**Brief Review: From Bench to Bedside**

**Endothelial Progenitor Cells**

**New Hope for a Broken Heart**

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Mr S. is a 62-year-old restaurant owner who has had recurrent stable angina that has not improved despite maximal medical therapy and an earlier coronary bypass. On angiography, Mr S. has diffusely diseased coronary vessels and is no longer considered a candidate for further direct revascularization. An alternative treatment strategy for revascularizing ischemic tissue is therefore required. One potential strategy, therapeutic neovascularization, aims to promote the formation of natural bypasses or collaterals within the ischemic tissue by harvesting the potential of endothelial progenitor cells (EPCs). This From Bench to Bedside article will explore the current concept of an EPC, the role EPCs play in revascularization. An alternative treatment strategy for revascularization of ischemic tissue by harvesting the potential of endothelial progenitor cells (EPCs). This From Bench to Bedside article will explore the current concept of an EPC, the role EPCs play in revascularization.

### What Are EPCs?

Progenitor cells are primitive bone marrow (BM) cells that have the capacity to proliferate, migrate, and differentiate into various mature cell types. EPCs, in particular, possess the ability to mature into the cells that line the lumen of blood vessels. The first evidence indicating the presence of EPCs in the adult circulation emerged when mononuclear blood cells from healthy human volunteers were shown to acquire an endothelial cell–like phenotype in vitro and to incorporate into capillaries in vivo. These putative EPCs were characterized via expression of CD34 and vascular endothelial growth factor receptor-2 (VEGFR-2), 2 antigens shared by embryonic endothelial progenitors, and hematopoietic stem cells (HSCs). Subsequent studies confirmed that CD34+ cells isolated from BM or umbilical cord blood also had the capacity to differentiate into mature endothelial cells. However, both CD34 and VEGFR-2 are expressed on various cell types. Thus, the search for more unique EPC markers continued.

In addition to CD34, early hematopoietic progenitor cells express CD133 (AC133), which is not expressed after differentiation. Using CD133 expression to define a very early subpopulation of progenitor cells, Peichev et al isolated a VEGFR-2+/CD133+/CD34+ subpopulation of cells able to differentiate into mature endothelial cells. More recently, hematopoietic progenitor cells that express CD34 and fibroblast growth factor receptor-1, or coexpress CD34, CD133, and VEGFR-3, have been shown to behave as EPCs.

Currently, most studies that estimate the number of EPCs in the circulation measure the number of CD34+/VEGFR-2+ or CD133+/VEGFR-2+ cells. However, the expression of CD34 does not appear to be an absolute requirement for EPC identification. Populations of CD34- HSCs and CD34+/CD133+VEGFR-2+ mesenchymal stem cells have been demonstrated to give rise to EPCs and generate mature endothelial cells. Thus, the lineage and exact phenotype of EPCs are not yet known.

### What Roles Do EPCs Have in the Adult?

The endothelium is a single-cell lining covering the internal surface of blood vessels, cardiac valves, and numerous body cavities that plays an important role in thrombogenicity, anticoagulation, leukocyte and platelet adherence, and vessel wall contraction and relaxation. Endothelial dysfunction predisposes to vasoconstriction, thrombosis, and atherosclerosis. EPCs may play an important role in endothelium maintenance, being implicated in both reendothelialization and neovascularization.

### Restoration of the Endothelial Lining

The physiological significance of EPC mobilization was illustrated in studies in which the thoracic aortae of adult dogs that underwent BM transplantation were implanted with Dacron grafts. After 3 months, the grafts were retrieved and found to be colonized with CD34+ endothelial cells of donor origin, suggesting that endothelialization arose exclusively from BM-mobilized EPCs. In humans, the surfaces of left ventricular assist devices were found to be colonized with CD133+/VEGFR-2+ cells. Together, these studies suggest the existence of a population of EPCs in the peripheral circulation that contributes to rapid endothelialization to prevent thrombotic complications.
The mobilization and incorporation of BM-derived EPCs has recently been shown to modulate reendothelialization at sites of endothelial cell damage.\textsuperscript{18,19} Both studies induced carotid artery endothelium injury and subsequently identified the incorporation of BM-derived endothelial cells at the site of injury. Thus, by restoring an intact endothelium, EPCs may participate in the maintenance of vascular homeostasis.

**EPCs and Neovascularization**

In the past, the vascularization of adult tissue was considered to occur via angiogenesis, the sprouting of new vessels from existing ones. It relies on the proliferation, migration, and remodeling of fully differentiated endothelial cells.\textsuperscript{20,21} However, with the discovery of circulating EPCs, it is currently believed that a combination of angiogenesis and a modified type of vasculogenesis contributes to neovascularization (Figure 1). Vasculogenesis refers to the formation of blood vessels de novo out of primitive EPCs during embryological development.\textsuperscript{6,21} With evidence suggesting EPC incorporation into the vasculature, EPC-dependent neovascularization in adults may represent a third means of blood vessel formation, paralleling developmental vasculogenesis in the embryo. EPCs may serve as the substrate for new vessel formation and simultaneously exert a paracrine effect to promote angiogenesis.

**Figure 1.** EPCs facilitate neovascularization and angiogenesis. Mobilization of EPCs from BM is enhanced by VEGF, angiopoietin-1, fibroblast growth factor (FGF), GM-CSF, MMP-9 activity, pharmacological agents such as statins, and erythropoietin (EPO).
Evidence for the potential of EPCs to participate in new blood vessel formation emerged from studies that demonstrated the formation of capillary-like structures from isolated HSCs or ex vivo expanded EPCs. The contribution of BM-derived cells to neovascularization, after ischemic injury in vivo, was shown in experiments using labeled populations of stem cells to reconstitute lethally irradiated mice. Jackson et al demonstrated the incorporation of labeled endothelial cells in regions of myocardial infarction. Using a mouse retinopathy model, Grant et al demonstrated that the recruitment of endothelial precursors to sites of ischemic injury plays a significant role in neovascularization. Using a single green fluorescent protein (GFP)-expressing stem cell, the group successfully reconstituted lethally irradiated mice. Three weeks after phacoemulsiﬁcation of the retinal vasculature to induce retinopathy, the damaged retinal vessels were replaced with a new, developing GFP+ capillary network. BM-derived EPCs also contribute to neovascularization during the newborn period, are present in corpus luteal and endometrial neovascularization after inductive ovulation, and are incorporated into vessels during wound healing. However, pathological tumor angiogenesis also depends on the recruitment of hematopoietic and circulating endothelial precursor cells. Thus, blockade of EPC migration may serve as a new target for antiangiogenesis therapy. If EPC-mediated neovascularization could be medically inﬂuenced and optimized to restore sufﬁcient blood ﬂow to ischemic areas, therapeutic revascularization would be possible. But before the therapeutic potential of cell-based therapies is realized, the mechanisms underlying EPC differentiation, mobilization, and recruitment must be identiﬁed.

What Factors Influence the Number of EPCs in the Circulation?
The majority of EPCs reside in the BM in close association with HSCs and BM stromal cells that provide the microenvironment for hematopoiesis. Within the BM, the progenitor cells are at different stages of differentiation. Under steady-state physiological conditions, EPCs represent only 0.01% of circulating mononuclear cells. For the progenitor cells to leave the BM, mobilization, which is a dissociation of the contact between the stromal cell and the EPC, is required. This process is termed recruitment.

EPC Recruitment and Mobilization
Endogenous stimuli such as tissue ischemia as well as exogenous cytokine therapy have been demonstrated to promote the mobilization of EPCs. The development of regional ischemia in both rabbits and mice resulted in an increase in the number of circulating EPCs. Similarly, EPCs were mobilized in patients with vascular trauma or acute myocardial infarction (AMI). Flow cytometry revealed that EPC counts signiﬁcantly increased in AMI patients compared with control subjects, an increase that was positively correlated to the elevated plasma levels of vascular endothelial growth factor (VEGF) in these patients. This capability of activating the endogenous regenerative capacity of EPCs is most likely mediated by chemokine and cytokine release.

Several studies have shown that the angiogenic growth factor VEGF promotes mobilization of EPCs and their incorporation into sites of neovascularization. VEGF promotes angiogenesis by inducing proliferation, differentiation, and chemotaxis of endothelial cells. It is essential for hematopoiesis and angiogenesis as illustrated by the death of mice in utero that have a single VEGF allele. Multiple isoforms of VEGF are secreted—VEGF165 being the most abundant—that exert their biological effects through interaction with 2 tyrosine kinase receptors, VEGFR-1 and VEGFR-2. Both of these receptors are expressed on HSCs and EPCs. The secretion of VEGF by HSCs and other cells in the BM compartment may induce EPC proliferation, BM vascular remodeling, and modulation of adhesion molecule expression, promoting EPC migration from the BM. After VEGF administration in mice, EPCs were signiﬁcantly increased in the circulation and displayed enhanced proliferative and migratory activity. In patients with lower limb ischemia who received VEGF gene transfer, there was an overall increase by >200% in circulating EPCs. These results suggest that VEGF overexpression can mobilize EPCs in humans. In addition to VEGF, other angiogenic growth factors, including angiopoietin-1, ﬁbroblast growth factor, and stromal cell–derived growth factor 1 (SDF-1), stimulate EPC mobilization and recruitment. Placental growth factor, a member of the VEGF family, was shown to stimulate collateral vascular formation in ischemic heart and limbs by acting via VEGFR-1 and inducing the recruitment of BM-derived EPCs. In addition to the direct effects of VEGF on EPC mobilization, VEGF can also induce the release of hematopoietic growth factors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) by BM endothelial cells.

Several chemokines and cytokines have been shown to promote the mobilization of EPCs. In mice, exogenously administered GM-CSF speciﬁcally mobilized EPCs that contributed to improved vascularization in ischemic hindlimbs. Similarly, a clinical trial administering GM-CSF to patients with extensive coronary artery disease (CAD) documented an overall improvement in myocardial collateral ﬂow. Although not determined, EPC recruitment to the myocardium may have contributed to this favorable outcome. Granulocyte colony stimulating factor (G-CSF), like GM-CSF, has been shown to increase the number of CD34+ cells that could stimulate neovascularization in regions of ischemic myocardium. Injection of the chemokine stem cell factor (SCF, sKitL) has also been shown to mobilize stem cells.

The recruitment of progenitor cells from the BM appears to require matrix metalloproteinase-9 (MMP-9) activity. MMP-9+ mice have impaired stem cell mobilization and MMP inhibitors effectively block EPC mobilization. MMP-9 activity causes the shedding of SCF, which favors recruitment of c-kit+ progenitors, such as EPCs, from the BM. The release of stem cell–active cytokines by metalloproteinase activity promotes the motility of otherwise quiescent EPCs. The mechanism by which placental growth factor recruits BM-derived progenitor cells was also demonstrated to involve upregulation of MMP-9 activity, mediating SCF
release. Once recruited and mobilized, the EPCs must home to and differentiate at the site of endothelialization.

**EPC Homing and Differentiation**

The same factors responsible for mobilization may also play a role in EPC migration and incorporation. Increased expression of VEGF, after either vascular injury or exogenous delivery, recruits EPCs. EPC migration to the site of vascular injury paralleled plasma VEGF elevation. Similarly, implantation of either a VEGF-loaded Matrigel plug or a VEGF pellet stimulated neovessel formation at the implantation site with a significant contribution by EPCs. This suggests that EPCs were drawn to the site of implantation by VEGF. SDF-1 may also contribute to EPC homing. SDF-1, which is elevated after myocardial infarction, binds to the chemokine receptor CXCR-4, which is highly expressed on EPCs. To support homing to and incorporation into sites of vascular injury, the adhesiveness of EPCs may be altered.

After human EPCs were exposed to simvastatin, the expression of integrin subunits α5β1 and αvβ3 was upregulated. Both α5β1 and αvβ3 are known to play a role in angiogenesis. These simvastatin-treated EPCs displayed increased incorporation into the neointimal of balloon-injured carotid arteries, which was abrogated on integrin receptor blockade. These results suggest that homing to and incorporation into foci of ischemic or vascular injury is determined not only by the number of circulating EPCs but also by the adhesiveness of EPCs, which changes during maturation. The mechanisms mediating EPC homing and differentiation are just beginning to be elucidated. Future work understanding these pathways may lead to new targets that can be exploited to maximize the efficiency of EPC-mediated neovascularization.

**Influence of Cardiovascular Risk Factors on EPCs**

EPC characterization in CAD patients was conducted by Vasa et al. The study reported an inverse correlation between the number of cardiovascular risk factors and the number and migratory activity of EPCs. Smoking was determined to be the major independent predictor for reduced EPC levels, whereas impairment of EPC migration was mainly influenced by hypertension. The mechanisms by which atherosclerotic risk factors reduce EPC number and function remain to be determined. However, Vasa et al speculate that these mechanisms may involve increased apoptosis of EPCs or interference with pathways regulating EPC differentiation and mobilization. Recently, it has been suggested that the levels of EPCs may serve as a surrogate biological marker for vascular function and cumulative cardiovascular risk.

**What Pharmacological Strategies Augment EPC Mobilization and Differentiation?**

Aside from the use of exogenously administered growth factors, cytokines, or chemokines, HMG-CoA reductase inhibitors or statins have been shown to enhance EPC mobilization and function. Statins possess favorable effects such as endothelial function improvement independent of cholesterol reduction. Dinninger et al and Llevadot et al both demonstrated that statins increased EPC differentiation in vitro and enhanced mobilization of EPCs in vivo. These favorable effects were likely mediated via the phosphatidylinositol-3-kinase/Akt pathway, a pathway also utilized by angiogenic growth factors to influence HSC differentiation. Treatment with statins promoted angiogenesis in normocholesterolemic animals and resulted in the accumulation of marrow-derived endothelial cells at sites of neovascularization. The ability of statins to mobilize EPCs is further illustrated by the 3-fold increase in circulating EPCs in stable CAD patients after 4 weeks of atorvastatin treatment. Thus, the potential of statins to increase the number of circulating EPCs that can participate in repair after ischemic injury may contribute to the mechanism by which statins enhance coronary blood flow in patients with stable CAD.

Statin therapy has also been shown to accelerate reendothelialization after vascular injury. Statins increased EPC mobilization and their incorporation into sites of damaged endothelium, minimizing the development of intimal hyperplasia. Modulation of integrin receptor expression was also induced by statin therapy, altering the adhesiveness of cells, which may promote EPC homing. Thus, statins may limit neointimal formation and ultimately the occlusion of diseased vessels in part by enhancing the mobilization and homing of EPCs after vascular injury.

**What Evidence Is There That EPCs Are Useful for Regeneration and Repair?**

With the identification of EPCs as important players in adult neovascularization, several studies have attempted to utilize EPCs to restore blood flow to ischemic tissue. Most have involved the transplantation of ex vivo expanded EPCs.

**EPC Transplantation for Peripheral Ischemia**

The transplantation of EPCs significantly improved blood flow recovery and capillary density in several animal hindlimb ischemia models. Kalka et al demonstrated that ex vivo expanded human EPCs (hEPCs) could be used successfully to promote neovascularization of ischemic hindlimbs in athymic nude mice. Mice receiving hEPCs had increased capillary density and significantly improved blood flow in the ischemic limb after transplantation. The perfusion in the hEPC-transplanted mice was sufficient to significantly increase the chance of successful limb salvage. Subsequent studies showed that autologous BM cell transplantation in a rat ischemic hindlimb model and BM mononuclear cell transplantation in a rabbit model of hindlimb ischemia were able to improve collateral vessel formation and blood perfusion in the ischemic limb. Furthermore, EPC transplantation induces blood flow recovery in the ischemic hindlimbs of both diabetic mice and rats, suggesting that EPC-mediated neovascularization can still occur under disease conditions and thus be applied as a therapeutic treatment in the patients who would benefit most.

**EPC Transplantation for Ischemic Myocardium**

Just as progenitor cell transplantation restored blood flow to ischemic hindlimbs, EPC transplantation after myocardial infarction induced neovascularization. Kawamoto et al demonstrated that transplanted, ex vivo expanded hEPCs had
a favorable impact on the preservation of left ventricular function. After the induction of myocardial ischemia, labeled hEPCs were injected intravenously. The hEPCs were shown to accumulate in the ischemic area and to participate in myocardial neovascularization. Echocardiography revealed ventricular dimensions that were significantly smaller and fractional shortening that was significantly greater in the hEPC transplant. The transplant group also had less ventricular scarring and better-preserved wall motion. Similar findings were documented by Kocher et al after the transplantation of G-CSF–mobilized CD34+ human cells containing both HSCs and EPCs. Transplantation was shown to improve myocardial function, prevent cardiomyocyte apoptosis, and limit myocardial remodeling. Neovascularization was observed both within the myocardial infarct and along the infarct border. However, endothelial cells of human origin tended to localize at the infarct core, whereas rat-derived endothelial cells contributed to new vessel formation at the infarct border. This suggests that BM-derived progenitor cells may directly contribute to in situ vessel formation in the core region and stimulate angiogenic sprouting from existing endothelium at the infarct border.

A subsequent study using a swine myocardial infarction model showed that the implantation of BM-derived mononuclear cells, cells that actively differentiated to endothelial cells in vitro, improved regional cardiac function. Three weeks after implantation, regional blood flow, capillary density, and the number of visible collateral vessels were significantly higher in transplant recipients compared with controls, with no malignant ventricular arrhythmias or bone formation by osteoblasts detected. Kamihata et al conclude that BM implantation represents a novel and safe strategy to achieve therapeutic angiogenesis. The beneficial effects are likely mediated by the ability of the transplanted cells to become incorporated into new vessels and to promote angiogenesis through angiogenic ligand secretion. However, a recent study has shown that EPCs can transdifferentiate into functionally active cardiomyocytes and thus may potentially assist with cardiomyocyte regeneration after ischemia.

**EPC Transplantation in Humans**

Initial results from clinical trials assessing the safety and feasibility of autologous progenitor cell transplantation are rather promising. The Therapeutic Angiogenesis using Cell Transplantation Study involved autologous implantation of BM mononuclear cells in patients with ischemic limbs as a consequence of peripheral arterial disease. At 4 weeks after the random injection of BM mononuclear cells into the gastrocnemius of one leg and peripheral blood mononuclear cells into the other leg as a control in patients with bilateral leg ischemia, various outcomes were measured. Legs into which the BM mononuclear cells were injected had a significantly improved ankle-brachial index, an improved transcutaneous oxygen pressure, a significant reduction in rest pain, and an increase in pain-free walking time. Improvements were sustained at 24 weeks with no serious complications. These promising results were attributed to the ability of marrow cells to initiate therapeutic angiogenesis by supplying both EPCs and angiogenic factors.

Two recent studies demonstrate that intracoronary infusion of autologous progenitor cells after AMI appears to be safe and effective in limiting postinfarction remodeling processes. The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) Trial was established to assess the safety and feasibility of autologous progenitor cell transplantation in patients with ischemic heart disease. Twenty patients with reperfused AMI were randomly assigned to receive an infusion of either BM-derived or circulating blood–derived progenitor cells into the infarct artery. At 4 months, transplantation of progenitor cells resulted in a significant increase in global left ventricular ejection fraction, improved regional wall motion in the infarct zone, reduced end-systolic left ventricular volumes, and increased myocardial viability in the infarct zone compared with a nonrandomized matched reference group. No differences were detected between the blood-derived and BM-derived progenitor cell groups, and no malignant arrhythmias or inflammatory reactions were observed. A second controlled clinical trial analyzed 10 patients with AMI who were treated by intracoronary transplantation of autologous mononuclear BM cells in addition to standard therapy. After 3 months, the group receiving cell transplantation showed a significant decrease in infarct region size and a significant increase in infarcted wall movement velocity compared with AMI patients treated with standard therapy alone. The transplant group also showed significant improvements in stroke volume index, left ventricular end-systolic volume, contractility, and perfusion of the infarct region. Taken together, the results of these 2 studies suggest that intracoronary infusion of progenitor cells can improve outcome after AMI (Figure 2). However, the results from larger-scale, randomized trials are required before the long-term clinical outcome of AMI patients receiving cell transplantation is known.

**What Does the Future Hold?**

With the promising results from both animal studies and early human clinical trials, the future for therapeutic neovascularization mediated by EPC transplantation appears bright. Since the discovery of EPCs in the adult circulation 6 years ago, a great deal of information related to the mechanisms controlling EPC recruitment, mobilization, homing, differentiation, and function has been obtained. However, more research in these molecular and biochemical pathways is required to shed light on how biological conditions may be optimized to increase neovascularization in patients with CAD or peripheral arterial disease. Future multipronged approaches that combine EPC transplantation with growth factor, cytokine, and pharmacological treatments may improve the therapeutic outcome in patients. One potential strategy may involve genetically modifying EPCs before transplantation. Iwaguro et al demonstrated that gene transfer of vectors encoding VEGF into EPCs ex vivo and injection of these modified EPCs into the ischemic hindlimbs of mice significantly increased neovascularization and hindlimb perfusion compared with mice transplanted with nontransduced EPCs. These VEGF-transduced EPCs displayed enhanced proliferation, adhesion, and incorporation and may operate not only...
as substrate for new vessels but also as VEGF secretors promoting the migration, proliferation, and remodeling of differentiated endothelial cells residing in the target ischemic tissue. Thus, by enhancing the angiogenic and regenerative properties of EPCs before transplantation, a more potent therapeutic strategy for postnatal neovascularization in patients with severe ischemic disease will be developed. However, strategies enhancing EPC function may not prove to be beneficial in all population groups and may even have detrimental effects, such as enhancing tumor angiogenesis. Although EPC transplantation is not currently available for patients, it is our contention that this treatment modality will come of age in the near future.

Note Added in Proof
During the review process, a few key advances in the field of EPC biology were made. Erythropoietin was demonstrated to enhance EPC mobilization (Heeschen et al, unpublished observations, 2003), while Assmus et al71 showed that the beneficial effects of statins extend to the inhibition of EPC senescence and the induction of EPC proliferation. Results from Aicher et al72 suggest that radioactive labeling may provide a useful technique for monitoring the homing of transplanted EPCs. Finally, data from our group indicate that the inflammatory marker C-reactive protein, at concentrations known to predict vascular events, potently inhibits EPC survival and differentiation (S. Verma et al, unpublished observations, 2003).

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References


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