Vein graft failure

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After the creation of an autogenous lower extremity bypass graft, the vein must undergo a series of dynamic structural changes to stabilize the arterial hemodynamic forces. These changes, which are commonly referred to as remodeling, include an inflammatory response, the development of a neointima, matrix turnover, and cellular proliferation and apoptosis. The sum total of these processes results in dramatic alterations in the physical and biomechanical attributes of the arterialized vein. The most clinically obvious and easily measured of these is lumen remodeling of the graft. However, although somewhat less precise, wall thickness, matrix composition, and endothelial changes can be measured in vivo within the healing vein graft. Recent translational work has demonstrated the clinical relevance of remodeling as it relates to vein graft patency and the systemic factors influencing it. By correlating histologic and molecular changes in the vein, insights into potential therapeutic strategies to prevent bypass failure and areas for future investigation are explored. (J Vasc Surg 2015;61:203-16.)

Clinical Relevance: **The autogenous vein bypass graft remains the gold standard revascularization method for the ischemic limb. Newly implanted vein grafts undergo dramatic structural changes in response to the new high-flow, high-pressure environment. These changes, which are commonly referred to as remodeling, include a pronounced inflammatory response accompanied by the development of a neointima and significant changes in matrix composition. Similar to how maturation of arm veins predicts the performance of an arteriovenous fistula, recent translational work has demonstrated that remodeling of the vein graft is important for subsequent patency of the lower extremity bypass graft.**

The autogenous vein bypass remains the most effective and durable revascularization strategy for patients with lower extremity ischemia despite the seemingly exponential proliferation of endovascular devices and techniques. In the United States, ~250,000 coronary artery and 80,000 lower extremity vein grafts are implanted per year.[1] Vein grafts, in contrast to inanimate stents or prosthetic grafts, are living and evolving conduits that respond to hemodynamic stimuli and to signals from the local environment.[2] Recent randomized controlled trials inform us that 30% to 40% of coronary and lower extremity vein grafts occlude or develop significant stenosis within the first year after implantation.[3,4] These figures have largely remained unchanged for the past several decades.[5] On one hand, this is a cause for optimism because results remain constant despite ever more challenging and complex patients.[5] On the other hand, it is discouraging to consider that 5 decades of high-powered science has not effectively changed bypass graft outcomes.

Endophlebectomy of vein graft stenosis, described first in 1965 at the University of Rochester, was used to treat a 56-year-old man whose femoropopliteal bypass developed a 1-cm stenosis 16 months after its construction.[6] Here, the authors described a white fibrous tissue that was sharply excised, and the graft was repaired with a vein patch angioplasty. This all-too-familiar description betrays the underlying inflammatory mayhem that conspired to produce such a bland-appearing lesion. We now characterize the lesion as intimal hyperplasia, which is present to some extent in all vein grafts.

Unlike coronary bypass grafts, duplex surveillance of lower extremity vein grafts can detect hemodynamically significant stenosis due to the vein graft's superficial location within the leg. The distribution of ultrasound-detected stenosis is diffuse in ~12% vein grafts, but most stenotic lesions are focal, often occurring in the perianastomotic regions or at valve sites.[7-9]

LIMITATIONS OF EXISTING ANIMAL MODELS

Growth factor inhibitors, transcription factors, cell cycle regulators, immunomodulators, and nitric oxide (NO) donors, among others, have all been effective at reducing intimal hyperplasia in experimental models.[10] Yet surprisingly, very few of these have entered into phase 1 human clinical trials. The lack of translation may be because existing animal models do not adequately

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represent human counterparts. They are generally constructed with short interposition grafts in high-flow environments, produce minimal to moderate stenosis, and rarely develop the severe occlusive lesions seen in the human vein grafts. Most preclinical programs have relatively short end points, commonly 28 days, which may not be sufficient to account for the late lumen loss due to fibrous expansion.[11-14] The healing of human vein grafts is known to occur well beyond this time frame, suggesting more long-term models are necessary to fully study complex mature lesions.

THE REMODELING OF HUMAN VEIN BYPASS

Although the extent and time frame of development of intimal hyperplasia in animals substantially differs from humans, one important similarity is the ability of the vein to rapidly remodel to stabilize hemodynamic stress.[12,15] The idea of human vein graft remodeling is hardly novel. Szilagyi et al[16] noted in the 1960s studying autopsy specimens that vein grafts had increased their diameter by as much as 50% to 75%. More recently, serial ultrasound studies in patient cohorts have demonstrated in vivo changes in human vein grafts.[17]

Remodeling of the vein graft can be thought of as the morphologic and geometric changes in the vein that happen through luminal dilation, reorganization of matrix and collagen, and the development of a neointima. The effects of the arterial environment on the vein have been best characterized by Dobrin et al[18,19] and others as four pairs of deformations and counteracting stresses—circumferential, longitudinal, radial (compressive), and pulsatile—in addition to the well known shear stress. Hence, exposing a vein graft to arterial pressure subjects it simultaneously to deformations and stresses in nine different directions.[18,19]

We hypothesized that the early geometric remodeling of the vein graft is a crucial determinant to successful long-term function of the bypass graft. To test this hypothesis, we initiated a prospective cohort study to systematically determine remodeling characteristics of lower extremity bypass grafts during the first year of implantation.[20-23] High-resolution ultrasound images were used to characterize luminal and wall changes from a defined region of the vein graft. We also used pulse-wave velocity (PWV) analysis to determine stiffness changes in the vein over time. PWV is the speed at which the flow pulse propagates through the conduit and is one measure of stiffness that is relatively independent of the outflow. Because it was impractical to map the entire bypass graft, we used a 5-cm segment (no branches or valves) of the graft as a surrogate for the behavior of the entire graft. High-resolution M-mode ultrasound was used to conduct vein graft lumen measurements at predetermined times, beginning in the operating room after the anastomoses were complete and then at 1, 3, 6, 9, and 12 months thereafter.

In these same patients, we collected demographic information and cardiovascular risk factors and tracked bypass-related and limb-related outcomes. Preoperative blood samples were obtained to measure lipids, biomarkers, and cytokines associated with inflammation and thrombosis to assess their clinical value and also to provide insights into mechanisms of vein graft failure. To ensure that these markers were not spuriously elevated, any patient with active infection, a recent procedure, or concurrent systemic illness was excluded from biomarker evaluation.

Our early findings of this study were largely descriptive in nature. We determined that most of the luminal and wall remodeling of the graft occurs in the first 30 days, followed by relative stability. There was an increase of ~25% in lumen change of the vein graft between the operating room and 1 month, but the luminal remodeling response varied substantially.[20,21] Although the lumens of most of the grafts increased, about one-quarter decreased in size. Similarly, wall thickness increased an average of 35% during this same period. As expected from animal data, the initial shear stress at the time of implantation was the single biggest hemodynamic factor accounting for the variability in luminal remodeling, but even so, only explained ~10% of luminal remodeling. This begins to get at some of the discrepancy between animal and human data, because most animal models of vein grafts use juvenile healthy animals without severe systemic illness such as advanced diabetes mellitus, hypertension, or dyslipidemia.

Our PWV studies determined that bypass grafts developed an increase in stiffness but were unexpectantly temporally delayed from the wall thickness changes. In fact, stiffness initially decreased and then rapidly rose, reflecting reorganization of matrix proteins.[21] The arterialized vein dramatically increased in stiffness by an average of ~65% from 3 to 6 months. Considering the vein wall consists of three principle components—cells and proteoglycans, elastin, and collagen—only an increase in the fibrous protein collagen could account for this.[20] This observation nicely complements animal data, whereby the wall thickness changes during the first 6 months were accompanied by a marked increase in collagen production.[11,12,24]

These early observations began to paint a picture that early luminal and wall thickness changes were followed by a period of stiffening of the graft, and changes could be measured for at least 6 months after implantation. Because we encountered so much variability in lumen caliber that was not explained by the graft's inflow, outflow, or hemodynamic stress, other explanations were sought. By assessing the patient's baseline level of inflammation, determined by preoperative measurement of high sensitivity C-reactive protein (hsCRP), an inverse correlation was noted between inflammation and the magnitude of luminal remodeling.[22] Specifically, veins placed in patients who had elevated preoperative hsCRP levels ($\geq$5 mg/L) dilated substantially less than those with hsCRP <5 mg/L and were an average of 0.5-mm smaller by the end of the first month. This was true despite having similar initial size at the time of implantation to those patients with hsCRP <5 mg/L.

Other significant demographic and clinical factors found to be associated with the early remodeling of vein

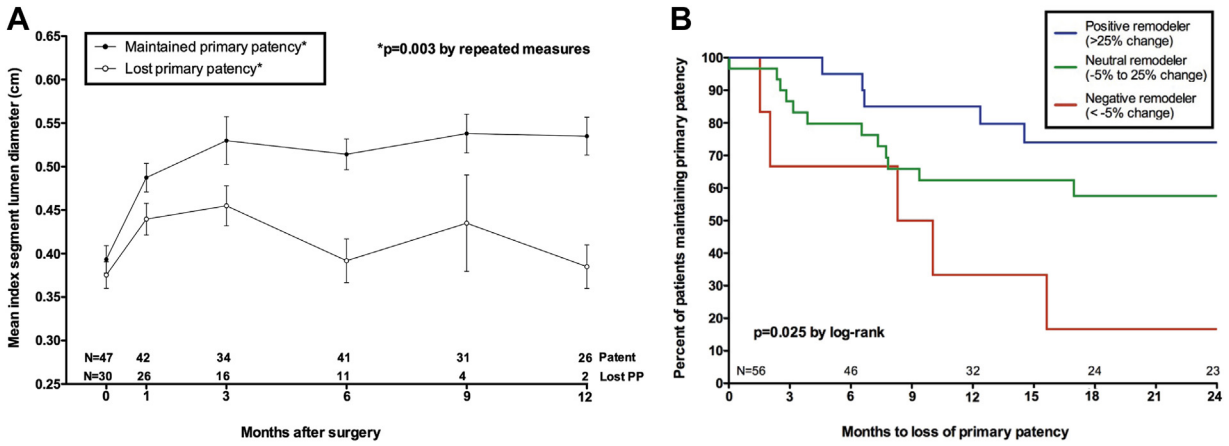

Fig 1. A, Index segment diameter based on eventual loss of primary patency. Values shown represent the mean diameter ± standard error of the mean at each interval. Although the starting diameter was at a similar size as those that remained patent, vein grafts that eventually lost primary patency failed to dilate and had a significantly smaller lumen size over time. **B,** Early (30-day) remodeling is associated with primary patency ($P = .02$). Adapted from Gasper et al.[23]

grafts included the patient's race and the use of a statin at the time of the operation, both of which have been shown to be associated with vein graft patency.[25,26] Specifically, African American race was associated with less positive remodeling during the first month of implantation, and vein grafts implanted in these patients never achieved the diameter of those in Caucasians.[23] Just as importantly, diabetes mellitus, hypertension, and hyperlipidemia were not associated with remodeling, none of which have been shown to be associated with reduced patency of lower extremity vein grafts.

By linking bypass outcome data with serial imaging data, we next determined that early vein graft remodeling is associated with midterm vein graft patency independent of initial vein size or other risk factors. Veins that do not enlarge or get smaller during the first postoperative month, referred to by us as poor remodelers, have a 13-fold increased risk of failure at 2 years compared with "robust remodelers," that is, those demonstrating >25% change in lumen diameter (Fig 1).[23] To put this in perspective, the use of small veins for bypass only has about a 2.5-fold increased risk of failure at 1 year,[27] suggesting that the remodeling of the vein in the first 30 days is at least as important as vein implantation size.

Thus, vein graft failure cannot be thought of as simply a segmental hyperproliferative disease that develops within a static tube. But rather, intimal hyperplasia develops within a dynamic conduit, molded by hemodynamics under the influence of systemic and regional factors. Thus, inflammation,[22] race,[25] gender,[28] and genetics[29] can act globally on the entire vein graft to influence its adaptation in the arterial circulation. However, should local levels of shear stress and wall tension be impeded from reaching or re-establishing baseline conditions due to local environmental conditions, flow disturbances, or intrinsic vein disease, the proliferative intimal reaction would be expected to continue and stenosis to supervene.[30,31] Therefore one explanation for segmental stenosis may be a hyperproliferative response superimposed on a restrictive pattern of inadequate outward remodeling (Fig 2).

Given the critical importance of early vein graft remodeling, mechanistic insights may inform future local or systemic therapy to improve patency. Studies specific to lower extremity venous bypass remodeling are relatively scarce, however. Examining other experimental models, such as arteriogenesis (collaterogenesis), arteriovenous fistula (AVF) maturation, and even varicose vein development, may provide important clues to direct future research.[32-34] There are, however, fundamental differences between these models and vein bypass remodeling, as denoted in Table I. We believe that revisiting some of the early histologic and ultrastructural studies of experimental vein grafts through the lens of recent molecular biology is informative in understanding inciting pivotal events.[35-37]

HISTOLOGIC REMODELING

The injury associated with venous harvesting and implantation into the arterial environment is unlike any other known vascular injury, including development of atherosclerosis, balloon angioplasty and stenting, and even creation of the AVF. It is abrupt, severe, and affects the entire length of the bypass graft. The histologic and ultrastructural consequences of this have been well described and are depicted in Fig 3.[35-41] Within 24 hours to 3 days after pressurization, vein graft endothelial cells (ECs) are focally absent or appear attenuated and elevated by subendothelial edema and infiltrating inflammatory cells that are present in the subendothelial space as early as 4 hours after implantation.[42,43] Platelets, inflammatory cells, and fibrin are adherent to denuded areas of endothelium where they release growth factors such as platelet-derived

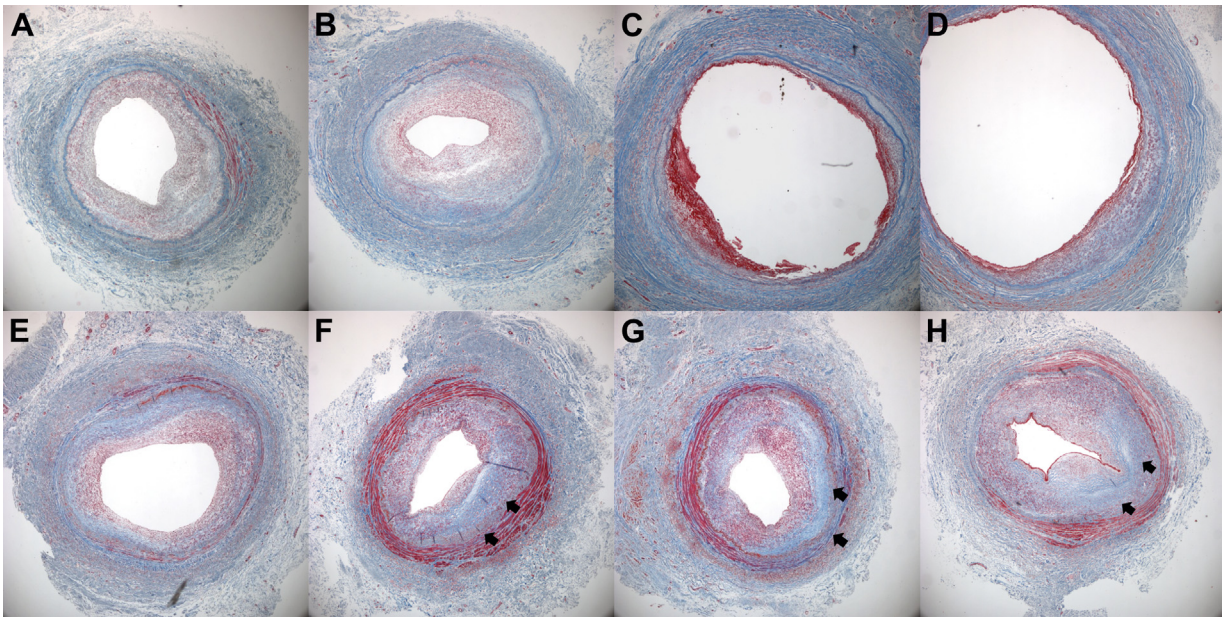

Fig 2. Histologic evidence for negative remodeling and intimal hyperplasia as a cause of late lumen loss in a great saphenous vein bypass graft. Masson Trichrome sections are from an 8-month-old femoral-posterior tibial artery vein bypass graft that was explanted due to hemodynamically significant stenosis identified by surveillance duplex ultrasound imaging. The repair was constructed by an interposition graft, and an 8-cm piece of diseased segment was explanted, registered, and serially sectioned from **A** (proximal graft) to **H** (distal graft). Note two areas of significant stenosis, sections **A** and **B,** and sections **F**-**H** with an intervening area of relatively normal vein. Although the vein was uniform in size and in luminal caliber at the time of the original surgery, the stenotic areas demonstrate loss of total vessel area, indicating lumen loss is due not only to intimal hyperplasia but also to negative remodeling. Note the amount of fibrous protein, as shown by the blue stain (*arrows*), in the stenotic segments of the graft.

Table I. Characteristics of human models of vascular remodeling

Variable	*Predilation diameter*	*Postdilation diameter*	*Percent remodeled*	*Flow after 1 month of remodeling*	*Pressure*	*Importance of endothelial function in remodeling*
Collateral vessels	30-50 μm	600-1000	2000%	???	<Arterial	+ + + +
Vein graft	>3.0 mm	4.5-5.0 mm	20%-30%	Medium (100-200 mL/min)	Arterial	+/−
AVF	>3.0 mm	5-7 mm	100%	High (600 mL/min)	Low (tapers quickly)	+ + +

AVF, Arteriovenous fistula.

growth factor BB, basic fibroblast growth factor, vascular endothelial growth factor, and insulin-like growth factor-1, among many others.[36,44,45] Areas of intact ECs subsequently slough or lose their barrier function so that they are permeable to plasma proteins, macromolecules, lipids, and growth factors. The remaining ECs demonstrate vacuolation and increase in Golgi and rough endoplasmic reticulum, indicative of conversion to a proinflammatory phenotype.[36] By 3 days, bare collagen, elastin, and other matrix proteins are visible with adherent platelets, red blood cells, and fibrin.

Depending on the animal model, the endothelial monolayer is largely restored by 10 days to 2 weeks, but functional restoration in a 60-cm-long human bypass graft likely takes far longer than it does in the short interposition grafts used in animals.[36,39,46] Although the exact time frame of human vein graft re-endothelialization is currently not known, we do know that mature (>12 months) vein grafts exhibit endothelium-dependent relaxation mediated by NO.[2] Evidence is emerging that the production of endothelium-derived relaxing factors may be delayed for up to 6 months after bypass grafting—long after the critical geometric remodeling period is complete—suggesting that early luminal remodeling is independent of this process (unpublished data from C.O., 2013). We now believe that reconstitution of a physiologically functional endothelium represents the third and final clinical stage of vein graft remodeling after luminal and wall thickness changes and stiffening. Programs focusing on earlier restoration of a functional EC monolayer and clinical measurements of

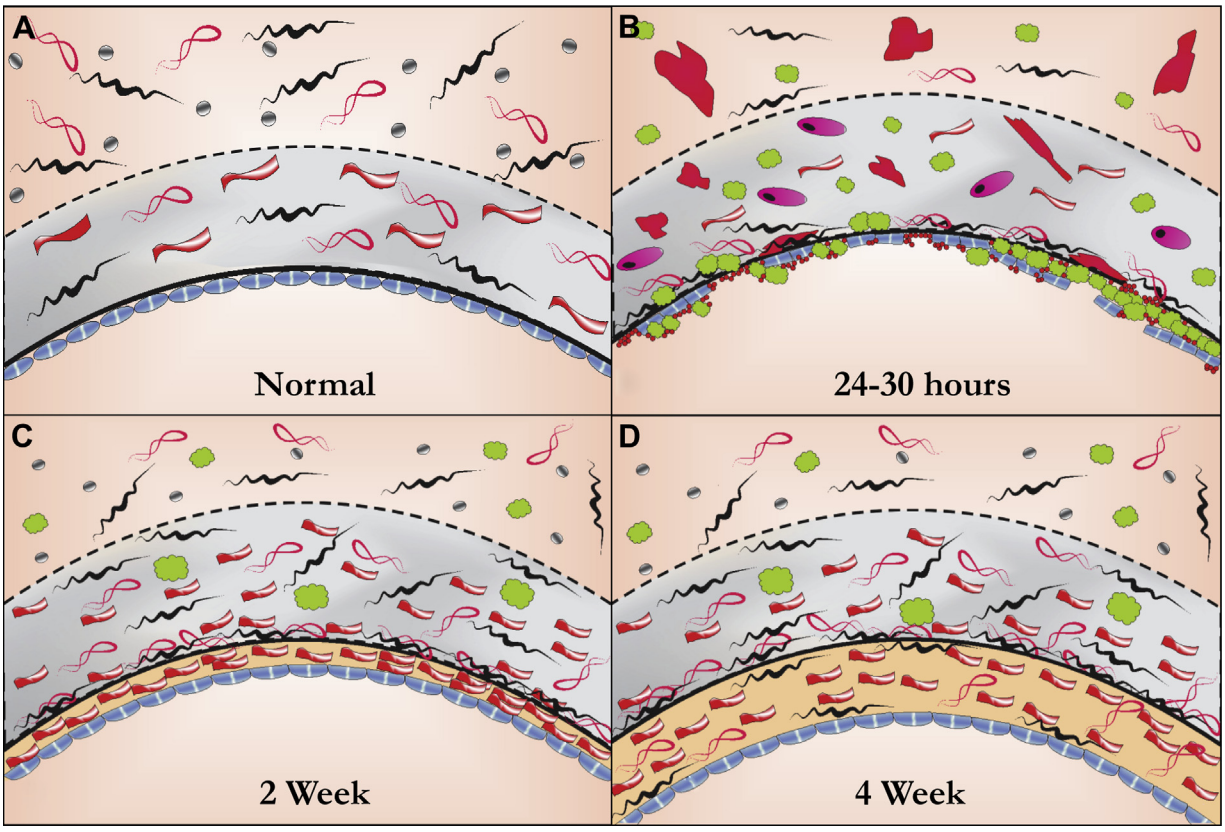

Fig 3. The histology of the healing autogenous vein graft. **A,** The intima in the normal vein is lined by large flat endothelial cells that are more permeable than those in arteries. The intima is separated from the media by fenestrated internal elastic laminae. The tunica media is thin compared with an artery, with two or more layers of smooth muscle cells (SMCs), whereas the adventitia is relatively thick, consisting of a loose collagenous network interspersed with fibroblasts, vasa vasorum, and small autonomic nerves. **B,** Within 24 hours after implantation, the vein grafts exhibit significant endothelial cell (EC) loss and subendothelial edema. Inflammatory cells, platelets, and fibrin are seen adherent to the surface and infiltrating underneath the attenuated endothelial cell monolayer. There is edema in the tunica media, with extensive SMC necrosis or swelling and hypertrophy of the remaining SMCs, with infiltration of inflammatory cells. **C,** By 2 weeks, there is re-endothelialization of the luminal surface and a developing neointima. Although the endothelium is continuous, it remains dysfunctional, as evidenced by organelle hypertrophy and adhesion molecule expression. The medial edema and inflammation is reduced, and there is increased collagen content. Surviving medial SMCs appear hypertrophic, with increased rough endoplasmic reticulum and Golgi apparatus indicating synthetic transformation. Over time, the adventitia becomes incorporated in surrounding tissue and vasa vasorum, and adrenergic nerve fibers grow in from adjacent arteries and connective tissue. **D,** By 4 weeks, there is a predominant layer of intimal thickening characterized by SMCs embedded in a matrix of collagen and ground substance. Although early medial thickening is caused predominantly by edema and inflammation, fibrous transformation is responsible for late medial thickening. Areas of the medial wall are devoid of cells and entirely replaced by fibrosis. Clinically, stiffening of the vein graft is likely from the increase in fibrous protein as well as increased cross-linking of extracellular matrix proteins.

re-endothelialization are likely to provide valuable data to our understanding of vein graft failure with immediate translational impact.[47]

Early after implantation, the media is marked by edema and focal hemorrhage that likely account for the early thickness changes, which can be measured by ultrasound imaging.[36] The increased radial (compressive) stress of pressurization and disruption from the vasa vasorum creates a zone of ischemia in the vein media. Almost immediately, smooth muscle cells (SMCs) show evidence of apoptosis or frank necrosis, as evidenced by marked vacuolated and pyknotic nuclei. The remaining SMCs, like the ECs, demonstrate severe structural changes, including cellular hypertrophy, mitochondrial swelling, and bleb formation, along with increased rough endoplasmic reticulum and Golgi. Inflammatory cells, particularly macrophages, gradually increase in the media and engulf the necrotic SMCs, where as many as 70% of medial SMCs are lost during this early period.

Despite the substantial acute loss of medial SMCs, most remaining SMCs resist apoptosis and enter the cell

cycle as early as 48 hours after injury.[48-50] The early injury response transcription factors c-fos and c-jun of the activator protein 1 (AP-1) complex can be seen to be induced in SMCs subjacent to the intima, where the first-wave growth and serum factors from adherent platelets and inflammatory cells emerge.[51,52] PDGF, insulin-like growth factor-1, and other growth factors signal increases in SMC migration and proliferation via phosphoinositol-3-kinase (PI3K)-dependent pathways by binding to receptor tyrosine kinases and G protein-coupled receptors.[53] PI3K, in turn, activates numerous downstream pivotal effector molecules related to cell proliferation, including mammalian target of rapamycin (mTOR), p38 mitogen-activated protein kinase, extracellular signal related kinases-1 and -2, and Akt/protein kinase B, which collectively lead to neointimal hyperplasia.[54,55] Inhibition of c-jun and PI3K reduced vein graft stenosis in experimental models.[51,56] Many excellent reviews have addressed SMC proliferation and migration with respect to vascular injury and intimal hyperplasia.[55,57-59]

The adventitia is characterized by fibroblasts within a loose connective tissue stroma with occasional vasa vasorum and vasa nervorum.[39] Adventitial fibroblasts, rich in nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, have been shown to be a source of reactive oxygen species (ROS) in blood vessels after mechanical stretch and injury.[60] In models of balloon injury and vein grafts, perivascular fibroblasts can be converted into myofibroblasts, SMC-like cells that have migratory and synthetic capacity.[61-63] In vivo marker gene transfer studies show that these cells can migrate into the developing intima and contribute to intimal hyperplasia.[63] Further evidence is supported by disruption of transforming growth factor-β or platelet-derived growth factor-BB signaling pathways that can attenuate myofibroblast migration into the neointima, reduce collagen content, and reduce constrictive remodeling after balloon angioplasty.[64,65]

Soon after implantation, breaks in collagen fibers, thrombosis of the vasa vasorum, and fragmentation of the adventitia can be seen. The vasa vasorum, initially disrupted by harvesting the vein, has been shown to return fully functional to the adventitia and outer media as early as 7 days after implantation, where it participates not only in nourishing the healing vein but also in inflammatory cell trafficking into and out of the vein graft.[40,66] The newly implanted vein graft has been generally assumed to receive oxygenation by passive diffusion from luminal arterial blood.[67] However, the vasa vasorum in veins penetrates close to the intima and possibly through to the lumen, so that retrograde filling by oxygenated blood may be possible.[68,69]

The in situ bypass, originally described by Hall[70] in 1962, and more recently advanced by Leather et al[71] and Shah et al,[72] has several theoretic advantages over the reversed saphenous vein graft: First, there is less dissection and therefore less disruption of the vasa vasorum, which should reduce the ischemia reperfusion injury. Second, a small AVF could increase the shear stress through the vein and improve outward remodeling. Third, there is a reduced size disparity at the femoral and distal anastomosis. In practice, however, these theoretic advantages have not translated to increased patency. It is likely that traumatic lysis of the valve leaflets, mobilization of the proximal and distal swing segments, and ligation of the numerous AVFs offset these advantages.

The adventitia is also a compartment housing progenitor cells that contribute to vascular repair by differentiating into a myofibroblast phenotype and possibly other cell types such as pericytes or ECs.[73,74] Because the adventitia lies between the vessel wall and surrounding tissues, it likely contributes to vein graft remodeling by integrating diverse signals from the vessel wall and the local environment. Indeed, a number of experimental programs have exploited adventitial delivery of therapeutic agents to the vein graft to take advantage of these mechanisms.[75-78]

By 3 weeks, the media and adventitia demonstrate extensive fibrous replacement with collagen and a much smaller amount of elastin. Histologic studies of mature grafts demonstrate normally appearing endothelium, a thick neointima composed of abundant collagen and ground substance, and a relatively thinner media.[41]

MOLECULAR REMODELING

The cellular stress and tissue damage associated with venous implantation activate the innate immune system through several different mechanisms. Necrotic cellular debris exposes modified lipids and proteins such as phosphoethanolamine and phosphorylcholine, which is recognized by CRP, a member of the pentraxin family of proteins. Pentraxins can be thought of as primitive antibodies that circulate at normally low levels and police for a limited repertoire of damage patterns commonly seen in invading microbes or damaged cell surfaces.[79] In this sense, they can be thought of as the humoral arm of the innate immune system.[80] CRP can bind to these normally cryptic epitopes, activate the complement system, and recruit inflammatory cells to the injured vein, which exacerbates injury and necrosis. CRP may also activate local SMCs to promote migration. Thus, although frequently viewed as a nonspecific biomarker of inflammation, CRP may act directly as a modulator of acute vascular injury.[81-86]

The release of endogenous stress-response proteins, such as heat shock protein 60, extracellular high mobility group box 1, tenascin-C, and biglycan, are some of the first mediators of immune activation.[87-91] These proteins, collectively referred to as damage-associated molecular patterns, are released by shear stress and matrix remodeling and are the endogenous ligands of the Toll-like receptors (TLRs).[92] TLRs transmit stress signals through adaptor proteins, such as myeloid differentiation protein-88, or the Toll or interferon domain-containing adapter-inducing interferon, to orchestrate the inflammatory response through transcription factors, including nuclear factor-κB (NF-κB).[93,94] TLR-4 and its endogenous ligands are found in human vein grafts, demonstrating its relevance to the current discussion. In TLR-4-deficient mice, vein grafts

demonstrate a reduction in outward remodeling.[95] However, TLR-4-deficient mice also exhibit reduced wall thickening and reduced SMC content, causally implicating this pathway in the formation of intimal hyperplasia and making it difficult to separate lumen dilation and wall thickening.

To separate remodeling from intimal hyperplasia, carotid ligation models have been used. After carotid artery ligation, flow in the contralateral carotid artery increases in a compensatory manner, resulting in flow-mediated vasodilation without the induction of intimal hyperplasia. In mice lacking TLR-4, there is defective flow-induced outward remodeling of the carotid artery and an increase in collagen content, suggesting that TLR-4 is necessary for the matrix turnover required for expansile remodeling.[96] By contrast, mice deficient in the NF-κB p50 subunit demonstrate increased flow-induced outward remodeling and reduced collagen content.[97] Homodimers of p50 bind to DNA but inhibit transcription activity by other NF-κB dimers, thereby acting as a brake on NF-κB.[98] Therefore, p50-null mice have a more pronounced inflammatory reaction in response to a flow stimulus.[97] Other studies show that TLR-4 is integral to osteocalcin-induced myofibroblast transformation from adventitial fibroblasts, which involves the inflammatory mediators, protein kinase C-δ, and cyclooxygenase 2.[99] These studies highlight the link between innate immune activation and matrix turnover with respect to vascular remodeling.

ROS and nitrogen species are also integral messengers in the innate immune system and important mediators of hemodynamic remodeling in the vein. Shear stress in the great saphenous vein, normally about 0 to 4 dynes/cm^2, abruptly rises to as high as 25 to 30 dynes/cm^2 after implantation,[21] which is more than sufficient for induction of ROS.[100] ROS can be generated from NADPH oxidases, NO synthase isoforms, including inducible NO synthase, xanthine oxidase, cyclooxygenase 2, cytochrome P450, and the mitochondrial electron transport chain.[101] Hydrogen peroxide generated from the electron transport chain is essential for flow-mediated dilation of the microcirculation and is a recognized hyperpolarizing factor.[102] ROS, particularly peroxynitrite, generated from the NADPH oxidase subunit p47^{phox}—a cytosolic component of the NADPH oxidase complex—in response to a high-flow fistula, mediates matrix metalloproteinase (MMP) gelatinase induction and outward remodeling of murine AVFs.[103] NADPH oxidase and superoxide are abundant in the early vein graft wall because they are produced not only by infiltrating neutrophils but also by SMCs and ECs.[104] In vein grafts and AVFs, increased superoxide production has been directly correlated with dedifferentiated cells in the neointima as well as with a reduction in free radical scavenging enzymes, superoxide dismutase, and Cu/Zn superoxide dismutase activity.[105,106] The presence of peroxynitrite, a product of superoxide and NO, suggests uncoupling of NO synthase isoforms and demonstrates the altered redox state that exists in the healing vein.[103,106]

ROS are essential for activation of MMPs by cleaving their prodomain and unmasking the active site.[100,103,107] The MMPs consist of a family of ~20 related proteins that collectively can degrade all of the core proteins and proteoglycans in the venous wall during remodeling.[108] Because of this potential destructiveness, MMPs are tightly regulated at several levels, including transcription, activation of protease proforms, secretion of stored MMPs, and through binding to their natural inhibitor, tissue inhibitor of MMP.[109] Thrombin, plasmin, and neutrophil proteases activate MMP-2 bound to membrane type 1 metalloproteinase, whereas the signal transducer extracellular signal related kinases-1 and -2 control MMPs in experimental vein graft models.[43,110] In addition, shear stress results in phosphorylation of the p65 subunit of the inflammatory transcription factor NF-κB, which along with AP-1 and other transcription factors, stimulate MMP transcription through coordinated promoter binding.[100,109] MMP-2 and MMP-9, referred to as gelatinases due to their ability to break down gelatin and several other collagens, in particular, the type IV collagen of the basement membrane, are upregulated within as little as 3 hours after venous implantation.[43,111-113]

Ultrastructural studies have documented progressive loss of type IV collagen in the early bypass graft, indicating destruction of the basement membrane (BM) that normally surrounds 95% of saphenous vein medial SMCs.[114,115] The SMC BM not only represents a physical barrier to SMC migration but also focal contacts between SMC and BM laminin that keep the cell in a quiescent, differentiated phenotype.[116] Therefore, destruction of the BM by MMPs liberates the SMC to migrate and also results in loss of differentiation.[117,118] Experimental inhibition of MMPs limits flow-mediated arterial enlargement and elastin degradation in rat and rabbit models,[119,120] whereas various in vivo and ex vivo techniques to increase tissue inhibitor of MMPs in the venous wall have been successfully used to mitigate intimal hyperplasia.[121-123] These studies highlight the fact that matrix turnover, intimal hyperplasia, and vascular remodeling are inextricably linked to one another.

The local renin-angiotensin-aldosterone system may be an important link between vein graft collagen production and inflammation. Aldosterone, signaling through the mineralocorticoid receptor (MR), has been shown to be an important mediator of vascular inflammation, oxidative stress, and fibrosis in clinical and experimental settings.[124-126] Aldosterone promotes vascular fibrosis in response to injury, and the MR regulates a number of profibrotic genes in SMCs, including type 1 and type 3 collagens and connective tissue growth factor.[127] The MR has been demonstrated to be upregulated in experimental vein graft models as well as in human explanted vein grafts, establishing its clinical relevance.[128] More recently, antagonism of the MR with spironolactone reduced vein graft wall thickening, fibrosis, and inflammation in a mouse vein graft model.[126] In that study, spironolactone treatment reduced the vein graft intima-media collagen area by 53% and

reduced the number of infiltrating polymorphonuclear cells (PMNs) by threefold.

The cytokine tumor necrosis factor-α (TNF-α) is also involved in immune-mediated vascular remodeling in animal models. In arteriogenesis, TNF-α colocalizes with macrophages located in a perivascular cuff surrounding remodeling arteries, and mice lacking TNF-α or the p55 TNF receptor show significant reduction in collateral blood flow. By contrast, inhibitors of TNF-α attenuate collateral artery development.[129] Although flow-mediated vascular remodeling was once thought to be a phenomena intrinsic to the vascular wall, recent work has demonstrated that macrophages are prerequisite for dilation or shrinkage of conduit vessels in response to flow stimuli.[130-132]

INFLAMMATORY CELL PHENOTYPE AND THE FATE OF REMODELING

The homing, trafficking, and retention of inflammatory cells into the vein graft wall is unlikely to be uniform along the entire graft length but rather is dependent on local flow disturbances and injury variance.[133,134] In general however, monocytes and PMNs can bind to the vein graft in high shear conditions (20-40 dynes/cm^2) through the leukocyte β_2-integrins, $\alpha_M\beta_2$ and $\alpha_L\beta_2$ receptors, allowing firm adhesion to vascular cells and >30 proteins of the extracellular matrix (ECM), including fibrinogen.[135,136] Early induction of MMPs to breakdown BM and other ECM barriers not only permits SMC migration out toward the intima but also facilitates entry of PMNs and monocytes into the vessel wall.[43] Although macrophages are essential for vessel remodeling, they also contribute to the inflammatory state of the newly implanted vein, as evidenced in macrophage depletion studies in which there is a reduction in inflammatory cytokines as well as intimal hyperplasia.[137,138]

Monocyte chemotactic factor-1 (MCP-1) and its receptor CC-chemokine receptor-2 is the classic chemoattractant pathway for monocyte invasion in the vein graft. Its importance in arteriogenesis is evidenced by the fact that MCP-1 administration augments collateral artery development after femoral artery ligation in experimental models.[139] Conversely, transgenic mice deficient in MCP-1 have reduced collateral artery formation.[140] MCP-1 has been shown to be induced in SMCs and ECs with ≤24 hours of exposure to increases in circumferential wall tension or stretch (cyclic stress) in a manner that depends on the inflammatory transcription factor AP-1.[141] AP-1 regulates the expression of many stress response genes, including those associated with the proinflammatory phenotype of ECs and vascular SMCs and is activated by ROS.[142] Increasing venous pressure in vivo increases SMC proliferation and MMP-2 activation in an AP-1-dependent manner.[143] MCP-1 also directly participates in growth and migration of SMCs by targeting cyclins through the inflammatory transcription factor, nuclear factor of activated T cells.[144-147] Hence, cyclic stress may induce MCP-1 production in vascular cells through AP-1-dependent mechanisms, and in turn, MCP-1 can induce neointimal formation through nuclear factor of activated T cells. Inhibition of MCP-1 or CC-chemokine receptor-2 reduces vein graft intimal hyperplasia.[145,148]

Other pathways are involved in inflammatory cell recruitment to the vein graft besides MCP-1. The CXC chemokine ligand-12, also called stromal cell-derived factor-1α (SDF-1α), is an essential cytokine for stem cell mobilization and is involved in homing circulating cells to the vein graft. Theoretically, SDF-1α and its receptor, CXCR4, could be beneficial by recruiting progenitor cells to repair the healing vein graft.[149] However, CXCR4$^{+/-}$ heterozygote mice have significantly lower CXCR4 cell surface receptor levels on bone marrow-derived mononuclear cells and are less responsive to SDF-1α. Vein bypasses placed in these mice exhibit less inflammatory cell infiltrate and less neointimal formation than those in wild-type controls.[150] A CXCR4 small molecule antagonist inhibits neointimal formation and smooth muscle progenitor cell mobilization after arterial injury in an apolipoprotein E$^{-/-}$ mice model, providing further circumstantial evidence of the importance of this pathway in vein graft failure.[151]

That inflammation and macrophages are involved in inward and outward remodeling suggests that we should not be looking solely at the magnitude of inflammation but rather at the nature of the inflammatory response.[131] Receptor characteristics and densities on the macrophage cell surface dictate what it "sees" and contribute to preferential trafficking and retention into blood vessels. For example, longer-lived resident macrophages expressing the chemokine receptor CXCR3 have been found in humans within the adventitia of abdominal aortic aneurysms and in areas of flow disturbance, such as the carotid bifurcation, suggesting that they are associated with pathologic states.[130] CXCR3 is the receptor for interferon (IFN)-induced protein of 10 kD, as well as monokine-induced IFN-γ. IFN-γ, in turn, is the classic T-cell helper (T_H1) cytokine that induces macrophage activation and production of the prototypical cytokines, TNF-α, interleukin (IL)-1, and IL-6. These macrophages are further classified by a low expression of the lymphocyte antigen 6c (Ly6c^{low}) and CXCR3$^+$; Ly6c^{low} macrophages are known to patrol the microvasculature, including adventitia microvessels.[152] In mice, CXCR3 signaling contributes to accumulation of adventitial macrophages and is also involved in negative remodeling associated with reduced flow.[130] Signaling through the CXCR3 receptor stimulates the transglutaminase cross-linking enzyme, factor XIII subunit a, which exerts its effects by cross-linking ECM proteins and serves as a biologic glue.[130,153,154] The combination of fixation of the ECM by cross-linking and increased collagen production clearly would prohibit luminal expansion. Might persistent flow disturbance around valve sites lead to a more pathologic inflammatory cell subset accumulation into the wall, leading to focal stenosis or failure of luminal expansion?

Polarization of the initial immune response may decide the ultimate fate of the vein graft. Although infiltrating monocytes contribute most conspicuously to vein graft

Table II. Three clinical stages of vein graft healing

Clinical stages	*Time frame*	*Ultrastructural correlates*	*Molecular correlates*
Lumen dilation and increased wall thickness	0-30 days	Endothelial denudation, medial wall edema	Inducers: DAMPs, neoantigens, ROS, serum growth factors
		Cellular homing/trafficking	Amplifiers: TNF-α, IL-1
		Apoptosis/necrosis	Receptors: TLRs, NLRs, IL-1R, and TNF-αR -1, -2
		Proliferation	Transducers: AP-1, NF-κB, NFAT
		Matrix remodeling	
Stiffness increase in Young's elastic modulus	3-6 months	Myofibroblast transformation	Fibrosis (TGF-β, CTGF, SMAD1/2, TGFR1, TGFR2)
		Collagen deposition	Cross-linking ECM transglutaminases (factor XIIIa)
Endothelial recovery, vein graft exhibits flow mediated vasodilation	>6 months	Complete endothelial coverage, barrier function, and return of vasoreactivity	EDRF: eNOS, NO, prostacyclin, EDHF

AP-1, Activated protein-1; *CTGF,* connective tissue growth factor; *DAMPs,* damage associated molecular patterns; *ECM,* extracellular matrix; *EDHF,* endothelium-derived hyperpolarizing factor; *EDRF,* endothelium-derived relaxing factors; *eNOS,* endothelium nitric oxide synthase; *IL,* interleukin; *NFAT,* nuclear factor of activated T cells; *NF-κβ,* nuclear factor-κβ; *NO,* nitric oxide; *NLR,* nod-like receptor; *ROS,* reactive oxygen species; *SMAD,* mothers against decapentaplegic homologue; *TGF-β,* transforming growth factor-β; *TGFR,* transforming growth factor-β receptor; *TLR,* Toll-like receptor; *TNF,* tumor necrosis factor.

remodeling, lymphocyte subpopulations and macrophage phenotype switching might ultimately set the stage for resolution or chronic inflammation and fibrosis. The professional antigen-presenting cell, the dendritic cell, and $CD3^+$ T lymphocytes have all been identified in human vein graft specimens.[155,156] Studies involving lung and liver fibrosis indicate that T_H2-type dominant cytokine responses involving IL-4, IL-5, IL-13, and IL-21 are profibrotic, whereas T_H1-associated cytokines, dominated by IFN-γ and IL-12, may be important in the resolution of inflammation and possess antifibrotic properties.[157] IFN-γ and IL-12 treatment attenuates fibrosis in experimental pulmonary and renal models of fibrosis.[158-160]

Of note, recent work has demonstrated that the resolution of inflammation, formerly viewed as a passive decrescendo of proinflammatory signals, is in fact an orchestrated process driven by specific "proresolving mediators" (PRMs). Using unbiased lipidomics in models of self-limited inflammation, researchers have discovered that novel lipid mediators derived from polyunsaturated fatty acids (PUFAs) are generated by specific biosynthetic pathways.[161-163] Four distinct classes of PRMs have been recognized: the lipoxins, derived from the ω-6 PUFA arachidonic acid, and the resolvins, protectins, and maresins, derived from the ω-3 PUFAs eicosapentaenoic acid and docosahexaenoic acid.[164-166] Resolvins act via specific G-protein-coupled receptors[167-169] to reduce expression of proinflammatory cytokines and adhesion molecules, increase expression of anti-inflammatory cytokines, and increase clearance of cellular debris.

Emerging evidence from our group and others has demonstrated biologic activity of PRMs on vascular cells.[170,171] Lipoxins and resolvins regulate leukocyte-endothelial interactions, reduce the formation of ROS, and regulate the production of prostacyclin and NO.[172-176] More recently, we have demonstrated a broad spectrum of beneficial actions of D-series resolvins on vascular cells.[177] These include:

1. Inhibition of leukocyte adhesion and adhesion molecule expression;
2. Inhibition of cytokine expression;
3. Inhibition of vascular SMC proliferation and migration;
4. Reduction in oxidative stress; and
5. A reduction in neointima formation after balloon angioplasty in rabbit arteries.

These studies suggest that endogenous resolution mechanisms may be an important element of the homeostatic process of vascular remodeling and may offer a new therapeutic target to manipulate vascular healing.

CONCLUSIONS

We have described three measurable, temporally distinct clinical stages of vein graft remodeling: luminal and wall thickness changes, changes in stiffness, and the return of endothelial function (Table II). To gain some mechanistic insights into these clinical stages, we have attempted to relate them to histologic and molecular changes associated with vein graft implantation. Of course, this is an oversimplification; for example, stimulating macrophages alone through the TLR-4 and its two adaptor proteins, as discussed, produces 775 unique proteins, including 52 cytokines![178] Regulation of this single receptor and its effectors not only occurs at the genetic and epigenetic layers but also depends on adaptor protein interplay, which can be synergic or redundant. As demonstrated in Fig 2, intimal hyperplasia and constrictive remodeling often occur together to reduce lumen area, suggesting common signaling pathways. However, we have chosen to highlight specific examples to create a temporal molecular framework of the innate immune system's role in the clinical stages vein graft remodeling.

Although inflammation is clearly crucial for remodeling of the new vein graft, we must be specific when characterizing inflammation. It is paradoxic that diabetes mellitus and renal failure, two diseases hallmarked by

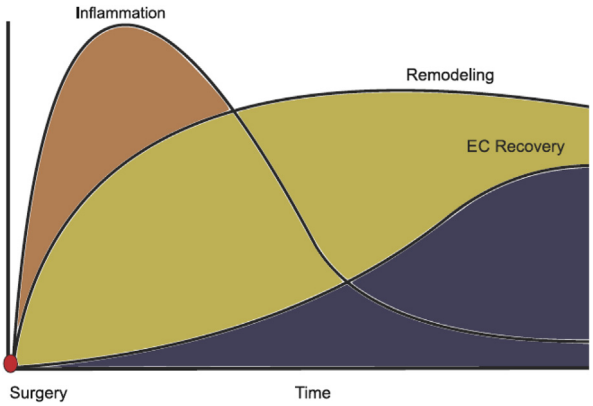

Fig 4. Hypothetic sequence of vein graft healing: Inflammation peaks early after implantation and then subsides. The critical period of vein graft luminal remodeling is largely complete by the first 30 days. Functional endothelial cell (*EC*) recovery is temporally delayed by several months. Mature vein grafts exhibit an endothelial layer overlying a stable neointima. Endothelium-dependent relaxation in mature grafts is mediated by nitric oxide (NO).

systemic inflammation and oxidative stress, have not been shown to directly affect vein graft remodeling or patency.[179,180] It is possible that an intense tissue-level inflammatory response to implantation that quickly subsides is most conducive to vascular wall remodeling, as denoted in Fig 4. However, should vein graft wall inflammation fail to resolve, then the stage is set for pathologic remodeling, fibrosis, and vein graft stenosis. Likewise, time and again, hemodynamic stress has been shown in animal models to influence the development of intimal hyperplasia.[18,19,181] High shear conditions skew the cytokine repertoire to a T_H2-type response, with lower inflammatory and higher anti-inflammatory cytokines compared with low shear stress.[182] However, blood flow and shear stress are largely unmodifiable factors dictated by the inflow and outflow conditions and vein diameter. Vascular surgeons know that vein grafts remain patent and function successfully in conditions of extremely low flow, such as the pedal bypass.[179] Even more extreme is the bypass to an isolated popliteal artery segment, initially described by Davis et al,[183] which provides testimony to the functionality of vein grafts whose outflow to the leg is solely through popliteal artery collateral circulation. Hemodynamic stresses are the blunt forces of global remodeling, but it is the nature of the inflammatory response that finely sculpts vein graft geometry.

AUTHOR CONTRIBUTIONS

Conception and design: CO, MC
Analysis and interpretation: CO, MC, WG, AR
Data collection: CO, MC, AR, WG
Writing the article: CO
Critical revision of the article: CO, MC
Final approval of the article: CO, MC, AR, WG
Statistical analysis: CO
Obtained funding: CO
Overall responsibility: CO

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