Therapeutic Angiogenesis and Vasculogenesis for Ischemic Disease

Part II: Cell-Based Therapies

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Cell Therapy For Therapeutic Vascularization

Endothelial Progenitor Cells and Postnatal Vasculogenesis: Experimental Evidence

The option of performing full-scale endothelial cell transplantation to optimize local neovascularization is daunting if even feasible. An alternative, attractive strategy is designed to exploit the conceptual notion that endothelial cells and hematopoietic stem cells were ultimately derived from a common precursor, the putative hemangioblast. Hematopoietic stem cells had been shown previously to be present in circulating blood, in quantities sufficient to permit their harvesting and readministration for autologous, in lieu of bone marrow, transplantation. The related descendants, endothelial progenitor cells, can be detected in the peripheral circulation. Initially, Flk-1 and a second antigen, CD34, shared by angioblasts and hematopoietic stem cells were used to isolate putative angioblasts from the leukocyte fraction of peripheral blood. Meanwhile, endothelial progenitor cells were isolated from human umbilical cord blood, bone marrow–derived mononuclear cells, or CD34+/CD133+ hematopoietic stem cells and were successfully ex vivo expanded with the use of human peripheral blood mononuclear cells. These cells differentiated into endothelial cells, as shown by expression of various endothelial proteins (KDR, von Willebrand factor, endothelial nitric oxide synthase, VE-cadherin, CD117) and uptake of Dil-acetylated LDL and binding of lectin. In animal models of ischemia, heterologous, homologous, and autologous endothelial progenitor cells were shown to incorporate into sites of active neovascularization in ischemic and tumor tissue. Blood flow recovery and capillary density were markedly improved, and the rate of limb loss was significantly reduced after transplantation of human peripheral blood–derived endothelial progenitor cells or bone marrow mononuclear cells. Likewise, infusion of peripheral blood–derived endothelial progenitor cells, bone marrow mononuclear cells, or purified CD34+ cells improved neovascularization and myocardial function after infarction. Isolated CD34+ cells also increased impaired blood flow in diabetic mice. These findings provide evidence that exogenously administered endothelial progenitor cells augment naturally impaired neovascularization in an animal model of experimentally induced ischemia.

Mobilization of Endothelial Progenitor Cells

Previous investigators have shown that wound trauma causes mobilization of hematopoietic cells, including pluripotent stem or progenitor cells from the bone marrow. Consistent with endothelial progenitor cell/hematopoietic stem cell common ancestry, data have shown that mobilization of bone marrow–derived endothelial progenitor cells constitutes a natural response to tissue ischemia. Endothelial progenitor cells may thus constitute a reparative response to ischemic injury, controlled by the bone marrow via circulating cytokines and soluble receptors and/or adhesive molecules. Meanwhile, several mobilizing factors have been identified. Vascular endothelial growth factor (VEGF) may be considered one of the most predominant mediators of ischemia-induced mobilization of endothelial progenitor cells in mice. Furthermore, in a clinical trial, gene therapy with VEGF plasmid transiently increased the number of endothelial progenitor cells in humans. After the initial report that the cytokine granulocyte-macrophage colony-stimulating factor stimulates mobilization of endothelial progenitor cells from the bone marrow, additional factors have been identified, including angiopoietin-1, stromal-derived factor-1 (SDF-1), placenta-derived growth factor (PlGF), and erythropoietin. Furthermore, the vasculoprotective 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) promoted mobilization and survival of endothelial progenitor cells under experimental conditions and in clinical trials (Figure).

Open Questions

Despite the consensus on the important role of endothelial progenitor cells and their usefulness for potential therapeutic vasculogenesis, several issues remain unanswered. One critical point is the definition of these endothelial progenitor cells, which is reflected by struggles with abbreviations in the literature. As mentioned above, endothelial progenitor cells from various sources and with dramatically different expres-
sion patterns of surface markers have been used successfully to improve neovascularization. One interpretation of these results is that different cells share a similar capacity. Alternatively, one may speculate that, at present, we lack the appropriate marker(s) to define the effective population of cells. Given that only a minor percentage of the cells are incorporated into the capillaries, a contaminating population common in the different cell sources may account for the biological effects in vivo.

The second critical point is how these endothelial progenitor cells enhance neovascularization. Various studies showed that bone marrow–derived cells incorporate into the newly formed capillaries and express endothelial markers, suggesting that endothelial progenitor cells enhance neovascularization by physically contributing to the newly formed capillaries. However, the absolute number of incorporated endothelial progenitor cells dramatically varies between 0% and 90% in the different studies. The experimental animal model (tumor angiogenesis versus limb ischemia), the time point assessed after ischemia, and the use of the endothelial marker proteins, which are more or less specific for endothelial cells, may have contributed to these dramatically different numbers. Interestingly, Lyden et al. demonstrated that the incorporation ratio is changed if different tumors were implanted, suggesting that the environment affects homing, transmigration, and differentiation. The interesting point, however, is that even in the study, which shows that Tie-2–positive bone marrow–derived cells are not integrated into the tumor vessels but are detected adjacent to the vessel, activation of a suicide gene in these bone marrow–derived cells blocked tumor angiogenesis. Thus, in this particular model, Tie-2–positive bone marrow–derived cells support tumor neovascularization and tumor growth without physically building endothelial structures. The authors speculate that paracrine effects are responsible for the proangiogenic effect. Thus, infiltrated bone marrow–derived progenitor cells may additionally contribute to neovascularization, tissue/vessel remodeling, and cardiac regeneration by paracrine effects, as shown previously for the improvement of arteriogenesis by monocyte cells.

Cell Therapy for Critical Ischemia: Evidence From Human Trials

The promising results from experimental studies promoted the initiation of clinical pilot trials, with the first results published in 2002. Tateishi-Yuyama et al. and the Therapeutic Angiogenesis by Cell Transplantation (TACT) study investigators performed a randomized controlled trial in patients with peripheral artery disease. They reported a significant increase in transcutaneous oxygen pressure, rest pain, and pain-free walking time in 22 patients with leg ischemia after intramuscular injection of bone marrow–derived mononuclear cells. Interestingly, freshly isolated peripheral blood monocytes did not exert any effect. These findings are in agreement with experimental data showing that freshly isolated CD14+ monocytic cells are not capable of improving neovascularization after hindlimb ischemia. To achieve a functional improvement, endothelial progenitor cells need to be ex vivo cultured to enrich an active subpopulation (which is maximally approximately 0.5% of the total monocytes) out of the peripheral blood mononuclear cells.

### Table 1. Stem/Progenitor Cell Therapy (Intracoronary Application) in Patients With Acute Myocardial Infarction

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell Type</th>
<th>Patients</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strauer et al.</td>
<td>BMC (40 mL) vs control</td>
<td>n=10</td>
<td>Hypokinetic area ↓ (LVA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=40*</td>
<td>Contractility infarct region ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>End-systolic volume ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Perfusion ↑ (thallium scintigraphy)</td>
</tr>
<tr>
<td>TOPCARE-AMI</td>
<td>BMC (50 mL) vs CPC (250 mL) vs control</td>
<td>n=20</td>
<td>Global and regional EF ↑ (LVA, echo, MRI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=59*</td>
<td>End-systolic volume ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Viability ↑ (PET, MRI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coronary flow reserve ↑</td>
</tr>
<tr>
<td>BOOST</td>
<td>BMC (120 mL) vs control (prospective, randomized)</td>
<td>n=30*</td>
<td>EF ↑ (MRI)</td>
</tr>
</tbody>
</table>

TOPCARE-AMI indicates Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction; BMC, bone marrow cell; CPC, circulating progenitor cell; LVA, left ventricular angiogram; BOOST, Bone marrow transfer to enhance ST-elevation infarct regeneration; and EF, ejection fraction.

Additional studies addressed the safety and feasibility of intracoronary infusion of stem/progenitor cells in patients after acute myocardial infarction (Table 1). Strauer et al\textsuperscript{28} infused bone marrow--derived mononuclear cells, which were isolated by Ficoll gradient centrifugation and cultivated overnight. Bone marrow--derived mononuclear cells (mean $2.8 \times 10^7$) were infused 5 to 9 days after the acute myocardial infarction. In comparison to 10 nonrandomized control patients, who did not undergo cell therapy or additional catheterization, bone marrow--derived mononuclear cell infusion enhanced regional infarct region perfusion as assessed by thallium scintigraphy. Moreover, stroke volume, end-systolic volume, and contractility indices were improved after cell therapy.

In the Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) trial, patients were randomized to receive either bone marrow--derived mononuclear cells or endothelial progenitor cells. Bone marrow--derived mononuclear cells (50 mL aspirate) were isolated by Ficoll gradient centrifugation and immediately infused after preparation. Endothelial progenitor cells were ex vivo expanded out of peripheral blood mononuclear cells from 250 mL blood for 3 days. Bone marrow cells or endothelial progenitor cells were infused a mean of 4 days after myocardial infarction. Bone marrow cells and endothelial progenitor cells significantly improved the global ejection fraction, as assessed by left ventricular angiography, compared with a nonrandomized control patient collective.\textsuperscript{29} Functional improvement was confirmed by MRI in a patient subcollective.\textsuperscript{30} Cell therapy also increased coronary flow reserve, which indicates an enhancement of neovascularization. Endothelial progenitor cells and bone marrow cells showed similar effects in vivo. Furthermore, a first randomized trial was recently presented.\textsuperscript{31} In this study, 60 patients with acute myocardial infarction were randomized to receive intracoronary infusion of bone marrow--derived mononuclear cells or were not treated (no recatheterization, no placebo). The authors reported an increase in ejection fraction and a reduction of end-systolic volumes as assessed by MRI in the group of patients receiving bone marrow cells.\textsuperscript{31} Intramuscular injection of bone marrow--derived stem/progenitor cells was used as the route of application in 4 additional pilot trials (Table 2). Direct injection of isolated hematopoietic stem cells, which express AC133$^+$ (1 to $1.6 \times 10^6$ cells), was performed during bypass surgery in 6 patients.\textsuperscript{32} Three other studies injected bone marrow--derived mononuclear cells by catheter-based transendocardial delivery. Perin et al\textsuperscript{33} and Tse et al\textsuperscript{34} used the NOGA catheter. Injection was guided by electromechanical mapping. Perin et al showed a significant improvement of ejection fraction and end-systolic volume in 14 patients with chronic ischemic heart failure who were treated with bone marrow--derived mononuclear cells compared with 7 controls. Perfusion was determined by single-photon emission CT and was significantly improved in patients treated with bone marrow--derived mononuclear cells. Tse et al treated 8 patients with ischemic heart disease and reported improvement of wall motion and wall thickening as well as decreased hypoperfusion 90 days after cell therapy. Finally, Fuchs and coworkers\textsuperscript{35} tested the effect of total unfractionated total bone marrow in 10 no-option patients with advanced coronary artery disease. Canadian Cardiovascular Society angina score and stress-induced ischemia were significantly improved after 3-month follow-up.

All studies at present are limited by the small patient collective and by the design of pilot safety and feasibility studies, which precludes a randomized, placebo-controlled, double-blind design. However, increased perfusion was demonstrated in most of the studies.\textsuperscript{28,29,31,33} The TACT study,\textsuperscript{12} which showed improvement in peripheral leg ischemia, further supports the concept that cell therapy may augment neovascularization, leading to oxygen supply to the tissue. The question of whether the infused cells in the heart preferentially act via improving tissue perfusion or also regenerate cardiac myocytes remains unresolved. Obviously, stem or progenitor cells may exert synergistic effects by enhancing both neovascularization and cardiac regeneration. Additional release of paracrine mediators by incorporated stem or progenitor cells may amplify the response by attracting circulating progenitor cells and/or tissue-resident stem cells.

### Table 2. Stem/Progenitor Cell Therapy in Patients With Chronic Ischemic Heart Failure

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell Type</th>
<th>Application</th>
<th>Patients</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical application</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stamm et al\textsuperscript{34}</td>
<td>CD133$^+$ BMC (85–195 mL)</td>
<td>Injection during bypass surgery</td>
<td>n=6</td>
<td>Feasible EF ↑ in 4 pts (LVA) Perfusion ↑ in 5 pts (SPECT)</td>
</tr>
<tr>
<td>Percutaneous application</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tse et al\textsuperscript{36}</td>
<td>BMC (50 mL)</td>
<td>Intramyocardial/NOGA</td>
<td>n=8</td>
<td>Feasible Wall motion and thickening ↑</td>
</tr>
<tr>
<td>Fuchs et al\textsuperscript{37}</td>
<td>BMC</td>
<td>Intramyocardial/NOGA</td>
<td>n=10</td>
<td>Feasible</td>
</tr>
<tr>
<td>Perin et al\textsuperscript{38}</td>
<td>BMC (50 mL)</td>
<td>Intramyocardial/NOGA vs control</td>
<td>n=14</td>
<td>EF ↑; end-systolic volume ↓</td>
</tr>
</tbody>
</table>

BMC indicates bone marrow cell; LVA, left ventricular angiogram; and EF, ejection fraction.
Impact of Clinical Phenotype: Considerations for Therapeutic Neovascularization

Most of the preclinical studies are performed with healthy and young animals. To transfer the experimental findings into the clinic, we must consider that the patients usually have an established coronary artery disease, are of advanced age, and have various risk factors such as diabetes, hypercholesterolemia, or hypertension. These circumstances may limit the effect of cell or gene therapy.

Preliminary clinical findings in patients with critical limb ischemia indicated that the response to phVEGF gene transfer was most robust and expeditious in young patients with premature atherosclerosis involving the lower extremities, so-called Buerger’s disease.36 This clinical observation was supported by experiments performed in live animal models. Specifically, native neovascularization of the ischemic hindlimb was markedly retarded in old versus young animals. Retardation of neovascularization in old animals appeared in part to result from reduced expression of VEGF in tissue sections harvested from the ischemic limb.37,38 Similarly retarded neovascularization and reduced VEGF expression were observed in diabetic (NOD)39 and hypercholesterolemic (ApoE−/−) mice.40 These studies have 2 implications. First, the findings suggest that the fundamental mechanism by which therapeutic neovascularization augments collateral development is to provide cytokine supplements to individuals who, because of advanced age, diabetes, hypercholesterolemia, or other as yet undefined circumstances, are unable to appropriately upregulate cytokine expression in response to tissue ischemia. In this regard, ligand supplementation may be analogous to erythropoietin administration in patients with refractory anemia. Second, cytokine administration clearly constitutes only one aspect of the therapeutic intervention. Regardless of how much ligand is administered, the resident population of endothelial cells that is competent to respond to an available level of angiogenic growth factors may also constitute a potentially limiting factor in strategies designed to promote neovascularization of ischemic tissues. A reasonable goal may therefore consist of developing a complementary strategy that would provide substrate together with ligand, a “supply side” version of therapeutic neovascularization. Delivery of endothelial progenitor cells would be one strategy to overcome this problem.

However, the same circumstances that limit VEGF expression also harm progenitor cell number and function. Age severely impaired the capacity of bone marrow–derived mononuclear cells to enhance neovascularization in an animal model.41 Risk factors for coronary artery disease were associated with a reduced number and functional activity of endothelial progenitor cells in the peripheral blood of patients7 and healthy volunteers.42 Likewise, patients with diabetes showed lower endothelial progenitor cell numbers.43 Although the mechanism by which endothelial progenitor cell numbers and functional activity are impaired in animal models or patients is not yet clear, the impaired functional activity of endothelial progenitor cells and bone marrow–derived mononuclear cells may limit the clinical success of autologous stem/progenitor cell therapy.44 Novel strategies to counteract stem/progenitor cell dysfunction in aged patients with coronary artery disease or diabetes may include expression of protective and/or antiaging genes.

The Future: Combination of Gene and Cell Therapy?

Cell therapy and gene therapy have proven to be effective to promote neovascularization in various animal models. Moreover, some of the early clinical studies have provided intriguing results. Although the final proof with large randomized trials is missing, both strategies may in general have the capacity to lead to improved formation of new blood vessels as a treatment for myocardial or peripheral ischemia. Two recent examples provide evidence that the combination of cell and gene therapy may enhance therapeutic neovascularization. In the first study, ex vivo expanded endothelial progenitor cells were transfected with VEGF before transplantation in a hindlimb ischemia model. Ex vivo transfection of endothelial progenitor cells with VEGF was shown to improve their capacity to augment neovascularization.44 The increase in survival of the endothelial progenitor cells and potential paracrine mechanisms provided by the local secretion of VEGF may underlie this effect. In another study, the human active subunit of the telomerase reverse transcriptase (hTERT) was used for ex vivo transduction of endothelial progenitor cells. hTERT-transfected endothelial progenitor cells showed a 2- to 4-fold increased capacity to augment blood flow and capillary density.45 hTERT antagonizes cellular aging and prevents apoptosis of various cell types. Intriguingly, hTERT transfection modulates the gene expression pattern, leading to an augmented release of growth factors.46 Thus, hTERT may not only directly support endothelial progenitor cell survival and proliferation but may also enhance the release of growth factors, which then can act in a paracrine manner. The transfection of genes that antagonize cellular aging may be optimally suited for treatment of elderly patients with coronary artery disease, who are well known to have a reduced endothelial progenitor cell number and functional activity (see above).

Potential Caveats of Gene and Cell Therapy

When discussing the potential for angiogenic therapies in patients with ischemic disease, one must also reflect on the origins of angiogenesis as a therapeutic target, ie, the identification of VEGF as a factor augmenting tumor neovascularization.47 Likewise, progenitor cells can incorporate in tumor vasculature48 and may contribute to tumor neovascularization.49 VEGF-induced angiogenesis has also been shown to play a critical role in the advent of certain forms of retinopathy49 and has been implicated as a factor promoting atherosclerotic plaque growth.50,51 Accordingly, attempts to induce neovascularization have raised appropriate concerns regarding the possibility of inducing unwanted or pathological vessel growth. These safety concerns will undoubtedly influence the choice of vectors for gene therapy or gene modification of progenitor cells. Thus far, with ≥900 patients treated in various trials of angiogenic gene- and cell-based therapies, there has been not indication of increased cancer incidence, retinopathy, or acceleration of atherosclerosis.
Ongoing surveillance will continue to inform us regarding the potential for these complications.

Future Perspective

It is interesting to speculate that the role of angiogenic growth factor receptors, their cognate ligands, and bone marrow–derived endothelial cell precursors may be assuming increased importance in an era of increasing longevity and concurrently compelling evolutionary selection pressures. In the days when the lifespan of an average human was limited to 30 years, trauma and infection led to deaths well before individual ability to upregulate VEGF expression and/or mobilize endothelial cell progenitors became an issue. Several million years later, nature may begin to favor survival of those best equipped to adapt to the stresses and survival threats posed by tissue ischemia unrelated to snake bites or elephant stampedes. Looking forward to the long term, the genetic endowment permitting one to appropriately upregulate cytokine expression and mobilize endothelial progenitor cells in a fashion that is optimally suited to revascularize ischemic tissues may constitute a distinct survival advantage. In the short term, recognition of those elements that constitute the genetic profile of such individuals may permit us to identify those individuals who are least capable of mounting a satisfactory response and to develop appropriate therapeutic interventions.

References


**KEY WORDS:** angiogenesis  ■  angiogenesis  ■  neovascularization
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