreover, the finding that the endothelium of the IMA produces significantly more prostacyclin¹² and NO¹³ than that of the saphenous vein may explain why endothelium-dependent relaxation is more pronounced, thus allowing flow-dependent autoregulation to occur¹². The diameter of the IMA graft is usually a closer match to that of the recipient coronary artery than is the diameter of a saphenous vein.

In addition, platelet-derived growth factor (PDGF) stimulates the proliferation of veins but not IMA smooth muscle cells¹⁴. These differences have been confirmed in an organ culture model, where intimal proliferation was significantly increased in surgically prepared veins compared with the IMA and fresh saphenous veins, thus suggesting a greater susceptibility of veins to injury¹⁵. There are therefore a number of biological differences that may explain the superior long-term performance from graft disease of the arterial grafts.

HISTOLOGICAL STUDIES

Scanning electron micrographs

In vein grafts 3 days after grafting, scanning electron microscopy revealed a high degree of endothelial loss, but deformed remnant endothelial cells and platelets were present on the luminal surface of the grafts (Fig. 1). At 1 week after implantation, the surface of the endothelial cells had recovered, but cell junctions were incomplete. After 2 weeks, the endothelium completely covered the intima, and there were no apparent differences from the control endothelium¹⁶.

In arterial grafts 1 day after grafting, the intimal surface of the mid-portion of the arterial graft was covered by mildly damaged endothelial cells (Fig. 1). The intercellular crevices were expanded, the junction of endothelial cells was broken in part, and some adherent leukocytes were observed. But no adherent platelets were observed and the integrity of the endothelial cell layer was largely maintained with endothelial cell denudation found. On the 3rd day after grafting, a nearly intact endothelial cell layer had covered the whole luminal surface, although endothelial cell junctions and inflammatory cells were observed to be in a shabby state, following a line in the direction of blood flow and exhibiting nearly normal cell junctions. On the 7th day after grafting, electron photomicrograph scanning of the luminal surface of the arte-

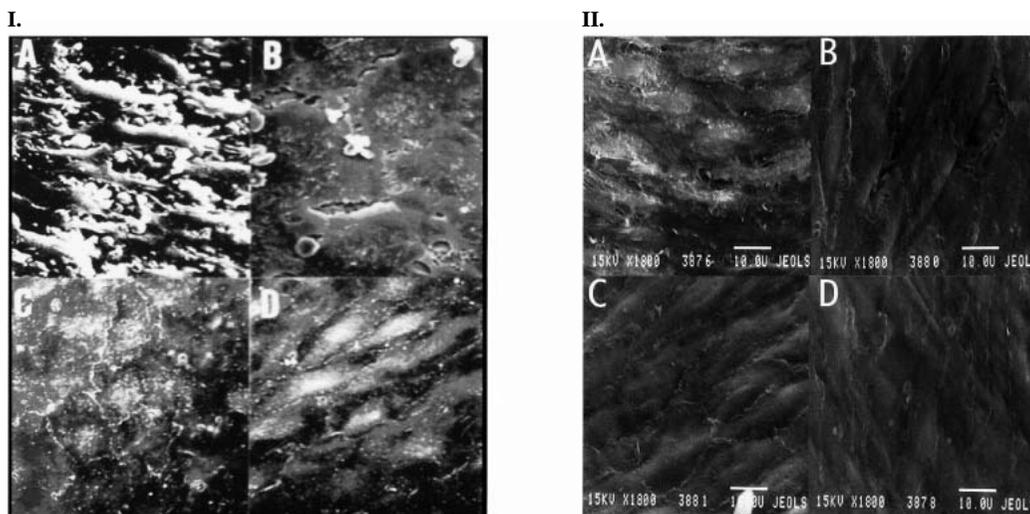


Fig. 1 Scanning electron micrograph^{16,18}.

I: Vein grafts

II: Arterial grafts

A 3 days after grafting, B 7 days after grafting, C 14 days after grafting,

D 28 days after grafting

In the vein grafts 3 days after grafting (I.A), scanning electron microscopy revealed a high degree of endothelial loss, with deformed remnant endothelial cells and platelets present on the luminal surface of the grafts. One week after implantation, the surface of the endothelial cells had recovered, but cell junctions were incomplete (I.B). After 2 weeks (I.C), endothelium completely covered the intima, and there were no apparent differences from the control endothelium.

In arterial grafts on the 3rd day after grafting (II.A), a nearly intact endothelial cell layer covered the whole luminal surface, although endothelial cell junctions and inflammatory cells were observed to be in a shabby state, which followed a line in the direction of blood flow and exhibited nearly normal cell junctions. On the 7th day after grafting (II.B), electron photomicrograph scanning of the luminal surface of the arterial grafts showed an endothelial cell layer with microvillus projections, and some microcavities were found on endothelial cells. These damaged endothelial cells seemed to be largely restored. On the 14th (II.C) and 28th day (II.D) after grafting, the luminal surface of arterial grafts was much the same as normal arterial endothelial cells.

rial grafts showed an endothelial cell layer with microvillus projections, and some microcavities on endothelial cells were found. These damaged endothelial cells seemed almost fully restored. On the 14th and 28th days after grafting, the luminal surface of arterial grafts was much the same as that in normal arterial endothelial cells^{17,18}.

These findings indicate that in canine vein grafts, the endothelium of the vein graft was once denuded¹⁶, and thereafter recovered within 3 or 4 weeks. In contrast, in arterial grafts, the denudation of the endothelium was minimal, and no platelet adherence was observed. Instead, a nearly normal intact endothelial cell surface had covered the intima within three days after grafting^{17,18}. The histological findings of the arterial grafts were quite different from those of vein grafts. This may be one of the reasons why the endothelium-dependent responses were preserved in the arterial grafts^{17,18}.

Time course of intimal thickening after grafting

In canine vein grafts and rabbit carotid vein grafts, the intimal thickening was observed¹⁹⁻²². Such thickening was not remarkable 3 days after implantation, and there was a gradual increase

at 4 weeks in the canine vein grafts¹⁶⁾.

On the other hand, in canine femoral arterial grafts, by each of the 1st, 3rd, 7th, 14th, and 28th postoperative days, no intimal thickening was observed^{17,18)}. The histological findings in terms of intimal thickening in the arterial grafts were thus quite different from those of the vein grafts.

Effects of flow changes on intimal thickening

Hemodynamic factors such as a low-flow velocity and low-shear stress result in a progression of late graft failure because of pronounced intimal thickening²³⁻²⁵⁾. Changes in the hemodynamic parameters have been shown to affect the structure of both normal and diseased vessels²⁶⁾.

The deformation of smooth muscle cells by arterial hemodynamics can lead to the activation of protein tyrosine kinases, thereby initiating smooth muscle cell proliferation²⁷⁾. Vein grafts with low flows are associated with greater intimal thickening²⁸⁾. Similarly, low-shear stress is also associated with an accelerated development of intimal thickening in vein grafts^{24,25,29)}. Morinaga reported that such a thickening develops in vein grafts under low-flow conditions (poor distal runoff) and is reversed when these vessels are then re-implanted into a system with normal flow parameters²⁵⁾.

In our previous studies, we classified the electromagnetically measured blood-flow waveforms at reconstructive surgery into five types. We reported a close relationship between the ultimate results of the arterial reconstruction and intraoperative blood-flow waveforms³⁰⁾. Grafts with a type 0 or I flow-wave pattern (normal-flow group characterized by steep acceleration and deceleration) showed a long-term patency. In grafts with type II, III or IV flow waveform patterns (abnormal-flow groups characterized by a gentle sloping), graft failure was more frequent than in the normal-flow group. A canine poor-runoff model was prepared according to Morinaga's method²⁵⁾, in which all tributary arteries distal to the saphenous artery in the unilateral posterior limb were ligated and severed, except for a superior branch of the posterior femoral artery. Four weeks after the first surgical procedure, the collateral vessels seemed to be fully developed

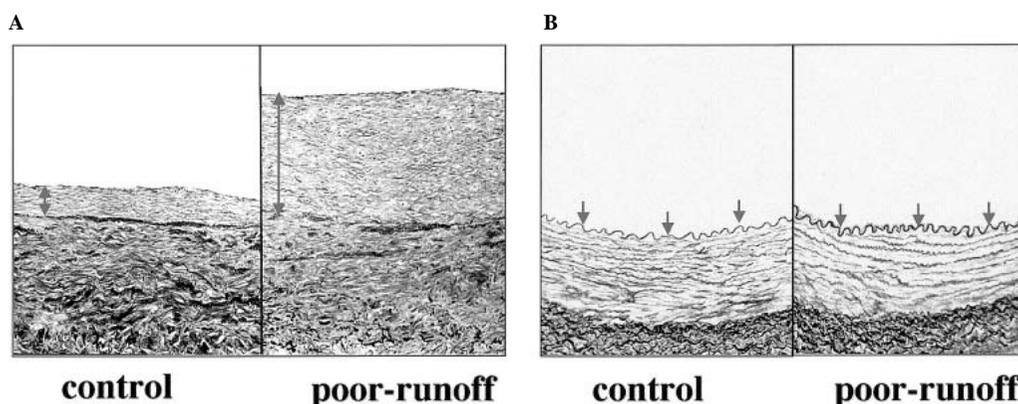


Fig. 2 Histological findings of the canine vein graft and arterial graft under control and poor-runoff conditions^{17,34)}.

Vein graft: The intimal thickening of the canine vein grafts in poor-runoff limbs was significantly thicker than that in control limbs.

Arterial graft: No apparent intimal thickening of the canine arterial grafts was observed in either normal or poor-runoff conditions.

in the right lower limb (the poor-runoff limb)²⁵). The femoral artery and vein were exposed at a site proximal to the previous operation, and the femoral vein and artery was interposed into the femoral artery in an end-to-end fashion.

The average value of intimal thickening of the canine vein grafts in poor-run off limbs was significantly thicker than that of control limbs,^{24,25} although no apparent intimal thickening of the canine arterial grafts was observed in the poor runoff^{17,18} (Fig. 2).

ENDOTHELIUM AND NITRIC OXIDE (NO)

Time course of endothelium-dependent relaxations

Previous studies have reported an impairment of the endothelium-dependent relaxations in autogenous vein grafts,^{16,19,20,21} although electron microscopy scanning demonstrated a morphologically intact endothelium^{16,19,21} (Figs. 1 and 2).

In canine arterial grafts, endothelium-dependent relaxations to ACh were maintained at each postoperative period examined^{17,18}. The maximum relaxations induced by ACh on the 3rd postoperative day after grafting were significantly impaired compared with the control, while on subsequent postoperative days, the relaxations in the arterial grafts and normal arteries were similar. The endothelium-dependent relaxation to A23187 was maintained at each postoperative period examined and was similar between the arterial grafts and normal arteries. The maximum relaxation induced by A23187 on the 1st and 3rd postoperative days after grafting was significantly impaired when compared with the control, while on subsequent postoperative days, the relaxations were similar between the arterial grafts and normal arteries^{17,18}.

Effects of flow changes on endothelium-dependent responses

The endothelium-dependent responses are known to be modulated by alterations in the blood flow³¹⁻³³. A chronically elevated blood flow is known to result in increased endothelium-dependent relaxation. In vein grafts, several studies have demonstrated that endothelium-dependent relaxations were impaired under normal-flow conditions¹⁹⁻²². In addition, we have reported that poor-runoff conditions in canine femoral vein grafts, which closely mimic those of clinical late-graft failure³⁰, cause more endothelial dysfunction than that which occurs with a normal runoff.^{30,34,35} On the other hand, in canine arterial grafts, relaxations to ACh, ADP and A23187 were comparable between the control and the poor-runoff femoral arteries^{17,18}.

The effects of lumbar sympathectomy on the properties of both endothelium and smooth muscle cells of the canine femoral artery and autogenous vein grafts under poor-runoff conditions were examined³⁶. Five weeks after the development of a poor-runoff model, a unilateral left sympathectomy was performed, and both femoral veins were also grafted to the femoral arteries on both sides. After 4 weeks, endothelium-dependent responses and intimal thickening of both autogenous vein grafts were then examined. The endothelium-independent contractions to acetylcholine and the endothelium-dependent relaxations to ADP and A23187 were all comparable between the right and left vein grafts. In addition, the intimal thickening of the vein graft was comparable between the two groups. These results suggest that lumbar sympathectomy does not alter endothelial function in terms of NO, although mean blood flow of the denervated femoral arteries and vein grafts was significantly higher than that of the innervated femoral arteries and vein grafts. These results suggest that continuous vasodilation following sympathectomy may be a more potent factor with respect to the regulation of vascular tonus than the physiologic regulation of NO.

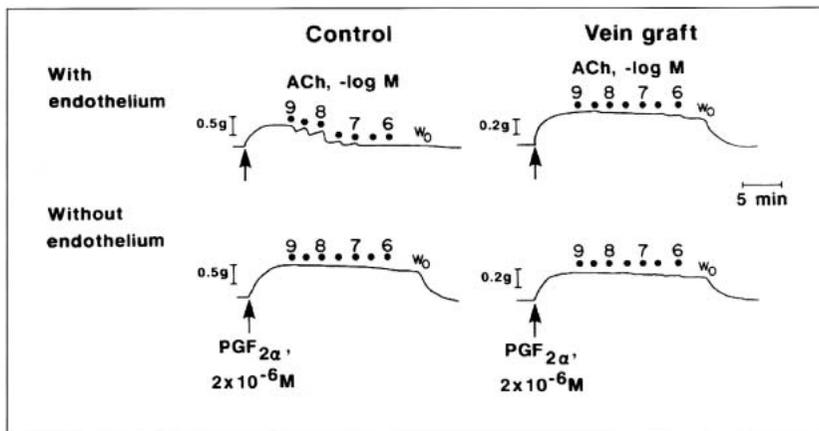


Fig. 3 Endothelium-dependent relaxations in rabbit jugular vein and vein grafts²²⁾.
 Control: In the rabbit jugular vein, ACh induced endothelium-dependent relaxations.
 Vein graft: In the rabbit jugular vein grafts 4 weeks after operation, ACh caused no relaxation irrespective of the presence of endothelium.

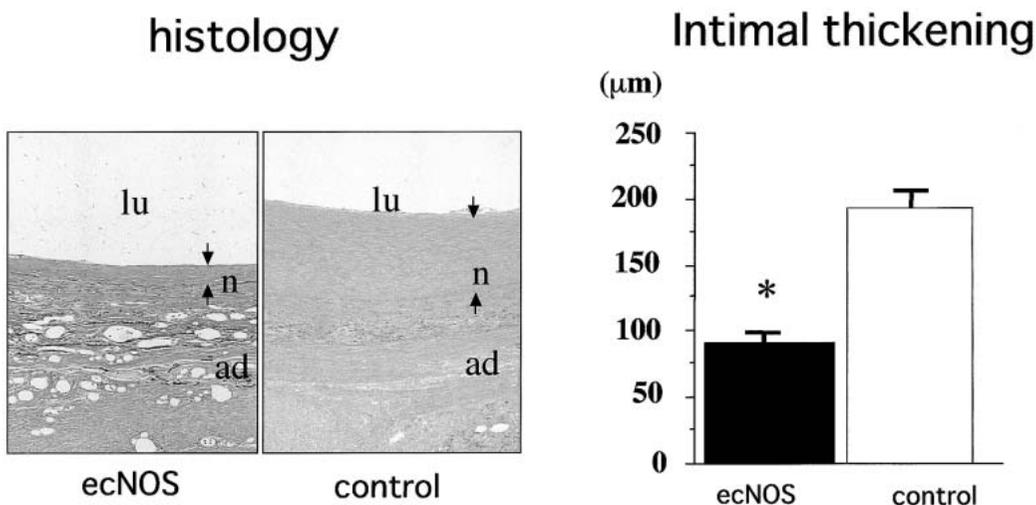


Fig. 4 Effects of ecNOS gene transfer on the intimal thickening of the canine vein grafts⁶²⁾.
 The gene transfer of ecNOS inhibited intimal hyperplasia of canine vein grafts caused by poor-runoff conditions in comparison with buffer-treated control.

Vein grafts and production of cyclic guanosine-monophosphate (cyclic GMP)

Next, we investigated the underlying mechanisms associated with the loss of responsiveness of veins grafted into the arterial circulation. In particular, we tested the possibility that the altered response is related to modifications in the biologic properties of the vascular smooth muscle, of the endothelial cells or both. After 4 weeks, vein grafts and unoperated veins were removed, and endothelium-dependent (acetylcholine: ACh) and endothelium-independent (nitric oxide, SIN-1 [the active metabolite of molsidomine]) relaxations were studied *in vitro*. In unoperated veins, ACh caused endothelium-dependent relaxations as well as NO- and SIN-1- in-

duced concentration-dependent relaxations in the presence and absence of the endothelium, respectively. These relaxations were associated with a time-dependent accumulation of guanosine 3':5'-cyclic monophosphate (cyclic GMP)²².

In vein grafts ACh induced only minor endothelium-dependent relaxations, whereas NO and SIN-1 evoked concentration-dependent relaxations in preparations without endothelium, which were shifted significantly to the right compared to unoperated veins. In vein grafts the endothelium-mediated production of cyclic GMP stimulated by ACh was significantly reduced when compared to that in unoperated veins, and that evoked by SIN-1 was not different. These results demonstrate that the production of endothelium-derived NO by endothelial cells is markedly impaired in vein grafts, and also that the responsiveness of the smooth muscle is reduced, although to a somewhat smaller extent²². These altered biologic responses of grafted veins may contribute to graft failure.

Comparison of endothelial function between in-situ and reversed vein grafts

Experiments were performed to determine whether the endothelium-dependent responses differed between *in situ* and reversed vein grafts. The influence of a valve disruption or a dissection of the adventitia was also examined³⁷. Segments of canine jugular veins were grafted into the carotid arteries during procedures such as reversed grafting, *in situ* grafting with valve disruption, *in situ* grafting without valve disruption, and *in situ* grafting with dissection of the adventitia.

In the reversed and *in situ* vein grafts with valve disruption, ACh caused endothelium-independent contractions, whereas in the *in situ* vein graft without valve disruption, ACh-induced endothelium-dependent relaxations were preserved. Adenosine diphosphate (ADP) caused comparable endothelium-dependent relaxations in the *in situ* vein graft irrespective of valve disruption. In the reversed vein graft, ADP-induced relaxations were significantly impaired. In the *in situ* vein graft with dissection of the adventitia, relaxations in response to ACh and ADP were significantly reduced.

These results suggest that endothelial function, in terms of NO in the *in situ* vein graft, can be preserved, and that adventitial dissection in *in situ* vein grafts should be minimized to preserve endothelial function.

CLINICAL MEANING OF ENDOTHELIAL DYSFUNCTION AND NITRIC OXIDE

Grafting with an autologous vein as a vascular substitute in the treatment of peripheral arterial occlusions is the surgical procedure that yields the best long-term results¹⁻³. However, late graft failure is still a significant problem, particularly in cases with poor-runoff vessels. Hemodynamic factors such as low-flow velocity and low-shear stress result in late graft failure due to a progression of intimal thickening^{23-25,28}.

Platelets probably play an important role in both thrombotic occlusion and intimal hyperplasia, and it was confirmed that intimal thickening may be platelet-mediated³⁸⁻⁴⁰, since certain anti-platelet drugs will reduce intimal hyperplasia^{41,42}. NO relaxes vascular smooth muscle, reduces platelet adhesion, and is in itself a potent anti-platelet-aggregatory substance^{4,5}. One characteristic of a diseased vascular wall is an impairment in endothelium-dependent relaxation. This has been repeatedly demonstrated in hypertensive animals, in animals with atherosclerosis, and in experimental cerebral vasospasm⁴³⁻⁴⁵. In humans, atherosclerotic arteries do not relax when infused *in situ* with ACh; instead, a paradoxical vasoconstriction occurs⁴⁶. In addition, an impairment in endothelium-dependent responses in the regenerated endothelial cells has also

been noted in experimental models of balloon denudation⁴⁷⁾ and reperfusion injury⁴⁸⁾. We obtained clear evidence that endothelium-dependent relaxations in response to ACh and A23187 are reduced slightly in arterial grafts in the early postoperative period, and that they are then quickly restored to normal, and no intimal thickening of arterial grafts was observed for the entire 4-week observation period^{17,18)}. In vein grafts, we found that a peak of intimal hyperplasia is reached 2 to 4 weeks after grafting¹⁶⁾. In addition, in vein grafts, endothelium-dependent relaxations were impaired in the early postoperative stage and continued until 6 weeks after grafting¹⁶⁾. However, the precise mechanism underlying the difference between the responses of vein grafts and arterial grafts remains unknown. The preserved endothelial function in canine arterial grafts in terms of endothelium-dependent relaxations, irrespective of the flow change, may thus be one of the mechanisms contributing to an absence of intimal thickening in arterial grafts with poor runoff conditions.

PREVENTION OF GRAFT ATHEROSCLEROSIS: ecNOS GENE TRANSFER

Although the etiology of long-term failure is still unclear, a combination of the progression of the host's underlying disease, increased graft intimal hyperplasia, and the development of atherosclerosis-like lesions within the vein graft appear to be the principal causes. Intimal hyperplasia is the universal response of a vein graft to insertion into the arterial circulation, and is considered to result from both the migration of smooth muscle cells out of the media into the intima and the proliferation of these smooth muscle cells; later the smooth muscle cells deposit an extracellular matrix. However, this concept is challenged by recent findings demonstrating that other sources of smooth muscle cells may contribute to vascular diseases.⁴⁹⁻⁵⁴⁾ Smooth muscle cells in vein grafts appear in the neointima earlier than in the media after cell death, which is an early cellular event in grafted vessels⁴⁹⁾. Shi *et al.*⁵⁰⁾ demonstrated that myofibroblasts in the adventitia of arteries may contribute to neointimal formation in response to endothelial cell injury. In addition, current evidence indicates that bone-marrow progenitor cells may be a source of smooth muscle cells for transplant arteriotomy⁵¹⁾, neointimal lesions of injured arteries,^{55,56)} and hypercholesterolemia-induced atherosclerosis⁵⁷⁾. Detailed examinations to elucidate the mechanisms of vein-graft intimal thickening need to be carried out, although recent a study suggested that bone marrow cells were not the source of smooth muscle cells in mice vein grafts⁵⁸⁾.

Therapies to limit the development of intimal thickening in vein grafts continue to attract considerable attention. However, no effective clinical regimen is presently available to counter the intimal hyperplastic response found in vein grafts. The use of aspirin is associated with a decrease in early thrombotic events in vein grafts, but it has not been documented to reduce the incidence of restenosis or the development of atherosclerosis⁵⁹⁾. We demonstrated that NO played a critical role in the impaired function and progression of lesions. For instance, we and others demonstrated that EDRF-dependent vasorelaxation and NO production were impaired in all phases of vein graft remodeling after implantation¹⁹⁻²²⁾, and we also showed this to be accelerated by the low-shear stress induced by poor-runoff canine models³⁴⁾. Similar to such functional impairment, the intimal thickening of vein grafts was also promoted by low-shear stress³⁴⁾, and increased synergistically to a greater degree due to hypercholesterolemia⁶⁰⁾. Considering the vasculoprotective properties of NO, a therapeutic strategy to induce NO production in the vessel wall thus seems reasonable. In fact, we have already shown that the systemic administration of L-arginin, a precursor of NO, inhibited vein graft intimal thickening with hypercholesterolemia,⁶¹⁾ and that poor-runoff-induced intimal progression was also suppressed by the

direct gene transfer of ecNOS⁶²). Recently, we also demonstrated that the ecNOS gene transfer suppressed intimal hyperplasia of vein grafts under hyperlipidemic conditions, although this effect may be limited because of the smooth-muscle cell loss related to the use of intraluminal delivery methods⁶³).

The strategy of ecNOS gene transfer seems to merit future study as a possible therapeutic approach to prevent late-graft failure in a clinical setting. Clearly, further studies are called for to clarify such factors as the gene delivery method and vehicles. In addition, proper sites to be transferred should be investigated to achieve more effective NO production and to genetically manipulate modified vein grafts which are resistant to both intimal thickening and atherosclerosis.

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