Role of endothelial progenitor cells in cardiovascular pathology

Gross P, Herbrig K

Department of Nephrology, University Medical Center, TU Dresden, Germany

Abstract

Replacement of injured endothelial cells by bone marrow derived endothelial progenitor cells (EPC's) is a new pathway of vascular repair after ischemia. Endothelial progenitor cells contribute less than 0.01% to the peripheral venous compartment of mononuclear cells. The detection of EPC's requires a demonstration of CD 34 and VEGFR-2 (vascular endothelial growth factor receptor-2) antigenic cell membrane determinants and proof of endothelial characteristics after outgrowth and differentiation in cell culture. The most important stimuli to the mobilization and proliferation of EPC's are VEGF, GM-CSF (granulocyte-macrophage colony stimulating factor), erythropoietin, HMG-CoA-reductase inhibitors and tissue ischemia. In vivo in patients EPC's appear to contribute to endothelialization of vascular grafts, the formation of collaterals of ischemic limbs and the healing of myocardial infarcts. The role of EPC's in uremia is currently under investigation.

Key words: endothelial progenitor cells, vascular endothelial growth factor, erythropoietin.

ADDRESS FOR CORRESPONDENCE:
Peter Gross M.D.
Department of Nephrology,
University Medical Center,
Fetscherstraße 74,
D - 01307 Dresden, Germany
Fax: +49 351 458 53 33
e-mail: petergross@mailbox.tu-dresden.de

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Introduction

Arteriosclerotic cardiovascular disease is a principal cause of death in the industrialized countries. An accelerated form of arteriosclerosis is found in patients with renal insufficiency or with end-stage renal failure. Endothelial injury is believed to be the first step in arteriosclerosis [1]. Endothelial injury is characterized by endothelial dysfunction, i.e. ineffective vascular dilatation in response to acetylcholine, thrombogenicity, enhanced adhesiveness of the endothelial surface and a loss of antiproliferative control over the adjacent layer of vascular smooth muscle cells [1]. It is likely that dysfunctional endothelial cells may recover upon cessation of the injurious insult(s) or they may be replaced by outgrowing endothelial cells from their neighbourhood. However, there may be an additional pathway: it was recently observed in recipients of a kidney transplant that the endothelial cells of capillaries in the graft were partly recipient derived, especially after an episode of rejection [2]. The cells were held to be derived from the recipient's bone marrow [2]. This and other observations opened up a new range of possibilities in endothelial regulation and repair. Much current research is devoted to the steps presumed to occur between the bone marrow stem cells and the vascular periphery as well as to the actual roles that bone marrow derived progenitor cells may assume in a mature endothelium.

Endothelial progenitor cells

Adult bone marrow contains (rare) pluripotent stem cells as well as stromal cells. Both cell types can be induced by activation of matrix metalloproteinase-9 to generate angioblast precursor cells, which in turn give rise to endothelial progenitor cells (EPC's) [3]. Asahara et al. [4] were the first to demonstrate the presence of EPC's in human peripheral blood. On the basis of known antigenic determinants found on cells involved in embryonic vasculogenesis – CD 34 and VEGFR-2 (vascular endothelial growth factor receptor-2); they were able to select

Table 1. **Marker proteins used in the differentiation of various endothelial progenitor cells (EPC's) and mature endothelial cells (EC's)**

	Bone marrow EPC's	Peripheral blood EPC's	Mature EC's
CD 133	+	+	-
CD 34	+	+	+
CD 31	-	+	+
VEGFR-2	+	+	+
VE Cadherin	-	+	+
vWF	-	+	+
CD 146	-	-	+

Abbreviations: CD 31: PECAM; vWF: von Willebrand factor; VEGFR-2: vascular endothelial growth factor receptor-2; Table modified after [4,10,23].

a fraction of cells from peripheral blood mononuclear cells that differentiated into endothelial cells under the conditions of cell culture – hence the term endothelial progenitor cells (EPC's). In addition the authors demonstrated homing of EPC's to sites of trauma and angiogenesis in vivo [4]. Reyes et al. [5] reported studies in vivo of bone marrow from healthy volonteer donors in which they showed the presence of stem cells within the samples and the subsequent differentiation of such stem cells into EPC's in response to cultivation in the presence of VEGF (vascular endothelial growth factor). Again, these EPC's contributed to wound healing in vivo later. Using subjects that had received a bone marrow transplant Lin et al. [6] also provided evidence of (donor) bone marrow derived EPC's in peripheral venous blood of recipients. In addition these authors showed a remarkable potential of their EPC's to multiply in culture. Over a time of 4 weeks they had a 1023-fold expansion of endothelial cells derived from EPC's [6].

Detection of endothelial progenitor cells

While it is clear that bone marrow stem cells give rise to EPC's which then leave the bone marrow, traverse the vascular compartment and home to the vascular endothelium of individual organs or tributaries there is debate on the surface markers to be used in the definition of EPC's. Most authors are in agreement that cells from the monocytic fraction of peripheral venous blood exhibiting CD 34 and VEGFR-2 antigenic determinants qualify as EPC's, i.e. as cells that later differentiate into mature endothelial cells and multiply prodigiously (*Tab. 1*). However, CD 133, VEGFR-3 and fibroblast growth factor receptor 1 have been suggested as additional characteristic surface markers of EPC's in some studies [7]. All of these marker proteins were originally known as antigens of embryonic hematopoetic stem cells and hematopoetic progenitor cells.

In order to study the number and function of EPC's it is customary to count circulating EPC's by FACS analysis. Their numbers in the peripheral circulation are very small. EPC's account for roughly 0.01% of circulating mononuclear cells. A second approach is to propagate them in culture [8]. The latter is used to determine their proliferation rate, their migratory capacity, their ability to form primitive tubes (termed: angiogenesis assay), their adherence function, and other qualities [8]. Cultured EPC's tend to lose their early progenitor markers – such as CD 133 – and aquire characteristics of endothelial cells – such as uptake of acetylated LDL, staining with Ulex Europeus agglutinin, expression of VE-cadherin and of CD 31.

Regulation of endothelial progenitor cells

The mobilization of EPC's from bone marrow and their circulating numbers are influenced by endogenous and exogenous factors as well as by pathological changes. The angiogenic growth factor VEGF has been shown in many studies to correlate with EPC counts and with EPC incorporation into sites of endothelial repair [9]. Injection of recombinant VEGF 165 in mice induced a rapid mobilization of hematopoetic stem cells and of EPC's, while a neutralizing monoclonal antibody to the VEGF-receptor-2 (VEGFR-2] inhibited these responses [9]. Other angiopoetic growth factors stimulating EPC mobilization are the following ones: angiopoietin-1, fibroblast growth factor, stroma cell derived growth factor-1 and placental growth factor [10]. 'The cytokines G-CSF and GM-CSF and the chemokine stem cell factor were also shown to increase EPC proliferation [10]. Bahlmann et al. studied the effects of erythropoietin in vivo in patients with moderate renal insufficiency and renal anemia [11]. They found a three-fold increase in the number of circulating EPC's together with a large augmentation of the proliferation rate in culture occurring within 2-6 weeks after the start of erythropoietin therapy [11].

Amongst exogenous factors HMG-CoA reductase inhibitors (statins) were shown to augment the numbers of hematopoietic stem cells in the bone marrow of mice and the proliferation rate of the corresponding EPC's in culture [12], effects that were mediated by the protein kinase Akt. The effects of statins were held to be at least as potent as those of VEGF [12]. Vasa et al. [8] extended these kinds of observations to human patients with coronary artery disease. Four weeks of therapy by 40 mg of atorvastatin daily increased the numbers of circulating EPC's threefold, augmented their proliferation rate in culture approximately fourfold and improved their migratory ability significantly [8].

Amongst pathological conditions with effects on bone marrow stem cells and EPC's ischemia has been demonstrated to provide a major stimulus [13]. Acute myocardial infarction in human patients, limb ischemia or vascular trauma during coronary artery bypass grafting were all associated with a rapid increase of EPC's in the circulation [14]. On the other hand there may also be downregulating effects at work in certain circumstances [15]. Studying 45 patients with stable coronary disease and 15 healthy volonteers, Vasa et al. [15] showed significant reductions of circulating EPC's and of the proliferation rate of EPC's in the patients with coronary artery disease. Of note they were able to correlate the number of atherosclerotic risk factors with the reductions of EPC

counts. In their study, smoking, a positive family history for coronary artery disease and hypertension were the major negative regulators of EPC's. The authors speculated that apoptosis was the mechanism involved in the observed effects. In another report – involving mice – it was suggested that age may lead to endothelial progenitor cell exhaustion and hence arteriosclerosis [16].

Potential roles of endothelial progenitor cells

We shall not address the issue of homing of EPC's in this communication. Assuming that there are ways to accomplish homing of EPC's to sites of involvement the question is: what purposes might be served by EPC's?

EPC's may contribute to rapid endothelialization. It was found in human patients that the surfaces of left ventricular assist devices had been colonized with CD 133+/VEGFR-2+ endothelial cells. Comparable observations have also been made on the internal surfaces of Dacron grafts in the aortic position of bone marrow transplanted dogs.

Werner et al. induced carotid artery injury in bone marrow transplanted mice resulting in the formation of a neointima [17]. They could show that bone marrow derived EPC's were involved in reendothelialization. However, in the presence of treatment by a HMG-CoA-reductase inhibitor the circulating pool of EPC's was enhanced, the homing of EPC's to the injured vessel wall was increased, reendothelialization was accelerated and neointima formation lessened [17].

Urbich et al. [18] studied neovascularization in a model of ligation induced hind-limb ischemia in the mouse. They gave intravenous infusions of EPC's that had undergone previous culture using VEGF or that were freshly isolated from blood. Only the former condition (culture and pretreatment with VEGF) yielded functionally improved neovascularization and augmented capillary density with demonstrable integration of EPC's into the respective vessels [18].

Glomerular endothelial cell injury and its repair in the Thy-1.1 model of the rat has also been studied [19]. In these observations an allogenic bone marrow transplant model was used to permit tracing of bone marrow-derived cells by MHC class I specific mAB. It was found that the damaged glomerular endothelium was repopulated by bone marrow-derived EPC's [19].

Potentially a very important area for EPC treatment may be coronary artery disease. Kamihata et al. [20] implanted EPC's in a porcine myocardial infarction model. Three weeks after implantation the treated animals had improved regional blood flow, increased capillary density and a higher number of collateral vessels at the infarct zone than control animals [20].

In a recent study in humans after acute myocardial infarction 20 patients received an infusion of autologous progenitor cells into the infarct artery [21]. Several months later the treated group was reported to show a significant increase in left ventricular ejection fraction and improved regional wall motion in the infarct zone as compared to untreated controls [21]. Another study in human patients was able to confirm these results [22]. Three months after intracoronary application of autologous progenitor cells the treated patients had a significantly decreased infarct region and a significant increase of myocardial wall motion in the infarct region compared with control.

In moderate renal insufficiency mildly reduced counts of circulating EPC's have been demonstrated [11]. Ongoing work in our own group serves to delineate the functional properties of EPC's in patients with uremia undergoing treatment by hemodialysis.

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