Endothelial Progenitor Cells
Mobilization, Differentiation, and Homing
Mihail Hristov, Wolfgang Erl, Peter C. Weber

Abstract—Postnatal bone marrow contains a subtype of progenitor cells that have the capacity to migrate to the peripheral circulation and to differentiate into mature endothelial cells. Therefore, these cells have been termed endothelial progenitor cells (EPCs). The isolation of EPCs by adherence culture or magnetic microbeads has been described. In general, EPCs are characterized by the expression of 3 markers, CD133, CD34, and the vascular endothelial growth factor receptor-2. During differentiation, EPCs obviously lose CD133 and start to express CD31, vascular endothelial cadherin, and von Willebrand factor. EPCs seem to participate in endothelial repair and neovascularization of ischemic organs. Clinical studies using EPCs for neovascularization have just been started; however, the mechanisms stimulating or inhibiting the differentiation of EPC in vivo and the signals causing their migration and homing to sites of injured endothelium or extravascular tissue are largely unknown at present. Thus, future studies will help to explore areas of potential basic research and clinical application of EPCs. (Arterioscler Thromb Vasc Biol. 2003;23:1185-1189.)

Key Words: bone marrow • angioblast • repair • angiogenesis • differentiation

The vascular endothelium represents a dynamic border between circulating blood and the surrounding tissue. This monolayer of endothelial cells (ECs) acts as a nonadhesive surface for platelets and leukocytes and produces a variety of important regulatory factors, such as prostaglandins and NO. In healthy subjects, a low basal level of endothelial turnover, respectively very low amounts of circulating, vessel wall–derived ECs (1 to 3/mL blood), has been described. However, acute stress injury of the vascular endothelium, which is often followed by programmed cell death (apoptosis) of ECs and leads to the loss of the antithrombotic properties of the vessel wall, rapidly enhances the number of circulating ECs. In addition, EC dysfunction is a critical event in the initiation of atherosclerotic plaque development. Thus, regeneration of the vascular endothelium is of particular importance. This endothelial reconstruction can occur by migration and proliferation of surrounding mature ECs. However, mature ECs are terminally differentiated cells with a low proliferative potential, and their capacity to substitute damaged endothelium is limited. Therefore, the endothelial repair may need the support of other cell types. Accumulating evidence in the past 5 years indicates that peripheral blood of adults contains a unique subtype of circulating, bone marrow–derived cells with properties similar to those of embryonal angioblasts. These cells have the potential to proliferate and to differentiate into mature ECs. Therefore, they were termed endothelial progenitor (precursor) cells (EPCs). Recent studies in animals and humans suggest the ability of EPCs to ameliorate the function of ischemic organs possibly by both induction and modulation of vasculogenesis and angiogenesis in areas with reduced oxygen supply or by stimulating the reendothelialization of injured blood vessels.

Characterization of Circulating EPCs
Asahara et al published the first detailed description of isolation of putative progenitor endothelial cells for angiogenesis in 1997. CD34-positive cells were isolated from human peripheral blood using magnetic microbeads. After plating on fibronectin-coated surface, the cells grew into cells with endothelial characteristics. Some time later it was discovered that 3 markers characterize the functional early EPC, CD133, CD34, and the vascular endothelial growth factor receptor-2 (VEGFR-2), termed also kinase insert domain receptor (KDR) or Flk-1. CD133 (termed originally AC133), an early hematopoietic stem-cell marker, is a 120-kDa transmembrane polypeptide, expressed on hematopoietic stem and progenitor cells from human bone marrow, fetal liver, and peripheral blood. A possible mix of both early progenitor and endothelial phenotype is CD133+/CD34+/VEGFR-2+ cells, which do not express vascular endothelial (VE) cadherin and von Willebrand factor. Cells with these characteristics are localized predominantly in the bone marrow. In the peripheral circulation of adults, more mature EPCs are found that obviously have lost CD133 but are positive for CD34 and VEGFR-2. Mature ECs show a high expression of VEGFR-2, VE-cadherin, and von Willebrand factor. CD133 is not detectable on the surface of human umbilical vein ECs. It seems, therefore, that the loss of
CD133 reflects the transformation of circulating EPCs into more mature endothelial-like cells. However, it is unclear at which time point the EPCs begin to lose CD133—during their transmigration from the bone marrow into the systemic circulation or later during their circulation. Furthermore, circulating EPCs express with different intensity a variety of markers, which are typical for the endothelial lineage. These markers include platelet endothelial cell adhesion molecule-1 (CD31), CD146, VE-cadherin, von Willebrand factor, endothelial NO synthase, and, on stimulation, E-selectin4,8,9 (Table 1). In general, early EPCs in the bone marrow or immediately after their migration into the systemic circulation are positive for CD133/CD34/VEGFR-2. Circulating EPCs obviously lose CD133 and are positive for CD34/VEGFR-2/CD31/VE-cadherin/von Willebrand factor.

Recruitment, Mobilization, and Differentiation of EPCs

The release of EPCs from the bone marrow is regulated by a variety of factors. The activation of matrix metalloproteinase-9 (MMP-9), which promotes the transformation of membrane-bound Kit ligand (mKitL) to a soluble Kit ligand (sKitL), is an early step in this process. Subsequently, cKit-positive stem and progenitor cells, including also a common hematopoietic and angioblast precursor cell (hemangioblast, HABL), move to the vascular zone of the bone marrow microenvironment. This translocation activates the cells from a quiescent to a proliferative state. Early EPCs in the bone marrow are positive for CD133/CD34/VEGFR-2. Circulating EPCs obviously lose CD133 and are positive for CD34/VEGFR-2/CD31/VE-cadherin/von Willebrand factor (vWF).

Figure 1. The mobilization of EPCs from the bone marrow is a complex process, regulated by a variety of factors. The activation of matrix metalloproteinase-9 (MMP-9), which promotes the transformation of membrane-bound Kit ligand (mKitL) to a soluble Kit ligand (sKitL), is an early step in this process. Subsequently, cKit-positive stem and progenitor cells, including a common hematopoietic and angioblast precursor cell (hemangioblast, HABL), move to the vascular zone of the bone marrow microenvironment. This translocation activates the cells from a quiescent to a proliferative state. Early EPCs in the bone marrow are positive for CD133/CD34/VEGFR-2. Circulating EPCs obviously lose CD133 and are positive for CD34/VEGFR-2/CD31/VE-cadherin/von Willebrand factor (vWF).

To date, no clear definition exists as to when an endothelial progenitor cell turns into a mature, fully differentiated endothelial cell in vivo. One possibility could be the loss of CD133 and a parallel or subsequent expression of von Willebrand factor in conjunction with the appearance of other endothelial characteristics. The starting point of this differentiation process may by the migration of EPCs from the bone marrow to the systemic circulation. After homing, ie, after adhesion and insertion into the monolayer of surrounding mature vascular ECs, this process may be completed.

Homing and Regenerative Potential of EPCs

After thrombotic microangiopathy or after balloon angioplasty,20–22 EPCs seem to participate in the repair of injured endothelium (Figure 2). It is not known which physiological
or pathological factors influence the homing and the differentiation of EPCs, and little is known about the signals that direct circulating EPCs to sites of injured vessels. Recent studies addressing the integration of EPCs into the mature endothelium found that a small fraction of these cells can also transdifferentiate into smooth muscle cells in vitro.23 This process seems to be dependent on the presence of transforming growth factor-β, and cell-cell contact. If this were the case, strategically located EPCs within the vascular endothelium could be an emerging standby tool for the regeneration of surrounding ECs or smooth muscle cells subsequent to an injury. Furthermore, EPCs may be involved in the regeneration of ischemic myocardium by modulation of angiogenesis and myogenesis, cardiomyocyte apoptosis, and remodeling in the ischemic cardiac tissue.24–26 EPCs have also been reported to participate in cerebral neovascularization after ischemic stroke.27

Thus, EPCs derived from the hematopoietic tissue of postnatal bone marrow may possess highly regenerative potential and some characteristics of embryonal stem cells. How these cells, if migrating from the bone marrow to the periphery, remain restricted in the circulation and which signals cause their homing to sites of injured endothelium or extravascular tissue remains open question (Figure 2).

Isolation and Culture of EPCs
EPCs can be isolated from bone marrow or peripheral blood. In addition, EPCs have also been isolated from fetal liver or umbilical cord blood.5,28,29 The classical isolation methods include the use of adherence culture of total peripheral blood mononuclear cells or the use of magnetic microbeads, coated with anti-CD133 or anti-CD34 antibodies. After isolation, the cells are cultured in medium with specific growth factors (eg, VEGF, bovine brain extract, and epidermal growth factor), which facilitate the growth of endothelial-like cells. The incubation in vitro with a mixture of growth factors, the adhesion on specific substrates (eg, fibronectin), and the contact with the extracellular matrix or the surrounding mature ECs in vivo will probably influence the proliferation or differentiation of bone marrow–derived EPCs. After initial adhesion in vitro, EPCs begin to lose their progenitor characteristics and start to differentiate. EPCs form within 3 to 4 weeks monolayers with endothelial appearance.8,28 Additionally, EPCs have the capacity to form capillary tubes in basement matrix gel, alone or when cocultured with CD34-negative cells, to incorporate acetylated LDL and to bind endothelial-specific lectin.43,29 Differentiation of human bone marrow–derived multipotent adult progenitor cells toward the endothelial lineage was induced only by seeding the cells at high density in serum-free or low-serum–containing medium with the addition of VEGF, whereas the culture in a medium enriched with FCS (≥10%) directed the differentiation into other cell types, including osteoblasts, chondroblasts, and adipocytes.30 A culture period of several days to 3 to 5 weeks of separated human EPCs may change their progenitor properties and their proliferative potential. EPCs have shown an exponential proliferation after 30 to 60 days in culture, which contrasts to the early outgrowth of vessel wall–derived ECs, which have a limited proliferative capacity.31 The isolation of a stable endothelial phenotype during 20 passages by using EPCs from 1- to 2-week-old sheep has also been described.9 The most important characteristics of human EPC isolated from different sources are summarized in Table 1.

Conditions Influencing the Number of EPCs
No systematic studies exist regarding physiological variations in the number of EPCs in healthy subjects. No published data describe the lifetime of circulating EPCs in vivo under physiological or pathological conditions. Data regarding the number of EPCs in the peripheral circulation of healthy adults are scant. For example, one study has described the isolation of 645 CD34-positive cells per milliliter of blood using anti-CD34 antibody–coated magnetic microbeads.32 Another study reported the number of circulating early CD133+/CD34+/VEGFR-2+ postnatal EPCs in healthy human subjects to be in the range of 0.002% of total peripheral blood mononuclear cells, corresponding to approximately 70 to 210 cells/mL.5 All in all, the number of EPCs in healthy subjects is low and correlates with the low number of circulating vessel wall–derived ECs.2

Some studies have described the influence of pathological conditions on the number of EPCs in vivo (Table 2). For example, the number of circulating EPCs and their migratory activity have been reported to be reduced in patients with risk factors for ischemic cardiovascular disease or to be negatively correlated with the Framingham cardiovascular risk factor score.33,34 EPCs from patients with diabetes mellitus type II were characterized by a decreased proliferation capacity and reduction of their adhesiveness and ability to form capillary tubes in vitro.35 The following 3 different mechanisms, either separate or in combination, could possibly explain the findings in these studies: first, a decreased mobilization from the bone marrow; second, an increased consumption of EPCs at sites of vascular injury; and third, a reduced half-life of circulating EPCs.

In contrast, limb ischemia and acute myocardial infarction were associated with a rapid increase of EPCs in the circulation.15,16 Vascular trauma, such as coronary bypass grafting or burn injury, also induces a rapid transient mobilization of

Figure 2. EPCs may contribute to the repair of injured vessels. However, the intercellular signaling between damaged endothelial cells and EPCs remains unclear. Some of the EPCs possibly integrate into the endothelial monolayer and may transdifferentiate into smooth muscle cells.
TABLE 2. Physiological and Pathological Factors and Therapeutic Compounds Have Been Found to Affect the Number of EPCs in the Systemic Circulation

<table>
<thead>
<tr>
<th>Conditions or Factors</th>
<th>Changes in the No. of EPCs</th>
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</thead>
<tbody>
<tr>
<td>Embryonal development (eg, umbilical cord blood)</td>
<td>Increased</td>
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<tr>
<td>Cardiovascular risk factors</td>
<td>Decreased</td>
</tr>
<tr>
<td>Limb ischemia</td>
<td>Increased</td>
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<tr>
<td>Acute myocardial infarction</td>
<td>Increased</td>
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<tr>
<td>Vascular trauma</td>
<td>Increased</td>
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<tr>
<td>Drugs</td>
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<tr>
<td>HMG-CoA reductase inhibitors</td>
<td>Increased</td>
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<tr>
<td>Growth factors</td>
<td></td>
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<tr>
<td>VEGF</td>
<td>Increased</td>
</tr>
<tr>
<td>G(M)-CSF</td>
<td>Increased</td>
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</tbody>
</table>

G(M)-CSF indicates granulocyte (macrophage) colony stimulating factor.

EPCs. Treatment with different HMG-CoA reductase inhibitors (statins) has been reported to increase the number of EPCs in vivo, possibly by mobilization from the bone marrow. This may contribute to angiogenesis and reendothelialization, observed after statin treatment. Release of EPCs in vivo has also been stimulated after application of growth factors such as G(M)-CSF or VEGF.

Potential Clinical Application

Three areas of therapeutic application of EPCs could be discussed: the repair of injured vessel wall, the neovascularization or regeneration of ischemic tissue, and the coating of vascular grafts. A critical limitation, so far, for the therapeutic application of postnatal EPCs is their low number in the circulation, which is even lower in patients with cardiovascular risk factors. Approaches to overcome this problem include the use of umbilical cord blood or the mobilization of EPCs by cytokines, growth factors, or drugs. For example, umbilical cord blood or G(M)-CSF-mobilized blood of adults contains up to 10-fold higher amounts of EPCs compared with nonmobilized blood of adults. The cord blood-derived cells are also characterized by a greater proliferative capacity. Recent studies have demonstrated a significant expansion of EPCs after ex vivo transfection with adenovirus encoding VEGF, and VEGF gene transfer in vivo has been shown to mobilize EPCs in human subjects. Application of drugs such as statins not only enhances the number of circulating EPCs but also stimulates their incorporation at sites of endothelial injury by increasing the expression of adhesion molecules on the EPC surface.

The possibility for infusion of an autologous bone marrow mononuclear cell suspension containing EPCs has recently been explored for the neovascularization and repair of ischemic organs. In a pilot study in patients with limb ischemia, the significant improvement of the function of the ischemic limbs observed after autologous transplantation of bone marrow cells was suggested to be the result of both CD34-positive EPCs and growth factors, released from the CD34-negative bone marrow fraction. Other clinical studies described the capacity of autologous bone marrow–derived cells or ex vivo expanded autologous EPCs for the repair of human myocardium after infarction. Of particular importance might be the local application of progenitor cell suspensions, eg, by intracoronary infusion after myocardial infarction. This specific treatment may facilitate the homing of the progenitor cells to the ischemic tissue and may help to avoid the potential risk for enhanced but unwanted vasculogenesis systematically or in tumor tissue. An additional clinical application might be the coating of vascular grafts with EPCs, thereby creating functionally active vessel prostheses, which could help to circumvent the problem of graft vasculopathy.

Summary and Perspectives

EPCs obviously participate in the regeneration of injured endothelium and of ischemic organs. A standardization of the procedures used for the isolation, phenotypic characterization, and culture of these cells will be a prerequisite for the use of EPC quantification in vivo as a diagnostic or prognostic tool or as a surrogate marker in clinical or pharmacotherapeutical studies. Besides other open questions, the role of CD34-negative cells in the process of vessel wall or tissue remodeling needs to be clarified. In addition, additional experimental, clinical, and cell biological studies are needed to increase the understanding of the function of EPCs and of the factors that determine their number and turnover rate as well as the mechanisms that stimulate or inhibit their mobilization, differentiation, and homing in vitro and in vivo. Such investigations are required to explore areas of future basic and clinical research, particularly because the first clinical trials using progenitor cells have just been started.

Acknowledgments

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References

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