Commentary

The Source of Neointimal Cells in Vein Grafts: Does the Origin Matter?

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One century ago, in 1906, the first successful clinical vein graft was performed using an *in situ* popliteal vein to bypass a popliteal aneurysm (reviewed in¹). The first free vein graft was performed one year later when a 10-cm-long saphenous vein segment was used to restore circulation after excision of a traumatic axillary aneurysm. In 1967, the first coronary artery bypass grafting was performed to treat life-threatening arterial occlusions by re-establishing vascularization. This first coronary artery bypass grafting initiated an explosive increase in clinical use of vein grafting,¹ and in 2005 an estimated 469,000 coronary artery bypass procedures were performed within the United States.² Besides coronary artery bypass surgery, vein grafting is used in the treatment of mesenteric ischemia and peripheral artery occlusive disease and in the creation of arteriovenous fistulas for hemodialysis. For short-segment lesions in both ischemic hearts and lower extremities, balloon angioplasty with or without stenting (ie, endovascular therapy) has become the interventional therapy of choice. Although major advancements have been made in the last two decades with regard to endovascular therapy (eg, introduction of drug-eluting stents), for longer-segment lesions, bypass surgery remains the needed treatment. At the level of the heart, the use of arterial grafts (left/right internal mammary artery, radial artery or gastro-epiploic artery) is preferred above vein grafts because arteries show higher patency rates. Appropriate autologous arteries are not always available, though; thus, the use of vein grafts is still very prominent in the treatment of ischemic heart disease. However, intimal hyperplasia after vein grafting remains an important problem, the process of which is further delineated by Diao et al³ in this issue of *The American Journal of Pathology*.

Intimal Hyperplasia (Neointima Formation) after Vein Grafting

Although coronary artery bypass surgery is an effective treatment for ischemic heart disease, approximately 50% of the vein grafts fail within 10 years after grafting, and in the case of vein grafting in the lower extremities, 30% to 50% fail even within 5 years after grafting as a result of occlusion of the vessel lumen. The most common cause of these occlusions is intimal hyperplasia, combined with ongoing atherosclerotic disease in the arterial outflow tract, especially in the lower extremities. Intimal hyperplasia is characterized by the formation of a neointima consisting of predominantly vascular smooth muscle cells (VSMCs) and extracellular matrix. Because of the arterial pressure and altered shear stress profile within vein grafts, endothelial cells (ECs) covering the luminal side of the vein become activated and damaged or are even lost. EC damage/loss is considered to be the inciting event for the development of intimal hyperplasia. Activated ECs, but also platelets and macrophages, release growth factors and cytokines that cause VSMC proliferation and migration into the subendothelial space of the intima. The subendothelial VSMCs continue to proliferate, and they synthesize and secrete extracellular matrix components, eventually culminating in the formation of an occlusive neointima.

Treatment after Vein Grafting

Currently, patients suffering from atherosclerosis and undergoing vein grafting are treated with anticoagulants, statins, and angiotensin-converting enzyme inhibitors to prevent thrombosis, lower cholesterol levels, and control blood pressure, respectively. This treatment regimen is

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primarily directed at reducing the atherosclerotic risk and is not efficacious in reducing intimal hyperplasia, as is underscored by the high rates of graft failure after vein grafting. A potential alternative strategy to treat ischemic disease would be restoration of vascularization of ischemic cardiac or peripheral tissue by stimulating the biological processes of angiogenesis (ie, new vessel formation from pre-existing mature ECs) and vasculogenesis [ie, new vessel formation from recirculating endothelial progenitor cells (EPCs)]. A successful alternative strategy as such would make vein grafting superfluous. However, although pro-angiogenic pharmacological agents are currently under development and some progress in this field is being made, it is anticipated that the need for vein grafting will remain at the same level for the coming years. Therefore, improvement of long-term patency of vein grafts is needed, and studies on the development of effective therapies that target the development of intimal hyperplasia are thus warranted since no adequate therapy is currently available.

As mentioned above, current treatment of vein graft recipients aims at reducing the atherosclerotic risk rather than the pathogenesis of intimal hyperplasia. Therapies aimed at preventing intimal hyperplasia should ideally have a binary action, ie, maintenance of vein graft endothelial integrity and inhibition of both VSMC recruitment/proliferation and extracellular matrix synthesis. EC integrity can be maintained by reducing the severity of EC damage, which is, however, hard to achieve in clinical practice. Alternatively, EC integrity can be restored by enhancing re-endothelialization mediated by neighboring mature ECs and/or recirculating EPCs, the latter possibly acting in a paracrine manner by creating a microenvironment that facilitates re-endothelialization by mature ECs. Enhanced EPC-mediated re-endothelialization using statins⁴ and estrogen⁵ has indeed been shown to attenuate neointima formation in experimental models of restenosis, and this most likely occurs also in vein grafts. Since VSMCs are the predominant cellular constituent of the neointima in vein grafts, prevention of recruitment of VSMCs, or their progenitors, to the injured vascular wall is a potentially efficacious strategy to attenuate intimal hyperplasia. However, when aiming at reducing recruitment of VSMCs to the injured vascular wall, one needs to know the anatomical origin of the neointimal VSMCs or their ancestors. Although it is well established that the bone marrow (BM) harbors a population of EPCs that can contribute to endothelial repair under pathogenetic conditions,⁶ the role of BM-derived VSMCs in the development of intimal hyperplasia in vein grafts is still a matter of debate.

Origin of Neointimal VSMCs in Vein Grafts

Although previous studies suggest that BM-derived cells do not participate in intimal hyperplasia in vein grafts, in this issue of the *AJP* Diao and colleagues³ elegantly show that both BM-derived VSMCs and ECs contribute to intimal hyperplasia after vein grafting. Using their experimental mouse model of intimal hyperplasia in autologous vein grafts (external jugular vein grafted into the infrarenal

abdominal aorta) using growth factor protein (GFP)-BM-chimeric mice, Diao and colleagues were able to demonstrate by high-resolution confocal analysis that at 2, 8, and 16 weeks after vein grafting, BM-derived ECs were incorporated in the endothelial layer of the vein graft. At 2 and 8 weeks after grafting, interruptions in the endothelium were detected, whereas at 16 weeks after grafting, the endothelial integrity was fully restored. Although the exact percentages of BM-derived ECs were not determined in this study, these results suggest that BM-derived ECs do contribute to re-endothelialization of vein grafts. However, the novelty of this article is the observation that a substantial number of neointimal VSMCs in vein grafts are of BM origin. When analyzing smooth muscle-myosin heavy chain expressing neointimal VSMCs for co-expression of GFP at two and eight weeks after vein grafting, ~5% and ~12%, respectively, of neointimal VSMCs appeared to be BM derived. This may be an underestimation since the average level of chimerism was ~70%, and the efficiency of detecting GFP expression in GFP-transgenic VSMCs was 'only' 50%. Unexpectedly, at 16 weeks after grafting neointimal GFP protein expression was no longer detected, suggesting loss of neointimal BM-derived VSMCs due to apoptosis or lack of robust GFP expression at later time points. To address this issue, neointimal VSMCs were microdissected using a laser capture microscope followed by real-time PCR for the presence of GFP DNA. Results indicate that 16 weeks after grafting, ~20% of the neointimal VSMCs contained GFP DNA and were thus of BM origin. Apparently, expression of GFP in neointimal VSMCs is not robust, and one should be aware of this technical limitation when using GFP-transgenic mice for tracking studies of vascular cells, since this can potentially lead to false-negative results. Taken together, Diao and colleagues provide clear experimental evidence that BM-derived VSMCs contribute substantially to neointima formation after jugular vein grafting in the abdominal aorta in mice.

The observation that BM-derived ECs are involved in re-endothelialization of vein grafts confirms results reported by others.^{7,8} Zhang and colleagues⁷ showed in their vena cava to carotid artery vein grafting model in mice that neointimal ECs are a mixed population of vein-intrinsic (graft-derived) and vein-extrinsic (recipient-derived) ECs, of which ~10% are derived from the BM. Using the same vein grafting model, Xu and colleagues⁸ observed complete replacement of graft endothelium with recipient-derived cells, of which one-third was derived from the BM. Since BM-derived EPCs are clearly involved in re-endothelialization of vein grafts, these cells might serve as a target for therapeutic intervention to enhance re-endothelialization. Mobilization of EPCs from the BM is stimulated by factors released from sites of vascular damage,⁹ after which EPCs are recruited to the site of injury, where they mature and incorporate into the tissue to restore endothelial integrity. A reduced capacity to maintain endothelial integrity in response to vascular damage might be related to reduced numbers of circulating EPCs. Various pathological conditions, like diabetes mellitus, and the presence of cardiovascular risk factors (smoking, hyperlipidemia, etc) have indeed been

associated with reduced number and function of circulating EPCs^{10,11,12} and may reflect reduced EC repair capacity. Especially under these pathological conditions, increasing the number (eg, by granulocyte colony-stimulating factor) and improving angiogenic potential of EPCs is expected to enhance re-endothelialization, resulting in reduced rates of atherosclerosis as well as in-stent restenosis and vein graft intimal hyperplasia.^{13,14}

BM origin of at least 20% of the neointimal VSMCs in vein grafts, as demonstrated by Diao and colleagues,³ is a novel finding that certainly adds to the current knowledge on the plasticity of the origin of neointimal VSMCs. In previous studies, using a vena cava to carotid artery vein grafting model in mice, both Hu and colleagues¹⁵ and Zhang and colleagues⁷ showed significant contributions (up to 40%) of host-derived VSMCs to intimal hyperplasia in their model. However, in both studies, BM-derived neointimal VSMCs were not detected. The difficulties of detecting GFP protein expression (but not the presence of GFP DNA) in VSMCs as demonstrated by Diao and colleagues may also hold true for the cell-tracking systems used by Hu and Zhang^{7,15} and might explain the different results obtained in these studies. Despite the fact that Diao and colleagues found a substantial contribution of BM-derived neointimal VSMCs, the vast majority (~80%) of neointimal VSMCs are derived from the non-BM compartment. The precise anatomical origin of the majority of neointimal VSMCs in vein grafts remains to be elucidated. Since up to 40% of the neointimal VSMCs in vein grafts is derived from outside the graft, potential sources of these graft-extrinsic cells include migrating medial VSMCs from the adjacent artery as well as non-BM-derived vascular progenitor cells that infiltrate the graft via the blood (through the vasa vasorum or through transendothelial diapedesis) or via postsurgical adventitial adhesions.⁷ Recently, Aicher and colleagues¹⁶ identified a population of adult non-BM-derived vascular progenitor cells residing in the small intestine and liver that were mobilized into the circulation and contributed to neovascularization after ischemic injury. Since the authors of this study focused on vasculogenesis mediated by these tissue progenitor cells, it must be determined whether these cells can also differentiate into VSMCs and contribute to intimal hyperplasia.

Hu and colleagues demonstrated that ~60% of neointimal VSMCs in vein grafts are derived from a vein-intrinsic source,¹⁵ and similar findings were reported by others.^{7,17} These data indicate that the vein graft itself is also a major source for neointimal cells, although the exact source of these cells remains to be elucidated. However, potential sources of these graft-intrinsic cells include adventitial myofibroblasts,¹⁸ but also, recently identified localized vascular progenitor cells residing in the media¹⁹ (phenotype: Sca-1⁺c-kit^{-/low}Lin⁻CD34^{-/low}) and adventitia²⁰ (phenotype: CD34⁺CD31⁻VEGFR2⁺TIE2⁺) may contribute to the development of vein graft intimal hyperplasia. Although these vascular wall progenitor cells were identified in the arterial wall, similar progenitor cell niches may also exist in the vein vascular wall. The contribution of vascular wall-derived progenitor cells in the development of vein graft atherosclerosis is supported by the obser-

vation that adventitia-derived Sca-1⁺ progenitor cells indeed contribute to vein graft atherosclerosis in the ApoE^{-/-} mouse model of atherosclerosis.²¹

Taken together, these experimental data indicate that neointimal VSMCs in vein grafts can be recruited from various anatomical locations, including the BM, the adjacent arterial media, the circulation (containing also vascular progenitors that originate from non-BM compartments), and the vein graft vascular wall itself.

Therapeutical Perspectives

As already mentioned above, enhanced restoration of endothelial integrity in vein grafts is expected to result in reduced intimal hyperplasia. Embryonic stem cells are able to differentiate toward the endothelial lineage and can be induced in the presence of vascular endothelial growth factor (VEGF) and mechanical force generated by the intrinsic flow of blood.²² Particularly Sca-1⁺ progenitors isolated from embryonic stem cell cultures have the capacity to differentiate toward functional ECs *in vitro*. Furthermore, embryonic stem cell-derived ECs were shown to enhance re-endothelialization and attenuated neointima formation when locally transplanted into an injured mouse artery.²² Although embryonic stem cells thus have potential to reduce vein graft intimal hyperplasia in the experimental setting, clinical use of these cells is most likely not feasible in the near future because of technical and ethical restrictions. However, the adult individual also harbors progenitor cells that are committed to differentiate toward the EC lineage. These EPCs have been shown to home to sites of vascular damage and contribute to re-endothelialization, resulting in reduced neointima formation. However, a critical limitation for EPC-based therapies is their low number in the circulation. This low number seemingly reflects insufficient quantity of endogenous EPCs to be biologically relevant in endothelial repair on vascular injury. Moreover, in pathological conditions like diabetes mellitus, the frequency of EPCs is even further reduced.^{10,11} To overcome this problem, the number of circulating EPCs can be increased through pharmacological mobilization using, for example, granulocyte colony-stimulating factor, statins, or the peroxisome proliferator-activated receptor- γ agonist rosiglitazone. Alternatively, numbers of EPCs can be increased by systemic or local transfusion after *ex vivo* expansion of mononuclear cell-derived EPCs. To further promote recruitment of EPCs to the injured vascular wall, local expression of chemoattractants (eg, VEGF or nitric oxide) at the site of injury can be induced.²² Increased local expression of chemoattractants may, however, result in recruitment of inflammatory cells and VSMC progenitor cells and may eventually result in enhanced neointima formation.

Diao and colleagues³ demonstrated that at least 20% of the neointimal VSMCs 16 weeks after grafting are derived from the BM. These data suggest that the BM harbors a population of VSMC progenitor cells that are recruited to the injured vascular wall (like EPCs) and initiate a remodeling process that proceeds beyond the

needs of functional repair, eventually resulting in luminal occlusion. Although the exact phenotype of BM-derived VSMC progenitor cells is as yet unknown and these cells can therefore not be easily detected in the circulation, Simper and colleagues²³ demonstrated that the human peripheral blood indeed contains a VSMC progenitor cell that can give rise to smooth muscle outgrowth cells *in vitro*. Identification of the exact phenotype of BM-derived VSMC progenitor cells as well as characterization of the molecular pathways involved in recruitment, differentiation toward VSMCs, and proliferation will reveal new molecular targets that can be used for therapeutic intervention to attenuate vein graft intimal hyperplasia.

The majority of neointimal VSMCs in vein grafts did, however, originate from the non-BM compartment³ and may actually have been derived from (progenitor) cells residing in the vein graft vascular wall itself. This brings opportunities from a treatment perspective, since grafts can be modified *ex vivo* before grafting to target VSMCs (progenitor cells), resulting in reduced differentiation, migration, and proliferation.

Ex vivo gene transfer using, for example, adenoviruses into the vascular wall is a possibility to introduce genes involved in angiogenesis to enhance re-endothelialization. Alternatively, local peri-adventitial application of antisense oligonucleotides or small interfering RNAs specific for gene products involved in VSMC differentiation, migration, and proliferation can block translation or induce degradation of respective mRNA transcripts²⁴ and potentially result in reduced intimal hyperplasia. Finally, another strategy to reduce vein graft intimal hyperplasia is the introduction of a transcription factor decoy, as was tested in a phase 3 randomized, double-blind, placebo-controlled efficacy and safety trial.²⁵ In this PREVENT (Project of Ex Vivo Vein Graft Engineering via Transfection) trial with the E2F decoy edifoligide, patients undergoing coronary bypass surgery received a saphenous vein graft that was pretreated *ex vivo* with edifoligide or placebo. Edifoligide binds the binding site of the transcription factor E2F, which is involved in controlling the expression of multiple genes responsible for cell cycle progression. Results from this study, however, revealed that edifoligide was no more effective than placebo in preventing vein graft failure 12 to 18 months after surgery, and long-term follow-up is needed to determine whether this strategy has delayed beneficial effects.

Experimental and clinical studies are thus ongoing in which novel therapeutic targets (including growth factors and mediators of signaling and cell cycle pathways) are tested for their efficacy to reduce VSMC differentiation, migration, and proliferation. However, although many such therapeutic targets have been identified and many agents have been directed toward them, few of these agents have reached the clinic.

Conclusion

Diao and colleagues³ convincingly demonstrate for the first time that a substantial number of BM-derived VSMCs are involved in the development of experimental vein graft

intimal hyperplasia. Together with BM-derived EPCs, which are involved in re-endothelialization of the injured vascular wall, these cells may serve as potential targets for therapeutic intervention. However, the majority of neointimal ECs and VSMCs were derived from a non-BM source and can be recruited from both vein graft-extrinsic and -intrinsic niches. These data illustrate that the development of vein graft intimal hyperplasia is a complex process displaying a lot of plasticity with respect to the origin of neointimal cells. Since, apparently, multiple cell types are involved in the development of vein graft intimal hyperplasia, designation of a single cell type as a target for intervention is most likely not sufficient to attenuate intimal hyperplasia, and a multi-target approach is required. These approaches should be binary in such a way that they simultaneously enhance re-endothelialization and reduce VSMC recruitment and proliferation. The development of such an approach is challenging but is required to be able to fight vein graft intimal hyperplasia in the future.

Finally, the origin of neointimal VSMCs and ECs in human vein grafts is as yet unknown. Although experimental animal models certainly provide insights into the processes underlying development of intimal hyperplasia (like the study by Diao and colleagues³) and are necessary to test the efficacy of new therapeutic compounds, these models are unlikely to represent the exact pathophysiology of the development of human vein graft intimal hyperplasia. Therefore, to our opinion, identifying the origin of neointimal VSMCs and ECs in human vein grafts and characterization of the molecular pathways involved is the next step in the quest for an adequate therapy to prevent the development of vein graft intimal hyperplasia. This will eventually result in prolonged vein graft patency with reduced patient morbidity and mortality.

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