Editorial

Vascular Matrix and Vein Graft Failure
Is the Message in the Medium?

Joseph Loscalzo, MD, PhD

The pioneering work of Favaloro4 established the era of surgical revascularization for the treatment of ischemic heart disease. The benefits of this procedure were recognized early, and the use of the saphenous vein as a bypass conduit rapidly gained widespread acceptance as an effective treatment for angina. However, surgical revascularization has significant shortcomings, principal among which is the high rate of accelerated atherosclerosis that develops in vein grafts: at 10 years after surgery, 40% of grafts are occluded and only 50% are free of significant disease.5

Vein graft failure is a consequence of three related processes. Early after implantation, thrombosis plays a critical role, both by predisposing to acute occlusion and by serving as a stimulus for neointima formation and, ultimately, graft atherosclerosis. Within 6 weeks after implantation, virtually all arterialized vein grafts develop intimal hyperplasia, which, although it only modestly compromises the lumen of the graft, renders the graft susceptible to atherosclerosis.6 The cellular events that account for this intimal response include migration and proliferation of vascular smooth muscle cells, perhaps derived from adventitial fibroblasts (myofibroblasts),7 followed by extracellular matrix synthesis and deposition. This latter process ultimately leads to progressive intimal fibrosis with relatively reduced intimal cellularity.5

Vein grafts are susceptible to intimal hyperplasia and atherosclerosis for two main reasons. First, explanted vein grafts are prone to vessel wall ischemia owing to a loss of functional vasa vorum; regrowth of these nutrient vessels appears as early as 5 days after graft placement, but a rich network sufficient to provide blood flow to the thickened neointima takes up to 6 months to develop.6 Second, an arterialized vein graft is subject to an acute, marked increase in wall stress. These two processes, ischemia and wall stress, induce endothelial dysfunction or loss, which reduces the availability of endothelial factors that prevent inflammatory cell adhesion, thrombosis, and smooth muscle cell proliferation, including chiefly prostacyclin, adenosine, and nitric oxide.7 Importantly, the natural history of the functional changes in the endothelium of an arterialized vein graft indicates that endothelial function improves after several weeks; however, the extent of restoration of function is incomplete and never achieves that of the prearterialized vein or a normal artery.8 In view of this pathophysiology, Cable and colleagues9 recently demonstrated the successful transfection of the endothelial nitric oxide synthase gene in human saphenous vein grafts as a means to preserve vascular nitric oxide production and attenuate the extent of endothelial dysfunction over time.

Another molecular approach to the prevention of vein graft disease has focused on inhibiting smooth muscle cell proliferation. As in studies of molecular targets for preventing restenosis after angioplasty,10 early work in this area has focused on modulating expression of cell cycle–regulatory genes. Using antisense oligodeoxynucleotides to block expression of proliferating cell nuclear antigen and cell division cycle 2 (CDC2) kinase, Mann et al11 were able to prevent vascular smooth muscle proliferation in an arterialized jugular vein graft in the rabbit. This approach led to a reduction in both intimal hyperplasia and diet-induced atherosclerosis in this animal model.

In this issue of Circulation, George et al12 used a different approach to the prevention of neointimal hyperplasia in vein grafts. Recognizing that matrix degradation by a family of extracellular proteases, the matrix metalloproteinases (MMPs), is essential for smooth muscle cell migration and proliferation in the neointima, these investigators attempted to reduce MMP activity by transfecting the gene for an inhibitor of MMPs. MMPs compose a superfamily of 66 known zinc peptidases that degrade collagen, gelatin, and elastin. These enzymes are found in organisms ranging from Bacteroides fragilis to humans,13 and 17 human types have been identified to date. In mammals, MMPs are critical for cell growth and proliferation, cell migration, organ development, reproduction, and tissue remodeling. In all of these biological phenomena, matrix degradation is essential to facilitate changes in cell phenotype: ligand-dependent cell-matrix associations are critical for modulating cell function, and matrix degradation can thereby modulate responses of the cell to its microenvironment.

Vascular smooth muscle cells, monocytes/macrophages, and endothelial cells have all been shown to express MMPs. Vein graft stenosis appears to be associated with increased expression of MMP-9 and increased activation of MMP-2,14 and pharmacological inhibitor studies show that MMPs are, indeed, involved in the formation of the neointima.15 In this regard, it appears that MMPs are critical for smooth muscle

See p 296

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cell migration and proliferation, which serve as the cellular basis for neointimal proliferation in vivo.\textsuperscript{16}

The family of tissue inhibitors of metalloproteinases (TIMPs) comprises 4 naturally occurring proteins that bind to and inactivate the MMPs. Only recently have the TIMPs been implicated in the pathobiology of vein graft disease: Kranzhofer et al\textsuperscript{17} showed that TIMP-1, -2, and -3 are expressed during neointima formation in organ cultures of human saphenous vein.

One might wonder how enzymes that degrade matrix and proteins that inhibit their action act in concert to promote neointima formation. Clearly, the cellular and molecular events are complex and depend on the temporal and spatial expression of these proteins, which is dependent on several regulatory mechanisms. The genetic regulation of the expression of these proteins occurs at several levels. Cytokines (interleukin-1\textalpha) and growth factors (platelet-derived growth factor-BB) act synergistically through a protein kinase C–dependent mechanism to increase expression of MMP-9, whereas transforming growth factor-\beta and platelet-derived growth factor-BB induce TIMP-3 expression in vascular smooth muscle cells; cytokines and growth factors have no effect on MMP-2, TIMP-1, and TIMP-2 expression.\textsuperscript{18} By contrast, interleukin-4, interferon-\gamma, and interleukin-10 inhibit the synthesis of MMP-1, MMP-3, and MMP-9, respectively, and interleukin-10 stimulates TIMP-1 production by monocytes.\textsuperscript{19}

In addition to regulation of gene expression, MMP activity is regulated by conversion of the inactivezymogen precursor into active enzyme. The inactive conformation of the proenzyme is maintained by the formation of a coordination complex between the active-site zinc ion and a cysteine residue in the (conserved) propeptide sequence PRCGXD.\textsuperscript{20} Stromelysin (itself a member of the MMP family), trypsin, kallikrein, chymase, and plasmin are known activators of MMPs,\textsuperscript{21} and regulation of the expression and activation of each of these proteolytic enzymes, in turn, indirectly modulates MMP activity.

On the basis of the growing body of evidence implicating MMPs in the smooth muscle cell proliferative response to vascular injury, George et al\textsuperscript{12} chose to inhibit MMP activity in vein grafts as a potential therapeutic approach to the prevention of intimal hyperplasia. In earlier work, they showed that transfer of the TIMP-1 gene inhibits smooth muscle cell migration and neointima formation in human saphenous vein; however, they were unable to demonstrate any effect on smooth muscle cell proliferation or viability with this particular TIMP.\textsuperscript{22} In the present study, these investigators transfected grafts with the gene for TIMP-3 hoping to exploit two unique properties of this TIMP, namely, its insolubility and matrix affinity and its ability to induce vascular smooth muscle cell apoptosis.\textsuperscript{23} Using this approach, they observed an 84% reduction in neointima at 14 days and a 58% reduction at 28 days in porcine vein grafts transfected with the TIMP-3 gene. As a control, they found that TIMP-2 gene transfection had no effect on neointima formation in this model.

This study has important implications for the treatment of vein graft disease that deserve emphasis. Intimal hyperplasia is a diffuse and uniform process in the explanted vein graft, and the application of a viral vector bearing a therapeutic gene to the entire graft is essential to ensure that the graft will be uniformly free of disease. In addition, the importance of TIMP-3 as an agent that induces vascular smooth muscle cell apoptosis cannot be overemphasized. In its dual role as an inhibitor of MMPs and an agent of apoptosis, TIMP-3 has a potentially unique advantage in vein graft therapy. In addition, its matrix affinity ensures appropriate, durable expression of activity at the critical site of action, ie, the vascular growth “medium” for smooth muscle cells, the matrix itself; other TIMPs are soluble and are released into the fluid phase, which limits their locus and duration of activity. In addition to these benefits, other, as yet unknown, advantages may derive from preservation of endothelial function both by impairing myofibroblast proliferation\textsuperscript{24} and by enhancing endothelial cell mitosis.\textsuperscript{25}

Notwithstanding these unique properties of TIMP-3, there are potential problems that have yet to be addressed. In patients with coexistent atherosclerotic disease, especially contiguous to the site of TIMP-3 gene expression (ie, distal to an anastomotic graft site), TIMP-3–induced apoptosis may lead to adverse plaque remodeling with weakening of the plaque. This possible adverse effect would be a consequence of reduced vascular smooth muscle mass and matrix production. Similar concerns have been raised in models of MMP overexpression, which also leads to plaque weakening but does so by proteolysis of infrastructural matrix.\textsuperscript{26} The longer-term benefits of this therapy also remain unknown: without persistent, durable expression and activity of TIMP-3, the possibility that this therapy delays, but does not prevent, intimal hyperplasia must be considered.

The problem of aortocoronary saphenous vein graft disease remains an important one for the broad population of patients undergoing surgical revascularization for coronary atherothrombotic disease.\textsuperscript{27} Novel approaches to this persistent problem are certainly warranted, and the growing area of vascular gene therapy provides one potential paradigm by which to apply these therapies. To date, most investigators in the field of vascular gene therapy have focused their attention on the problems of restenosis and angiogenesis. Restenosis, although morphologically similar to vein graft disease, has a different vascular pathobiology; however, there are similarities that, by analogy, have served as the basis for the choice of therapeutic target. The most obvious targets in this disorder, namely, cell cycle genes governing smooth muscle cell proliferation, have been the major emphasis of the first wave of studies in this field. These early studies, in my view, represent a proof of principle for vascular gene therapy rather than a specific treatment for vein graft disease (or restenosis, for that matter) with a high likelihood of success. It is timely to identify more rational therapeutic approaches in this embryonic field and to do so with an eye toward specificity, unique and targeted mechanism, and durability of effect. TIMP-3 overexpression represents a fine example of such a rationally designed approach to the problem of vein graft disease. Still, the data presented by George et al\textsuperscript{12} are preliminary, and owing to the many other practical issues that must be addressed in the development of this agent, only time
References


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