Endothelial progenitor cells in pregnancy

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Abstract

The discovery of endothelial progenitor cells has generated considerable interest in the field of vascular biology. These cells arise from a population of circulating mononuclear cells and have the capacity to form new blood vessels and contribute to vascular repair. Circulating endothelial progenitor cell numbers are reduced in patients with cardiovascular risk factors and in the presence of endothelial dysfunction, but are increased in response to ischaemia, oestrogens and drug therapy. They have been studied in pathologies from cardiovascular and renal disease to rheumatoid arthritis and pre-eclampsia. Pregnancy is a challenge to the maternal vascular system, requiring systemic adaptation and pronounced local changes in the uterus. Diseases of pregnancy such as pre-eclampsia and gestational diabetes increase the risk of pregnancy complications and are associated with endothelial dysfunction. We propose that endothelial progenitor cells have an important role in the regulation and maintenance of the vasculature during pregnancy. This review summarises our current understanding of endothelial progenitor cells, with specific reference to their role in angiogenesis and human pregnancy.

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Introduction

The observation of an affinity between endothelial cells and haematopoietic cells was first reported in 1920 by Florence Sabin. Until recently, differentiation of endothelial cells from angioblasts was thought to exclusively occur within the developing embryo. In 1997, Asahara and colleagues isolated a population of bone marrow-derived cells from human peripheral blood capable of ex vivo expansion, differentiation into a mature endothelial phenotype and neovascularisation in response to acute tissue ischaemia (Asahara et al. 1997). Shortly after this, Shi and colleagues observed that bone marrow cells were important for the endothelialisation of intra-aortic Dacron grafts in dogs (Shi et al. 1998). These studies suggest the existence of a population of circulating endothelial progenitor cells (EPCs), which are mononuclear, cells arising in bone marrow with the capacity to differentiate into mature endothelial cells. Normal human pregnancy involves adaptation of maternal vasculature to accommodate and sustain the developing foetus. Abnormal adaptation of the uterine vasculature is associated with deficient placentation and some diseases of pregnancy including pre-eclampsia and gestational diabetes are associated with systemic endothelial dysfunction (Brosens et al. 1972, McCarthy et al. 1993, Knock et al. 1997). We postulate that EPCs play an important role in development, regulation and maintenance of the vasculature during pregnancy. This review summarises our current understanding of the origin and function of EPCs, and highlights their potential role in angiogenesis and vascular repair in human pregnancy.

Characterisation of circulating EPCs

EPCs are characterised by their expression of both haematopoietic and mature endothelial cell antigens, and by their ability to proliferate, migrate and differentiate into mature cell types. Asahara and colleagues exploited two antigens shared by endothelial cells and haematopoietic stem cells (HSCs) to isolate putative EPCs from peripheral blood (Asahara et al. 1997). CD34 is expressed by most mature endothelial cells (Fina et al. 1990) as well as all HSCs but is lost by haematopoietic cells as they differentiate (Civin et al. 1984). Kinase insert domain receptor (KDR), the extracellular domain of vascular endothelial growth factor receptor (VEGFR) (Shalaby et al. 1995), is also expressed by both early HSCs and endothelial cells but is lost on haematopoietic cell differentiation (Matthews et al. 1991). CD34⁺ and KDR⁺ cells, isolated from peripheral blood leucocytes
form vascular structures in vitro and incorporate into the vessel wall in experimental models of neovascularisation (Asahara et al. 1997).

Co-expression of CD34 and KDR has been used in a number of experimental and clinical studies to identify circulating EPCs. No surface marker unique to endothelial progenitors has been identified and so it remains difficult to distinguish EPCs from mature endothelial cells that have been swept into the circulation or haematopoietic cells. CD133 is expressed by haematopoietic cells, but not by mature endothelial cells. Identification of CD133, KDR and CD34 co-expression may differentiate between circulating mature and progenitor endothelial cells (Peichev et al. 2000). The rarity of EPCs in peripheral blood (100–200 cells/ml), has made their study difficult.

Alternative methods have been described for the characterisation and quantification of EPCs based on the culture of endothelial cells from circulating mononuclear cells. A number of functional assays have been reported, most involving the isolation of peripheral blood mononuclear cells by density centrifugation of blood and subsequent culture on fibronectin coated plates. After 5–7 days in culture, adherent colonies are seen, where spindle shaped cells emerge from a cluster of round cells (EPC colony forming units, EPC-CFUs). These adherent cells display a variety of endothelial-like properties including the uptake of acetylated low density lipoprotein (AcLDL) and staining with UEA-1 (Fig. 1), a lectin of Ulex europaeus, specific for endothelial cells in a variety of tissues binding to the carbohydrate moiety α-l-fucose (Stephenson et al. 1986).

Whilst counting EPC-CFUs measures the capacity of circulating mononuclear cells to form endothelial cells, the colonies may not directly arise from the CD34⁺ stem cells. The exact phenotype of EPC-CFUs remains a matter of debate in part because the purity of CD34⁺ cells used in the initial Asahara study was only 15% (Asahara et al. 1997). Peripheral blood contains several cell types that can differentiate into endothelial-like cells in vitro, including haematopoietic stem cells, mononuclear phagocytes (monocyte-macrophages), and mature endothelial cells (Ingram et al. 2005). In a recent methodological paper by George and colleagues no correlation was found between the number of peripheral blood CD34⁺ cells and the number of EPC-CFUs (George et al. 2006).

Studies addressing the origin of EPCs have demonstrated that monocytes express endothelial lineage markers such as KDR and can differentiate into endothelial cells (Schmeisser et al. 2001). Rehman and colleagues found that the majority of EPC-CFUs expressed monocyte markers such as CD14, Mac-1, and CD11c, suggesting that peripheral blood EPCs are derived from monocyte-like cells (Rehman et al. 2003). The concept that functional endothelial cells may originate from a CD14⁺ progenitor is supported by

Figure 1 (A) A typical colony-forming unit (EPC-CFU) with a characteristic core of round cells and sprouting spindle cells at the periphery (×100 magnification). (B) Overlay with immunofluorescence staining of EPC-CFU demonstrating uptake of Dil-acetylated-LDL (red) (×100 magnification). (C) Fluorescent micrograph of cultured EPCs 4 days after isolation from peripheral blood, stained with lectin Ulex europaeus-FITC (green) and demonstrating uptake of Dil-acetylated-LDL (red) (×40 magnification). Figure 1(C) reproduced with permission from Kalka et al. (2000).
reports that mature endothelial cells from human umbilical vein express CD14 (Jersmann et al. 2001) and that isolated CD14<sup>+</sup> cells can improve neovascularisation after mouse hind limb ischaemia (Urbich et al. 2003). We present a diagram outlining two potential ways that endothelial cells might arise from haematopoietic stem cells via myeloid or endothelial progenitor subtypes (Fig. 2).

In this emerging field, many different methods have been used to characterise and quantify putative endothelial progenitors, preventing straightforward comparison. Reduced levels of both phenotypic (Schmidt-Lucke et al. 2005) and functional EPCs (Werner et al. 2005) predict adverse outcome in patients with coronary artery disease. Whilst questions remain as to the origin and phenotype of EPCs, evidence that they contribute to vascular repair and neoangiogenesis in animal models is compelling (Table 1).

**Mobilization and differentiation of EPCs**

The vascular endothelium is a monolayer of cells central to the regulation of blood vessel tone and permeability. It acts as a non-adhesive surface for leucocytes and platelets, and produces important factors in the regulation of fibrinolysis and blood flow. Endothelial dysfunction caused by mechanical or biochemical stress and inflammation is characterised by reduced nitric oxide bioavailability and a progressive loss of endothelial cells (Libby 2002, Wassmann & Nickenig 2003). Endothelial denudation is one of the earliest pathophysiological features of vascular disease, with persistent endothelial dysfunction responsible for the progression and clinical manifestations of atherothrombosis (Ross 1999, Weissberg & Bennett 1999). Integrity of the vascular endothelium is a dynamic equilibrium between endothelial degeneration and repair (Karter et al. 2004).

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**Figure 2** Possible pathways by which endothelial cells may arise from haematopoietic stem cells. Haemangioblasts give rise to circulating haematopoietic stem cells of myeloid or endothelial progenitor subtypes, with endothelial cells potentially derived from either pathway.
Mechanisms of EPC mobilisation in conditions other than ischaemia may be more relevant to understanding the role of EPCs in maternal circulation during pregnancy. The effect of oestrogens in maintaining endothelial function may be related to EPC mobilisation and enhanced vascular repair. In a rat-carotid injury model, exogenous oestradiol accelerates re-endothelialisation and attenuates medial thickening via mobilization and proliferation of bone marrow-derived EPCs. This response was absent in endothelial nitric oxide synthase (eNOS) knock out animals (Iwakura et al. 2003). Recent mouse studies with oestrogen receptor (ER) α and β knockout animals demonstrate roles for both receptors α and β in EPC-mediated neovascularisation in response to ischaemia. In addition, ERz messenger RNA expression was higher than ERβ messenger RNA expression in EPCs. VEGF expression was significantly down-regulated on EPCs from ERz knockout mice compared with EPCs from wild type animals (Hamada et al. 2006).

### Vascular remodelling in pregnancy

Growth of endometrial vasculature in preparation for implantation begins in the proliferative phase and continues in the secretory phase of the menstrual cycle. This physiological angiogenesis is thought to occur primarily through elongation and intussusception of existing small vessels (Gargett & Rogers 2001). Sprouting is important in placentation and is also a feature of angiogenesis associated with pathology such as ischaemia (Reynolds & Redmer 2001). A role for EPCs in physiological endometrial angiogenesis has been implicated by Asahara and colleagues where EPCs were demonstrated within vasculature and stroma of the endometrium and myometrium after induced ovulation in mice (Asahara et al. 1999). Increased circulating levels of EPCs are seen during the secretory phase of the menstrual cycle in human subjects (Matsubara et al. 2006).

The uterine vasculature undergoes dramatic remodelling during pregnancy. In addition to vasodilation of the uterine artery, remodelling of maternal spiral arteries provides a large vascular bed perfusing the placental intervillous space with maternal blood (Brosens et al. 1967). During placentation, this remodelling is mediated by interstitial and endovascular trophoblast invading maternal vessels (for a review see Pijnenborg et al. 2006). During the wave of trophoblast invasion, maternal spiral artery endothelium is extensively damaged and then repaired. This results in a fresh layer of endothelium (Pijnenborg et al. 2006). The repair is thought to be effected by local endothelial cells though it is plausible that circulating EPCs are involved.

### Vascular and endothelial function in pregnancy

Pregnancy necessitates cardiovascular adaptation to sustain the developing foetus. Initially, peripheral

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**Table 1** Characteristics of endothelial progenitor cells

| Phenotypic | Cell-surface expression of haematopoietic stem-cell (CD34, CD133) and endothelial-cell antigens (KDR) |
| Functional | Produce characteristic colonies and tubular structures on fibronectin in vitro |
| | Migrate and incorporate into areas of vascular damage in vivo |
| | Differentiate into mature endothelial cells |

The concept of a pool of endothelial progenitors in the bone-marrow, capable of moving to effect angiogenesis or vascular repair in response to ischaemia or vascular injury is supported by both *in vitro* and *in vivo* studies. In a mouse model, Asahara and colleagues demonstrated differentiation of donor bone marrow cells into endothelial cells and their subsequent incorporation into the vasculature during processes such as ovulation, wound healing, recovery from hind limb ischaemia and neoplasia (Asahara et al. 1999).

Reduced circulating EPC levels are observed when established cardiovascular risk factors are present suggesting a role for EPCs in the maintenance of endothelial function. Decreased numbers of EPCs have been demonstrated in cigarette smokers (Vasa et al. 2001) and in patients with diabetes mellitus (Tepper et al. 2002) or rheumatoid arthritis (Grisar et al. 2005). Hill et al. (2003) observed a strong correlation between the Framingham cardiovascular risk score (which uses cardiovascular risk factors to predict future risk of coronary artery disease (Wilson et al. 1987)) and circulating EPC numbers. EPC numbers predicted systemic endothelial function more accurately than the Framingham risk score (Hill et al. 2003). Furthermore, lower levels of EPCs are associated with adverse outcome in patients with coronary artery disease (Schmidt-Lucke et al. 2005, Werner et al. 2005) and impaired myocardial remodelling after infarction (Leone et al. 2005).

The postulated factors responsible for mobilization of EPCs from the bone marrow are the subject of an intense search. Such a factor might form a therapeutic strategy to enhance vascular repair. EPCs are released in the context of acute ischaemic injury, such as myocardial infarction (Leone et al. 2005, Massa et al. 2005) and following vascular injury as a consequence of coronary artery bypass grafting (Gill et al. 2001). Vascular endothelial growth factor (VEGF) and stromal cell-derived factor-1 (SDF-1), both released from ischaemic tissue, are thought to be important factors in the mobilisation of EPCs. Other cytokines such as granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) mobilise both haematopoietic progenitors and EPCs. These have been used for many years to harvest progenitors for autologous bone marrow transplantation in the context of haematological malignancy. However, they also induce a pro-inflammatory state which may limit their therapeutic potential.

Endothelial function is preserved in spite of the systemic inflammatory response associated with normal pregnancy. There is increased production of pro-inflammatory cytokines, including interleukin (IL)-6, IL-12, and tumour necrosis factor (TNF)-α with associated leucocytosis. The neutrophil count rises steadily throughout gestation, to peak at term (Austgulen et al. 1994, Rebelo et al. 1995, Melczer et al. 2003, Sacks et al. 2003). As well as being a pro-inflammatory state, normal pregnancy is associated with increased insulin resistance and hyperlipidaemia, controlled by hormonal changes. Pregnancy might be considered a ‘stress’ test for the maternal vascular endothelium (Sattar & Greer 2002).

Potential role of EPCs in normal pregnancy

Only three cross-sectional studies of circulating EPCs during normal human pregnancy have been reported to date. Different methods have been used to characterise and quantify putative endothelial progenitors, preventing straightforward comparisons between studies. The first, by Sugawara et al. (2005a) studied circulating EPCs in the peripheral blood of 20 pregnant women. They observed greater numbers of colony-forming units (EPC-CFUs) at greater gestational age. EPC-CFUs correlated with serum oestradiol concentrations (Sugawara et al. 2005a). Oestrogens are known to have vasculo-protective effects, in part due to increasing production of nitric oxide and by decreasing reactive oxygen species (Mendelsohn & Karas 1999). Oestrogens also mobilise EPCs from the bone marrow in vivo. They inhibit the senescence of EPCs and stimulate VEGF production in vitro (Strehlow et al. 2003, Imanishi et al. 2005). Mobilisation of EPCs may be an important mechanism by which oestrogens protect the vascular endothelium during pregnancy.

A second study by Gussin et al. (2002) supports this hypothesis. They cultured peripheral blood mononuclear cells from non-pregnant and pregnant women. Early outgrowth endothelial cells were formed from both groups. Late outgrowth cells, which have a higher proliferative potential, were only formed by the cells from pregnant women. The authors initially hypothesized that these cells were of fetal origin and their original intention was to optimise the culture of fetal cells. To identify fetal cells, they stained for X and Y chromosomes and discovered that none of the colonies contained fetal cells. They concluded that endothelial cells were of maternal origin and that pregnancy is associated with mobilization of EPCs into the circulation (Gussin et al. 2002).

In contrast, a study by Matsubara and colleagues of 36 healthy pregnant women observed decreasing numbers of circulating EPCs with increasing gestational age. They directly quantified EPCs by flow-cytometry, selecting for co-expression of CD34, CD133 and KDR. They also assessed EPC proliferation by counting cells that stained for both lectin Ulex europaeus and the uptake of

![Figure 3](image-url) Proposed mechanisms by which EPCs may contribute to angiogenesis in human endometrium. Mature resident endothelial cells (purple) and proliferating mature resident mature endothelial cells (blue). Circulating EPCs (yellow) may incorporate into endothelial monolayer at points of elongation and intussusception, or proliferate to form new micro vessels (sprouting). Circulating EPCs may have a supportive paracrine effect on adjacent mature endothelial cells, through the release of angiogenic factors including VEGF. EPCs release VEGF in vitro and in vivo murine studies (Urbich et al. 2005). Adapted with permission from Gargett CE & Rogers PA 2001 Human endometrial angiogenesis. Reproduction 121 181–186.
acetylated-LDL after 7 days in culture. With increasing gestation, they found decreased numbers of these cells and decreased responses when stimulated by TNF-α and angiotensin II (Matsubara et al. 2006).

It is difficult to compare the results of these studies because different methods were used to count EPCs. It remains uncertain whether the cells measured by flow-cytometry are responsible for forming endothelial-like structures in cell culture. It is possible that the reduction in CD34+/CD133+/KDR+ cells is caused by dilution in expanding plasma volume. A limit of all three studies is the cross-sectional study design. Many factors affect circulating EPC numbers were not described for the study subjects. Prospective studies following women through gestation would provide more information about EPCs in pregnancy.

Vascular dysfunction and EPCs in diseases of pregnancy

Pre-eclampsia and gestational diabetes are associated with maternal endothelial dysfunction (McCarthy et al. 1993, Knock et al. 1997). Pre-eclampsia is the only disease of pregnancy in which EPCs have been studied. Pre-eclampsia, occurring in 5–7% of first pregnancies with 20–25% recurrence is the most common medical complication of pregnancy. The disease, characterised by hypertension and proteinuria, is a significant cause of maternal and perinatal morbidity and mortality worldwide (Stone et al. 1994).

Despite recent advances, the pathogenesis of pre-eclampsia remains unclear. Deficient placentalisation (decreased trophoblast invasion and subsequent spiral artery remodelling) is seen histologically in most women with pre-eclampsia (Broens et al. 1972, Pijnenborg et al. 1991). Maternal endothelial dysfunction is a core feature of pre-eclampsia. Vascular cellular adhesion molecule-1 (VCAM), intercellular adhesion molecule-1 (ICAM), E-selectin, endothelin-1 and cellular fibronectin, all soluble markers of endothelial dysfunction are raised in the blood of women with pre-eclampsia. Some are evident before the clinical features of the disease (Taylor et al. 1991, Schiff et al. 1992, Kraayenbrink et al. 1993, Higgins et al. 1998, Bretelle et al. 2001). Other markers of endothelial dysfunction including asymmetric dimethylarginine (an endogenous inhibitor of nitric oxide synthesis) (Savvidou et al. 2003), plasminogen activator inhibitor type 1 (PAI-1) (Roes et al. 2002) and tissue plasminogen activator (t-PA) (Belo et al. 2002), also rise before clinical symptoms appear with t-PA correlating with the degree of proteinuria (Belo et al. 2002). Women with pre-eclampsia are more likely to have impaired uterine artery doppler waveforms (Campbell et al. 1983) and reduced flow-mediated dilation of the brachial artery at 23–25 weeks gestation, suggesting that endothelial dysfunction precedes pre-eclampsia (Savvidou et al. 2003). Endothelial dysfunction, observed in pre-eclampsia, persists beyond pregnancy (Lampinen et al. 2006) and epidemiological data suggest an increased maternal risk of hypertension, coronary and cerebro-vascular disease (Wilson et al. 2003).

Pre-existing conditions associated with endothelial dysfunction, such as hypertension, renal disease, and diabetes, increase the risk of developing pre-eclampsia. Although there are extensive studies reporting decreased levels of EPCs or abnormal function of EPCs in men and non-pregnant women with these conditions, there are few available data about EPCs in pre-eclampsia. In addition, as with the studies in normal pregnancy, the published data are conflicting. Matsubara and colleagues reported no difference in the number of circulating EPCs measured by flow-cytometry in pre-eclamptic women although culture of mononuclear cells resulted in more endothelial-like cells. These cells had increased proliferative response following stimulation with TNF-α and angiotensin II compared with cells from women without pre-eclampsia (Matsubara et al. 2006). This increase may be a physiological response to ischaemia in the placenta and other organs, similar to that seen in myocardial infarction (Leone et al. 2005, Massa et al. 2005). In contrast, Sugawara et al. (2005b) demonstrated decreased numbers of EPC-CFUs and increased senescence of EPCs in patients with pre-eclampsia compared with gestationally matched controls (Sugawara et al. 2005b). These studies are not easily comparable because of the different methods used, and it is difficult to draw a conclusion from the discordant observations. It is likely that EPC function is more important than quantity, and ideally a subsequent study of EPCs in pre-eclampsia would assess number and function prospectively prior to the onset of disease.

Postulated role of EPCs in pre-eclampsia

The role of EPCs in the pathogenesis of pre-eclampsia is as yet unknown. However from studies outside of pregnancy, EPCs have a potential role in maintaining vascular integrity. Decrease in number and function is associated with endothelial impairment. We hypothesise that EPCs represent a common cellular pathway linking cardiovascular risk factors and endothelial dysfunctional states, with pre-eclampsia. They may be involved in homeostasis of the pregnant uterine and systemic vasculature. Their deficiency or impairment may render the individual vulnerable to the inflammatory and metabolic insults of pregnancy, leading to widespread endothelial dysfunction and pre-eclampsia. Fig. 4 represents this hypothesis schematically.

Future directions

Future research strategies evaluating the role of EPCs in pregnancy will hopefully address the following...
questions: Are EPCs useful markers or predictors for pre-eclampsia? Do levels reflect severity of disease?

EPCs may also have a role in other pathologies of pregnancy such as gestational diabetes. Prospective longitudinal studies are required to assess EPC quantity and function during and after pregnancy. In addition, animal studies and in vitro models will provide us with a greater understanding of the role of EPCs in the maintenance of the endometrial vasculature and in placentation.

Summary

The discovery of a circulating endothelial progenitor cell that is capable of contributing to physiological and pathological angiogenesis has generated intense interest in the field of vascular biology. To date this has focused particularly in the areas of vascular injury and atherosclerosis where the therapeutic possibilities of EPCs are already being explored. We have summarised the current literature on EPCs in pregnancy and postulate that these progenitors may have an important role in regulating vascular homeostasis in pregnancy. Their impairment or deficiency may underlie maternal susceptibility to pre-eclampsia.

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