



Review

Endothelial progenitor cell therapy in atherosclerosis: A double-edged sword?

Pengxia Liu^a, Bin Zhou^{a,b}, Dongsheng Gu^a, Lei Zhang^a, Zhongchao Han^{a,*}^a State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, National Engineering Research Center of Cell Products, 288 Nanjing Road, Tianjin 300020, PR China^b Department of Cardiology, Children's Hospital Boston, Harvard Medical School, 300 Longwood Ave, Boston, MA, USA

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ABSTRACT

Atherosclerosis, an inflammatory process that selectively affects arteries, is highly prevalent in human. Thrombo-occlusive complications of atherosclerosis, including stroke and myocardial infarction, are becoming major causes of morbidity and mortality in the industrialized world. Atherosclerosis develops in response to local endothelial injuries. Endothelial dysfunction and cell loss are prominent features in atherosclerosis. Restoring the endothelial lining to normal is critical for slowing or reversing the progression of atherosclerosis. Increasing data suggest that endothelial progenitor cells (EPCs) play a significant role in reendothelialization of the injured blood vessels. This review focuses on the effects of EPC mobilization and transfusion in the condition of atherosclerosis. The aim of the review is to provide an update on the progress in this research field, highlight the role of EPCs in atherosclerosis and discuss the possible mechanisms and potential risks of progenitor cell-based therapy in atherosclerosis.

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1. Introduction

Atherosclerosis is a multifactorial process with complicated aetiology. It is considered as a chronic inflammatory condition that can be converted into an acute clinical event by plaque rupture and thrombosis. The response-to-injury hypothesis presupposes that atherosclerosis is a chronic inflammatory process following localized injury to the vessel wall, in particular to the endothelial layer lining the lumen of the vessel. Common conditions predisposing to atherosclerosis, such as hyperlipidemia, hypertension, diabetes, and smoking, are associated with endothelial dysfunction (Calles-Escandon and Cipolla, 2001; Cardillo et al., 2002; Casino et al., 1995; Celermajer et al., 1996). The failure of endothelial repair process is intimately linked to atherosclerotic inflammation and lesion formation. Therefore, vascular endothelium is an alternative promising therapeutic target for restoring vascular integrity. Traditionally, it was believed that the damaged/lost endothelial cells (ECs) in the arterial wall would be replaced by neighboring endothelial replication (Ross et al., 1977). However, this dogma is challenged by the findings showing that EPCs contribute to vascular repair (Gulati et al., 2003b; He et al., 2004; Rauscher et al., 2003; Wassmann et al., 2006). These studies have generated excitement about the possible use of EPCs as a novel preventative and/or treatment strategy for atherosclerosis. Here

we discussed in detail the role of EPCs in atherosclerosis, the possible mechanisms of vascular repair by EPCs and the potential risks of progenitor cell-based therapies in the context of atherosclerosis.

2. Endothelial progenitor cells and atherosclerosis

Since Asahara et al. (1997) first described that purified CD34⁺ hematopoietic progenitor cells within human peripheral blood can differentiate ex vivo to an endothelial phenotype in 1997 and Rafi found circulating bone marrow-derived EPCs in the adult in 1998 (Shi et al., 1998), considerable progress has been made in the past 10 years regarding EPC biology and the role of EPCs in cardiovascular diseases.

Currently, the definition of EPCs and their functional characteristics are under debate. EPCs were originally thought to derive exclusively from the bone marrow, but recent studies have indicated alternative sources of EPCs, including umbilical cord blood from healthy puerperas, adipose tissues, skeletal muscle, heart, vessel wall and spleen (Beltrami et al., 2003; Ingram et al., 2005; Majka et al., 2003; Murohara et al., 2000; Planat-Benard et al., 2004; Werner et al., 2003). Various studies provided compelling evidence that EPCs can derive from CD34⁺ or CD133⁺ hematopoietic stem cells (Asahara et al., 1997; Gehling et al., 2000; Peichev et al., 2000; Shi et al., 1998) and peripheral blood mononuclear cells (MNCs) or CD14⁺ monocytes (Fujiyama et al., 2003; Rehman et al., 2003; Schmeisser et al., 2001; Urbich et al., 2003). However, recent refinements have revealed that

* Corresponding author. Tel.: +86 22 27210717; fax: +86 22 66211430.

E-mail address: tihzchan@public.tpt.tj.cn (Z. Han).

CD34⁺CD133⁺KDR⁺ cells include haematopoietic rather than endothelial progenitors, and that true EPCs are not derived from CD133⁺ or CD34⁺CD45⁺ haematopoietic precursor cells, but from CD34⁺CD45⁻ cells (Bertolini et al., 2006; Case et al., 2007; Timmermans et al., 2007). Based on the information provided by various studies, Fadini et al. (2008) recently suggested CD34⁺KDR⁺ represents the best compromise of EPC phenotype in terms of detection accuracy, biological meaning and clinical usefulness. In addition, accumulating data show that two major types of EPCs can be distinguished in culture: early EPCs and late EPCs (Gulati et al., 2003a; Hur et al., 2004; Yoon et al., 2005). Early EPCs are consistent with the original cells described by Asahara et al. (1997), whereas late EPCs, namely outgrowth endothelial cells (OECs), are first demonstrated by Lin et al. (2000). The characteristics of two types of cells are summarized in Table 1, early EPCs contribute to neovasclogenesis mainly by secreting angiogenic cytokines, whereas late EPCs enhance neovasclogenesis by providing a sufficient number of ECs (Hur et al., 2004; Yoder et al., 2007). Mixed transplantation of early EPCs and late EPCs has synergistic effects (Yoon et al., 2005). As discussed in two elegant reviews, both flow cytometry and cell culture used in EPC quantification are not yet standardized and slight modifications in technique could result in the measurement of different cell populations, which prevents straightforward comparisons between different studies (Bertolini et al., 2006; Rouhl et al., 2008). Further studies are necessary to establish a golden standard methodology to identify bona fide EPCs and to understand the functional characteristics of EPC subtypes and the optimal cell type or cell combination for cell therapy under certain pathological conditions.

EPCs play an important role in vascular injury and atherogenesis. Injury of endothelial monolayer by mechanical removal of the endothelium or chronic inflammatory mobilizes EPCs from the bone marrow, which in turn mediates the regeneration of ECs and the repair of injured sites (Condon et al., 2004; Hunting et al., 2005; Libby, 2002; Ross et al., 1977; Shoji et al., 2004). Increasing data indicate that adherent platelets and fibrin, the early response to vascular injury, recruit bone marrow-derived progenitor cells to arterial thrombi in vitro and in vivo and induce their subsequent differentiation towards an endothelial phenotype (Daub et al., 2006; de Boer et al., 2006; Massberg et al., 2006; Stellos et al., 2008). Moreover, coagulation factors thrombin and protease-activated receptor-1 also stimulate bone marrow progenitor cell proliferation and EPC differentiation (Tarzami et al., 2006). Circulating EPCs may thus provide an endogenous repair mechanism to counteract ongoing risk factor-induced endothelial

injury and to replace dysfunctional endothelium under physiological conditions. However, various studies have demonstrated that in humans, cardiovascular risk factors, such as hyperlipidemia, hypertension, diabetes, smoking, physical inactivity and aging, impair number and function of EPCs (Bahlmann et al., 2005; Chen et al., 2004, 2007; Imanishi et al., 2004; Kondo et al., 2004; Krankel et al., 2005; Laufs et al., 2005; Loomans et al., 2004; Rauscher et al., 2003; Scheubel et al., 2003; Tepper et al., 2002; Wang et al., 2004). Our work also documented that both oxidized low density lipoprotein (oxLDL) and diabetes have detrimental effects on EPCs quantity and quality (Ma et al., 2006; Zhou et al., 2006, 2007). A number of reports provide evidence that levels of EPCs in healthy men may be a surrogate biologic marker for vascular function and cumulative cardiovascular risk and that reduced levels of circulating EPCs independently predicts early subclinical atherosclerosis, atherosclerotic disease progression, occurrence of cardiovascular events, death from cardiovascular causes and prognosis after ischemic stroke (Fadini et al., 2006; Hill et al., 2003; Schmidt-Lucke et al., 2005; Werner et al., 2005; Yip et al., 2008). In addition, the factors demonstrated to be protective against atherosclerosis have been correlated with increased numbers of EPCs, such as high-density lipoprotein, estrogen, statins, and angiotensin II inhibitors (Dimmeler et al., 2001; Iwakura et al., 2003; Pellegatta et al., 2006; Strehlow et al., 2003; Vasa et al., 2001a; Walter et al., 2002; Werner et al., 2002). Functional studies in atherosclerotic apolipoprotein E (ApoE) deficient mice indicate that progressive progenitor cell deficits may contribute to the development of atherosclerosis and that EPC administration can improve endothelium-dependent vasodilation (Rauscher et al., 2003; Wassmann et al., 2006). However, Xiao et al. (2007) recently reported in a population-based study that EPC number increases in subjects with higher Framingham risk scores (FRS). The discrepancy may be due to different methods used to characterize and quantify putative EPCs. They used EPC-colony forming unit (EPC-CFU) and double staining with Dil-Ac-LDL and lectin to determine EPC number, but not CD34⁺KDR⁺ cells count with flow cytometry as Vasa et al. (2001b) did. Results from the different enumerative method are not exchangeable. Dil-Ac-LDL uptake and lectin binding are typical functional properties of ECs and both early EPCs and late EPCs are acLDL⁺Lectin⁺, whereas CD34⁺KDR⁺ cells are mainly contained in early EPC subtype (Table 1). EPC-CFU is another culture method for isolating and enumerating EPCs, which may be decreased in patients at risk for vascular disease and increased during acute cardiovascular stress (Prater et al., 2007). However, Yoder et al. (2007) demonstrated recently that EPC-CFU are not EPCs but hematopoietic-derived

Table 1
Characteristics of early EPCs and late EPCs.

Classifications	Early EPCs	Late EPCs (OEC)	Publication
Origin	CD14 ⁺ and CD14 ⁻ -derived cells, which containing high levels of CD34 ⁺ KDR ⁺ cells	CD14 ⁻ -derived cells	Gulati et al. (2003a), Yoder et al. (2007), Yoon et al. (2005)
Morphology	Spindle shape	Cobblestone shape	Gulati et al. (2003a), Hur et al. (2004), Yoder et al. (2007)
Growth pattern in vitro	Appearing at 3–7 days after seeded, peak growth at 2–3 weeks and death at 4 weeks	Appearing late at 2–3 weeks after seeded, exponential growth at 4–8 weeks and living up to 12 weeks	Gulati et al. (2003a), Hur et al. (2004), Yoder et al. (2007)
AcLDL uptake and lectin binding	Yes	Yes	Gulati et al. (2003a), Hur et al. (2004), Yoder et al. (2007)
Cytokine secretion	More VEGF, IL-8, MMP9	More MMP2	Hur et al. (2004), Yoon et al. (2005)
NO production	Low	High	Gulati et al. (2003a), Hur et al. (2004)
Incorporation into HUVECs	Good	Better	Hur et al. (2004)
Tube formation	No	Yes	Gulati et al. (2003a), Hur et al. (2004), Yoder et al. (2007)
Vessel formation in vivo	No	Yes	Yoder et al. (2007)

Abbreviations: EPCs, Endothelial progenitor cells; OECs, outgrowth endothelial cells; acLDL, acetylated low-density lipoprotein; VEGF, vascular endothelial growth factor; IL-8, interleukin-8; MMP9, matrix metalloproteinase-9; MMP2, matrix metalloproteinase-2; NO, nitric oxide; HUVECs, human umbilical vein endothelial cells.

monocyte/macrophage cell colonies. Moreover, EPC number might be increased as a result of compensation in the subjects with lower risk factors. In conclusion, competent EPCs in bone marrow may play a critical role in maintaining the functional activity of the endothelium and preventing atherogenesis, whereas the impairment of EPCs by risk factors may contribute to atherogenesis and atherosclerotic disease progression.

3. Therapeutic manipulation of endothelial progenitor cells

3.1. EPC mobilization

Accumulating data demonstrate that EPCs can be mobilized and recruited to sites of injury, which might contribute to vascular reendothelialization or plaque regression.

There are many physiological modulators that can change the circulating EPC levels, including physical training, erythropoietin (EPO), estrogen, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), stromal cell-derived factor-1 (SDF-1) and vascular endothelial growth factor (VEGF). Physical training increases the production and circulating numbers of EPCs via a partially nitric oxide (NO)-dependent, antiapoptotic effect in mice and in humans (Laufs et al., 2004; Rehman et al., 2004). EPO, which is routinely used for stimulation of erythropoiesis, also potentially augments EPC levels (Bahlmann et al., 2004; Heeschen et al., 2003; Urao et al., 2006). Plasma levels of EPO are significantly associated with EPC levels in patients with coronary artery disease (CAD) (Heeschen et al., 2003). In vascular injury mice, neointimal formation was inhibited by EPO to 48% of the control (Urao et al., 2006). Estrogen has significant vasoprotective and atheroprotective effects, which are mediated in part through mobilizing EPCs (Bakir et al., 2000; Hamada et al., 2006; Iwakura et al., 2003; Krasinski et al., 1997; Strehlow et al., 2003; White et al., 1997; Xing et al., 2004; Yue et al., 2000). Estrogen deficiency by ovariectomy in mice significantly decreases EPCs circulating in the peripheral blood and residing in the bone marrow, whereas estrogen replacement can significantly increase circulating Sca-1⁺Flk-1⁺ EPCs and bone marrow-derived EPCs at reendothelialized areas compared with placebo (Iwakura et al., 2003; Strehlow et al., 2003). G-CSF is typically used for mobilization of CD34⁺ cells in patients, and has also been proved to be one of the cytokine that can be used in mobilizing EPCs (Cho et al., 2003; Fujita et al., 2007; Honold et al., 2006; Kong et al., 2004a; Papayannopoulou, 2004; Powell et al., 2005; Takahashi et al., 1999; Takamiya et al., 2006; Yoshioka et al., 2006). Injection of recombinant human G-CSF increases the number of circulating MNCs that express endothelial cell lineage markers, facilitates reendothelialization and inhibits neointima development following balloon angioplasty or intravascular radiation (Cho et al., 2003; Kong et al., 2004a; Takamiya et al., 2006; Yoshioka et al., 2006). However, recent evidence indicates that G-CSF treatment does not attenuate neointimal hyperplasia and restenosis formation in a canine model of renal arterial balloon injury (Mei et al., 2008). Similarly, the effect of G-CSF administration in the context of atherosclerosis is also controversial. Although there are some reports indicating G-CSF administration prevents the progression of atherosclerosis in rabbits and improves the clinical signs and symptoms of patients with intractable atherosclerotic peripheral artery disease (Arai et al., 2006; Hasegawa et al., 2006), recent data from other groups provide conflicting conclusions. Haghighat et al. (2007) demonstrated in ApoE deficient mice model of atherosclerosis, not only did short-term administration of G-CSF or GM-CSF fail to demonstrate any beneficial therapeutic effect, but both resulted in a worsening of atherosclerosis. Hence, the effect of EPC mobilization by G-CSF or GM-CSF in vascular injury and atherosclerosis should be further investigated. Furthermore,

stromal cell-derived factor-1 (SDF-1) and vascular endothelial growth factor (VEGF) also mobilize EPCs and enhance atherosclerotic plaque progression (Abi-Younes et al., 2000; Celletti et al., 2001; Kalka et al., 2000; Lucerna et al., 2007; Moore et al., 2001). Proinflammatory response may be a cause of the deleterious effects, which hampers the use of these mobilizing factors for endothelial regeneration in the condition of atherosclerosis.

Furthermore, EPCs can be mobilized by HMG-CoA reductase inhibitors (statins). Statins have been developed as lipid-lowering drugs and are well established to reduce morbidity and mortality from CAD. Primary and secondary prevention trials suggest that statins possess favorable effects independent of cholesterol reduction. EPCs are one of the targets of statins (Assmus et al., 2003; Dimmeler et al., 2001; Kusuyama et al., 2006; Llevadot et al., 2001; Spyridopoulos et al., 2004; Thyberg, 2002; Vasa et al., 2001a; Walter et al., 2002; Werner et al., 2002). Statin treatment enhances the circulating pool of EPCs, propagates the advent of bone marrow-derived ECs in the injured vessel wall, and, thereby, accelerates reendothelialization of the balloon-injured arterial segments and significantly reduces neointimal thickening compared with saline-injected controls (Walter et al., 2002; Werner et al., 2002). Interestingly, a recent report depicted while statin treatment transiently mobilized EPCs, initiation of statin therapy significantly diminished the number of circulating and isolated EPCs after 3 months in patients with chronic CAD. The statin dose during chronic and continuous treatment independently predicted reduced numbers of circulating as well as isolated EPCs in patients with CAD (Hristov et al., 2007). This may be explained by exhausted mobilization or by improved incorporation at sites of tissue hypoperfusion with potentially beneficial effects in therapeutic angiogenesis. Further research is welcome to elucidate the mechanisms.

The molecular mechanisms of EPC mobilization remain unclear thus far. However, several studies have indicated that the activation of the phosphatidylinositol 3 kinase (PI3K) and protein kinase B (Akt) pathway may play an important role in mobilization of EPCs. PI3K/Akt pathway is a signaling pathway known to regulate downstream NO production and cellular survival. Activation of the PI3K/Akt pathway is a putative mechanism by which statins enhance the mobilization, proliferation, migration, and survival of EPCs (Assmus et al., 2003; Dimmeler et al., 2001; Kureishi et al., 2000; Kusuyama et al., 2006; Llevadot et al., 2001; Spyridopoulos et al., 2004). EPO, estrogen and exercise are also well known to mobilize EPCs at least partially via PI3K/Akt signaling pathway (Bahlmann et al., 2004; Iwakura et al., 2003; Laufs et al., 2004; Urao et al., 2006). Furthermore, ischemia-induced mobilization of EPCs is defective in Akt1 knockout mice (Ackah et al., 2005). Lacking endothelial nitric oxide synthase (eNOS) in mice is related to a defect in EPC mobilization (Aicher et al., 2003). The marked delay in endothelial regeneration in aged rats is mediated in part by a reduced Akt and eNOS activity (Torella et al., 2004).

3.2. EPC transfection

Infusion of different EPC populations and ex vivo expanded EPCs contributes to reendothelialization and inhibits intimal hyperplasia after mechanical vascular injury (Fujiyama et al., 2003; Griesse et al., 2003; Gulati et al., 2003b, 2004; He et al., 2004; Nowak et al., 2004; Werner et al., 2003), thereby, providing a novel therapeutic option (Table 2).

However, controversy exists with respect to the effects of intravenous transfusion of EPCs in the condition of atherosclerosis. Chronic treatment with bone marrow-derived EPCs from young non-atherosclerotic ApoE^{-/-} mice prevents atherosclerosis progression in ApoE^{-/-} recipients despite persistent hypercholesterolemia (Rauscher et al., 2003). Short-term intravenous transfusion

Table 2

Studies on vascular repair by progenitor cell in vascular injury models.

Animal model	Cell used	Cell characteristics	Therapeutic effects	Publication
Rabbit model of balloon carotid injury	Autologous cultured EPCs from peripheral blood	acLDL incorporation, isolectin binding, Flk1 ⁺	Accelerated reendothelialization, improved endothelial function	He et al. (2004)
Rabbit model of balloon carotid injury	Autologous culture-modified MNCs from peripheral blood	acLDL incorporation, expression of eNOS, CD31 ⁺	Accelerated reendothelialization, improved endothelium-dependent vasoreactivity, reduced neointimal formation	Gulati et al. (2003b)
Athymic nude rat model of balloon carotid injury	Bone marrow-derived monocyte lineage cells	CD34 ⁺ /CD14 ⁺	Accelerated reendothelialization, inhibition of intimal hyperplasia	Fujiyama et al. (2003)
Mice model of balloon carotid injury	Spleen-derived mouse MNCs or cultured EPCs from spleen-derived MNCs	Cultured EPCs: acLDL incorporation, isolectin binding, Sca-1 ⁺ , CD34 ⁺ , c-kit ⁺ , VEGFR-2 ⁺	Accelerated reendothelialization, reduced neointimal formation in both cells treatment	Werner et al. (2003)
Rabbit model of balloon carotid injury	Autologous cultured EPCs from peripheral blood	acLDL incorporation, vWF ⁺ , Flk-1 ⁺ , VE-cadherin ⁺	Accelerated reendothelialization, inhibition of intimal hyperplasia	Griese et al. (2003)
Nude mouse model of balloon injury	Freshly isolated cells from peripheral blood	VEGFR-2 ⁺ or Tie-2 ⁺	Accelerated reendothelialization	Nowak et al. (2004)
Rabbit model of balloon carotid injury	Cultured OECs from rabbit PBMCs	acLDL incorporation, expression of eNOS and caveolin-1	Delivery of OECs confers greater vascular protection than PBMCs	Gulati et al. (2004)

Abbreviations: EPCs, Endothelial progenitor cells; acLDL, acetylated low-density lipoprotein; eNOS, endothelial nitric oxide synthase; MNCs, mononuclear cells; Sca-1, stem cell antigen-1; c-kit, stem cell factor receptor; VEGFR-2, vascular endothelial growth factor receptor-2 or KDR in humans, flk-1 in mice; vWF, von willebrand factor; VE-cadherin, vascular endothelial cadherin; Tie-2, angiopoietin receptor; OECs, outgrowth endothelial cells; PBMCs, peripheral blood mononuclear cells.

of spleen-derived MNCs improves endothelium-dependent vasodilation in atherosclerotic ApoE^{-/-} mice (Wassmann et al., 2006). These studies implicate that injection of ex vivo generated EPCs or MNCs is associated with an enhanced reendothelialization, an improvement of endothelial function and reduction of atherosclerotic burden, suggesting that EPC infusion holds great promise for the treatment of atherosclerosis. However, Silvestre et al. (2003) reported that transplantation of bone marrow-derived MNCs in ApoE knockout mice with hindlimb ischemia promotes further atherosclerotic plaque progression in an ischemic setting. George et al. (2005) demonstrated that administration of total bone marrow cells or spleen cell-derived EPCs results in an increase in atherosclerotic lesion size in the ApoE knockout mouse, whereas EPC transfer reduces markers associated with plaque stability. Particularly, a recent study reported that chronic EPC injection has no effect on atherosclerosis development or progression, but chronic smooth muscle progenitor cells (SPCs) injection limits plaque development and promotes changes in plaque composition towards a stable phenotype in mice (Zoll et al., 2008). It seemed that ischemic setting is unfavorable for EPC therapy in atherosclerotic model and SPCs are better than EPCs in stabilizing the atherosclerotic plaque at least in mouse model (Table 3).

These ambiguous findings call for caution in the cell characteristics, dosing and scheduling protocols of progenitor cell therapy in future studies.

3.3. EPC-based gene therapy

Aging in the presence of severe risk leads to depletion of competent progenitor cells. The partial depletion of EPCs is found in the marrow of older ApoE knockout mice and in patients of various degrees of cardiovascular risk (Hill et al., 2003; Rauscher et al., 2003). How to resurrect or enhance the ability of EPCs is a promising area of study. Here we provide examples of gene therapy.

Vasculoprotective gene eNOS is closely related to the number and function of EPCs (Aicher et al., 2003; Thum et al., 2005). Our group has demonstrated that oxLDL impairs EPCs at least partially by decreasing Akt phosphorylation and eNOS protein as well as mRNA expression in EPCs (Ma et al., 2006). Gene transfer of eNOS in rats can inhibit vascular SMCs proliferation and neointimal formation after balloon injury (Cooney et al., 2007; Janssens et al.,

1998). Transplantation of autologous EPCs overexpressing eNOS enhances the vasculoprotective properties of the reconstituted endothelium, leading to inhibition of neointimal hyperplasia in rabbits following balloon angioplasty, indicating that eNOS overexpression enhances the vasculoprotective effect of EPCs (Kong et al., 2004b). The finding suggested transplantation of genetically modified EPCs expressing vasculoprotective genes may be a feasible therapeutic option to vascular repair.

Although it may be premature to rush any conclusions based on the preliminary finding, genetic modifications of EPCs might improve EPC-mediated vascular protection via various possible mechanisms, thereby modulating the endothelial regeneration process.

4. Possible mechanisms of EPC-mediated vascular repair

The mechanisms underlying vascular repair by EPCs are yet to be made clear. However, numerous studies in animals have suggested that the vasoprotective and atheroprotective effects of EPC mobilization or transfusion are associated with cell replacement effect and non-cellular differentiation effects like trophic support and enhancement of endogenous repair process. From the characteristics of early EPCs and late EPCs discussed above, we hypothesize that early EPCs might mainly by trophic support and enhancement of endogenous repair process, whereas late EPCs mainly by cell replacement, contribute vascular repair (Fig. 1).

4.1. Cell replacement effect

Atherosclerosis is largely attributed to chronic inflammation following localized injury to the vessel wall. As a general rule, a rapid and successful repair of the arterial wall is required to contain the inflammatory reaction within physiological limits. EPCs derived from bone marrow or other tissues have an intrinsic capacity for differentiation into ECs (Asahara et al., 1997; Beltrami et al., 2003; Majka et al., 2003; Murohara et al., 2000; Planat-Benard et al., 2004; Shi et al., 1998; Werner et al., 2003). Over the past years a number of studies have described that there are ECs in neointima derived from mobilized or transferred progenitor cells which contribute to vascular repair in the vascular injury model (Griese et al., 2003; Gulati et al., 2003b; Kong et al., 2004a; Takamiya et al., 2006; Werner et al., 2003, 2002). Takamiya et al. (2006) depicted circulating c-Kit⁺/Flk-1⁺ EPCs mobilized by G-CSF

Table 3

Studies on the effect of progenitor cell mobilization or transfusion in atherosclerotic model.

Animal model	Cell used	Dosage and timing of delivery	Therapeutic effects	Publication
Cell mobilization				
Myocardial infarction-prone Watanabe heritable hyperlipidemic rabbits		G-CSF administrated at a dose of 10 µg/kg/day s.c. daily for 7 days	Reduced stenosis score of coronary artery and lipid plaque area of thoracic aorta	Hasegawa et al. (2006)
Atherosclerotic ApoE ^{-/-} mice		G-CSF or GM-CSF administered at a dose of 10 µg/kg/day s.c. daily for 5 days per week on alternating weeks for a total of 20 doses	Increased atherosclerotic lesion extent, increased adventitial vascularity, similar total cholesterol levels	Haghighat et al. (2007)
Cell transfusion				
Atherosclerotic ApoE ^{-/-} mice	BM-derived progenitor cells from young or old ApoE ^{-/-} mice	Chronic treatment Intravenous injection of 10 ⁶ cells every two weeks beginning at 3 weeks of age until 14 weeks of age	Treatment with BM-derived progenitor cells from young ApoE ^{-/-} mice prevents atherosclerosis progression, but has no effect on elevated plasma cholesterol levels	Rauscher et al. (2003)
Atherosclerotic ApoE ^{-/-} mice	Spleen-derived MNCs isolated from wild-type mice	Intravenous transfusion of 2 × 10 ⁷ cells on 3 consecutive days	Increased vascular eNOS activity, improved endothelium-dependent vasodilation Atherosclerotic lesion formation is not significantly altered	Wassmann et al. (2006)
Atherosclerotic ApoE ^{-/-} mice	BM cells or spleen cell-derived EPCs from ApoE ^{-/-} mice at 10 weeks of age	Intravenous injection 10 ⁶ cells for 3 times at 2 weekly intervals	Increased atherosclerotic lesion size in both groups, decreased plaque stability in mice injected spleen cell-derived EPCs, similar total cholesterol levels in all groups	George et al. (2005)
ApoE ^{-/-} mice with or without arterial femoral ligature	BM-derived MNCs from wild-type mice	Intravenous injection 10 ⁶ cells at the time of ischemia induction	BM-MNC transplantation without ischemia does not affect atherosclerotic plaque size BM-MNC transplantation with ischemia increases lesion size Similar total cholesterol levels and plaque composition in all groups	Silvestre et al. (2003)
ApoE(-/-)RAG2(-/-) mice	EPCs and SPCs cultured from human umbilical cord blood	Intravenous injection 5 × 10 ⁵ SPCs or EPCs every other week, one or 12 weeks after starting a high fat diet	SPCs limit plaque development and promote changes in plaque composition towards a stable phenotype in mice EPC injection had no effect on atherosclerosis development or progression	Zoll et al. (2008)

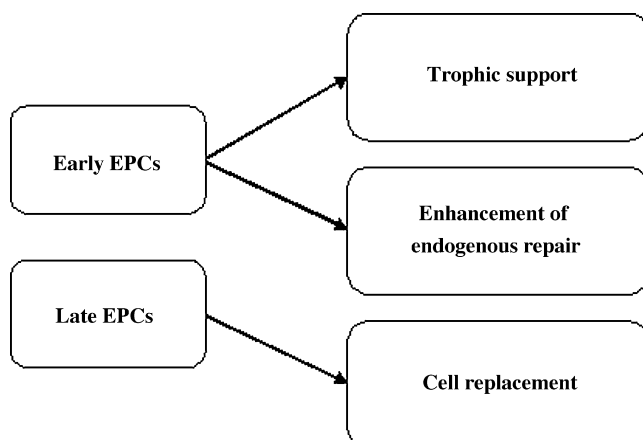
Abbreviations: G-CSF, Granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; BM, bone marrow; MNCs, mononuclear cells; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell.

occupied 39% of the total luminal length in the neoendothelium. Griese et al. (2003) demonstrated that LacZ-transduced EPC transplantation leads to about 60% reendothelialization of balloon-injured rabbit carotid arteries as early as 4 days after

transplantation and LacZ-positive cells costaining with the endothelial cell marker CD31 are seen lining the lumen of injured vessel. Furthermore, it has also been documented that in atherosclerotic ApoE^{-/-} mice, intravenous transfusion of spleen-derived MNCs improved endothelium-dependent vasodilation, and histological analyses identified exogenously applied progenitor cells expressing the endothelial cell marker CD31 in the endothelial cell layer of atherosclerotic lesions (Wassmann et al., 2006). In a recent study, Foteinos et al. reported that endothelial turnover and repair by EPCs are, at least in part, derived from bone marrow during development of atherosclerosis in ApoE^{-/-} mice (Foteinos et al., 2008). Hence, cell replacement is known as one of the mechanisms of vascular repair by progenitor cells.

4.2. Non-cellular differentiation effects

More and more researchers pay close attention to the trophic effects of EPCs. Accumulating data show that EPC-mediated vascular protection probably does not totally result from transdifferentiation into ECs (Fazel et al., 2006; He et al., 2004; O'Neill et al., 2005; Santhanam et al., 2007; Tse et al., 2007; Yoshioka et al., 2006). In vitro, early EPCs release angiogenic cytokines including G-CSF, GM-CSF, VEGF, platelet-derived growth

**Fig. 1.** Possible mechanisms of vascular repair by EPC.

factor-BB (PDGF-BB), epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), interleukin-8 (IL-8), transforming growth factor- β 2 (TGF- β 2), etc. (He et al., 2004; Hur et al., 2004; Rehman et al., 2003). Early EPCs exert a strong paracrine mitogenic effect on mature ECs in part via IL-8 secretion (He et al., 2005). In hypoxia, mobilization of bone marrow-derived cells enhances the angiogenic response to hypoxia through paracrine release of growth factors but not transdifferentiation into ECs (O'Neill et al., 2005). Beneficial effects of progenitor cells in cardiac repair are partially by trophic support (Fazel et al., 2006; Tse et al., 2007). In cerebral arteries, paracrine effect of EPCs promotes vasoprotection by increasing prostacyclin (PGI₂) production and intracellular concentration of cAMP (Santhanam et al., 2007). Furthermore, Cho et al. recently reported that myocardial EPC transplantation induces humoral effects, which are sustained by host tissues. They found up-regulation of multiple humoral factors is sustained for longer than 2 weeks, though most of the transplanted EPCs disappear within a week. By injecting human EPCs into ischemic myocardium of immunodeficient mice, they proved that the expression of human EPCs (donor)-derived cytokines rapidly decrease to a non-detectable level within a week, but up-regulation of mouse (recipient)-derived cytokines, including factors that could mobilize bone marrow cells, is sustained. Moreover, they found bone marrow-derived stem or progenitor cells are increased in the peripheral circulation and incorporated into the site of neovascularization and myocardial repair after EPC transplantation. They suggested that EPC transplantation simulates endogenous repair progress, which plays a crucial role in repairing myocardial injury (Cho et al., 2007).

Although non-cellular differentiation effects of EPCs in the condition of atherosclerosis is yet to be studied, from these findings, we can hypothesize that trophic support and enhancement of endogenous repair process may play a role in vascular repair by EPCs.

5. Potential risks of EPC mobilization and transfusion in atherosclerosis

Despite these encouraging results that mobilization or transfusion of EPCs contributes to vascular repair, potential unfavorable effects on plaque progression and stability should not be neglected.

Firstly, mobilization or transplantation of early EPCs might promote atherosclerosis by augmenting disease-associated unfavorable angiogenesis. Early EPCs secrete many angiogenic cytokines and play an important role in angiogenesis. Proinflammatory properties harbored by these cells may contribute to enhanced atherogenesis. Silvestre et al. (2003) reported bone marrow-derived MNCs transplantation promotes further atherosclerotic plaque progression in an ischemic setting. Ischemia induced release of growth factors predominantly accounts for this effect. George et al. (2005) described that transfer of a large number of spleen cell-derived EPCs and bone marrow cells accelerates atherosclerosis in ApoE knockout mice and that the plaques from ApoE knockout mice infused with spleen cell-derived EPCs contain smaller fibrous caps, larger lipid cores and a larger number of CD3 cells, suggesting that angiogenic effect of a large number of cells transfer partially accounts for the possible proatherogenic effect. In a recent study, Haghighat et al. (2007) documented that in ApoE deficient mice maintained on a high-fat diet, both G-CSF and GM-CSF actually demonstrate an increase in atherosclerotic lesion extent and the increase in atherosclerotic extent is associated with an increase in adventitial vascularity, suggesting a mechanistic role for vasa vasorum neovascularization. The functions of the vasa vasorum neovascularization are proposed to be important regulators of plaque growth and lesion instability, for these plaque

capillaries sustain perfusion, deposit proatherogenic plasma molecules, recruit immune cells and progenitors, and promote intraplaque hemorrhage (Moreno et al., 2006; Moulton, 2006). In this respect, late EPC subtype is probably a better candidate for atheroprotection.

Secondly, EPCs might contribute atherogenesis in the presence of platelets that adhere to the injured vessel wall. Platelets could induce differentiation of CD34⁺ progenitor cells into foam cells (FCs) and most CD34⁺ cells do not differentiate into ECs in the presence of platelet. This indicates that platelet-dependent CD34⁺ cell homing in peripheral organs is critically involved in the regeneration of vascular lesions or ischemic tissue (Daub et al., 2006; Stellos et al., 2008).

Thirdly, EPCs might promote atherogenesis by recruiting SMCs. Controversy exists on the exact origin of recruited SMCs in atheroma (Bentzon et al., 2007, 2006; Caplice et al., 2003; Mayr et al., 2008; Sata et al., 2002). Sata et al. (2002) depicted that bone marrow-derived cells give rise to most of the smooth-muscle-like cells that contribute to arterial remodeling in hyperlipidemia-induced atherosclerotic mice. Caplice et al. (2003) documented by autopsy that SMCs in human coronary atherosclerosis arise from bone marrow. It is well known that recruiting of SMCs to the intima of the vessel wall is a significant contributor to atherosclerotic plaque progression (Schwartz, 1997).

Taken together, although circulating EPCs normally repair and rejuvenate the arteries under physiological conditions, EPCs contribute not only to vascular healing but also to lesion formation under certain pathological conditions. EPCs may protect against lesion formation in early lesion, decrease plaque stability in advanced lesion and promote atherosclerotic plaque progression in ischemic setting. The regeneration of the endothelium by EPCs is atheroprotective, whereas plaque angiogenesis induced by EPCs and SMC or FC differentiation of EPCs may also contribute to atherogenesis, which has to be considered for a potential therapeutic application (Fig. 2). Understanding of the regulatory network that controls the EPCs for healing damaged endothelium or recruits EPCs to the growing atherosclerotic plaque is a biologically interesting question and clinically relevant work in future.

6. Therapeutic implications and perspective

Evidence that EPC mobilization or transfusion might play a role in preventing atherosclerosis is provided by basic research of mechanical vascular injury and hyperlipidemia-induced atherosclerosis. Although it seems that there is broad consensus in EPCs contributing to vascular repair in mechanical vascular injury model, the effect in atherosclerotic model arouses controversy. A major limitation of most experimental studies in mechanical vascular injury is that injury is assessed in healthy arteries. Given the complexity of human atherosclerotic lesions, none of the vascular injury models would represent the exact pathogenesis and much more aspects should be taken into account when choosing EPC mobilization or transfusion as anti-atherosclerotic therapy.

As discussed above, angiogenic cytokines like G-CSF, GM-CSF and VEGF mobilize not only EPCs but also inflammatory cells. Many leukocyte subsets, including monocytes, mast cells and neutrophils, involve in the pathology of atherosclerosis in mice and there is complex equilibrium and interplay between progenitor cells and other cells that contribute to the process of atherosclerosis (Weber et al., 2008; Zernecke et al., 2008). Proinflammatory response provoked by the inflammatory cells would promote further atherosclerotic plaque progression, which hampers the use of these mobilizing factors as anti-atherosclerotic therapy. Evidence indicated that short-term administration of G-CSF and

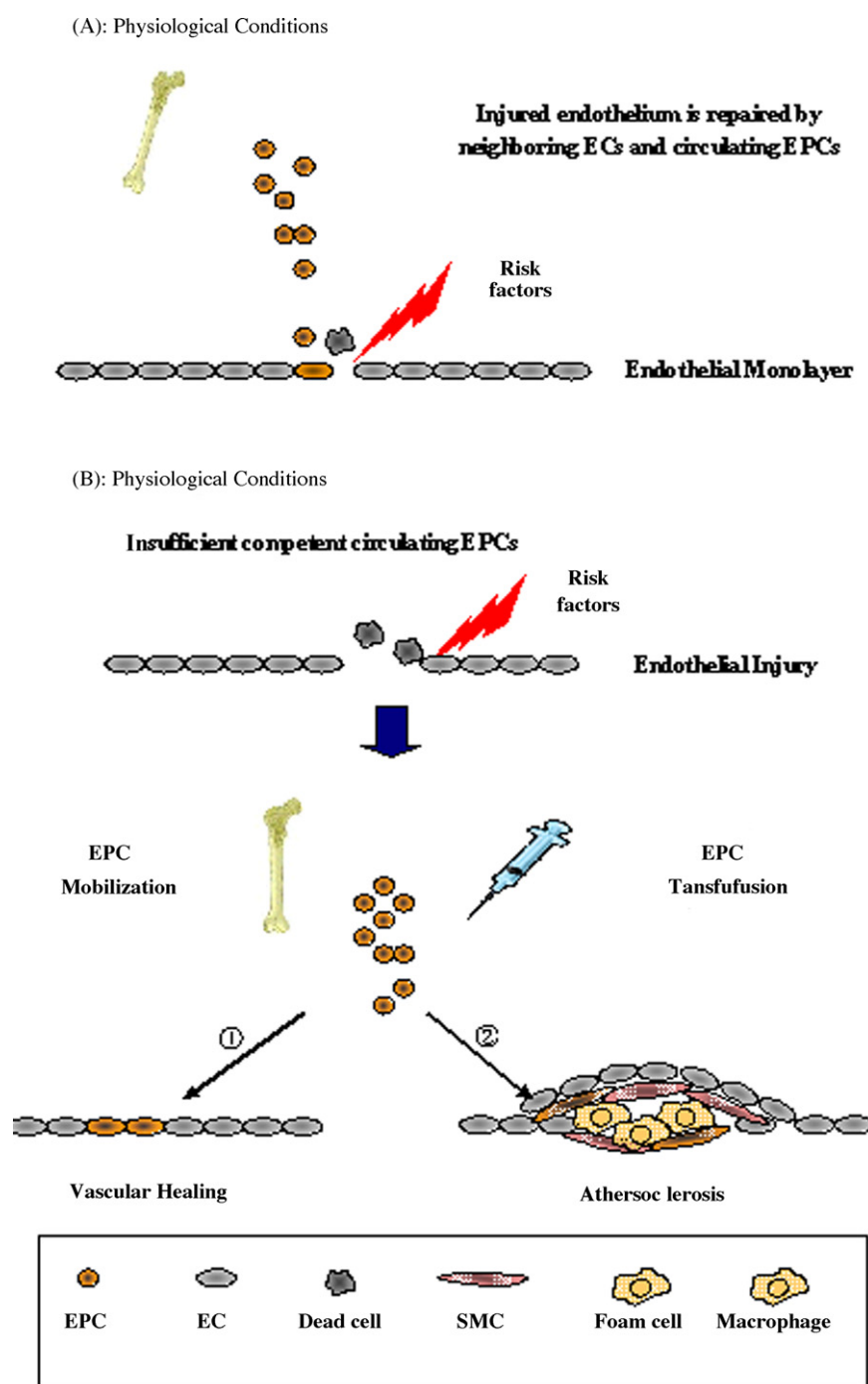


Fig. 2. Contributions of EPCs to vascular repair and lesion formation under physiological and pathological conditions. (A) Under physiological conditions, ongoing risk factor-induced endothelial injury is repaired by neighboring ECs and circulating EPCs. (B) Under pathological conditions, EPC insufficiency and impaired function are a possible cause of the insufficient repair of endothelial injury. EPC mobilization or transfection contributes not only to vascular healing but also to lesion formation under certain pathological conditions. The regeneration of the endothelium by EPCs protects against lesion formation (①), whereas plaque angiogenesis by EPCs and FC or SMC differentiation of EPCs may also contribute to atherogenesis (②).

GM-CSF has atheroprotective effects, but more frequent administration of these cytokines enhances atherosclerotic plaque progression, suggesting that short-term administration may be a better option (Table 3). Furthermore, body condition is a major limiting factor for mobilization of EPCs. Aging in the atherosclerosis milieu may accelerate the consumption of bone marrow EPCs (Zhu et al., 2007). Elderly patients show a limited response towards EPC mobilizing/differentiation stimuli (Dimmeler and Vasa-Nicotera, 2003; Scheubel et al., 2003). Bone marrow cells

from young and wild type, but not old ApoE^{-/-} mice are capable of preventing atherosclerosis (Rauscher et al., 2003). Partial depletion of EPC number and function is related to risk factors (Vasa et al., 2001b). The migratory response to SDF-1 and in vivo capacity of G-CSF-mobilized EPCs is significantly reduced in patients with chronic ischemic heart disease (Honold et al., 2006). Of note, these issues should be taken into account when therapeutic mobilization of autologous EPCs is applied for treatment of atherosclerosis. In a word, the body condition and the proinflammatory effects of

therapeutic EPC mobilization by cytokines in the condition of atherosclerosis have to be considered for a potential therapeutic application.

In regard to EPC transfusion, we should take into account the characteristics of therapeutically acceptable cells and the body condition of receptor. At present, there is no consensus on the exact phenotype of the EPCs and therefore the best candidate cell for transplant has not been established. The nature of the administered cells is different in each of the studies as well as scheduling protocols, which prevents an accurate comparative analysis and may be largely responsible for discrepancies between published data regarding this cell type. Given the proinflammatory properties harbored by early EPCs, late EPCs might be a better candidate (Table 1). Recent evidence indicated SPC may favor plaque stability during advanced atherosclerosis (Zoll et al., 2008). Further research is necessary to identify the best cell that has atheroprotective effect in different disease stage. Furthermore, the atherosclerosis milieu would affect the functional activity of transferred cells. A suitable body condition scoring according to EPC transfusion is required. As shown in Table 3 ischemic setting may unfavorable for EPC therapy. In different stages of atherogenesis there are a series of different changes in vessel (Lusis, 2000). In a recent review Hristov and Weber (2008) discussed the double-edged effect of circulating vascular progenitor cells in primary atherosclerosis. Hence, we can presume that the effects of progenitor cell infusion at different time points are different. Suitable scheduling protocols of progenitor cell therapy need to be established.

On the whole, there are many unresolved questions regarding EPC therapy in atherosclerosis. We must clarify these issues before we translate these novel strategies into clinical application.

7. Conclusions

In conclusion, much remains to be learned about the vascular repair process in the condition of atherosclerosis. The improper use of EPC mobilization or transfusion might promote the atherosclerotic disease progression. Further understanding of the biology of EPC subtypes, elucidating the definitive contribution and the mechanisms responsible their putative role in atheroprotection/atherogenesis will be challenging and informative.

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