Understanding the Role of Endothelial Progenitor Cells in Percutaneous Coronary Intervention

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Percutaneous coronary intervention is associated with mechanical endovascular injury and endothelial denudation. Re-endothelialization is essential for restoration of normal vascular homeostasis and regulation of neointimal hyperplasia. The endothelial progenitor cell recently emerged as an important component of the response to vascular injury, having the potential to accelerate vascular repair through rapid re-endothelialization. There remains considerable uncertainty over the precise identity and function of endothelial progenitor cells, and harnessing their therapeutic potential remains a challenge. A better understanding of the role of circulating progenitors in the response to vascular injury is necessary if we are to develop effective strategies to enhance vascular repair after percutaneous coronary intervention. In this review, we examine the preclinical and clinical evidence of a role for bone marrow-derived putative endothelial progenitor cells after iatrogenic vascular injury associated with balloon angioplasty and stent deployment. Therapies designed to mobilize endothelial progenitors or to increase their ability to home to the site of stent implantation may have a role in the future management of patients undergoing percutaneous coronary intervention. (J Am Coll Cardiol 2010;55:1553–65) © 2010 by the American College of Cardiology Foundation

Although percutaneous coronary intervention (PCI) improves myocardial perfusion and clinical outcomes in patients with ischemic heart disease, the treated coronary artery segment inevitably undergoes significant mechanical trauma. Endothelial denudation and endovascular laceration by rigid stent struts and high-pressure balloon inflations disturb vascular function and initiate an intensive local inflammatory response. Vascular injury may result in neointimal hyperplasia, in-stent restenosis (ISR), and the potentially catastrophic complication of acute stent thrombosis. Re-endothelialization, a necessary component of restoring vascular homeostasis, was previously thought to occur purely through the migration and proliferation of mature endothelial cells adjacent to regions of endothelial denudation (1). However, attention has recently focused on a novel mechanism of vascular repair involving a bone marrow-derived precursor or stem cell: the endothelial progenitor cell (EPC). It has been proposed that EPCs are mobilized in response to vascular injury and can home to sites of endothelial denudation (2) and facilitate re-endothelialization (3). The discovery of the EPC has launched a new field of cardiovascular research, changed our understanding of vascular repair mechanisms, and offers exciting and novel approaches to manage cardiovascular disease and the complications associated with modern revascularization strategies.

PCI and Vascular Injury

Adverse cardiac events after PCI continue to be problematic despite advances in stent design and adjunctive pharmacotherapy. The incidence of angiographic restenosis after PCI is approximately 11% and has been reported to be as high as 29% in higher risk populations using bare-metal stents (4). Stent thrombosis, although a relatively rare complication of PCI, can still occur in as many as 2% of cases and has potentially devastating and fatal consequences (5). These complications arise, in part, as sequelae of the vascular trauma that occurs during PCI as a result of the necessary high-pressure balloon inflations and forceful apposition of comparatively rigid stent struts against the vessel wall that leave the treated segment of vessel denuded of its endothelium with a consequent disruption of normal vascular function. Stent struts may cause laceration of the internal elastic lamina that may extend through the tunica media to the external elastic lamina (6). Vessel injury causes activation and aggregation of platelets and thrombus formation, which may lead to acute or subacute stent occlusion (7). Vascular injury triggers both local (6) and systemic (8) inflammation with a typical cellular response of a rapid influx of neutrophils followed by the migration of monocytes and macrophages into the vessel wall. Cytokine and growth factor release stimulates the migration and prolifer-
Endothelial Progenitor Cells and PCI

The process of stent integration into the vessel wall has been elegantly characterized using scanning electron microscopy (10). During the first 6 weeks, a thin multilayered thrombus is present between the vascular lumen and arterial wall, and an increasing concentration of smooth muscle cells and extracellular matrix can be detected as time progresses. Six to 12 weeks from stent implantation, the thrombus resolves and endothelial cells begin to cover the stented segment. Complete re-endothelialization takes place approximately 3 months after stent implantation, at which time the number of smooth muscle cells begins to decrease. The endothelium is not only integral to normal endogenous fibrinolysis and vasomotor function, but also provides a protective barrier for smooth muscle cells against circulating growth factors and secretes a number of inhibitory factors that prevent smooth muscle cell proliferation. Rapid re-endothelialization is therefore of critical importance to restore normal vascular function, reduce vascular inflammation, and prevent adverse remodeling after PCI (11). It is now clear that bone marrow-derived cells are an important component of the cellular response to vascular injury with the potential to enhance re-endothelialization after coronary intervention.

EPCs

Bone marrow-derived cells with the capacity to differentiate into mature cell types exist within the adult circulation. This concept has been confirmed primarily through studies of bone marrow transplant recipients in whom appropriately differentiated cells of donor origin integrate into host structures, such as the heart and lung (12,13). Contrary to the traditional paradigm of re-endothelialization, it is now clear that this same principle applies to vascular endothelial cells. The term EPC was first used by Asahara et al. (2) in 1997 and has subsequently been applied interchangeably to a variety of cell populations by different investigators. Considerable ambiguity regarding the optimal definition of an EPC continues to cause problems in the field. Broadly speaking, 2 approaches to identify EPCs have predominated: 1) identifying cells bearing surface markers that indicate both cellular naïveté and endothelial origin; and 2) inferring the presence of endothelial precursors within a given cell population by the identification of cells bearing mature endothelial characteristics after a period of culture under angiogenic conditions.

Surface markers. A detailed discussion of the controversies surrounding EPCs and their definition is outside the scope of this review. EPCs were originally defined by the expression of CD34 and kinase domain receptor (KDR), the extracellular domain of vascular endothelial growth factor receptor-2, as these cell surface markers were thought to indicate cellular naïveté and a vascular phenotype, respectively (Table 1) (2). Just as endothelial function is integral to the maintenance of normal vascular homeostasis, EPC regulation seems to be similarly important. Concentrations of circulating CD34+KDR+ cells are decreased in patients with traditional cardiovascular risk factors including cigarette smoking, elevated low-density lipoprotein cholesterol (14), diabetes mellitus (15), and hypertension (16). Indeed, there seems to be a cumulative effect of multiple cardiovascular risk factors on concentrations of CD34+KDR+ cells (14), with levels markedly decreased in patients with overt atherosclerotic disease of the coronary and peripheral circulation (17). A robust association has been demonstrated between a high circulating concentration of CD34+KDR+ cells and freedom from myocardial infarction, hospitalization, revascularization, and cardiovascular death in patients with coronary artery disease. Additionally, the predictive value of CD34+KDR+ cell concentration is independent of traditional cardiovascular risk factors (18,19). Both CD34+KDR+ cells (14) and CD133+KDR+ cells (20) decrease with advancing age, although, interestingly, when patients are matched for biological characteristics, in particular, angiographic severity of coronary disease, chronological age seems less important (21). Finally, it seems that EPC biology is in part inherited, with apparently healthy offspring of patients with coronary artery disease having reduced levels of CD34+KDR+ cells compared with the offspring of healthy controls (22).

Because CD34 and KDR are also expressed on circulating mature endothelial cells (23), an additional marker of cellular naïveté, CD133, has been suggested as a means of ensuring the identity of an EPC. CD133 is a precursor of an endothelial cell-like phenotype and has been used either alone or in combination with CD34 and KDR as a definition for EPCs by numerous investigators (24–26). However, the rarity of these cells in the circulation makes them difficult to identify reliably and therefore a less practical candidate for therapeutic use. Furthermore, recent studies suggest that CD133 identifies a purely hematopoietic cell line and that these cells are in fact not capable of forming a true endothelial phenotype (27,28).

Cells expressing CD14, a receptor for endotoxin (29), have a predominant monocytic phenotype and have been the subject of much interest in the EPC literature. CD14+ cells are 10 times more abundant in the peripheral blood than CD34+ cells, are highly plastic, and have the ability to adopt a variety of mature phenotypic and functional characteristics, including those of endothelial cells under the right environmental cues (30–35). Several studies have indicated a role for monocytes in the repair of endothelial

Abbreviations and Acronyms

EC-CFU = endothelial cell colony-forming unit
ECFC = endothelial colony-forming cell
eNOS = endothelial nitric oxide synthase
EPC = endothelial progenitor cell
G-CSF = granulocyte colony-stimulating factor
ISR = in-stent restenosis
KDR = kinase domain receptor
PCI = percutaneous coronary intervention
VEGF = vascular endothelial growth factor

Endothelial Progenitor Cells and PCI

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injury and restoration of function (32,34,36–41); however, it is our view that CD14+ cells accelerate vascular repair through the secretion of angiogenic growth factors rather than by differentiating into true endothelial cells.

**Functional phenotype.** Functional assays of EPCs have been widely used to enumerate EPCs (Fig. 1). The most common is the endothelial cell colony-forming unit (EC-CFU) assay popularized by Hill et al. (42). EC-CFUs are generated from a nonadherent population of mononuclear cells cultured on fibronectin and exhibit endothelial cell-like characteristics such as expression of CD31, CD141, CD105, CD146, CD144, von Willebrand factor, Tie-2, KDR, CD34, and E-selectin and an ability to bind lectins and take up acetylated low-density lipoprotein (Table 1). EC-CFUs were therefore thought to be endothelial cells formed from circulating progenitor cells. However, it is now recognized that many of the surface antigens used to define mature endothelial cells are nonspecific and are shared by circulating monocytes in peripheral blood (43). EC-CFUs are in fact composed of monocytes and angiogenic lymphocytes (36,44) and have little capacity to form perfusing vessels or incorporate directly into vascular structures (37). EC-CFUs exhibit phagocytic activity and avidly secrete angiogenic factors, such as vascular endothelial growth factor (VEGF), stromal derived factor-1, matrix metalloproteinase-9, interleukin-8, hepatocyte-like growth factor, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor. All these factors have been implicated in the mobilization of precursors from the bone marrow and the acceleration of angiogenesis (32,44). Nevertheless, EC-CFUs are reduced in patients with coronary artery disease (45), diabetes mellitus (46), and hypertension (47). Increased EC-CFUs are associated with better vascular function (42) and are mobilized in response to cardiovascular stress, including discrete vascular injury caused by angioplasty (48–51). The EC-CFU assay therefore serves as a marker of cardiovascular health and possibly indicates an individual’s capacity to exert a hematopoietic and angiogenic response to tissue injury, but it is not a direct measure of differentiating or proliferating EPCs. As such, EC-CFU is an inappropriate name for this population of cells, but given the lack of an alternative, we use this nomenclature in the interests of maintaining consistency with existing literature.

Interest has recently been drawn toward the late outgrowth EPC or endothelial colony-forming cell (ECFC) (Fig. 1). ECFCs are derived from mononuclear cells but differ from EC-CFUs in that they arise from an adherent population cultured on type I collagen and appear after 2 to 3 weeks of culture. Although ECFCs share many phenotypic characteristics with EC-CFU (52), they do not express the hematopoietic marker CD45 or myeloid/macrophage

### Table 1: Characteristics Used to Identify Putative Endothelial Progenitor Cells

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Distribution</th>
<th>Function</th>
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<tbody>
<tr>
<td>CD45</td>
<td>Leukocytes</td>
<td>A signaling molecule regulating leukocyte differentiation and proliferation</td>
</tr>
<tr>
<td>CD14</td>
<td>Monocytes, macrophages, some neutrophils</td>
<td>Endotoxin receptor regulating inflammatory cytokine production such as TNF by monocytes</td>
</tr>
<tr>
<td>CD115</td>
<td>Monocytes, macrophages</td>
<td>Receptor for macrophage-CSF regulating myeloid proliferation and differentiation</td>
</tr>
<tr>
<td>CD117 (c-Kit)</td>
<td>Hematopoietic stem and progenitor cells</td>
<td>Receptor for stem cell factor; stimulates cellular proliferation</td>
</tr>
<tr>
<td>CD133</td>
<td>Hematopoietic stem and progenitor cells</td>
<td>Unknown</td>
</tr>
<tr>
<td>CD34</td>
<td>Hematopoietic stem and progenitor cells, capillary endothelium</td>
<td>Intercellular adhesion molecule; binds E- and L-selectins and is thought to regulate leukocyte/endothelial interactions</td>
</tr>
<tr>
<td>CD31</td>
<td>Leukocytes, platelets, endothelium</td>
<td>Adhesion molecule thought to be important for transendothelial cellular migration to sites on acute inflammation</td>
</tr>
<tr>
<td>Acetylated low-density lipoprotein uptake</td>
<td>Macrophages, monocytes, endothelium</td>
<td>N/A; phagocytic process occurring in myeloid and endothelial cells</td>
</tr>
<tr>
<td>Ulex binding</td>
<td>Macrophages, monocytes, endothelium</td>
<td>N/A; histochemical stain</td>
</tr>
<tr>
<td>CD105</td>
<td>Endothelium, activated macrophages, smooth muscle</td>
<td>Constituent of transforming growth factor-beta receptor 1; important regulator of angiogenesis</td>
</tr>
<tr>
<td>CD141</td>
<td>Endothelium, smooth muscle, monocytes, and neutrophils</td>
<td>Binds thrombin and activates protein C and initiates anticoagulant pathways</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>Endothelium, platelets</td>
<td>Hemostasis</td>
</tr>
<tr>
<td>Tie-2 (CD202)</td>
<td>Endothelium, monocytes, stem cells</td>
<td>Angiopoietin-1 receptor; regulates vessel remodeling and maintains vascular integrity</td>
</tr>
<tr>
<td>CD146</td>
<td>Endothelium, melanoma cells, dendritic cells</td>
<td>Intercellular adhesion molecule</td>
</tr>
<tr>
<td>E-selectin (CD62E)</td>
<td>Endothelium</td>
<td>Adhesion molecule regulating leukocyte/endothelial interactions and cell trafficking to sites of inflammation</td>
</tr>
<tr>
<td>CD144</td>
<td>Endothelium</td>
<td>Intercellular adhesion molecule regulating endothelial permeability and proliferation</td>
</tr>
<tr>
<td>VEGFR-2 (CD309, KDR, Flk1)</td>
<td>Endothelium</td>
<td>Regulation of endothelial adhesion and signaling; essential for embryonic vascular development</td>
</tr>
<tr>
<td>Endothelial nitric oxide synthase</td>
<td>Endothelium</td>
<td>Enzymatic generation of nitric oxide</td>
</tr>
</tbody>
</table>

CSF = colony-stimulating factor; N/A = not available; TNF = tumor necrosis factor.
markers such as CD14 and CD115 and do not exhibit phagocytic function. ECFCs arise from nonhematopoietic cells negative for CD133 and the panleukocyte marker CD45, but expressing CD34 and KDR (27). Enrichment of unsorted mononuclear preparations for CD45<sup>−</sup>/CD34<sup>+</sup> cells increases the frequency of ECFCs by approximately 400% compared with abolition of ECFC growth in the CD45<sup>−</sup> fraction (27). Of the populations so far identified, the CD45<sup>−</sup>/CD34<sup>+</sup>-derived ECFCs most accurately fulfills the criteria of an EPC. They conform to an endothelial phenotype and morphology, are derived from bone marrow (53), have robust proliferative potential (54,55), and are capable of forming perfusing vessels in vivo (37). ECFCs are mobilized within the first few hours after myocardial infarction (56,57) and have been positively correlated with the severity of coronary artery disease in patients undergoing coronary angiography (58). However, their pathophysiological relevance or therapeutic potential remains unclear. ECFCs and CD45<sup>−</sup>/CD34<sup>+</sup> cells represent a potentially important but understudied component of endogenous vascular repair mechanisms.

It is interesting to note that despite an incomplete understanding of the fundamental basic science governing EPC biology, numerous clinical studies utilizing EPC populations as cell therapy have been conducted in the setting of acute myocardial infarction, chronic left ventricular dysfunction, and peripheral vascular disease (59). Although frequently yielding encouraging results, autologous cell therapy has disappointingly, but perhaps unsurprisingly, failed to provide marked or sustained clinical benefit. A greater understanding of EPC biology is urgently required if we are to harness their therapeutic potential in the treatment of patients with cardiovascular disease.

**EPCs and Iatrogenic Vascular Injury**

The role of EPCs after the vascular injury resulting from balloon angioplasty and stenting has not been widely studied. Experimental models of arterial injury confirm that bone marrow–derived mononuclear cells home to areas of endothelial denudation, accelerate re-endothelialization, re-

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**Figure 1 Early Versus Late Outgrowth Endothelial Cell Colonies**

(A) The endothelial cell colony-forming unit (EC-CFU) assay. EC-CFUs are generated from a nonadherent population of mononuclear cells cultured on fibronectin and appear some 5 to 7 days into culture. EC-CFUs exhibit endothelium-like surface characteristics; however, it is now recognized that EC-CFUs are composed of monocytes and angiogenic lymphocytes and have little capacity to form perfusing vessels or incorporate directly into vascular structures. EC-CFUs exhibit phagocytic activity and avidly secrete angiogenic growth factors. EC-CFUs are depressed in association with cardiovascular disorders and are mobilized in response to cardiovascular stress. EC-CFUs therefore serve as a marker of cardiovascular health and possibly indicate an individual’s capacity to exert a hematopoietic and angiogenic response to tissue injury. The nomenclature for this population requires revision. (B) The endothelial colony-forming cell (ECFC) assay. ECFCs are generated from adherent mononuclear cells grown on type I collagen and appear after 2 to 3 weeks of cell culture. ECFCs are morphologically indistinguishable from mature endothelial cells and are derived from nonhematopoietic (CD45<sup>−</sup>) cells expressing CD34. ECFCs have robust proliferative potential and the capacity to form perfusing blood vessels in vitro. EPC = endothelial progenitor cell.
store endothelial function, and attenuate neointimal hyperplasia (Table 2) (60–65). To date, there have been few clinical studies that have specifically addressed the role of putative EPC populations after angioplasty (Table 3). Studies of EPCs in patients undergoing PCI have largely examined the behavior of EC-CFs. Unfortunately, many of the studies were small and did not include a control group for comparison. Initially, EC-CFs were measured in patients undergoing peripheral angioplasty by Bonello et al. (48), who reported an increase in circulating mature endothelial cells immediately after angioplasty, with a 2- to 3-fold increase in EC-CFs at 24 h. These findings were confirmed by Marboeuf et al. (49), who added that mobilization of EC-CFs correlated with plasma C-reactive protein concentrations. EC-CFs are also mobilized after PCI in patients with acute coronary syndromes (51). However, in the absence of a control group of patients managed conservatively, it is difficult to determine whether EC-CFs were mobilized in response to myocardial infarction or discrete vascular injury during PCI.

In our own studies, the number of EC-CFs were measured in 54 patients undergoing elective coronary angiography (8). EC-CFs were increased in patients who had follow-up intervention, but, importantly, were unchanged in those patients who underwent diagnostic angiography alone (8). The finding of an increase in EC-CFs after angioplasty alone may be another evidence that EC-CFs participate in the re-endothelialization of injured arteries.

### Table 2 Preclinical Studies of Putative Endothelial Progenitor Cells After Vascular Injury

<table>
<thead>
<tr>
<th>First Author, Year (Ref. #)</th>
<th>Model</th>
<th>Number of Subjects</th>
<th>Putative EPCs</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walter et al., 2002 (65)</td>
<td>Murine Tie-2/tieZ BM transplant recipients subjected to balloon-mediated arterial injury and pretreatment with simvastatin or placebo</td>
<td>18 placebo, 34 simvastatin</td>
<td>Di-Ac-LDL lectin+ MNCs</td>
<td>Simvastatin enhanced EPC mobilization after vascular injury and increased their adhesive capacity; re-endothelialization was accelerated by BM-derived cells and neointimal hyperplasia was reduced</td>
</tr>
<tr>
<td>Werner et al., 2002 (63)</td>
<td>Murine GFP BM transfection followed by wire-mediated arterial injury and pretreatment with rosuvastatin or placebo</td>
<td>5 placebo, 4 rosuvastatin</td>
<td>Sca 1+ KDR+ cells of BM origin</td>
<td>Rosuvastatin enhanced BM-derived EPC mobilization after vascular injury; re-endothelialization was accelerated by BM-derived cells and neointimal hyperplasia was reduced</td>
</tr>
<tr>
<td>Werner et al., 2003 (62)</td>
<td>Intravenous cell therapy after wire-mediated murine arterial injury</td>
<td>6 vascular injury</td>
<td>Spleen-derived Di-Ac-LDL lectin+ MNCs with (EPC) or without (MNC) a period of culture in endothelial growth medium</td>
<td>Cell therapy enhanced re-endothelialization in splenectomized animals and was associated with a reduction of neointima formation, but MNCs were more effective than EPCs</td>
</tr>
<tr>
<td>Fujiyama et al., 2003 (40)</td>
<td>Intravenous cell therapy versus saline after balloon-mediated murine arterial injury</td>
<td>12 vascular injury, 12 controls</td>
<td>BM: CD14+ CD34+, CD14-CD34+ PB CD14- CD34+</td>
<td>Compared with saline placebo, BM-derived CD34+ cells and both PB- and BM-derived CD14- cells up-regulated endothelial markers, accelerated neointimal-thelialization, and inhibited neointimal hyperplasia after activation with MCP-1</td>
</tr>
<tr>
<td>Kong et al., 2004 (61)</td>
<td>G-CSF versus control before balloon-mediated murine arterial injury</td>
<td>5 G-CSF, 5 controls</td>
<td>CD34+ KDR+</td>
<td>G-CSF enhanced EPC mobilization after vascular injury; re-endothelialization was accelerated by BM-derived cells and neointimal hyperplasia was reduced</td>
</tr>
<tr>
<td>Nowak et al., 2004 (41)</td>
<td>Intravenous cell therapy after balloon injury in mice using CD14/CD11b cells expressing Tie-2, Tie-2, KDR, KDR+, or saline control</td>
<td>6 cell therapy, 6 controls</td>
<td>Myeloid cells (CD14, CD11b) expressing KDR+ or Tie-2+ CD14+ KDR+ and CD14+ Tie-2+ displayed endothelial characteristics in culture and enhanced re-endothelialization of denuded arteries with an associated reduction in neointimal hyperplasia</td>
<td></td>
</tr>
<tr>
<td>Elsheikh et al., 2005 (39)</td>
<td>Intravenous cell therapy using GFP-transduced cells after balloon-mediated arterial injury in mice</td>
<td>10 CD14+ KDR+ cells, 10 CD14+ KDR+ cells, 6 controls</td>
<td>CD14+ KDR+ CD14+ KDR+</td>
<td>Unlike CD14+ KDR+ cells, CD14+ KDR+ cells exhibited an endothelial phenotype in culture and contributed to neointimal-thelialization</td>
</tr>
<tr>
<td>Yoshioka et al., 2006 (60)</td>
<td>G-CSF after wire-mediated arterial injury in mice; treatment pre- and post-arterial injury versus post-arterial injury alone</td>
<td>22 G-CSF, 20 controls</td>
<td>CD34+ KDR+</td>
<td>G-CSF reduced neointimal hyperplasia in association with mobilization of bone marrow-derived EPCs and accelerated re-endothelialization compared with control; the effect was enhanced if administered before injury; few BM-derived cells contributed to neointimal-thelialization</td>
</tr>
<tr>
<td>Takamiya et al., 2006 (64)</td>
<td>1. G-CSF before balloon-mediated arterial injury in rats 2. GFP BM-transfected mice subjected to balloon-mediated arterial injury</td>
<td>10 G-CSF, 5 placebo</td>
<td>CD117+ KDR+</td>
<td>1. G-CSF enhanced EPC mobilization after vascular injury; re-endothelialization was accelerated and neointimal hyperplasia was reduced 2. GFP expressing BM-derived cells contribute to neointimal-thelialization</td>
</tr>
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</table>

BM = bone marrow; Di-Ac-LDL = diacetylated low-density lipoprotein; EPC = endothelial progenitor cell; G-CSF = granulocyte colony-stimulating factor; GFP = green fluorescent protein; KDR = kinase domain receptor; MCP = monocyte chemoattractant protein; MNC = mononuclear cell.
CFUs within the first 24 h of angioplasty is consistent and suggests a role for these cells in the immediate response to vascular injury (8,48–51). Few studies have addressed whether EC-CFU mobilization influences the development of ISR and the need for target vessel revascularization. In a retrospective study, George et al. (66) demonstrated that in patients presenting with proliferative as opposed to focal restenosis, EC-CFUs were reduced both in number and adhesive capacity, suggesting that a deficiency in circulating progenitors might predispose to aggressive neointimal hyperplasia. This study did not detect a difference in the number of EC-CFUs between patients with and without angiographic restenosis. However, 2 other groups later reported a decrease in EC-CFUs in patients presenting with restenosis, and these decreased EC-CFUs had reduced proliferative and migratory capacity as well as increased senescence (67,68).

In addition to EC-CFUs, mobilization of ECFCs and CD45−CD34+ has been detected within hours of acute myocardial infarction in small preclinical and clinical studies. One might speculate that PCI will exert a similar effect, although no studies have specifically examined ECFC or CD45−CD34+ cells after PCI. The clinical outcome of patients with comparatively high or low ECFCs undergoing PCI is also unknown. Two small clinical studies demonstrated a decrease in CD34+KDR+ (CD45−/−) EPCs within the first few hours after coronary angioplasty, although neither had control groups and interpretation is challenging (69,70). Although homing of circulating progenitors to sites of denuded endothelium is a possible explanation, it is more likely that this decrease is due to the normal diurnal variation of EPCs (71). Egan et al. (72) measured a variety of surface markers expressed on mono-
nuclear cells including CD34, CD133, KDR, CD117, CD31, and CXCR4⁺ in patients undergoing PCI, diagnostic coronary angiography alone, and healthy controls. In this small study, patients with coronary artery disease had lower resting levels of CD34⁺, CD133⁺, CD117⁺, CD34⁺CD117⁺, CD34⁺CD31⁺, and CXCR4⁺ cells compared with healthy controls. After PCI, they observed an increase in those cells expressing CD133, CD117, CD34/CD31, CD34/CD117, and CXCR4 within 6 to 12 h (72). CXCR4 is thought to be integral to EPC homing and integration, and it is of interest to note that reduced levels of CXCR4 correlated with the incidence of angina at 1 year. Although underpowered to address clinical outcomes, this study suggests a role for CXCR4 in EPC homing and integration at the site of vascular injury after PCI.

CD34⁺CD45⁺ mobilization within the first 6 h after PCI has been reported, and, interestingly, the magnitude of CD34⁺CD45⁺ mobilization independently predicts subsequent ISR (73,74). It is challenging to interpret these findings in light of our understanding of the behavior of CD45⁻CD34⁺ cells in vitro. It is possible that an exaggerated response by hematopoietic precursors (CD45⁺) over endothelial precursors (CD45⁻) after PCI may favor a maladaptive response to vascular injury, therefore leading to restenosis. Further prospective studies are needed to determine the clinical relevance of these putative EPC populations in PCI-associated vascular injury.

**Hypothetical role of EPCs in vascular injury.** We suggest that circulating EPCs play a critical role in the response to iatrogenic vascular injury from PCI (Fig. 2). We hypothesize that EC-CFUs precursors, composed of angiogenic monocytes and lymphocytes, are mobilized early after vascular injury, home to sites of vascular injury, and avidly secrete angiogenic factors encouraging resident endothelial cell proliferation and migration. Over a period of weeks and months, bone marrow-derived progenitors proliferate and contribute to effective re-endothelialization and the restoration vascular homeostasis. A robust response by the bone marrow may lead to early homing of EC-CFU precursors to sites of vascular injury with subsequent recruitment and integration of EPCs to the endothelial monolayer, facilitating rapid recovery of normal endothelial function and vascular homeostasis. Alternatively, an inadequate EC-CFU/EPC response after PCI may result in delayed re-endothelialization and persistent inflammation, thereby potentiating smooth muscle hypertrophy and extracellular matrix deposition, leading to restenosis and symptoms of myocardial ischemia.

Prospective studies examining the relevance of EC-CFUs and ECFCs in angioplasty-related vascular injury and its complications, such as restenosis and stent thrombosis, are awaited.

**Therapeutic Potential of EPCs in Vascular Injury**

Current strategies to decrease the incidence of complications after PCI are based on suppressing cellular proliferation rather than enhancing vascular repair. Drug-eluting stents have dramatically reduced the incidence of early ISR, but local antiproliferative therapy may interfere with vascular healing and prevent formation of a functional endothelial layer (75). Therapies designed to mobilize endothelial progenitors or to increase their ability to home to the site of stent implantation and facilitate vascular repair are attractive and have the potential to improve clinical outcomes after PCI. These approaches fall into 3 broad categories: pharmacological, cellular, and stent-based therapies.

**Pharmacological therapy.** The mechanisms responsible for the mobilization of EPCs have been reviewed extensively elsewhere (76,77). EPC mobilization is thought to occur through activation of the phosphatidylinositol 3-kinase/Akt pathway by angiogenic factors that stimulate endothelial nitric oxide synthase (eNOS). Increased nitric oxide bioavailability leads to cleavage of intercellular adhesions between stem cells and stromal cells of the bone marrow by proteinases, such as elastase, cathepsin G, and matrix metalloproteinases, and mobilization of EPCs into the circulation (78). Reduced nitric oxide bioavailability is evident in patients with cardiovascular risk factors and is integral to the development of atherosclerosis (79). Disordered function of eNOS, such as that occurring in patients with diabetes mellitus, is thought to be in part responsible for impaired mobilization of EPCs in such conditions (80). Bone marrow cells treated with an eNOS transcription enhancer to increase eNOS activity exhibit enhanced migratory and neovascularization capacity, and, when administered in a model of hind-limb ischemia, can improve organ function. Furthermore, this beneficial effect is reversed by the use of an eNOS inhibitor, strongly implicating nitric oxide in the process of vascular repair and neovascularization (81). Detailed mechanistic data regarding EPC mobilization in the context of discrete vascular injury, however, are limited. There are numerous factors thought to induce EPC mobilization including VEGF, fibroblast growth factor, growth hormone, insulin-like growth factor, angiopoietin, stromal cell-derived factor-1, erythropoietin, eostrogens, G-CSF, statins, angiotensin receptor blockers, and peroxisome proliferator-activated receptor antagonists. Several of these have been examined in the context of discrete vascular injury as potential therapeutic strategies for the mobilization of EPCs.

**STATINS.** Statins mobilize CD34⁺ and KDR⁺ cells into the peripheral circulation (82) in a dose-dependent manner (83). Statins also increase the formation of EC-CFU (84) and ECFC (85) colonies and can induce in vitro differentiation of CD14⁺ and CD34⁺ cells toward an endothelial phenotype (86). This has prompted investigators to examine the effect of statins on EPCs in the context of acute vascular injury. Walter et al. (65) demonstrated an accelerated rate of re-endothelialization and a reduction in neointimal hyperplasia in rats treated with simvastatin after balloon-mediated arterial injury; an effect associated with an increase in quantity and adhesive capacity of Ac-LDL⁺BS-1-lectin⁺
cells in culture. Although this in vitro definition of an EPC is relatively nonspecific, these studies did use a Tie-2/LacZ bone marrow transplant model to demonstrate that the neoendothelium in statin-treated animals was derived from bone marrow, supporting the presence of an EPC. Werner et al. (63) performed similar studies with rosuvastatin and obtained confirmatory findings. Fuduka et al. (87) demonstrated that fluvastatin administration can abrogate im-

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**Figure 2** Hypothetical Role of Circulating Progenitor Cells After Iatrogenic Injury

The endothelial monolayer acts as a nonadhesive surface for platelets and leukocytes and regulates fibrinolysis and vascular tone. (A) Under resting conditions, circulating concentrations of the precursors of endothelial cell colony-forming units (EC-CFUs) and circulating endothelial progenitor cells (EPCs) are low, particularly in patients with atherosclerotic disease. (B) Intracoronary stent placement forcefully pushes the obstructive plaque out of the way, but causes damage to the endothelium and underlying artery, exposing collagen and tissue factor, activating platelets and the coagulation cascade, which may result in acute or subacute stent thrombosis. In the absence of an intact endothelium, local platelet/platelet and platelet/leukocyte complexes form, and intense local inflammatory infiltrates ensue with detectable systemic inflammation. We hypothesize a biphasic response to vascular injury caused by percutaneous coronary intervention. (C) The early response consists of mobilization of the precursors of EC-CFUs involving angiogenic monocytes and lymphocytes. EC-CFU precursors home to the site of injury and avidly secrete angiogenic factors, encouraging resident endothelial cell proliferation and migration and the mobilization and homing of bone marrow-derived and local endothelial progenitors to the site of vascular injury. (D) Over a period of weeks and months, progenitor cells proliferate, contributing to effective re-endothelialization and the restoration vascular homeostasis. (E) An inadequate EC-CFU/EPC response after percutaneous coronary intervention may lead to delayed re-endothelialization and persistent inflammation, thus potentiating smooth muscle hypertrophy and extracellular matrix deposition, leading to restenosis and symptoms of myocardial ischemia. (F) However, a robust response by the bone marrow leads to rapid homing of EC-CFU precursors to sites of vascular injury with recruitment and integration of EPCs to the endothelial monolayer, facilitating rapid re-endothelialization and recovery of normal endothelial function and vascular homeostasis. CFU = colony-forming unit; G-CSF = granulocyte-colony stimulating factor; GM-CSF = granulocyte-macrophage colony stimulating factor; HGF = hepatocyte-like growth factor; IL = interleukin; MMP = matrix metalloproteinase; VEGF = vascular endothelial growth factor; VEGFR2 = vascular endothelial growth factor receptor-2.
paired re-endothelialization caused by sirolimus-coated stents; however, in contrast to the above studies, this effect did not seem to be mediated through mobilization of bone marrow-derived progenitors but rather through enhancing proliferation of mature endothelial cells adjacent to the stented segment. On the basis of this small study, the investigators speculated that the beneficial effects of statins on EPCs were insufficient to counter the inhibitory effects of sirolimus. Enhanced mobilization and function of EPCs may in part explain the reduced rate of ISR after PCI that is observed in patients treated with statins (88).

G-CSF. G-CSF has no established role in the treatment of cardiovascular disease, but is used routinely for the mobilization of EPCs for autologous bone marrow transplant recipients. G-CSF induces release of elastase and cathepsin G from neutrophils that cause release of progenitors from the stem cell niche into the bloodstream. G-CSF also mobilizes a variety of putative EPC phenotypes including EC-CFUs (89,90), CD34+/CD133+ and CD133+/KDR+ (90) c-Kit+/KDR+ (64), CD34+/KDR+ (60), and CD34+/CD133+/KDR+ (89), and several studies demonstrated a beneficial effect of G-CSF on re-endothelialization (60,61,64,91). Kong et al. (61) and Takamiya et al. (64) used G-CSF to accelerate re-endothelialization and successfully attenuate neointimal hyperplasia in balloon-injured animals. Yoshioka et al. (60) also demonstrated that G-CSF mobilized CD34+/KDR+ cells and accelerated re-endothelialization, but, in contrast to the study by Takamiya et al., very few bone marrow-derived EPCs contributed to re-endothelialization. The mechanism by which G-CSF reduces neointimal hyperplasia is therefore unclear. Yoshioka et al. speculated that G-CSF may increase proliferation and migration of adjacent endothelial cells either directly or through stimulation by attaching EPCs. It is possible that even a marginal increase in attaching EPCs might be enough to increase the secretion of angiogenic factors (92–94).

Shi et al. (91) used G-CSF to accelerate endothelialization of Dacron grafts implanted in the aortas of dogs, but despite enhanced endothelialization, animals treated with G-CSF had considerably more neointimal formation than controls. Concordant with this preclinical study, intracoronary G-CSF administered to patients with myocardial infarction was associated with an increased incidence of ISR, despite improvements in left ventricular ejection fraction (95). This effect may have been mediated through the nonspecific proinflammatory actions of G-CSF and the mobilization of smooth muscle progenitors from the bone marrow. The nonspecific nature of the effects of G-CSFs hampers its translation into a targeted therapy for vascular repair.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR AGONISTS. Peroxisome proliferator-activated receptor agonists inhibit vascular smooth muscle proliferation and migration, improve endothelial function, and accelerate re-endothelialization. This can lead to an attenuation of neointimal formation in mice subjected to femoral angioplasty. Wang et al. (96) attributed this effect to the ability of roziglitazone to drive pleuripotent bone marrow-derived vascular progenitors toward an endothelial phenotype and away from a smooth muscle phenotype. Peroxisome proliferator-activated receptor agonists may therefore have a role to play in augmenting a maladaptive response to iatrogenic vascular injury, although concerns remain regarding the safety profile of these drugs in patients with ischemic heart disease (97).

ERYTHROPOIETIN. The effects of erythropoietin on the response to discrete vascular injury are similarly favorable in terms of re-endothelialization, possibly through eNOS-dependent mobilization of Sca-1+/KDR+ cells, enhanced proliferation of resident endothelial cells, and reduced apoptosis of the injured artery (98). Erythropoietin has also been associated with an increased incidence of neointimal proliferation despite adequate re-endothelialization in animal studies, again possibly as a result of the nonspecific mobilization of smooth muscle progenitors (99). Ongoing clinical trials of erythropoietin in patients with acute myocardial infarction, the REVEAL (Reduction of Infarct Expansion and Ventricular Remodeling With Erythropoietin After Large Myocardial Infarction) (100) and HEBE-III (A prospective, randomized, double blind, placebo controlled clinical study to examine the effects of a single bolus erythropoietin on left ventricular function in patients with an acute myocardial infarction) (101) trials, will address whether erythropoietin will have a favorable effect on vascular remodeling.

ESTROGEN. Estrogens enhance vascular repair in models of arterial injury through eNOS-dependent mobilization and proliferation of bone marrow-derived EPCs (102) and reduced apoptotic signaling through the actions of caspase-8 (103).

Cellular therapy. Intravenous and intracoronary infusions of bone marrow-derived cells have been used in an attempt to accelerate vascular healing. Using animal models of endovascular injury, transfusion of mononuclear cells cultured in angiogenic medium to produce EPCs has successfully accelerated re-endothelialization and attenuated neointimal hyperplasia in several studies (62,93,104,105). Although effective, it is difficult to be certain whether the cells infused in these studies truly differentiated to become endothelial cells. It remains possible that the beneficial effects on the development of neointimal hyperplasia observed were mediated through a paracrine influence of angiogenic monocyte and lymphocyte populations contained in mononuclear preparations. The populations used were selected using fairly nonspecific characteristics, and it is certain that the cell preparations infused were heterogeneous in their composition. There have been no clinical studies using infused progenitor cells specifically to influence restenosis. Data regarding the transfusion of progenitor cells in human studies are derived from studies examin-
ing the effect of mononuclear cell infusion on left ventricular dysfunction and myocardial ischemia (106). The results of these studies have been largely disappointing in terms of providing a sustained clinical benefit, although they do suggest that intracoronary transfusion of mononuclear cells is feasible and safe (107). Concerns that infusion of mononuclear cells may increase inflammatory signaling and smooth muscle proliferation are well founded theoretically, and there are some reports of an increased incidence of coronary events including restenosis and thrombosis after infusion of CD133+ progenitor cells (108).

**Stent-based therapy.** Technological advances and the evolution of intracoronary stents provide a potential vehicle to deliver novel therapies directly to the site of vascular injury. Attempts to coat intracoronary stents with endothelial mitogens, such as VEGF, have not been encouraging in terms of re-endothelialization (109). However gene-eluting stents directly delivering naked plasmid DNA encoding for VEGF-2 can accelerate re-endothelialization and reduce lumen loss in animal models (110).

The Genous Bio-engineered R stent (OrbusNeich, Hong Kong) is coated with monoclonal antibodies directed against CD34 and is designed to attract EPCs and therefore encourage re-endothelialization. Genous stents have already progressed to phase II and III clinical trials and have been deployed in >5,000 patients. Preliminary data from small registries reported major adverse cardiovascular event rates ranging from 7.9% to 13% (111–114). However, data from the e-HEALING registry on 4,996 patients suggest a favorable target lesion revascularization rate of 7.7%, with a low major adverse cardiovascular event rate of 7.7%, with a low incidence of subacute (0.5%) and late (0.3%) stent thrombosis (115). Interestingly, the HEALING II registry reported that patients with normal CD34+KDR+ EPC titers had lower rates of ISR compared with those patients with reduced circulating EPCs (late luminal loss 0.53 ± 0.06 mm vs. 1.01 ± 0.07 mm). Furthermore, a subgroup of 30 patients in this nonrandomized study underwent serial evaluation using intravascular ultrasound, and regression of neointimal volume was observed in patients with higher concentrations of EPCs (112).

The first randomized, controlled trial in a small cohort of patients with ST-segment elevation myocardial infarction was recently presented and demonstrated a trend toward increased restenosis with the Genous stent when compared with a standard chromium-cobalt stent (116). Restenosis with CD34 capture stents may occur as a consequence of nonspecific binding with non-EPCs. CD34 is common to a range of circulating progenitors contributing to vascular healing, it is also evident that their function is dependent on both the local microenvironment and a synergy between other populations mobilized in response to the vascular insult. For us to use EPCs as an effective treatment modality, further studies are required to elucidate not only the target cell type of interest, but also the involvement of the microenvironment on cellular behavior, the best mode of delivery or mobilization to the site of injury, and the need for adjunctive therapy to optimize EPC function.

**Conclusions**

Endothelial regeneration is a key component of an effective response to the vascular injury associated with PCI. Understanding the mechanisms involved in this process will provide the rationale for therapies that target the acceleration of vascular healing. Much progress has been made toward determining the variety of populations involved in the cellular response to vascular injury. Although it is clear that circulating progenitors contribute to vascular healing, it is also evident that their function is dependent on both the local microenvironment and a synergy between other populations mobilized in response to the vascular insult. For us to use EPCs as an effective treatment modality, further studies are required to elucidate not only the target cell type of interest, but also the involvement of the microenvironment on cellular behavior, the best mode of delivery or mobilization to the site of injury, and the need for adjunctive therapy to optimize EPC function.

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