The Promise of Cell-Based Therapies for Diabetic Complications: Challenges and Solutions

Yagna P.R. Jarajapu, Maria B. Grant

Abstract: The discovery of endothelial progenitor cells (EPCs) in human peripheral blood advanced the field of cell-based therapeutics for many pathological conditions. Despite the lack of agreement about the existence and characteristics of EPCs, autologous EPC populations represent a novel treatment option for complications requiring therapeutic revascularization and vascular repair. Patients with diabetic complications represent a population of patients that may benefit from cellular therapy yet their broadly dysfunctional cells may limit the feasibility of this approach. Diabetic EPCs have decreased migratory prowess and reduced proliferative capacity and an altered cytokine/growth factor secretory profile that can accelerate deleterious repair mechanisms rather than support proper vascular repair. Furthermore, the diabetic environment poses additional challenges for the autologous transplantation of cells. The present review is focused on correcting diabetic EPC dysfunction and the challenges involved in the application of cell-based therapies for treatment of diabetic vascular complications. In addition, ex vivo and in vivo functional manipulation(s) of EPCs to overcome these hurdles are discussed. (Circ Res. 2010;106:854-869.)

Key Words: diabetes ■ endothelial progenitor cells ■ bone marrow ■ angiogenesis

Diabetes is associated with a broad spectrum of vascular complications that constitute a major health care concern in the western world. Despite the prevalence and the debilitating nature of diabetic vascular complications, cellular/molecular/genetic mechanisms underlying vascular dysfunction remain unclear and vary with the cell type studied and the vascular bed examined; however, the literature to date reflects a consensus that endothelial dysfunction is a key initiator that precedes development of vascular complications. Almost 6000 research articles have been published since 1973 that directly link diabetic vascular complications to endothelial dysfunction. These publications report studies using animal models as well as those describing clinical trials and their outcomes.

The definition of endothelial dysfunction varies depending on the organ studied; however, in general, endothelial dysfunction can be characterized by impaired endothelium-dependent dilatation to agonists, to shear stress, or to local ischemia. In a particular context such as proliferative diabetic retinopathy (PDR), the dysfunction is described as endothe-
lial activation with proinflammatory and proliferative phenotype that ultimately disrupts vascular integrity resulting in increased permeability. In general, diabetic microvascular complications are typically associated with dysregulation of vascular remodeling and vascular growth with decreased responsiveness to ischemic/hypoxic stimuli and impaired or abnormal neovascularization.

Lack of endothelial regeneration and impaired angiogenesis contribute to the progression of diabetic micro and macrovascular complications. Formation of stable vasculature in response to tissue injury is an essential event for the restoration of blood flow and the repair of the affected tissue areas. Currently, clinical management of diabetic complications relies exclusively on pharmacological therapeutics that minimally affect endothelial repair or regeneration in most cases, and, therefore, these treatments have modest influence on end organ dysfunction. Hence, there is a need for therapeutic interventions that actually accelerate repair of dysfunctional endothelium in the end organ and restore blood flow to result in functional tissue generation.

After the identification of “putative” endothelial progenitor cells (EPCs),

1 diabetic vascular complications are now thought to be, in part, the result of the reparative dysfunction of these cells. In response to these intriguing findings, a rapid progression of EPCs from the “bench to the bedside” occurred via translational studies even in the absence of a consensus about the true identity of the “EPC.” These seminal discoveries indeed paved the way for cell-based therapeutics for patients with vascular complications; however, a few caveats must be considered for autologous transplantation of cells in diabetic patients. The cells themselves are known to be dysfunctional, and the diabetic environment poses a variety of challenges that need to be considered. Otherwise, cell-based therapies may not be beneficial and may actually result in worsening of existing vascular complications in a patient population most in need of help.

The present review provides a brief introduction to the characteristics of EPCs and their dysfunction in diabetes and focuses mainly on the challenges involved in the application of cell-based therapies for diabetic vascular complications. In addition, a variety of possible ex vivo and in vivo manipulation(s) of EPCs to overcome these hurdles are discussed.

Putative EPCs

Based on the observations that Dacron arterial prosthesis in humans were endothelialized,2,3 it was hypothesized that a subpopulation of cells in the circulation support the replenishment of endothelial cells (ECs) within the vasculature and, in part, sustained neovascularization in response to tissue injury. This concept was similar to the phenomenon of hematopoietic recovery and persistent replenishment of blood cells by circulating hematopoietic stem cells (HSCs). Before these observations, adult neovascularization or de novo formation of blood vessels was thought to occur exclusively by angiogenesis, which is formation of blood vessels by existing vessels, rather than by vasculogenesis, formation of blood vessels from stem or progenitor cells (Figure 1).4,5

This long-held paradigm regarding the origins of neovascularization was shifted by the observations of Asahara et al1

This study showed that the putative EC progenitor cells or angioblasts could be isolated from human peripheral blood by magnetic bead selection on the basis of cell surface antigen expression and in vitro these cells differentiated into ECs. Specifically, CD34+/VEGFR-2+ mononuclear cells were shown to express EC-associated markers such as CD31, vascular endothelial growth factor (VEGF) receptor (VEGFR)-2, Tie-2, and E-selectin when cultured on fibronectin. The EPC population of Asahara et al1 also expressed endothelial NO synthase (eNOS). However, both CD34 and VEGFR-2 antigens are present on ECs, and so, to exclude differentiated circulating ECs from a study population, now CD133 has been included as a marker limited to primitive cells but not expressed on ECs. To date, many antigenic markers including CD34, CD133, CD45, VEGFR-2, CD133, CXC chemokine receptor (CXCR)4, CD14, and CD31 have been used to identify EPC populations. Cell populations characterized by these surface markers have been shown to become angiogenic or to support angiogenesis in vivo or in vitro assays.6-11 An elegant study by Loomans et al12 in mice suggested eNOS expression is a reliable marker to identify bone marrow (BM)-derived EPCs and this concept has been strongly supported by other studies.1,13 In animal models of ischemia, heterologous, homologous, and autologous EC progenitors incorporated into sites of active angiogenesis, and this led to their use for augmentation of collateral vessel
Figure 1. Adult stem cells of the bone marrow: the BM hosts at least 2 known types of adult stem cells, the HSCs and the MSCs. The MSCs have been shown to differentiate into various cell types, including osteoblasts, adipocytes, chondrocytes, myocytes, fibroblasts and endothelial cells. The most prominent adult stem cell in the BM is the HSC. HSCs can give rise to the hematopoietic progenitor cells, which in turn give rise to the lymphoid, the myeloid and likely the EPC. The BM microenvironment is composed of BM stromal cells (which are the source of SDF-1), adipocytes, and cells of the bone matrix, osteoblasts, and osteoclasts. The vessels within the BM, composed of periocytes and endothelium, function to provide a barrier between the hematopoietic compartment and the circulatory system discussed in greater detail in Figure 2.

growth to ischemic tissues. These pivotal studies were corroborated by many investigators and by the discovery that these EPCs can be found in BM niches and mobilized to areas of tissue injury/repair (Figure 2).14,15 Later, the protocol of Asahara et al was slightly modified by eliminating the cell-sorting step and the so-called colony-forming unit (CFU)-Hill or CFU-EC was developed as a commercial kit to quantify EPCs and enumeration of CFU-EC correlated closely with cardiovascular risk factors.16

Recent studies examining the relationship between EPC number/function and presence of vascular disease support that CD34+ cells or cells that express both CD34 and VEGFR-2 are reliable markers for the “putative” EPC phenotype and that cells with these surface markers indeed predict defective vasoregenerative capacity in a given clinical condition. CD34+VEGFR-2 cell counts predicted the occurrence of cardiovascular events in a 10 month follow-up study involving 120 patients.17 CD133+ cell counts also predicted cardiovascular events, but to a lesser degree in a study involving 519 patients with 12-month follow-up.18 Patients with coronary artery disease showed a 5-fold reduction in three different subsets of circulating CD133+ cells: CD133+CD34+, CD133+CXCR4+, and CD133+VEGFR-2+ cells. Total CD34+ cells correlated with all cardiovascular parameters and risk estimates better than CD34+VEGFR-2 and CD133+-based phenotypes.19,20 Recently, Fadini et al11 reported that circulating CD34+ cell number was an independent risk biomarker of cardiovascular events and significantly correlated with outcomes in metabolic syndrome, based on their study involving 214 patients over a 34 month period. Taken together, CD34+ cells clearly represent a cell population of clinical utility. Although CD34+VEGFR-2+ represent an equally efficient marker, the number of these cells found in steady-state peripheral blood is extremely low.

Despite the convincing clinical evidence, no studies have demonstrated that CD34+VEGFR-2 or CD34+CD133+VEGFR-2+ cells differentiate into EC cells in vitro.22 Although direct vascular integration of CD34+ cells has been shown in some in vivo studies, more studies attribute the reparative function of these cells to direct integration but to paracrine mechanisms (Table).10,23–28 Recently, the assertion that CD34+VEGFR-2+ cells are bona fide EPCs has also been brought under fire.22 The cells growing as CFU-Hill colonies have been suggested to be mostly composed of monocytes and T cells29 and thus genetically linked to primitive hematopoietic cells.30 Later studies also revealed that CFU-ECs are not endothelial-committed and do not form perfused vessels in vivo.30 Monocytes have recently been shown to acquire endothelial markers in vitro by the uptake of platelet microparticles and thus a simple transfer of CD31 and vWF antigens can occur.31 Alternatively EPC populations can be isolated from peripheral or umbilical cord blood using in vitro culture producing 2 distinct subtypes which have been named early EPCs (eEPCs) and outgrowth ECs (OECs).32 This ex vivo analysis of cells by expansion culture has become popular and is now widely used for characterizing EPCs. These 2 phenotypically different populations are isolated from total mononuclear cells based on the time in culture and the matrices used: eEPCs originate as early as 4 days after plating and organize into clusters with very low proliferative potential and OEC (or late outgrowth EPCs) survive up to 2 to 4 weeks and exhibit endothelial morphology with a higher proliferative potential.32,33 eEPCs are a heterogeneous population of cells and are believed to be hematopoietic in origin because these cells display overlapping markers for ECs, monocytes, or macrophages; are phagocytic and antithrombogenic; and give rise to macrophages. These cells typically do not participate in vascular repair but have the potential to augment vascular network formation by secreting paracrine factors. OECs express endothelial markers, lack CD14 or CD45, form tubular structures de novo, and incorporate into developing vascular networks but are believed to be devoid of paracrine effects.30,32,34,35 EPCs analyzed by flow cytometry and those obtained from in vitro expansion cultures have phenotypic/functional characteristics in common; however, these characteristics have not been thoroughly compared.

Schofield16 suggested that “stem cell” properties can be imposed on cells that normally do not possess stem cell function by an “appropriate” microenvironment and a corollary we put forth is that an “inappropriate” microenvironment can result in a loss of a cell’s stem cell/progenitor properties. In health, a range of cells may have this stem cell potential to function by an “appropriate” microenvironment and a corollary we put forth is that an “inappropriate” microenvironment can result in a loss of a cell’s stem cell/progenitor properties. In health, a range of cells may have this stem cell potential to function by an “appropriate” microenvironment and a corollary we put forth is that an “inappropriate” microenvironment can result in a loss of a cell’s stem cell/progenitor properties. In health, a range of cells may have this stem cell potential to function by an “appropriate” microenvironment and a corollary we put forth is that an “inappropriate” microenvironment can result in a loss of a cell’s stem cell/progenitor properties. In health, a range of cells may have this stem cell potential to function by an “appropriate” microenvironment and a corollary we put forth is that an “inappropriate” microenvironment can result in a loss of a cell’s stem cell/progenitor properties. In health, a range of cells may have this stem cell potential to function by an “appropriate” microenvironment and a corollary we put forth is that an “inappropriate” microenvironment can result in a loss of a cell’s stem cell/progenitor properties.
endothelial differentiation from BM-derived cells from normal and 3 month streptozotocin (STZ)-diabetic rats. These studies raise questions about the value of culture-expanded EPCs as valid diagnostic or prognostic tools.

Despite the lack of consensus on the identity of EPCs based on surface expression of antigenic markers and the clinical relevance of in vitro expanded progenitor populations, most investigators agree that the following criteria must be satisfied by a cell in order for it to be considered an EPC. The cell must mobilize from niches in response to ischemic stimuli, homing to areas of ischemia and participation in neovessel formation. Although there is an ongoing debate about the exact identity of EPCs, evidence has been accumulating from preclinical and clinical studies that multiple populations of progenitor cells whether freshly isolated or in vitro expanded may have therapeutic efficacy (Table).

### Paracrine Function of EPCs

The degree of vascular engraftment by administered putative EPCs/BM-derived progenitor cells (BMCs) varies among studies but ranges from none to a high percentage. Regardless of the number of cells that physically integrate into the vessel wall and the antigenic/phenotypic characteristics they express, circulating BMCs participate in vascular repair and promote vascular growth by releasing proangiogenic factors. An elegant study by Majka et al reported that human CD34+ cells, myeloblasts, erythroblasts, and megakaryoblasts release numerous growth factors, cytokines, and chemokines that regulate the process of hematopoiesis by autocrine and paracrine mechanisms; however, this study did not evaluate vascular repair. Harraz et al showed that CD34+CD14+ or CD34+ cells can incorporate into the endothelium of blood vessels in mouse ischemic limbs; however, this required the presence/coinjection of CD34+ cells, clearly indicating paracrine modulation of CD14+ populations by CD34+ populations. Direct evidence for the paracrine interaction between subsets of EPCs was also provided by Krenning et al, who showed that CD34+ cells modulate proliferation and endothelial differentiation of CD14+ cells by releasing hepatocyte growth factor, interleukin-8, and monocyte chemoattractant protein-1. In yet another study, culture-expanded EPCs derived from human monocytes/macrophages exhibited low potential for proliferation and endothelial differentiation but were capable of

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**Figure 2. BM and HSC niches:** The maintenance of HSC self-renewal and differentiation is dependent on the specialized microenvironments or “niches.” Stem cells are known to reside in close proximity to endosteal linings of the BM cavities, endosteal niche, or close to sinusoidal endothelium, the vascular niche. Blood capillaries or BM sinusoids drain into a central sinus, the largest vascular structure in the BM, which contains more committed stem and progenitor cells than the relatively quiescent stem cells of the endosteal niche. Mobilization of BM cells involves the exodus of stem/progenitor cells into the circulation, whereas homing is the “opposite” of this event. HSCs mobilize from the endosteal niche move to the vascular niche and ultimately into the circulation. Mobilization is dependent on levels of cytokines or growth factors such as SDF-1, G-CSF, fibroblast growth factor (FGF), or VEGF in the BM and circulation with the involvement of matrix metalloproteinases such as MMP-2, MMP-9, cathepsin-G, and elastase. Homing involves interaction of integrins on HSCs/EPCs that are stimulated by SDF-1 with vascular cell adhesion molecule (VCAM), intercellular adhesion molecule, E-, and P-selectins expressed on BM endothelial cells (BMEC), followed by firm adhesion and subsequent endothelial transmigration into the hematopoietic compartment is mainly accomplished by very-late antigen-4 (VLA-4) interactions. HPC indicates hematopoietic progenitor cells; MPP, multipotent progenitor cells; mSCF, murine stem cell factor; Opn, osteopontin; SNO, S-nitrosothiol.
Circulating Stem/Progenitor Cells in Diabetes

Diabetic individuals with vascular complications would potentially benefit from cellular therapy with autologous cells; however, their EPCs are dysfunctional, because of a reduction in the number of circulating progenitors or attenuated function in in vitro angiogenic assays with or without an actual reduction in the number of cells.60,61

In a series of systematic studies, Egan et al59 evaluated cellular phenotypes in the peripheral blood of diabetic patients and observed a significant decline in the number of CD34+, CD34+KDR+, CD34+KDR+, CD34+CD133+KDR+, CD133+KDR+, CD117+KDR+,

Table. Antigenic Characteristics of Different Progenitor Populations That Have Been Evaluated for Therapeutic Angiogenesis

<table>
<thead>
<tr>
<th>Antigenic Markers</th>
<th>Donor</th>
<th>Test System(s)</th>
<th>Functional End Point(s)</th>
<th>Proposed Mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+/CD45−/Flk-1+</td>
<td>Mouse or Rabbit HLI</td>
<td>None</td>
<td>Direct Differentiation1</td>
<td></td>
</tr>
<tr>
<td>CD34+</td>
<td>Human Diabetic</td>
<td>Diabetic mouse HLI</td>
<td>Limb perfusion, Vascular incorporation26</td>
<td></td>
</tr>
<tr>
<td>CD34+CD117+GATA+</td>
<td>Rat MI</td>
<td>Capillary density, myocardial function and remodeling</td>
<td>Direct differentiation and paracrine effects161</td>
<td></td>
</tr>
<tr>
<td>CD34+ckIT−Sca1+</td>
<td>Mouse MI</td>
<td>None</td>
<td>Direct differentiation162</td>
<td></td>
</tr>
<tr>
<td>CD34− or CD34−</td>
<td>Mouse HLJ</td>
<td>Limb perfusion</td>
<td>Direct differentiation and vascular incorporation13</td>
<td></td>
</tr>
<tr>
<td>CD34− or CD34+</td>
<td>Diabetic mouse wounds</td>
<td>Vascularity</td>
<td>Paracrine effects23</td>
<td></td>
</tr>
<tr>
<td>CD34− or CD34+KDR+</td>
<td>Mouse MI</td>
<td>Infarct size, myocardial perfusion</td>
<td>Direct differentiation163</td>
<td></td>
</tr>
<tr>
<td>CD34+</td>
<td>Obese-diabetic mouse</td>
<td>Mouse HLJ</td>
<td>Limb perfusion and autoamputation54</td>
<td></td>
</tr>
<tr>
<td>CD133+</td>
<td>Diabetic ischemic wounds in mice</td>
<td>Wound closure</td>
<td>Paracrine effects48</td>
<td></td>
</tr>
<tr>
<td>CD34+ or CD14+</td>
<td>Diabetic mouse HLJ</td>
<td>Limb perfusion</td>
<td>Paracrine effects10</td>
<td></td>
</tr>
<tr>
<td>Culture-expanded cells</td>
<td>Mouse MI</td>
<td>Myocardial function</td>
<td>Direct differentiation and vascular incorporation164</td>
<td></td>
</tr>
<tr>
<td>Rabbit carotid balloon injury</td>
<td>Mouse HLJ</td>
<td>Endothelial function; neointima formation</td>
<td>Direct differentiation165</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mouse HLJ</td>
<td>Limb perfusion</td>
<td>Paracrine effects; direct differentiation or vascular incorporation166–169</td>
<td></td>
</tr>
</tbody>
</table>

Refer to the recent review by Sekiguchi et al42 for preclinical studies in models of MI and controlled clinical trials.
Figure 3. Diabetic dysfunction in the BM mobilization of stem/progenitor cells and paracrine regulation of ischemic vascular repair. In normal conditions, factors released by ischemic/injured tissue cause mobilization of BM cells, which, when transmigrated into areas of ischemia, release proangiogenic factors and physiological levels of NO and ROS that modulate repair mechanisms by activating vascular endothelium in the surrounding areas, by recruiting more BM cells and by modifying ischemic environment. In diabetic conditions, signals arising from the vascular injury are weaker, resulting in reduced mobilization of BM cells into circulation. Those few progenitor cells reaching the areas of ischemia are either not able to release proangiogenic factors or release antiangiogenic or proinflammatory factors and nonphysiological levels of NO and ROS that delay or inhibit vascular repair.

and CD34+/CD31+ cell populations compared to normal subjects. These results clearly indicate a generalized reduction in putative endothelial progenitors with different levels of maturity/differentiation. In contrast, Lee et al demonstrated an increase in c-Kit+ and CD34+ cells in advanced PDR; however, the results were based on a small sample size and a limited characterization of comorbidities and drug treatment that may have had an effect on progenitor numbers.

Kusuyama et al showed that in newly diagnosed patients with type 1 diabetes, the number of EPCs was significantly related to hemoglobin A1c and blood sugar levels before treatment and, therefore, concluded that diabetes reduces the number of circulating EPCs according to its severity. The decline in the number of circulating progenitor cells is linearly correlated with the severity of diabetes and degree of glucose control has been shown to be negatively correlated with circulating progenitor numbers. Therefore, it is clear that improvement of glycemic control significantly increases the number of circulating EPCs which are vascular protective.

Similar observations have been made in experimental studies that reported decreased numbers of BMCs in circulation of diabetic rodents in response to ischemic injury compared to nondiabetic animals and this was associated with reduced eNOS expression in these cells. The reduction in circulating progenitor cells may signal diabetic dysfunction of BM or an inability to respond to signals from injured vasculature/ischemic tissue (Figure 3). Selective depletion of functional hematopoietic progenitor cells in the BM was also observed in the patients with postinfarction heart failure, suggesting increased demand and turnover. In contrast, Nguyen et al found an increased number of myofibroblast progenitors in these patients, suggesting that the reduction may be limited to cardio- and vasculoprotective progenitors. Thus, reduced numbers of functional EPCs in circulation have been identified as an important pathology in diabetic patients.

Diabetic BM dysfunction has been poorly studied. Diabetic neuropathy may be a determinant of this diabetic BM defect. We recently reported that decreased numbers of BMCs in the circulation are accompanied by increased numbers of “trapped” cells in the BM of type-2 diabetic rats. In physiological conditions, the release of BMCs into the circulation follows a definitive circadian pattern, which is regulated by sympathetic neuronal activity. Diabetic peripheral neuropathy affects the BM and is manifested as a reduced number of nerve endings within the BM. This leads to an altered circadian rhythmicity of BMC release and reduced numbers of cells in the circulation.

Changes in signaling of matrix metalloproteinase (MMP)-9, eNOS (see below), and c-kit ligand–receptor may also mediate this BM dysfunction and involvement of additional factors cannot be ruled out. The diabetic defect in the release of BM cells is reversed by glucose normalization early in the disease course, suggesting that the mobilization mechanisms are sensitive to chronic hyperglycemic conditions and early on remain reversible.

In contrast, mobilizing cells from BM is not always an ideal option as cells obtained after G-CSF stimulation were found to have reduced in vivo vasoreparative function in patients with ischemic heart disease despite their higher clonal expansion capacity. Moreover, mobilization of BMCs for vasoreparation was shown to aggravate underlying vascular dysfunction in some clinical conditions. Mobilized cells from diabetic BM have not yet been characterized but need to be investigated systematically to identify the risk/benefit ratio.

**Diabetes and EPC Dysfunction**

Diabetic EPCs exhibit reduced proliferative potential and migratory function. Vascularization was depressed when diabetic EPCs were injected into normal mice. Moreover, diabetic EPCs show a reduced ability to integrate into EC tubes in vitro. A large number of studies point to the defective NO signaling as a mediator of diabetic EPC dysfunction.

**NO and Oxidative Stress**

NO is generated from the guanidino group of L-arginine and is an NADPH-dependent reaction catalyzed by a family of NO synthase (NOS) enzymes: eNOS, neuronal NOS, and iNOS. Deficiencies in L-arginine supply have been strongly implicated in vascular diseases, including diabetes. If the

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supply of L-arginine or the essential cofactor tetrahydrobiop- 
terin is inadequate, NOS becomes “uncoupled” and uses 
molecular oxygen as a substrate to form superoxide instead of 
NO. An imbalance between L-arginine availability and NOS 
activity can also occur when cellular transport of L-arginine is 
inhibited,73 recycling of L-citrulline back to L-arginine is 
reduced, catabolism of L-arginine by arginase is increased,74
or competitive inhibition for the active site of NOS by 
asymmetrical dimethylarginine occurs.75 In diabetic vascula-
ture, eNOS was decreased, whereas NADPH oxidase (a 
major source of superoxide in the vascular endothelium), 
iNOS, and ONOO− were all increased.76 The shift in redox 
state in diabetes with increased generation of reactive oxygen 
species (ROS) causes interaction of NO with superoxide, 
resulting in loss of bioavailable NO and formation of 
ONOO−. The reduced NO and elevated ONOO− can lead to 
microvascular dysfunction in diabetes.77–79 In diabetes, un-
coupling of eNOS has been found to be a source of ROS 
generation and, when blocked, mouse models show reduced 
signs of endothelial dysfunction.80 Diabetic vascular dysfunc-
tion in rats was shown to be prevented by administration of 
1400W, a specific iNOS inhibitor, suggesting a major role for 
the iNOS in diabetic complications.81,82

NO-mediated signaling pathways are essential for mobil-
ization of BMCs83,84; NO activates MMP-9, releasing soluble 
Kit ligand, which shifts resident BM cells from a quiescent to 
a proliferative niche and stimulates rapid mobilization into 
the circulation.85,86 NO regulates migration of stem/progeni-
tor cells into ischemic sites and promotes their survival.86,87 It 
has been shown that diabetic BMCs have decreased eNOS 
activity, and more importantly, that exogenous NO can 
correct the migratory defect in these cells.71

In addition to its effects on NO signaling, hyperglycemia 
has been shown to decrease circulating BMCs by increasing 
their senescence via activation of p38 mitogen-acti-

vated protein kinase (MAPK)88 or Akt/p53/p2189 pathways 
or by downregulation of SIRT1, the mammalian homolog of 
Sir2 (silent information regulator-2).90 A recent study showed 
that hyperglycemia shifts differentiation of BMCs into proin-
flammatory phenotype with decreased EPCs and increased 
macrophages.91

Increased ROS in EPC Dysfunction

In physiological conditions, ROS have been shown to be 
involved in cellular signaling mechanisms that result from the 
reversible oxidation of redox-sensitive target proteins. Protein 
tyrosine phosphatases are extremely sensitive to oxidative 
modification which leads to increased phosphorylation and 
activation of many receptor tyrosine kinases.92 Overproduc-
tion of ROS in diabetes caused by increased activation of 
NADPH oxidase has been shown to be involved in the 
initiation and progression of diabetic vascular complications 
by decreasing the bioavailability of NO.93,94

BMCs express NADPH oxidase isoforms and it has been 
suggested that low levels of ROS in the BM play an essential 
role in preserving primitive HSCs in the hypoxic environ-
ment; slightly elevated levels promote mobilization of HSCs 
in the early stages of postischemic neovascularization, but 
excessive ROS production causes senescence and impairs

self-renewal of HSCs.95–98 Expression of antioxidant en-
yzymes catalase, glutathione peroxidase, and manganese su-
peroxide dismutase (MnSOD) is higher in circulating EPCs, 
whereas basal levels of ROS are lower compared to that in 
ECs, suggesting that EPCs are resistant to oxidative stress.99,100 In contrast, Ingram et al101 reported that clono-
genic proliferative EPCs derived from adult peripheral blood 
are more sensitive to oxidative stress and exhibit decreased 
clonogenic capacity and angiogenic function in the presence 
of oxidants. Our studies show that human CD34+ cells of 
diabetic origin show higher levels of NADPH oxidase-
dependent ROS production and decreased NO bioavailability.

We further observed that decreasing NADPH oxidase activa-
tion results in reduced ROS production, increased bioavail-
able NO levels, and improved EPC migration in response to 
SDF-1 or VEGF.102

Advanced Glycation End Products

Advanced glycation end products (AGEs) are modified pro-
teins or lipids that are nonenzymatically glycated and oxи-
dized after chronic exposure to sugars associated with diabe-
tes. AGEs affect cellular function by altering intracellular 
mechanisms via activating the receptor for AGEs, RAGE, as 
well as by modifying the extracellular environment by the 
formation of cross-links between key molecules in the base-
ment membrane/extracellular matrix. Several studies sug-
gested that AGEs are not required to impair the vasoreparative function of EPCs. Incorporation of CD34+ cells into endothelial 
sprouting was impaired in the presence of pathological AGE 
concentrations because of enhanced apoptosis of cells via 
activation of p38 and p44/42 MAPKs or extracellular signal- 
regulated kinase 1/2 MAPK pathways with the activation of 
nuclear factor κB.103,104

Other studies implicated oxidative stress and downregula-
tion of Akt and cyclooxygenase-2 in the AGE-mediated 
apoptosis, impaired migration, and reduced tube formation in 
EPCs that were reversed by anti-RAGE antibody.105,106 In 
diabetes, the modification of extracellular matrix proteins by 
AGEs may impair or prevent interaction of EPCs with the 
vascular wall, thereby adversely affecting the initial trigger-
ing events in the process of re-endothelialization and angi-
genesis.107 This may be the case when autologous cells are 
“corrected” for their diabetic dysfunction ex vivo before their 
reinfusion for therapy. This is one of the challenges that need 
to be addressed when autologous cell transplantation is 
considered for therapeutic reendothelialization in diabetes. 
For a detailed account of the biological effects of AGES and 
strategies to alleviate their effects in diabetes refer to the 
recent review by Negre-Salvayre et al.108

Diabetic Microvascular Complications

Retinopathy

Retinopathy is the most common of all diabetic complica-
tions, with almost all diabetic patients developing background 
retinopathy or nonproliferative diabetic retinopathy (NPDR), 
the non–vision-threatening form of diabetic retinopathy that 
is characterized by “microaneurysms,” hemorrhages, and 
exudates. The later stages of NPDR are accompanied by 
vasodegeneration characterized by retinal ischemia attribut-
able to areas of nonperfusion. These ischemic areas appear as acellular capillaries in the histological examination, a hallmark feature of diabetic retinopathy.

As discussed in the preceding sections, diabetes is associated with both widespread dysfunction and reduced numbers of EPCs. Thus, it is not surprising that several studies have detected reduced EPC number in patients with NPDR. Brunner et al. conducted a case–control study that compared 90 patients with type 1 diabetes with and without retinopathy and concluded that in type 1 with retinopathy, EPCs underwent stage-related regulation. In NPDR, a reduction of EPC numbers was observed, whereas in PDR, a dramatic increase of mature EPCs was observed. This is in keeping with the hypothesis that the vasodegenerative phase of NPDR is associated with reduced reparative function of these cells. In contrast, Lee et al demonstrated an increase in c-Kit+CD34+ cells in advanced PDR; however, the study was small in sample size and there was limited characterization of comorbidities and drug treatments that could influence progenitor numbers. Kusuyama et al examined EPCs from peripheral blood obtained from 11 patients with type 1 diabetes with PDR, 12 age- and gender-matched type 1 diabetes without retinopathy, and 11 age- and sex-matched nondiabetic controls. They found that the number of colony-forming units per $1 \times 10^6$ monocytes was increased in patients with PDR when compared with patients without retinopathy. Nondiabetic controls showed an intermediate number of EPC counts that were nonetheless significantly increased with respect to patients without retinopathy.

Taken together, these results can be explained by the hypothesis that in contrast to EPCs in healthy individuals that rescue and maintain the existing retinal capillary bed, in diabetic patients, the reduced number and reduced clonogenic potential of EPCs might predispose these patients to development of the vasodegenerative phase of diabetic retinopathy. Once the damage is widespread, inflammation increases and chemokines are produced by the ischemic retina; the BM may respond/compensate by increasing the production of EPCs, which, in the pathological setting of a growth factor-rich vitreous and retina, may result in aberrant neovascularization characteristic of PDR.

No studies to date have evaluated the potential of EPCs in the treatment of patients with diabetic retinopathy. However, animal studies have provided a better understanding of possible vascular repair by EPCs in retinopathy. Participation of BM cells in the retinal repair has been documented in mouse models; homing to areas of retinal injury and differentiation of BMCs into ECs, microglia, and astrocytes have been observed. High numbers of EPCs contributing to both repair as well as pathological neovascularization in the eye have been observed in rodent models. This may be attributable to the particularly quiescent nature of the resident retinal vasculature (typical retinal EC turnover occurs every 4 years), thus facilitating the contribution of circulating cells to the newly forming vessel. In human–mouse chimeric models of ocular vascular injury, we observed that healthy human CD34+ cells effectively repair injured mouse/rat retina by directly participating in re-endothelialization of acellular capillaries (Figure 4).

**Figure 4. Human CD34+ of nondiabetic, but not diabetic, origin integrate into degenerate vasculature in mouse eyes.**

Two days after intravitreal or systemic administration, integration of diabetic cells into existing vasculature was not observed (A), whereas cells of nondiabetic origin show extensive integration into small and medium sized vessels (yellow in the composite images (B). Insets show separate red and green channels used to make the composite images. C, CD34+ cells home to an area of injury and traverse toward the ischemic region of the capillary (arrows). Copyright 2007 American Diabetes Association. From Diabetes 2007;56:960–967. Reprinted with permission from The American Diabetes Association.

**Nephropathy**

Whereas the evidence for EPCs taking part in the pathogenesis of diabetic retinopathy is quite robust, the evidence for involvement in diabetic nephropathy is less strong; although a role for endothelial dysfunction has been documented in the development of diabetic nephropathy. In addition to the 3 different types of endothelium (vascular, peritubular, and glomerular), the kidney contains glomerular mesangial and tubular epithelial cells. Studies are yet to be carried out to identify stem/progenitor cell phenotypes that regenerate/differentiate into one or more of these cell types. The participation of BMCs in kidney repair has been shown in different experimental studies; however, these findings were challenged by a study using a chimeric mouse model. In addition, Lee et al observed homing of very few of the human mesenchymal stem cells (MSCs) in the glomeruli of STZ-diabetic NOD/SCID mice following intracardiac administration of the MSCs. More systematic studies need to be carried out to support the use of cell-based therapies in diabetic nephropathy.

**Neuropathy**

Peripheral neuropathy is a common complication of diabetes, which may lead to foot ulcers that can result in amputation. Diabetes-induced impairment of the microcirculation precedes diabetic neuropathy. Therefore, re-endothelialization or vascular repair may in fact be essential for the restoration of nerve function. A few studies have tested the therapeutic potential of stem/progenitor cells in diabetic neuropathy. Using intramuscular injection of mononuclear cells derived from peripheral blood or BM, Hasegawa et al showed significant amelioration of diabetic neuropathy in rats with 4 weeks of STZ-diabetes. This study further showed this beneficial effect was not accompanied by angiogenesis but
Diabetic MACROVASCULAR COMPLICATIONS

Diabetic microvascular complications are a high risk factor for the progression of atherosclerosis, which, in turn, manifests as coronary artery disease or peripheral vascular disease. Recent studies observed close association between EPCs and incidence of atherosclerosis. It is not clear whether EPCs promote or alleviate the progression and severity of atherosclerosis. Controversial results from experimental studies could be attributable to the lack of uniformity in the age and gender of the animals used because these factors seem to influence the impact of BMCs on the development of atherosclerosis. Early case reports suggested that BMCs may worsen restenosis; however, later studies showed no evidence for this outcome.

Diabetic macrovacular complications are a high risk factor for the progression of atherosclerosis, which, in turn, manifests as coronary artery disease or peripheral vascular disease. Recent studies observed close association between EPCs and incidence of atherosclerosis. It is not clear whether EPCs promote or alleviate the progression and severity of atherosclerosis. Controversial results from experimental studies could be attributable to the lack of uniformity in the age and gender of the animals used because these factors seem to influence the impact of BMCs on the development of atherosclerosis. Early case reports suggested that BMCs may worsen restenosis; however, later studies showed no evidence for this outcome.

Diabetic erectile dysfunction is a neurovascular response involving angiogenesis, enhanced cellularity, reepithelialization, and glandularization, which is indicative of cutaneous regeneration. Specificity of cellular phenotypes governing these different events is not yet known, but the phenomenon of wound healing is severely impaired in diabetes. Earlier studies in cell-based therapies for diabetic wound healing were focused on using fibroblasts; however, recent preclinical studies explored the benefits of BMCs. Healthy, nondiabetic CD34+ cells were shown to accelerate vascularization and wound healing in diabetic mice; murine diabetic BMCs inhibited angiogenesis but produced wound healing in diabetic mice suggesting that these 2 events involve distinct mechanisms perhaps involving distinct cellular phenotypes. Murine BM-derived MSCs or their conditioned medium were shown to enhance wound healing with cutaneous regeneration that was not observed with CD34+ cells. BM stromal cells also have been shown to improve wound closure following topical or systemic administration in diabetic rats. Human fetal-derived CD133+ cells produced effective wound healing in diabetic mice. Recently, murine lin+ cells were found to be effective in diabetic wound healing and were shown to remain in wound areas for up to 28 days compared to lin- cells that were not found after 7 days. These studies suggest that healthy progenitors may serve to enhance wound healing in diabetic patients.

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Neovascularization: When Is There Too Little or Too Much?
Perhaps one of the most perplexing aspects of diabetes vascular dysfunction is how the retina can respond to the same insults with pathological neovascularization and the other vascular beds consistently demonstrate reduced angiogenesis. This “paradox” remains at the center of key questions in the field. So how can this be reconciled? One explanation is that the diabetic retina and vitreous represents a unique environment in which high concentrations of complementary growth factors (VEGF, insulin-like growth factor [IGF]-1, fibroblast growth factor, SDF-1, erythropoietin) act in a synergistic manner in contiguous environments (retina and vitreous) that provide scaffolding (remnant “hyaloid”) for new vessel formation. This unique structural arrangement and an accompanying plethora of inflammatory cells likely set up an ideal environment for neovessel formation. Once the damage is widespread, inflammation increases and chemokines are produced by the ischemic retina; the BM responds by increasing the production of EPCs, which in the pathological setting of the growth factor rich vitreous and retina may result in pathological neovascularization. Thus, the cooperation of circulating EPCs with resident retinal vasculature and circulating inflammatory monocytes results in “too much” neovascularization, leading to PDR. In contrast, in nonocular vascular beds, the level of neovascularization is markedly reduced, in part, because of progressive basement membrane modification by AGEs, which contributes to impairment of EPC reparative function after diabetes-related endothelial injury.107

Ex Vivo Manipulation of Diabetic Cells
Genetic Modification of Cells
Accumulating evidence indicates that biologically modified EPCs to release proangiogenic factors may be a potent therapeutic tool compared to unmodified cells. In this regard, Iwaguro et al143 transduced murine EPCs ex vivo with adenoviral vector encoding VEGF gene and demonstrated that VEGF-expressing cells had enhanced angiogenic potential in vitro and in vivo assays. Murasawa et al144 have used a similar approach to delay human EPC senescence by transducing progenitor cells with hTERT (human telomerase reverse transcriptase) and observed increased capillary density and blood flow in the mouse hindlimb ischemia. EPCs that were transduced to overexpress eNOS were shown to inhibit neointimal hyperplasia in a rabbit model of carotid artery balloon angioplasty.145 Choi et al146 have targeted glycosyn thase kinase-3β in human early EPCs and late outgrowth EPCs and reported that this genetic modification enhanced their vasoregenerative potential in mouse hindlimb ischemia. Human MSCs transduced with the prosurvival gene Akt-1 showed enhanced viability and greater functional repair in a mouse infarct model.147 We recently identified that expression of IGFBP-3 (IGF-1 binding protein-3) in stem/progenitor cells or in the area of vascular injury dramatically enhanced the homing and vascular repair.112

Ex Vivo Treatment With Small Molecules
Ex vivo treatment of BMCs with a novel molecule, AVE9488, that increases eNOS expression by stimulating eNOS promoter activity, enhanced the angiogenic function of EPCs in mouse hindlimb ischemia.148 Similar effects were produced by ex vivo treatment with IGF-1, which indeed reversed age-associated decrease in human and murine EPCs.149 However, one should be very cautious when adopting this approach in diabetic cells, because without correcting the diabetic oxidative environment, an increase in NO production by iNOS may result in higher production of reactive nitrogen species that further increase oxidative stress. Sphingosine-1-phosphate and its synthetic equivalent, FTY720, were shown to stimulate EPC function in restoring blood flow in mouse ischemic limbs via activating CXCR4-coupled signaling pathway.150 Active p38 MAPK downregulates the number of BMCs in response to high glucose or tumor necrosis factor–α and ex vivo treatment with the p38 MAPK inhibitor SB203580 has been shown to increase the proliferation of EPCs that can be used for transplantation.151 Sorrentino et al152 showed that treatment of human diabetic EPCs with the peroxisome proliferator-activated receptor-γ agonist rosiglitazone improved their angiogenic function in a mouse model of carotid injury by reducing NADPH oxidase–dependent ROS production and enhancing bioavailable NO levels. Agents or molecular maneuvers that can reduce oxidative stress as well as increase eNOS expression/activation would be of immense value in correcting diabetic EPC dysfunction.

Autologous Cell Therapy in Diabetic Vascular Complications
Despite strong lines of evidence supporting the concept of therapeutic angiogenesis by putative EPCs, few studies have used diabetic models, particularly, testing autologous transplants. As described above, diabetic cells have impared reparative function with complex underlying mechanisms. Cells for treatment of diabetic vasculopathy should be equipped with cellular and molecular armamentarium to withstand the in vivo diabetic microenvironment. Their migration to the site of repair and the ability to transmigrate, differentiate, and engraft into a blood vessel should be minimally affected by the diabetic environment. Several approaches have been proposed, but few have been tried in diabetic cells and evaluated in diabetic models. Hypoxic preconditioning of EPCs was shown to enhance their angiogenic potential.142 However, it is questionable whether diabetic EPCs would be as responsive as normal cells to hypoxia.
In Vivo Manipulation of Diabetic Cells

In vivo manipulation of EPCs may involve modifying the diabetic milieu or the EPC niches to release functioning cells for homing to the areas of repair; however, possible options are very few. Treatment with growth hormone has been shown to reverse age-associated impairment of EPC function with increased eNOS expression, and this treatment resulted in elevated IGF-1 levels in mice and humans.140 Although mobilization of BM cells using G-CSF for therapeutic angiogenesis requires more thorough safety evaluation,69,70 studies using AMD3100 appear to be encouraging.153 AMD3100 is a small molecule antagonist of chemokine receptor CXCR4 and has been shown to mobilize BM cells in mice and humans.154–156 In vivo treatment with AMD3100 or local administration of cells mobilized by AMD3100 accelerates blood flow to ischemic limbs in 12 week STZ-diabetic mouse.153 However, these studies need to be carried out in mice with different durations of diabetes, because it has been hypothesized that BM microenvironment may initially provide protection to resident EPCs from adverse diabetic milieu of the circulation and cells become dysfunctional only with longer durations of diabetes (Grant, MB, unpublished). Another CXCR4 antagonist, SDF-1BP2G, has better angiogenic function compared to AMD3100 but has not yet been studied in diabetic models.157

Manipulation of Diabetic Host Environment

A successful outcome of the cell-based therapy also depends on the host environment. An optimal “fertile” environment is essential for the adhesion and transmigration of cells that require interaction of cell surface molecules with extracellular matrix proteins. The oxidative diabetic milieu modifies the host environment, and the manipulation of which is required to enhance the reparative function of cells. Treatment with antioxidants or anti-AGE agents is an option. Treatment with insulin, glitazones, or statins may directly or indirectly makes the host environment favorable for the therapeutic behavior of the cells.

Future of Cell-Based Therapies

Attempting translational studies before understanding the full identity and characteristics of EPC phenotype is likely fraught with difficulties. Which cell type is appropriate for a particular complication is still a difficult question to be answered. Although the study by Tendera et al158 found no difference in the efficacy of unselected BMCs and CD34+/CXCR4+ cells, preclinical studies and ongoing clinical trials support the use of CD34+ cells compared to the total mononuclear leukocytes or unselected BMCs, although a higher dose of CD34+ cells was shown to be less efficacious than lower doses.42,159,160 As described above, autologous cell therapy in diabetics requires the ex vivo modification of EPCs for a better angiogenic outcome regardless of the cell type chosen. At the same time, PDR needs treatments inhibiting new retinal blood vessel formation, including blocking EPC recruitment and engraftment therein. Conversely, other sites and disease conditions may actually need an increase of EPC activity toward tissue repair, eg, in myocardial ischemia or wound healing. The previous sections have emphasized the role of NO in maintaining normal function of EPC/BMCs and ways to maintain balanced NO and ROS levels. All BMC populations may have a key role in the repair process, and it is likely that combinations of progenitor cells will be needed, as will the simultaneous optimization of the diabetic environment into which these cells will be placed. Such a complex approach will likely be needed to optimally treat diabetic patients with vascular complications.

Acknowledgments

We thank Dr Lynn C. Shaw for the preparation of schematics for this manuscript.

Sources of Funding

This work was supported by NIH grants EY007739 and EY012601 (to M.B.G.).

Disclosures

None.

References


21. Jarajapu and Grant Cell-Based Therapies for Diabetic Complications 865


Jarajapu and Grant


The Promise of Cell-Based Therapies for Diabetic Complications: Challenges and Solutions
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*Circ Res.* 2010;106:854-869
doi: 10.1161/CIRCRESAHA.109.213140

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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The Promise of Cell-Based Therapies for Diabetic Complications: Challenges and Solutions: Correction

In the article that appears on page 854 of the March 19, 2010, issue, the legend for Figure 2 should not contain a permission to reprint material from The American Diabetes Association. The legend for Figure 4 should contain a permission to reprint material from The American Diabetes Association. The legend for Figure 4 should read as follows:

Human CD34+ cells of nondiabetic, but not diabetic, origin integrate into degenerate vasculature in mouse eyes damaged by ischemia/reperfusion injury (Caballero et al25). Two days after intravitreal or systemic administration, integration of diabetic cells into existing vasculature was not observed (A), whereas cells of nondiabetic origin show extensive integration into small and medium sized vessels (yellow in the composite images) (B). Insets show separate red and green channels used to make the composite images. C, CD34+ cells home to an area of injury and traverse toward the ischemic region of the capillary (arrows). Copyright 2007 American Diabetes Association. From Diabetes 2007;56:960–967. Reprinted with permission from The American Diabetes Association.

Reference 49 is incorrect and should appear as follows:


The authors and publisher regret these errors. These errors have been noted in the online version of the article, which is available at http://circres.ahajournals.org/cgi/content/full/106/5/854.

Reference


DOI: 10.1161/RES.0b013e3181e1963e